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Evaluation of Blackgram Seed Viability Through Accelerated Ageing Test

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Abstract The Study has been carried out by exposing blackgram seeds to accelerated ageing test (45 × °C and 100% relative humidity) for 0 to 8 days. The evaluation of seed vigor was obtained by comparing aged seeds to un-aged (control) seeds. The results revealed that, a progressive decline in germination percent with increasing ageing period (from 98% in control to 66% after 8 days). However, reduction of germination percent was coincided with the reduction of seed quality parameters. As a conclusion, 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 Blackgram seeds, Germination, Vigor.

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Introduction

Seed being a biological entity, deterioration is inevitable, irreversible and inexorable. Seed deterioration is a phenomenon, which begins immediately after attaining physiological maturity even on the mother plant. One of the most important basic needs for higher productivity is quality seed is characterized with higher viability and vigor. 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 at warranted situation without entering into natural storage. Changes occurring in seed during accelerated ageing are significant with regard to quality and longevity of seed. Seed longevity is one of the components of seed quality. The speed at which ageing processes taken place depends on the seed's ability to resist degradation changes as well as its protection mechanisms. Accelerated aging is one of the vigor tests widely used to determine the quality of seed lots [1]. Under such storage conditions, seeds typically lose their viability within a few days or weeks. Hence in the present study the seeds obtained from different seed and crop management techniques were aged by providing 40°C and 100% RH to the storage atmosphere for eight days and the deterioration pattern was observed at initial and eighth day after accelerated age-

ing and evaluated for seed quality characters.

Materials and Methods

The freshly harvested seeds were collected, pre-cleaned and dried to 8.0% moisture content. The seeds were taken for accelerated ageing test 0 to 8 days at 40°C and 100% 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 seeds in triplicate were taken separately in a pre weighed (M_1) moisture estimation bottle and the sample weights along with the bottle were recorded (M_2). The bottles were kept in a hot air oven maintained at 105±2°C for 6h. Then the bottles were taken out and cooled in a desiccator with calcium carbonate for 30 minutes. The weight of bottle along with dried seeds were recorded (M_3) individually. The moisture content was calculated on wet weight basis adopting the following formula and expressed as percentage.

$$\text{Moisture content)\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Germination (%)

Germination test, in quadruplicate of 100 seeds, each with four sub replicates of 25 seeds were carried out in roll towel in a germination room maintained at temperature of 25 ± 1°C and RH of 96 ± 2% with diffused light. Final count bsed on normal seedling was recorded on seventh day and the mean recorded as germination in percentage.

Root length (cm)

After the germination period of seven days, ten normal seedlings were selected at random in each of the replication, and were measured for root length, from the collar region to the tip of primary root using mesuring scale. The mean expressed as root length in centimeter.

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

Accelerated seeds (days)	Germination (%)	Root length (cm)	Shoot length (cm)	Drymatter production (g/10 seedlings)	Vigor index
Initial	98 (81.86)	15.3	24.8	265	3930
1	93 (74.65)	14.5	23.2	252	3506
2	91 (72.54)	13.7	21.9	239	3240
3	87 (68.86)	13.0	19.1	215	2793
4	84 (66.42)	12.2	17.6	201	2503
5	79 (62.72)	11.6	15.9	182	2173
6	75 (60.00)	9.8	13.2	169	1725
7	70 (56.78)	8.7	12.0	152	1449
8	66 (54.33)	7.9	10.7	141	1228
Mean	83 (65.64)	11.86	17.60	202	2432
SEd	5.25	0.87	1.75	7.45	172.66
CD (*p*= 0.05)	11.10	1.78	3.20	13.57	350.58

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 centimeter.

Drymatter content (mg/10 seedlings)

Seedlings used for growth measurement were dried in an hot air oven maintained at 85±2°C for 24 h and cooled in a desiccator for 30 min. and weighed in an electronic balance and the mean expressed as drymatter production per 10 seedlings in milligram.

Vigor index

Vigor index (VI) was calculated by using the formula and the mean expressed in whole number.

VI=Germination (%) ×[root length (cm) + shoot length (cm)].

Electrical conductivity (dSm^{-1})

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 expressed as electrical conductivity in dSm^{-1}.

Protein content (%)

A quantity of 100 mg of ground seed material was taken in a 50 ml polyethylene screw cap bottle and 25 ml of 1N NaOH was added. The mixture was shaken for 10 min. in a wrist action shaker to disperse the protein. Then, 10 ml of the suspension was poured into a graduated test tube and 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% copper sulfate solution was added and the bottle was reshaken for an additional duration of five min to develop color 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 expressed as protein content in percentage.

$$\text{Protein content (\%)} = 3.78 + (61.6 \times \text{OD value})$$

Dehydrogenase enzyme activity (OD value)

A representative seed sample from each treatment and replication were taken and pre-conditioned by soaking them in water for 4 h at room temperature. Out of this, 10 seeds were taken at random and prepared by removing the seed coat. Then the seeds were steeped in 0.2% of 2, 3, 5-triphenyl tetrazolium chloride solution and kept for staining in dark at 40°C for 1 h. After staining, 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 color formazan completely. The extract was decanted and intensity of color was read in a spectrophotometer (ELICO SL 159) at 470 nm. The mean OD values were reported as dehydrogenase activity.

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

Accelerated aged seeds (days)	Moisture content (%)	Electrical conductivity (dSm^{-1})	Protein content (%)	Dehydrogenase activity (OD value)
Initial	8.0	0.082	20.2	0.317
1	8.2	0.089	20.0	0.311
2	8.5	0.092	19.7	0.301
3	8.8	0.099	19.2	0.290
4	9.0	0.109	19.0	0.282
5	9.4	0.120	18.9	0.275
6	9.6	0.127	18.7	0.263
7	9.9	0.135	18.4	0.250
8	10.0	0.144	18.0	0.239
Mean	9.0	0.111	19.1	0.281
SEd	0.20	0.004	0.50	0.003
CD (p=0.05)	0.38	0.009	0.99	0.007

Statistical analysis

The data obtained from different experiments were analysed fpr 'F' test of significance following the methods. Wherever necessary and the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5% probability level. The data were tested for statistical significance (*). If F test is non-significant, it was indicated as NS.

Results and Discussion

The results revealed that a significant increased were observed in moisture content after accelerated ageing at all periods of ageing from one to eight day compared to control. Under accelerated ageing condition, moisture content increased from 8.00% (in control) to 10.0% respectively. The increase could explain by increase in imbibe water due to the disorgnazation of the cell menbrane during ageing. The possible causes could be due to 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 in wheat.

Accelerated ageing resulted in progressively loss of seed viability and vigor. The germination percentage was decreased with increase in days of ageing, from 0 to 8th day (from 98 to 66). Reduction in germination is due to degradation of mitochondrial membrane, leading to reduction in energy supply necessary for germination. Sundaralingam et al. [2] observed similar decrease in germination with advances in days of accelerated ageing, while Natarajan [3] found that it also correlated well with viability rating of seed.

The evaluated seedling vigor characters were also in line with germination in observing a decreasing trend with advances in ageing, which might be due to the senescence observed with aged seeds after accelerated ageing. The decline in shoot length, root length and seedling vigor index might be attributed to DNA degradation with ageing which leads to impaired transeription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination [4] and also effect the lipid peroxidation on the protein content important for seedling growth with decrease in germination percentage and seedling length, lead to decrease in vigor index (Table 1).

Electrical conductivity of seed leachate was increased gradually over periods from initial to eighth day(from 0.082 to 0.144) of accelerated ageing. The electrical conductivity was the lowest in seeds from initial period which might be due to higher membrane integrity. The increased in electrical conductivity may be due to autooxidation of polyunsaturated fatty acids in the membrane liquid compound involving free radical chain reactions. The loss in membrane integraty and the leakage of electrolytes are the first symptoms of seed deterioration. While considering the biochemical characters, protein content was declined with increase in ageing period (Table 2).

The dehydrogenase enzyme activity which is responsible for respiratory action was varied significantly among the ageing period. Ageing have damaging effect on enzymes that are necessary to convert reserve food in the embryo to usable form [5]. The decrease in dehydrogenase enzyme activity observed due to storage. Seed quality characters showed similar changes in both naturally and accelerated aged seeds in rice hybrids [6]. According to Deshpande and Mahadevappa [7] reported four days of accelerated ageing (44±1^{o}C and 98 + 1% RH) period was equivalent to six months of natural ageing and eight days to one year in rice. Similar results were reported earlier [8].

Conclusion

It could be concluded that the storability of freshly harvested blackgram cv ADT 3 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.

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