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Effect of storage environments and storage time on germination of soybean (*Glycine max* (l.) Merr) seeds

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Abstract
Soya bean (*Glycine max* (L.) Merr) seed germination period, like other oil seed crops is very short-lived and often reduced prior to planting time. This study was carried out to investigate the influence of storage environment and storage time on the germination of soya bean seeds. Two accessions of soya beans (NGB/TO/02/12/148 and NGB00111) were used for the study. Five hundred grams of each of the two accessions were drawn and partitioned into three parts. Samples from each accession were kept separately in three different storage environments namely ambient (control), short term and medium term conditions over a period of ten months. The stored seed samples were drawn at regular intervals and evaluated for germination test. Results of analysis of variance (ANOVA) revealed that effects of accession, storage environment, storage time and their interaction were highly significant ($P<0.01$) on germination of soya bean seeds. However, there was no significant difference for germination of soya bean seeds stored under medium and short term storage conditions suggesting that efforts should be made to optimise storage conditions in these environments. Moreover, highly significant accession x storage environment x storage time interaction ($P>0.01$) indicates that in assessing germination potential of soya bean seeds, genotype, storage environment and storage time should be given prime consideration.

Keywords: Environment, Germination, Soya bean, Storage, Time,

Introduction
Seed storage is the maintenance of high seed germination and vigour from harvest until planting and this is important to get adequate healthy and vigorous plant stands. Germination period in soya bean (*Glycine max* (L.) Merr) seeds, like most other oil seed crops is very short-lived and germination level is often reduced prior to planting time (Agha *et al*., 2004). The short shelf life of soya bean seeds is the result of its high lipid content and high levels of polyunsaturated linolenic and linoleic acids. The longevity of the seeds during storage is controlled by several factors among which are nature of the seeds, temperature and relative humidity of the storage environment, seed moisture content and storage time. The amount of moisture in the seeds is the most important factor influencing seed viability during storage. The lower the moisture content, the longer the seeds can be stored provided the moisture level can be controlled throughout the storage time. Lovato and Balboni (1997) for instance reported maximum of 13% as the safe seed moisture for storage of cereals. Temperature can also affect the shelf life of seeds by altering the rate of certain reactions through inactivation of enzymes (Vertucci and Roos, 1993). With the use of temperature isotherms, Vertucci and Roos (1993) showed that optimum moisture content for storage of soya bean seeds varied with storage temperature. Storage potential of seeds are also influenced by the relative humidity of the surrounding atmosphere. Seeds are hygroscopic in nature. This means they pick up and release moisture from and into the surrounding air

until the vapour pressures of seed moisture and air reach equilibrium which can lead to rapid seed deterioration especially where humid conditions prevail. Duration of seed storage can be one of the factors that influence seed deterioration due to natural seed aging. Depending on the storage time, storing seeds beyond optimum storage time might result in reduction of germination potential, seedling establishment and final seed production (Sisman, 2005). Ravi *et al*. (2014) concluded that seed germination percentage in *Rheum australe* D Don., a high altitude endangered medicinal perennial herb declined with increasing storage duration irrespective of storage condition.

Germination is emergence and development from seed embryo of those essential structures which are indicative of the seed's capacity to produce a normal plant under favourable conditions. The process starts by the uptake water by the dry seed and is completed when a part of the embryo, usually the radicle, penetrates the seed coat. The Association of Official Seed Analysts (AOSA, 2009) defined seed germination as 'The emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions'. Standard germination test therefore forms an integral aspect of seed quality control and it is conducted under the best conditions possible for seed and seedling growth.

The National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria is the national focal point for genetic resources conservation and utilisation in Nigeria. NACGRAB holds many accessions of soya beans in her gene banks and over the years, has distributed soya bean accessions from her working collections to meet the requirements of researchers in the National Research System. The soya bean seed is well known for its short storage life and this problem is presently compounded by irregular power supply to genebanks in NACGRAB which often results in fluctuation in temperature and relative humidity in the short and medium term storage environments. This makes soya bean seed deterioration a major problem. Our assumption was that high-germination soya bean seeds (>70%) could be carried over at least to the second year if other storage conditions are optimised to reduce seed deterioration considering minimum of ten hours power supply to the genebanks.

This study might provide useful information for NACGRAB and other genebanks experiencing such challenges on how to plan for proper storage conditions to maximise shelf life of the soya bean seeds. Although several studies have been conducted on the storability of soya bean seeds using different storage environments (El Borai *et al*., 1993; Tatipata, 2009; Jantana *et al.,* 2005; Sajo and Tame, 2012; Tame and Mibzar, 2010; Tame *et al*., 2013), this study would furnish additional information in making decision on the regeneration cycle of soya bean under such conditions. The objective of this study therefore was to compare the effects of three storage environments namely ambient (control), short term and medium term conditions on germination of soya bean seeds.

Materials and Methods

Seeds of two accessions of soya beans (NGB/TO/02/12/148 and NGB00111) with

initial germination percentages of 92 and 94%, respectively were sourced from the seed gene banks of the National Centre for Genetics Resources and Biotechnology (NACGRAB), Ibadan. Seed regeneration was done during the late growing season of 2014. The materials were harvested in November while seed processing was carried out in December 2014. Five hundred grams of each of the accessions were drawn and further subdivided into three equal parts. Samples from each accession were kept separately in different storage environments: ambient (control), short and medium term storage conditions in February 2015 using plastic containers as packaging materials. The stored seed samples were drawn at regular intervals of 2, 4, 6 and 8 months (April, June, August and October) and evaluated for germination percentage. Electricity supply for minimum of ten hours was ensured in both short and medium term storage environments. Temperature and relative humidity of both short and medium term storage environments were taken daily but temperature of ambient environment was taken daily. Standard germination test was conducted in three replicates of 100 seeds. The experiment was arranged in a 2 x 3 x 4 factorial using completely randomised design (CRD). The three factors were accession of soya bean, storage environments and time of storage. The seeds were placed in germination plastic containers lined with four layers of tissue paper moistened with 15ml of distilled water. The containers were covered and placed in a germinating chamber at 25 ± 2°C. The seeds were kept moist every day for seven days. Germination counts were taken seven days after sowing. Data on germination percentage were subjected to analysis of variance (ANOVA) using Statistical Analysis Software, SAS Version 9.1. Data on percentages do not conform to normal distribution, the germination data were therefore log transformed before subjecting them to the ANOVA. However, since ANOVA did not detect any significant difference between transformed and untransformed values, untransformed values were presented. Pertinent means were separated by the use of the least significant difference (LSD) at 0.05 level of probability.

Results

The results of the mean temperature ranges in the three storage environments and mean relative humidity ranges in the short and medium term storage environments during the period of study are presented in table 1. Temperature under ambient conditions ranged from 24 to 29.5°C while that of the short term storage environment ranged from 15.07 to 22.59°C. The lowest temperature value was observed under the medium term storage environment which ranged from -8.18 to 4.12°C (Table 1). The mean relative humidity ranges in the short and medium term storage environments were 26.88% to 50.67% and 42.72% to 72.10% respectively (Table 1). Results of the analysis of variance (ANOVA) revealed that effects of accession (ACC), storage environment (ENV) and storage time (STR) were highly significant ($P<0.01$) on the germination of soya bean seeds (Table 2). Also, effect of accession by storage environment by time of storage interaction (ACC × ENV × STR) were highly significant ($P<0.01$) on the germination of soya bean seeds (Table 2). The germination percentage for accession NGB 00111 was higher (78.78%) than that of NG/T0/02/12/148 which exhibited a germination percentage of 51.22% (Table 3). Seeds stored under ambient storage conditions had the lowest mean germination percentage (46.83%) (Table 3). However, the results further revealed that mean germination percentages for soya bean seeds stored under short (74.33%) and medium (73.83%) term conditions were

Table 1: Mean temperature (°C) and relative humidity (%) ranges in short and medium term storage chambers and mean temperature range under ambient conditions during the experiment.

Storage environment	Temperature (°C)	Relative humidity (%)
Ambient	24 to 29.5	---
Short term	15.07 to 22.59	26.88 to 50.67
Medium	-8.18 to 4.12	42.72 to 72.10

not significantly different (Table 3). It was also observed that storability of soya bean seeds was highly dependent on storage time in this study. Although there was no significant difference between the mean germination percentages for April (74.44%) and June (73.33%), germination percentage started decreasing significantly with mean values of 67.55% and 44.67% in August and October respectively. In addition, germination of soya bean accessions was significantly affected by combined effect of storage environment and storage time. At almost one year after harvest (STR4), soya bean accession NGB/TO/02/12/148 (ACC1) had germination values of 53% and 40% while NGB00111 (ACC2) had germination values of 89% and 77% observed under the short and medium term storage environments respectively (Table 4).

Discussion

Storability of seeds is determined by a wide number of factors such as genotype, period of storage, initial seed quality, storage environment and seed moisture content. In this present study, effect of soya bean

Table 2. Mean squares, means, coefficient of determination (R^2) and coefficient of variation (CV) from the combined analysis of variance for laboratory germination test conducted at Seed Testing Laboratory, NACGRAB, Ibadan.

Source of Variation	DF	Germination (%)
Rep	2	200.00*
Accession (ACC)	1	13667.56**
Storage Environment (ENV)	2	5942.00**
Storage Time (STR)	3	3471.70**
ACC x ENV	2	13.56 ns
ACC x STR	3	268.44**
ENV x STR	6	1272.37**
ACC x ENV x STR	6	367.33**
Error	46	49.275
Total	71	694.42
R^2 (%)		95
CV		10.80
Mean		65.00

*, **, Significant at probability level of 0.05 and 0.01, respectively; ns = not significant

Table 3. Effect of accessions, storage environments and time in storage on seed germination of soybean at NACGRAB, Ibadan.

Factors	Seed germination (%)
Accession	
NG/10/02/12/148 (ACC1)	51.22b
NGB 00111 (ACC2)	78.78a
LSD	3.33
Storage Environment	
Ambient (ENV1)	46.83b
Short term (ENV2)	74.33a
Medium term (ENV3)	73.83a
LSD	0.33
Time (Months)	
April (STR1)	74.44a
June (STR2)	73.33a
August (STR3)	67.55b
October (STR4)	44.67c
LSD 0.05	4.71

Means with different letters within the column of the same factor are significantly different at P=0.05

accessions was significant on germination. This agrees with the findings of Tame and Elam (2015) who reported significant difference in germination of soya bean varieties. Similar results were reported by Omar *et al*. (2012) where highly significant differences were obtained among the tested cultivars of wheat for all the characters studied. The short shelf life of soya bean seeds is as a result of its high lipid content and high levels of polyunsaturated linolenic and linoleic acids. Hence, this study investigated the influence of storage environments and storage time on the germination of soya bean seeds. A non-significant difference was noticed between the germination percentages of soya bean seeds stored in the short and medium term storage chambers. This might be due to the inability to maintain uninterrupted power supply to these two storage environments which resulted fluctuation in temperature coupled with high relative humidity in the environments. There were differential germination responses of soya bean seeds at different storage times. From this present study, irrespective of the storage environment, germination started declining as the storage time increased. This finding is in agreement with the report of Verma and Tomer (2003). The authors stated that seed germination, emergence rate and seedling establishment decreased with increase in seed storage period in Brassica (*Brassica campestris*). Also, the result corroborated the findings of Yilmaz and Aksoy (2007) who reported a decrease in the germination of *Rumex scutatus* with increase in storage time irrespective of different storage conditions.

Table 4. Seed Germination of Soybean Seeds as Affected by Accession, Storage Environment and Storage Period at NACGRAB, Ibadan.

Accessions	Storage Environment	Storage Period	Mean germination (%)
ACC1	ENV1	STR1	41.33
ACC1	ENV2	STR1	61.33
ACC1	ENV3	STR1	64.00
ACC1	ENV1	STR2	54.67
ACC1	ENV2	STR2	64.00
ACC1	ENV3	STR2	72.00
ACC1	ENV1	STR3	29.33
ACC1	ENV2	STR3	66.67
ACC1	ENV3	STR3	64.00
ACC1	ENV1	STR4	4.00
ACC1	ENV2	STR4	53.33
ACC1	ENV3	STR4	40.00
LSD (0.05)			**13.93**
ACC2	ENV1	STR1	93.33
ACC2	ENV2	STR1	92.00
ACC2	ENV3	STR1	94.67
ACC2	ENV1	STR2	77.33
ACC2	ENV2	STR2	78.67
ACC2	ENV3	STR2	93.33
ACC2	ENV1	STR3	70.67
ACC2	ENV2	STR3	89.33
ACC2	ENV3	STR3	85.33
ACC2	ENV1	STR4	4.00
ACC2	ENV2	STR4	89.33
ACC2	ENV3	STR4	77.33
LSD (0.05)			**9.48**

There were differential germination responses due to interactive effect of storage environment and time on soya bean accessions. Soya bean accession NGB00111 (ACC2) had a higher mean germination percentage of above 75% when stored under short and medium term environments after ten months in storage. Similar results were recorded by Omar *et al*. (2012) where they reported highly significant interaction among storage periods, cultivars and environments for radical and plumule length in wheat germination. Hence, in assessing the germination of soya bean seeds, genotype, storage time and storage environment should be given prime consideration in order to avoid wrong conclusion on the germination potential of a particular accession.

Conclusion

In spite of the short shelf life of soya bean seeds, the longevity of soya bean seeds

depends on the interactive effect of genotype, storage environment and storage time. Hence, from this study, it can be concluded that with a minimum of ten hours of electricity supply, soya bean seeds can be stored safely for over one year at a temperature below 15°C and at a relative humidity below 40% with germination value of >75% depending on the genotype. There was no significant difference in the germination of soya bean seeds stored under both short and medium term conditions from this study. This suggests that the temperature and relative humidity in both short and medium term storage conditions were not effective to bring a significant difference in the germination value recommended for short and medium storage periods. In addition, irrespective of the storage environment, germination of soya bean seeds declined as the storage time increased.

Recommendation

Optimisation of the cold room conditions could result in high soya bean seed germination and longer storage times. The results of this study are important for soya bean seed producers operating under such conditions in their decision making. It is therefore recommended that soya bean stored in cold rooms under this conditions must be kept in a tightly closed moisture-resistant container. In addition, the relative humidity in the storage enviroments can be reduced to the barest minimum by using a dehumidifier especially in the medium term chamber. Nevertheless, soya bean seed viability monitoring should be done annually while adequate plan should be made for the regeneration at least once in two years to ensure minimum germination standard of 75%.

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