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Prediction of storability of greengram seeds through accelerated ageing test

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ABSTRACT

Studies were carried out in the Department of Seed Science and Technology, TNAU, Coimbatore by exposing seeds to accelerated ageing test for eight days (45±°C and 100% relative humidity). The evaluation of seed vigour was obtained by comparing aged seeds to control seeds. The results revealed that six days of accelerated ageing was equivalent to nine months of natural storage at the time maintained the germination above Indian Minimum Seed Certification Standards.

Keywords: Greengram seeds; germination; vigour

INTRODUCTION

Deterioration is an irreversible phenomenon in biological organisms which continues till they are completely dead. The loss in seed vigour is evidenced by delayed emergence, slower growth and ultimately the decline in seed germinability (Woodstock 1973). The deterioration of seed (biological entity) can be accelerated by provision of adverse temperature (40°C) and relative humidity (100%) to the storage atmosphere which is valuable in prediction of its storability (Delouche and Baskin 1973) at warranted situation without entering into natural storage. Seed and crop management technique may or may not influence the seed quality of progeny seeds. Hence to identify the performances of seeds obtained from seed and crop management techniques the progeny seeds were evaluated through accelerated ageing test.

MATERIAL and METHODS

The freshly harvested seeds were collected, pre-cleaned and dried to 8.0 per cent moisture content. The seeds were taken for accelerated ageing test 0 to 8 days at 40°C and 100 per cent relative humidity (RH) to the storage atmosphere and the deterioration pattern was observed. After 8 days of accelerated ageing test the following seed quality parameters were evaluated:

Moisture content (%): Five gram of seed in triplicate was taken separately in a pre-weighed (M1) moisture estimation bottle and the sample weights along with the bottle were recorded (M2). The bottles were kept in a hot air oven maintained at 105 ± 2ºC for 6 h. The bottles were taken out and cooled in a desiccator with calcium carbonate for 30 minutes. The weight of bottles along with dried seeds was recorded (M3) individually. The moisture content was calculated on wet weight basis adopting the following formula and expressed as percentage (Anon 1999):

$$\text{Moisture content (\%)} = \frac{M2 - M3}{M2 - M1} \times 100$$

Germination (%): Germination test in quadruplicate of 100 seeds each with four sub-replicates of 25 seeds was carried out in roll towel in a germination room maintained at 25 ± 1ºC temperature and 96 ± 2 per cent RH with diffused light. Final count based on normal seedlings was recorded on seventh day and the mean recorded as germination in percentage (Anon 1999).

Root length (cm): After the germination period of seven days ten normal seedlings were selected at random in each replication and measured for root length from the collar region to the tip of primary root using measuring scale. The mean was expressed as root length in centimetres.

Shoot length (cm): Seedlings used for measuring root length were also used for measuring shoot length. The length between the collar region to tip of the primary leaf (plumule) was measured and the mean expressed as shoot length in centimetres.

Dry matter content (mg/10 seedlings): Seedlings used for growth measurement were dried in a hot air oven maintained at 85 ± 2°C for 24 h, cooled in a desiccator for 30 min and weighed in an electronic balance. The mean was expressed as dry matter production per 10 seedlings in milligram (Gupta 1993).

Vigour index: Vigour index (VI) was calculated by using the formula suggested by Abdul–Baki and Anderson (1973) and the mean was expressed in whole number:

VI= Germination (%) x {root length (cm) + shoot length (cm)}

Electrical conductivity (dS/m): Four replicates of 50 seeds in each treatment and replication were taken, pre-washed and soaked in 50 ml of distilled water for 6 h at room temperature. The seed leachate was collected by decanting and the electrical conductivity (EC) was measured in a digital model conductivity meter (Elicotype Cm-82) possessing electrode at cell constant of 1.0 with calibration on EC mode. The mean was expressed as electrical conductivity in dS/m (Presley 1958).

Protein content (%): Ground seed material 100 mg was taken in a 50 ml polyethylene screw cap bottle and 25 ml of 1N NaOH was added to it. The mixture was shaken for 10 min in a wrist action shaker to disperse the protein. Out of it 10 ml of the suspension was poured into a graduated test tube and was used as a blank to compensate for the differences in the amount of natural pigments extracted and to the remaining suspension in the bottle 0.25 ml of 10 per cent copper sulphate solution was added. The bottle was reshaken for an additional duration of five min to develop colour complex. The sample solution was then poured into a separate test tube and left overnight along with its blank to allow the dispersed material to settle down. After centrifugation at 3000 rpm for 10 min the optical density (OD) of the clear supernatant solution was measured in an Optima UV-VIS spectrophotometer (Model SP-3000) using red filter (620 nm) with corresponding blank. From the mean OD value the protein content for each sample was calculated using the following formula and the mean was expressed as protein content in percentage (Ali-Khan and Youngs 1973).

Protein content (%)= 3.78 + (61.6 x OD value)

Dehydrogenase enzyme activity (OD value): A representative seed sample from each treatment and replication was taken and pre-conditioned by soaking the seeds in water for 4 h at room temperature. Out of this 10 seeds were taken at random and prepared by removing the seed coat; seeds were steeped in 0.2 per cent of 2, 3, 5-triphenyl tetrazolium chloride solution and kept for staining in dark at 40°C for 1 h. The stained seeds were soaked in methyl cellosolve solution @ 1 ml per seed for 4-6 h with occasional stirring till the extraction of red colour formazan completely. The extract was decanted and intensity of colour was read in a spectrophotometer (ELICO SL 159) at 470 nm. The mean OD values were reported as dehydrogenase activity (Kittock and Law 1968).

The data obtained from different experiments were analysed for F-test of significance following the methods described by Panse and Sukhatme (1985).

RESULTS and DISCUSSION

The results revealed that the moisture content was increased from initial to eighth day of ageing (from 8.0 to 9.7). The possible reason could be the hydrophilic nature which provided continuous and slow supply of moisture to the seed and increased the moisture which could be due to the prevention of moisture equilibration between the seed and atmosphere at higher frequency as dealt by Warham (1986) in wheat.

For subjecting seeds to accelerated ageing, germination was reduced from 0

Table 1. Assessing the ageing days for initial seed quality parameters in greengram seeds

Accelerated aged seeds (days)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigour index
Initial	96 (78.46)	17.3	23.5	275	3898
1	92 (93.92)	15.5	21.0	260	3357
2	89 (70.63)	13.2	19.7	237	2941
3	83 (65.65)	11.4	16.2	225	2299
4	80 (63.43)	10.9	14.3	213	2006
5	78 (62.02)	9.5	13.0	198	1764
6	75 (60.00)	8.0	11.9	182	1497
7	70 (56.79)	7.6	10.7	170	1278
8	64 (53.99)	6.9	9.4	158	1036
Mean	80 (63.43)	11.1	15.5	213	2230
SEd	4.45	1.22	0.99	6.46	164.14
$CD_{0.05}$	9.34	2.57	2.09	13.57	344.85

Figures in parentheses indicate arc sine values

to 8th day (from 96 to 64). The decline in germinability during storage could be attributed to the irreversible ageing characteristics of all living biological organisms causing deteriorative changes in the physical, physiological and biochemical characters of seed (Abdul-Baki and Anderson 1973, Roberts 1972). Kumar and Singhal (1991) found that accelerated ageing reduced the germination of parents and their crossed seeds in pea. Rao and Wagle (1981) subjected soybean seeds to accelerated ageing at 40^{o}C temperature and 94 per cent RH and recorded higher germination percentage to fall rapidly to zero after eighth day. Sundaralingam et al (2001) observed similar decrease in germination with advances in days of accelerated ageing while Natarajan (1998) found that it also correlated well with viability rating of seed.

The vigour parameters of the stored seeds in terms of shoot length, root length, dry matter production and vigour index were also in decreasing order with increase in ageing period (Table 1). McDonald and Phaneendranath (1978) concluded that soybean seed vigour evaluation gave the most consistent results if seeds were aged in a single layer for 48 h with standard condition of tray and box size and depth of water.

Table 2. Assessing the ageing days for biochemical parameters in greengram seeds

Accelerated aged seeds (days)	Moisture content (%)	Electrical conductivity (ds/m)	Protein content (%)	Dehydrogenase activity (OD value)
Initial	8.0	0.070	22.6	1.015
1	8.3	0.079	22.5	1.007
2	8.5	0.085	22.2	0.999
3	8.9	0.092	22.0	0.982
4	9.0	0.100	21.7	0.965
5	9.2	0.111	21.4	0.953
6	9.3	0.122	21.1	0.940
7	9.6	0.130	20.9	0.931
8	9.7	0.143	20.5	0.919
Mean	8.9	0.103	21.6	0.114
SEd	0.25	0.003	0.60	0.003
$CD_{0.05}$	0.54	0.008	1.28	0.008

Electrical conductivity of seed leachate was increased gradually over periods from initial to eighth day (from 0.070 to 0.143) of accelerated ageing. The electrical conductivity was the lowest in seeds from initial period which might be due to higher membrane integrity. The increase in electrical conductivity may be due to autooxidation of polyunsaturated fatty acids in the membrane liquid compound involving free radical chain reactions (Doijode 1988). While considering the biochemical characters protein content declined with increase in ageing period (Table 2).

The dehydrogenase enzyme activity which is responsible for respiratory action varied significantly among the treatments and ageing period. The decrease in dehydrogenase enzyme activity observed due to storage is in agreement with the findings of Moore (1972), Kittock and Law (1968), Woodstock (1973) and Sung (1996). Seed quality characters showed similar changes in both naturally and accelerated aged seeds in rice hybrids (Ramanadane and Ponnuswamy 2004). According to Deshpande and Mahadevappa (1994) four days of accelerated ageing (43 ± 1ºC and 98 + 1% RH) period was equivalent to six months of natural ageing and eight days to one year in rice.

CONCLUSION

The results of the present study showed that the storability of freshly harvested greengram cv CO 6 seeds could be predicted through six days of accelerated

ageing test. Six days of accelerated ageing is equivalent to nine months of natural ageing at the time maintained the germination percentage above Indian Minimum Seed Certification Standards.

REFERENCES

Abdul-Baki AA and Anderson JD 1973. Vigour determination of soybean seeds by multiple criteria. Crop Science **13:** 630-633.

Ali-khan ST and Youngs CG 1973. Variation in protein contents of field peas. Canadian Journal of Plant Science **53:** 37-41.

Anonymous 1999. International rules for seed testing. ISTA, Seed Science and Technology, Supplement Rules **27:** 25-30.

Delouche JC and Baskin CC 1973. Accelerated ageing technique for predicting seed relative storability of seed lots. Seed Science and Technology **1:** 427-452.

Deshpande and Mahadevappa 1994. Effect of accelerated ageing on rice seeds. Food Research International **42:** 674-681.

Doijode SD 1988. Comparison of storage containers for storage of French bean seeds under ambient conditions. Seed Research **16:** 245-247.

Gupta PC 1993. Seed vigour testing. In: Handbook of seed testing. Quality Control and Research Development, New Delhi, India, 243p.

Kittock DL and Law AG 1968. Relationship of seedling vigour to respiration and tetrazolium chloride reaction of germinating wheat seeds. Agronomy Journal **60:** 286-288.

Kumar S and Singhal NC 1991. Effect of accelerated ageing on parents and their crossed seeds in pea (*Pisum sativum* L). Seed Research **19(1):** 51-53.

McDonald, MB Jr and Phaneendranath BR 1978. A modified accelerated ageing seed vigour test for soyabean. Journal of Seed Technology **3(1):** 27-37.

Moore RP 1972. Previous history of seed lots and differential maintenance of seed viability and vigour in storage. Proceedings of the International Seed Testing Association **28(4):** 691-699.

Natarajan N 1998. Seed vigour, viability and production studies in blackgram (*Vigna mungo* (L) Hepper) cv CO3. PhD thesis. Tamil Nadu Agricultural University, Coimbatore, TN, India.

Panse VG and Sukhatme PV 1985. Statistical methods for agricultural workers. ICAR, New Delhi, India, pp 327-340.

Presley JT 1958. Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. Disease Reporter **42(7):** 852.

Ramanadane T and Ponnuswamy AS 2004. Ageing and anatomical influence of seed storability in rice (*Oryza sativa* L) hybrids and parental lines. Tropical Agricultural Research **16:** 37-50.

Rao AS and Wagle DS 1981. Beta-amylase activity in artificially aged soyabean seeds. Biologia Plantarum (Praha) **23(1):** 24-27.

Roberts EH 1972. Cytological, genetical and metabolic changes in seed viability associated with loss of viability of seeds. EH Roberts (ed). Chapman and Hall Ltd, London, pp 253-306.

Sundaralingam K, Srimathi P and Vanagamudi K 2001. Presowing seed management. In: Recent trends and participatory approaches on quality seed production (K Vanangamudi, A Bharathi, P Natesan, R Jerlin, TM Thiyagarajan and S Kannaiyan eds). Department of Seed Science Technology, Tamil Nadu Agricultural University, Coimbatore, TN, India.

Sung JM 1996. Lipid peroxidation and peroxidase scavenging in soybean seeds during ageing. Physiologia Plantarum **97(1):** 85-89.

Warham EJ 1986. The effect of different packing materials on moisture uptake by dry wheat in stimulated humid tropical conditions. Seed Science and Technology **14:** 641-655.

Woodstock LW 1973. Physiology and biochemical tests for seed vigour. Seed Science and Technology **1:** 127-157.

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