Pak. J. Bot., 38(4): 1071-1078, 2006.

RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.) VAR. CHAKWAL-97 TO ARTIFICIAL AGEING IN RELATION TO ITS VIABILITY UNDER MID-TERM CONSERVATION IN GENEBANK

ASJAD ALI[*], SADAR UDDIN SIDDIQUI[**], M. AFZAL[**] AND M. FAYYAZ CHAUDHARY[*]

[*]*Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[**]*National Agricultural Research Centre, Islamabad, Pakistan*
email-ssadar@hotmail.com

Abstract

Wheat is most important among cereal crops in terms of area and production and is a staple food for more than one third of the world's population. Seed storage for germplasm conservation is a big problem, as viability is decreased even under the optimal storage conditions in the genebank. About 85% viability is minimum requirement to store seeds in the genebanks for conservation purpose.

Seeds of some plant species loose viability faster than others and there may be varietal responses within species. Artificial seed ageing (AA) is used to check seed viability and germinability potential. The objective of the present study was to determine the affect of artificial seed ageing on seed viability, while outlining the contribution of different factors like temperature and moisture in seed ageing.

Wheat (*Triticum aestivum* L.) var. Chakwal-97 with two types of seed initial moisture contents i.e. low (LMC) and high (HMC) was used as experimental material. Treatments comprised of three temperature regimes (40, 50 and 60^{o}C) each with 4 types of incubation durations (24, 48, 72 and 96 h). Two type of relative humidity (RH) [ambient (low) or high up to 100%] was maintained in seed containers during incubation. Between paper towel (BP) method was used for germination at 25 ^{o}C (± 2) under light conditions to check the seed viability after artificial ageing.

It was concluded by visualizing the germination behavior of artificially aged seeds of wheat (*Triticum aestivum* L.) variety Chakwal-97 that the viability decreases with the increase in AA incubation temperature. Incubation of seeds under high relative humidity (RH) has more effect in decreasing the seed viability than low RH. Under high RH the seed viability decreases with increase in incubation period.

Introduction

Wheat is very important crop all over the world and especially in Pakistan it has also got the status of economic and political crop. Wheat contributes more calories and protein to the world's diet than any other food crop (Hanson *et al*., 1982). In the list of wheat producing countries Pakistan is the 10th largest wheat producing country and is presently contributing about 2% of global wheat supply. Wheat bears a key position in Pakistan's economy and is cultivated over an area of some 8,463,000-hectare (Anon., 2000). Wheat is adapted to a wide range of soil and climatic conditions in Pakistan. Ecotypes and diverse genotypic land races have developed over time, which needs to be conserved.

Presence of precious plant genetic resources in Pakistan is a subject of national and international importance for preservation of genetic diversity. Genebank of Plant Genetic Resources Programme maintains around 23000 accessions of different species. The

elements that perturb quality and quantity of wheat are diseases, pests, climatic influences and changing consumers demand. Seed storage is a big problem during long-term / medium-term seed storage; in view of germplasm conservation, the viability is decreased even under the optimal storage conditions in the genebank (Anon., 2005). The pace as well as process of ageing in stored seeds largely depends on chemical composition of seeds (Mayer & Poljadoff, 1982).

Seed moisture content (MC) and storage temperature are two of the most important factors affecting seed storage life as visualized by Harrington, (1973) who stated that "Each 1% reduction in seed moisture doubles the life of the seeds; and each 5^0C reduction in seed temperature doubles the life of the seeds". The International Board for Plant Genetic Resources (IBPGR) has recommended that seeds should be properly dried to 3-7% MC for most species (Tao, 1985). Moisture content of 10% or below is essential for safe cotton seed storage, even for a short time period (Banks *et al*., 1998).

The Handbook of Vigour Test Methods (Anon., 1996) lists AA test as a recommended test for soybean and as a suggested test for several other species. However, few investigations have been made of various combinations of ageing temperature and time for evaluating of wheat seed vigour.

Seed survival curves of all cultivars of wheat at 24% moisture content (mc) and 45^0C for up to 96 h tested at 21^0C show a clear separation in germination after 48h ageing. It is suggested that seed quality as well as genotype may be responsible for reducing final germination of cultivars. Screening of wheat genotypes for seed ageing under real storage and artificially accelerated ageing conditions would provide guidelines to ensure appropriate storage of germplasm, specific genotypes and commercial seed lots (Chabra *et al*., 1992).

About 85% viability is minimum requirement to store seeds for conservation purposes in the genebanks. Seeds of some plant species loose viability faster than others and there may be varietal responses within species. So due to these problems artificial seed ageing (AA) is used to check seed viability and germinability. The objective of the present study was to determine the affect of artificial seed ageing on seed viability with the contribution of different factors like temperature and moisture to seed ageing.

Materials and Methods

This study aimed to see the response of the artificially aged seeds of wheat (*Triticum aestivum* L.) in relation to its effect on germination as a consequence of seed deterioration. Wheat variety Chakwal-97 (commonly cultivated in arid area of Rawalpindi division) was provided by Wheat Programme of NARC for this study. This research work was done in Seed Preservation Laboratory of Plant Genetic Resources Programme (PGRP), Institute of Agri-Biotechnology and Genetic Resources (IABGR) at National Agricultural Research Center (NARC), Islamabad during the period of September 2004 to June 2005.

Seed moisture content determination: Seed moisture content of the variety was determined with the help of moisture content meter. Dry seed moisture content (MC) was determined which was assumed as low moisture content (LMC). The second seed lot was soaked in distilled water for 15 minutes and then excess moisture was blotted with paper towels. The seed moisture content of this lot was considered as high moisture content (HMC).

Incubation for artificial ageing (AA): Seeds were placed in a bottle, hanging over a nylon mesh under the bottle cap tightly closed to avoid gaseous exchange. Fifty seeds were used in each case of LMC and HMC. Fifty ml of water was added to bottle at base in samples where high relative humidity [(RH) up to 100%] was to be achieved. Seeds were incubated at different temperatures separately i.e., 60°C, 50°C and 40°C for different duration of time i.e., 96 h, 72 h, 48 h and 24 h. For this purpose three separate incubators were used. Treatments were replicated four times. Electric seed counter was used to count seeds. The following is the summary of the treatments:

Factor A = Temperature = 4 [3 ageing treatment (40, 50 and 60°C) with 1 (25 °C) control at room temperature for comparison of treatment results].
Factor B = Incubation Time = 4 (24, 48, 72 and 96h)
Factor C = Seed (Initial) Moisture Content = 2 (LMC and HMC)
Factor D = Container bottle RH = 2 [(Ambient or low) and (high up to 100%)]
Replications = 4 (each with 50 seeds)

Electrical conductivity: Seeds of all treatments were soaked in 50 ml distilled water at 25°C for 24 h before taking E.C. value (Tekrony, 2003).

Germination test for seed viability: Paper towel, between paper (BP) method was used for germination test (Anon., 1996). Victory paper towels of Shinbashi Scishi Company, Shizuoka, Japan with paper size of 22 X 23 cm were used. Fifty seeds were placed on two moist sheets of paper towel, covered with another sheet and rolled. These rolls were placed in plastic beakers after draining excess water and covered with polythene bags to maintain moisture. Beakers were put in an incubator maintaining temperature at 25^0C (±2) under light conditions. Germinated seeds were counted every day from 1st to 7th day.

Shoot weight (seedling vigor): After 7th day the shoots were weighed as an indicator of seedling vigour, 10 shoots from each treatment were collectively weighed on an electric balance.

Data recorded: Data was recorded for the following parameters after incubation:

Seed weight (grams), electrical conductivity (ms/m), germination percentage (%) and shoot development (on weight basis) as an indicator of seed vigour.

Statistical analysis: The statistical analysis was applied on data, which was recorded in the form of percentage. In this regard simple percentage and average computations were applied on the pooled data (Mario, 1994). MS Excel package was used to enter and analyze data.

Results and Discussion

This work was intended to study wheat (*Triticum aestivum* L.) Var. Chakwal-97 as a case to determine the effect of artificial seed ageing (AA) and to envisage seed viability during storage for conservation purposes in the genebanks for mid-term period. The results obtained for seed weight on incubation for AA, corresponded to EC values for all temperature regimes whereas the germination percentage and shoot weight for 50°C and 60°C show clear demarcation when kept under high relative humidity (RH).

Seed weight (for moisture content determination): Storage conditions, particularly temperature and moisture have been indicated as the main factors influencing seed longevity. High temperature and moisture accelerate loss of viability in most species (Barton, 1961). Therefore the measurement of seed moisture content [where increased seed weight was an indicator of attained moisture content (MC)] was studied. The seed weight (g) was recorded to know the amount of moisture content in seeds as indicated by increase in weight over control. The weight in control was 1.73g (Fig. 1). High seed weight was recorded in seeds incubated under high relative humidity (HMCW) as compared to that at low RH or control. Seed weight was different within the variety at different temperature treated seeds. Seeds incubated at 50^{o}C showed high weight (3.35g) on 4th day of incubation while the highest in seeds incubated at 60^{o}C (2.32g) and 40^{o}C (2.28g).

The moisture content of the seeds (Chakwal-97) incubated at different temperatures (40, 50 and 60^{o}C) did not differ significantly among themselves when incubated at low (ambient) RH Fig. 1; and remained at par to control. Whereas, when the seeds were incubated at high RH (about 100%) their MCs were increased as indicated by increase in seed weight. Temperature and moisture play a significant and fundamental role in determining the storage longevity of seeds. The pattern of seed ageing is, in general, described in terms of its water content during storage (Walters, 1998). The highest increase was observed at 50^{o}C at 4th day of incubation period. It was also interesting to note that at 50^{o}C the seed MC was higher for the soaked seeds i.e., seeds having higher initial seed moisture content for the respective incubation period. However, under high RH the elevated moisture intake at 50^{o}C was indicator of lost viability while the slight increase at 40^{o}C incubation was indicator of produced ageing effect during storage that was revealed on germination test.

Electrical conductivity (EC): EC value of seeds was different at three incubation temperatures. It ranged from 4.26 to 17.11 ms/m. Only the seeds kept incubated under high RH showed significant differences in EC. Seeds treated at 50^{o}C showed high value of EC (17.11 ms/m) after 4 days of incubation time. The highest EC in seeds incubated at 60^{o}C (11.49 ms/m) and 40^{o}C (7.06 ms/m) was also achieved on 4 days of incubation under high RH (Fig. 2). The EC value of control was in range of 4.6 to 5.0 ms/m.

EC value followed the same trend as in MC behavior with respect to RH during incubation. The temperature during soaking and /or the evaluation will influence the results of the conductivity test for several species (Loeffler *et al*., 1988; Hampton *et al*., 1992). EC values were comparable to control for all temperatures and incubation duration regimes when incubated at low RH. The initial seed MC (either LMC or HMC) did not differ significantly. Whereas, all EC values were higher to control except for few values for 40^{o}C temperature and in most cases the EC value at 50^{o}C and high RH were the highest at all incubation duration (Fig. 2). For variety Chakwal-97 under high RH at 40^{o}C the EC of the seeds with HMC were lower than that of respective EC of LMC seeds. Almost same trend was followed by EC for seeds at 60^{0}C and high RH. However, seeds at 50^{o}C and high RH followed this trend up to 2nd day and afterwards though the EC values were raised but higher for seeds with HMC than LMC (Fig. 2). Our results suggest that EC values are indicative of seed deterioration only when the seeds were incubated at high RH therefore soaking the seed to enhance initial seed MC is not necessary. However, this behavior needs to be studied across a wide range of genotypes to develop a standard protocol. Similarly the incubation temperature for AA should be more than 40^{o}C to achieve signified difference to control.

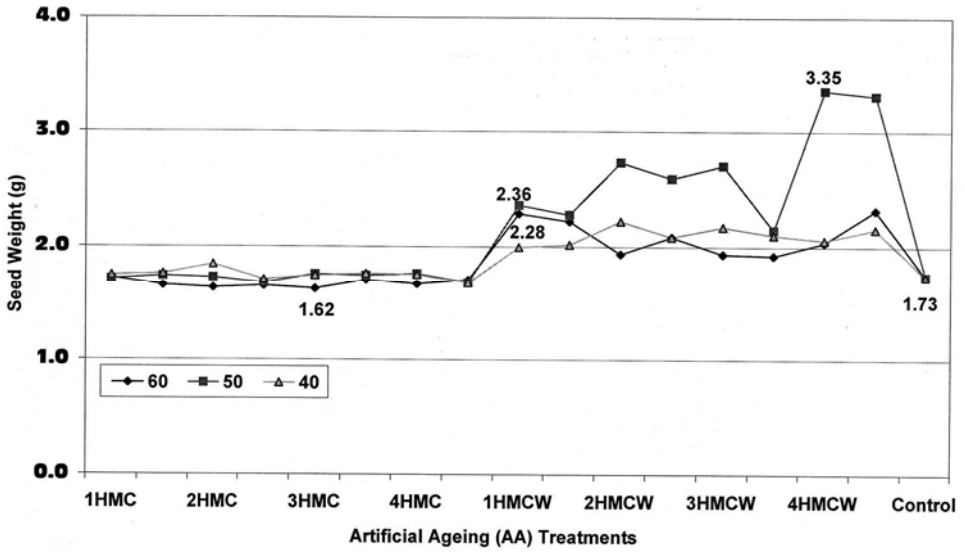

Fig. 1. Effect of AA on seed weight of wheat seeds (Chakwal-97)(1-4 = Days, MC = Seed moisture content, L/H = Low/High, W = water added to container for 100% RH).

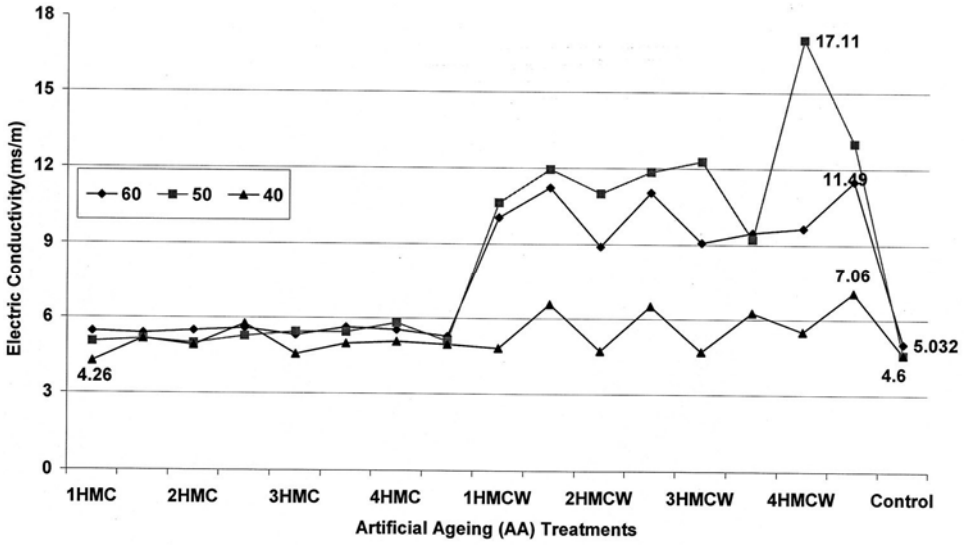

Fig. 2. Effect of AA on EC value of wheat seeds (Chakwal-97) (1-4 = Days, MC = Seed moisture content, L/H = Low/High, W = water added to container for 100% RH).

Seed germination percentage: After artificial ageing, seed germination percentage ranged from 0 to 84% for treated seeds and up to 88% for control. Seeds treated at 40°C and 50°C gave high germination percentage (84%) with LMC on 4th day (Fig. 3). The decline in germination percentage was in order of increase in incubation temperature provided the RH was low. In case the RH was increased germination dropped to zero at both high temperatures i.e., 50 and 60°C.

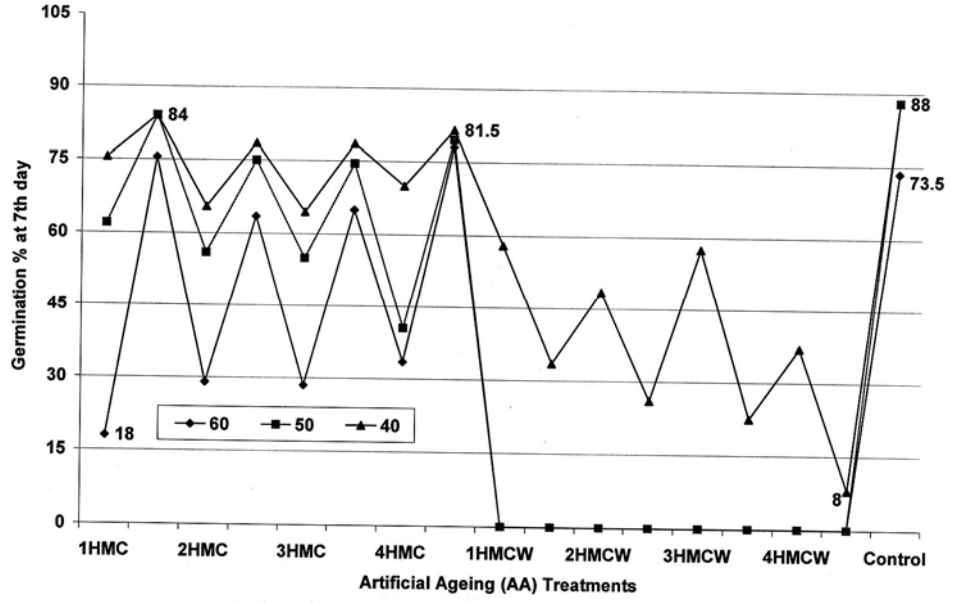

Fig. 3. Effect of AA on germination % of wheat seeds (Chakwal-97) (1-4 = Days, MC = Seed moisture content, L/H = Low/High, W = water added to container for 100% RH).

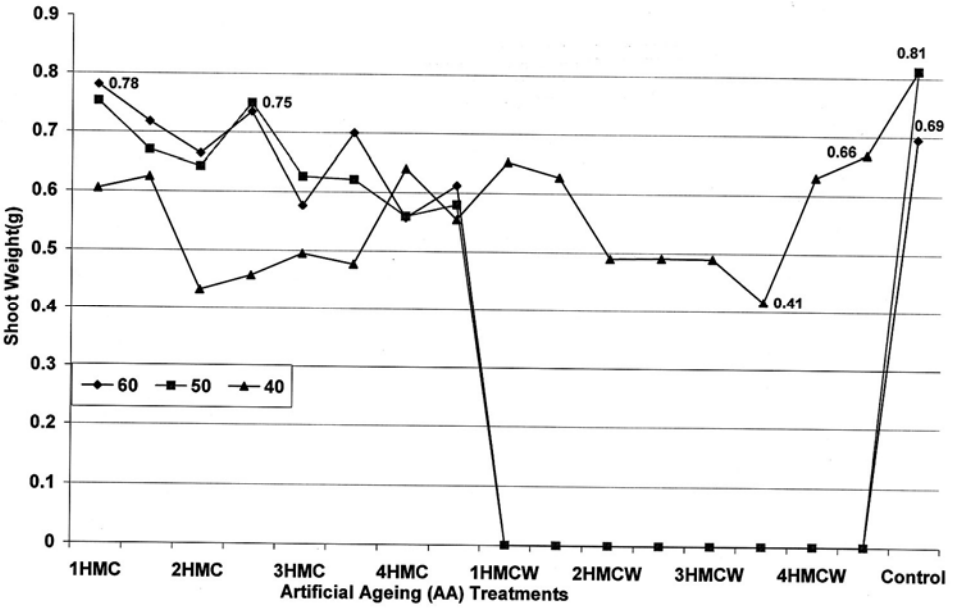

Fig. 4. Effect of AA on shoot weight of wheat seeds (Chakwal-97) (1-4 = Days, MC = Seed moisture content, L/H = Low/High, W = water added to container for 100% RH).

Studies on germination percentages of different years old wheat and barley seeds indicated a decrease of seed viability with the increase of age (Barton, 1961). Genotypic variation for seed ageing among wheat genotypes exists (Madan *et al*., 1989). Screening of wheat genotypes for seed ageing under real storage and artificially accelerated ageing conditions would provide guidelines to ensure appropriate storage of germplasm, specific genotypes and commercial seed lots. The germination response to artificial ageing was most influenced by the RH during the incubation period particularly at higher temperatures of 50°C and above (Fig. 3). The initial MC of seeds resisted ageing when being low. The heat treatment (40°C) affected the germination after the seed MC had reached a threshold (boundary) level (Maurel, 1997).

In case of Chakwal-97 the germination response was quite different under the different RH conditions. The germination was higher for corresponding LMC seeds under low RH and lower for the same under high RH. The germination declined with the increase in temperature. The effect was pronounced if the seed initial MC was high when incubated at low RH. When incubating seeds at high RH the germination was lost at higher temperatures i.e., 50°C or above and even at 40°C the germination declined with the increase in duration of incubation. Ageing causes stress on seeds, which leads to, impaired germination and loss of seedling vigor (Purkar *et al*., 1980). However, the improvement in germination percentage for seeds incubated under low RH and 60°C needs to be studied over a long duration and diverse temperature regimes at variable seed MC. The results of EC value and germination data indicate that even 24 h incubation at 50°C and high RH could deteriorate the seed. Loosing the germinability will render this method ineffective for evaluating the vigor. Thus the present study suggests that incubation of seed at 40°C under high RH for at least 4 days would be suitable for comparing genotype behavior. The study also directs to investigate range of temperatures between 40°C and 50°C.

Shoot development (weight basis): The shoot weight was recorded as an indicator of the seed vigour. Shoot weight of seedlings developing from seeds incubated at 60°C for 24 h was high (0.78g) with HMC treatment followed by 50°C and 40°C (0.75g, 0.60g) respectively. Shoot weight of control treatment was as high as 0.81g in both 50 and 40°C tested groups (Fig. 4).

As the seeds were grown between paper towels and supplied only with distilled water and light condition, it was only the seed potential vigour to support the growth. In all cases shoot developed from seeds incubated at 40°C irrespective of initial MC, RH or duration of incubation. Shoots were developed at all temperatures when incubated at low RH, irrespective of temperature, duration of incubation or MC. In case of Chakwal-97 no shoot development occurred at 50 or 60°C showing its susceptibility to high temperature under high RH conditions (Fig. 4). However, the variability of data for this character makes it irrelevant for inferring any seed vigor relationship. Studying shoot weight at low RH for longer durations or at high RH at other temperatures between 40 to 50°C may reveal inferable data.

References

Anonymous. 1996. International rules for seed testing: rules (International Seed Testing Association) 1996. *Seed Sci. Tech*., 24, Suppl., 29-156.

Anonymous. 2000. *Agriculture Statistics of Pakistan.* Ministry of Food, Agriculture and Livestock division (Economic Wing), Islamabad. P. 47-52.

Anonymous. 2005. Annual report 2005, *Institute of Agri-Biotechnology and Genetic Resources*, NARC, Islamabad.

Banks, J.C., O.H. Williams and N.B. Thomas. 1998. *Cotton - The Crop and Plant*. Web site, http://clay.agr.okstate.edu/plantsoilsci/faculty/jcb/extension.html.

Barton, L.V. 1961. *Seed preservation and longevity*. Leonard Hill, London, Interscience NY, 216.

Chabra, B.S., T.P. Yadava and R.K. Behl. 1992. Variability for seed ageing and storability in wheat. *Wheat Info. Ser*., 75: 68-71.

Hampton, J.G., K.A. Johnstone and V. Eua-Umpon. 1992. Bulk conductivity test variables for mungbean, soybean and french bean seed lots. *Seed Sci. Tech*.., 20: 677-686.

Hanson, H., N.E. Borlang and R.G. Anderson. 1982. *Wheat in the third world*. West View Press, Boulder, Colorado.

Harrington, J.F. 1973. Problems of seed storage. In: *Seed Ecology*. (Ed.): W. Heydecker. 251-264. Butterworths, London, UK.

Loeffler, T.M., D.M. TcKrony and D.B. Egli. 1988. The bulk conductivity test as an indicator of soybean seed quality. *J. Seed Tech*., 13: 781-794.

Madan, S., G. Talwar, R.K. Behl and U. Verma. 1989. Effect of accelerated ageing on germination seedling vigour and beta-amylase activity in wheat. *Res. & Dev. Reporter*, 6: 48-54.

Mario, F.T. 1994. *Analysis of variance, Elementary statistics.* Mc Graw Hill, New Dahli. :574-576.

Maurel, C. 1997. Aquaporins and water permeability of plant membranes. *Annu. Revue of P. Phy. and P. Mol. Bio*., 48: 399-429.

Mayer A. M. and A. Poljadoff-Mayber. 1982. *The germination of seed*. 3rd Ed. Pergamon Press Ltd. Headington Hill Hall. Oxford OX3 OBW. England. p: 10-37.

Purkar, X., R.B. Mehra and S.K. Banerjee. 1980. Seed ageing induced variability in quantitative characters of Pea and Wheat. 19th ISTA Congress, Preprint No.60.

Tao, K.L. 1985. Standards for genebanks. FAO/IBPGR. *Pl. Genet. Resources Newsl*., 62: 36-41.

Tekrony, D.M. 2003. Precision is an essential component in seed vigour testing. *Seed Sci. Tech*., 31: 435-447.

Walters, C. 1998. Understanding the mechanisms and kinetics of seed ageing. *Seed Sci. Res*., 8: 223-244.

(Received for publication 13 October 2005)