GERMINABILITY OF VARIETIES OF BEAN (*Phaseoulos vulgaris* L.) AND OKRA (*Abelmoschus esculentus* L. Moench) UNDER LOW TEMPERATURE STORAGE CONDITION

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Abstract: Bean (Phaseoulos vulgaris L.) and okra (Abelmoschus esculentus L. Moench) are common vegetables cultivated in the most regions of Sri Lanka. The seeds of these crops are mainly stored in cold storage as bulk before releasing retail lots to sales outlets. Although these certified seeds are frequently tested by seed testing laboratories, there are frequent complaints from farmers that they do not guarantee maximum field emergence. We hypothesized that the prolonged cold storage affects the vigour of the stored seeds. Certified seed lots of two pole bean varieties, 'Keppetipola Nil' (KN) and 'Bandarawela Green' (BG) and two okra varities, 'MI-5' and 'Haritha' were stored in poly sack and polypropylene bags under controlled temperature (17±1 °C) and relative humidity (RH) (52-55%) over a two-year period. Longevity of seed lots P₇₅ and P₅₀ were calculated using probit analysis. In the study, the bulk seeds of bean and okra varieties packed in polypropylene and poly sack bags resulted in a high germination percentage throughout the storage period for two years. Seed quality parameters, seed germination, seed moisture, field emergence and vigour index significantly varied with the storage duration and declined in all four varieties. Significant differences in moisture, field emergence and vigour index were recognized as affected by the packing materials in all varieties except MI-5. Both poly sack and polypropylene packing materials were found suitable to maintain viability at the minimum seed certification standards for two years of storage period. Poly sack was more appropriate than polypropylene to keep viability for more than 2 years of storage under low temperature and RH condition. These findings would help seed handlers including seed producers and seed sellers to decide the optimum conditions to store crop seeds between growing seasons in the tropical environment.

Keywords: cold storage; packing materials; prolonged storage; quality deterioration; seed longevity

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1. INTRODUCTION

It is of paramount importance to provide advice to seed handlers, including seed producers and seed sellers on how best the crop seeds can be stored between growing seasons, particularly in the tropical environment. Seed storage plays a major role in storing seeds as quality planting material for propagation in the subsequent seasons, as food storage and as conservation of genetic resources. During storage, process of seed aging is very complicated (Walters, 2007). It is affected by multiple factors such as seed quality at harvest, (Probert et al., 2009), pre-storage treatments such as processing and drying (Hong et al., 1998). According to several research findings, seed moisture content, temperature and seed container types used to keep optimal moisture level also affects the quality of seeds (Walters, 1998; Willis et al., 2012; Gomez-Campo, 2006; Walters, 2007). Among all, the moisture content in seeds, temperature and gaseous environment at which the seeds are stored, are the three main important environmental factors considered in seed storage (Probert et al., 2009).

Fate of seeds in the storage can be assigned to three main classes based on the seed population responses to dehydration or desiccation tolerance. They are, i) desiccation tolerant and long lived as orthodox, ii) desiccation sensitive and short lived as recalcitrant (Roberts, 1973) and iii) response to tolerate moderate desiccation as intermediate (Ellis et al.,1990, 1991). The seed storage responses of a range of species have clearly shown that the critical moisture content and temperature delimiting the three classes is dependent on a number of factors including the growth age of a material, processing method (especially drying temperature and speed) and post-storage methods (Prichard, 2003). According to Singh et al (2016), effective storage time is difficult to estimate because seed storability depends on the initial quality and storage conditions of the seed lots and may vary between different seed species. By monitoring seed deterioration, the longevity of seeds can be maximized and keep operating cost in practicable which is an essential function of commercial seed management.

Bean (*Phaseoulos vulgaris* L) and okra (*Abelmoschus esculentus* L. Moench) are common vegetables cultivated in several regions in Sri Lanka, creating a considerable seed demand annually. The responsibility of supplying certified quality seeds to the farmers in Sri Lanka lies on the Department of Agriculture, which produces the required seeds for bean and okra farmers. These seeds are stored in cold storage as bulk before releasing to the sales outlets. Commercial farmers and home gardeners purchase seeds as bulk or in the packed form from retail outlets. Laboratory test results of certified seeds do not represent the actual field germination which poses some qualitative difficulties to farmers. Farmers frequently make complaints on poor performance in their fields. The only possibility for the aforesaid outcome is the poor storage conditions that the seeds face until the seeds are used by the farmers. Therefore, in the present study, we attempted to assess the changes in the viability and germinability of bean and okra seeds packed in two different packing materials under the low temperature and RH conditions and stored as bulks for two years. This type of long-duration studies on seed storage are also scarce.

2. MATERIALS AND METHODS

Two varieties each of bean (*Phaseoulos vulgaris*) and okra (*Abelmoschus esculentus* L. Moench) which are high yielding and more popular among Sri Lankan farmers, were investigated

in the study. The initial germination percentage and moisture content were tested and recorded Table 1.

Crop	Variety	Initial germination (%)	Initial moisture (%)			
Bean	Bandarawela green	93	10.1			
	Keppetipola Nil	90	9.8			
Okra	MI-5	94	9.9			
	Haritha	93	10.5			

Table 1 Initial germination (%) and seed moisture (%) of varieties of bean and okra

Certified seed lots of two pole bean varieties, 'Keppetipola Nil' (KN) and 'Bandarawela Green' (BG) and two okra varities, 'MI-5' and 'Haritha' were used for this study. Seed lots of each variety were packed separately in polypropylene and poly sack bags in the farmer ware house and transported to the processing center at Kundasale. Seeds were Processed and packed in polypropylene as 10 kg bulk and packed in poly sack as 30 kg bulk separately. Those were kept under the controlled temperature $(17\pm1 \ ^{\circ}C)$ and relative humidity (RH) (52-55%) conditions for two years. Samples were drawn every one month and subjected to the standard seed quality tests.

According to the guidelines of the International Seed Testing Association (ISTA, 2015), Laboratory germination test was conducted using sand media method. Hundred seeds of each variety were used to plant 25×4 seeds per germination tray. Then kept them in germination chamber at 25 ± 1 °C with 8 hours of light to germinate. Number of germinated seeds was counted on the 5th day as the first count and considered as standard germination and expressed as a percentage.

Field emergence test conducted as in Randomized Complete Block Design with three replicates. 100 seeds per replicate were sown in well-pulverized soil and the normal seedlings count was taken in the 14th day of the planting as per the ISTA guidelines. Number of emerged plants were expressed as a percentage of field emergence.

The samples were ground and the high constant temperature oven method with a temperature 130-133 °C drying over 1 hour period was used as per the guided by the ISTA.

3. DATA ANALYSIS

Data were analyzed using SAS (Windows 9) statistical analytical package and treatment means separated using Duncan and LSD mean separation at probability level of 0.05. The Probit analysis first described by Roberts (1973) was used to model the data from seeds stored under the controlled environmental conditions. Changing viability with the storage period show liner relationship. Value of σ was represent by the slope of the line and initial viability of seeds denoted as Ki showed by the intercept. According to Ellis and Roberts (1980), relationship of $v = \text{Ki} - p/\sigma$ was used to calculate the viability (v) in probit values after p years in storage. Storage constants of P₇₅ and P_{50 termed} as the longevity of seeds according to Ellis and Roberts (1980). Where P₇₅ and P₅₀ are the time for viability to fall 75% and 50% respectively.

4. RESULTS AND DISCUSSION

4.1. Effect of packing materials and storage time on seed germination.

It is well established that the laboratory seed germination test usually provides an indication of the ability of seeds in a lot to germinate and produce seedlings that will emerge from the soil and grows into healthy and vigorous plants. Therefore, it is necessary to maintain high germinability during commercial seed production.

Bulk seeds of two bean varieties, Bandarawela Green (BG) and Keppetipola Nil (KN) packed in polypropylene and poly sack bags displayed a high germination percentage throughout the storage period for two years (Figure 1). Storage time significantly (p<0.001) affected the germination percentage while packing material \times variety interaction effect too (p<0.0001). Although packing material affected significantly for the variety KN, it was not so for the variety BG. According to the Roberts (1999), the patterns of loss of viability are similar for all orthodox seeds, for any given set of storage conditions and different species may show considerable differences in longevity. Both varieties in both packing materials-maintained seed certification standards (75% germination) over two-year period in this study.

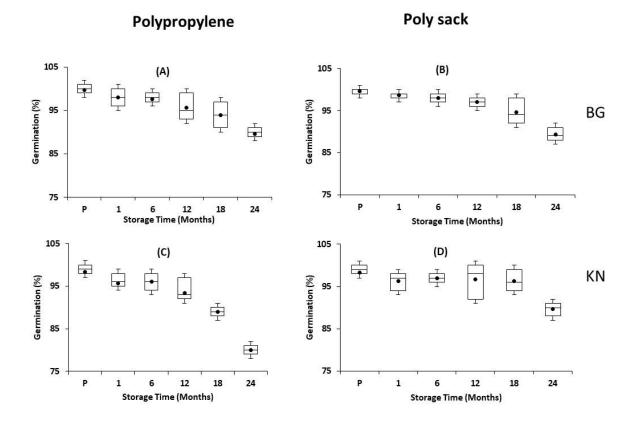


Figure 1 Box plot of mean germination of bean variety BG (A,B) and KN (C,D) in packing materials polypropylene (A,C) and poly sack(B,D) over the two-year storage period.

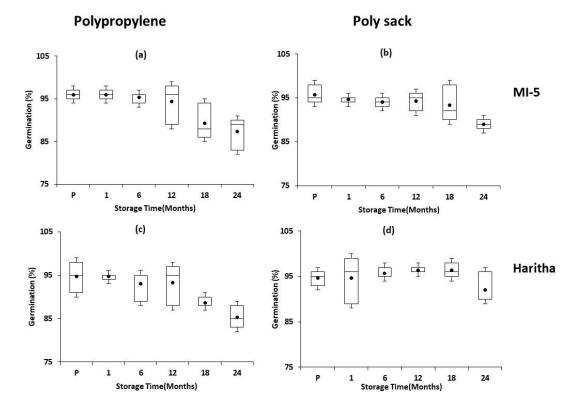


Figure 2 Box plot of mean germination of okra variety MI-5 (A,B) and Haritha (C,D) in packing materials polypropylene (A,C) and poly sack (B,D) over the two-year storage period

Seed storage conditions affected seed quality and in final seed yield after cultivation. The storage potential of seed lot is related to their stage of deterioration on initial conditions (Morad, 2013). In the seeds of okra stored under low temperature conditions in two separate packing materials during the 2-year period, there was a significant effect of time of storage (p<0.0001) and packing material (p<0.0001) on seed germination (Figure 2). Throughout the study period, irrespective of the variety, the germination percentage of okra seeds maintained the values above seed certification standards (75%) successfully.

Seed longevity is simply defined by Rajjou and Debeaujon (2008) as the total seed life span or the duration to which the seed viability is maintained after seed storage (storability). During seed storage, seeds tend to deteriorate while losing the vigour. As a result, seeds become more sensitive to stresses during germination and ultimately die. The rate of this aging depends on the seed moisture content, temperature and initial seed quality (Walters et al., 2005b). The seed longevity varies among families, species, genotypes, seed lots, and even among individual seeds inside the same container and depends on the storage conditions too (Walters et al., 2005a; Nagel et al., 2009; Probert et al., 2009; Van et al., 2013; . Ho-Sun et al., 2013; Desheva, 2016).

Results from the assessment of probit longevity (the time taken for germination to fall to 75% (P₇₅) and 50% (P₅₀) of seed lots of germination under constant temperature and RH conditions are presented in Table 2. Seeds with high initial viability and low moisture conditions will also

survive longer in storage. Even though good storage conditions maintained the viability of seeds, it declines with the duration of the storage (Walters et al, 2005a). Seed aging or seed deterioration is commonly described as the loss of seed viability over time (Coolbear, 1995). Hence, the viability of commercial seeds is needed to be assessed periodically to detect the loss in viability during storage before it falls below the standard level.

Table 2 Seed longevity of Bean and Okra varieties after two-year storage in low temperature and RH

		Polypropylene						Poly sack					
Сгор	Variety	Moisture content (%) variation during storage	Ki	1/σ	σ (Years)	P 75 (Years)	P50 (Years)	Moisture content (%) variation during storage	Ki	1/σ	σ (Years)	P75 (Years)	P50 (Years)
Bean	BG	13.8±0.506	1.0009	-0.0036	23.15	5.81	11.60	12.0±0.617	1.0000	-0.0032	26.04	6.51	39.06
	KN	14.2±0.369	0.9972	-0.0062	13.44	3.24	6.68	12.0±0.6333	0.9846	-0.0034	24.51	5.75	11.88
Okra	MI-5	10.2±0.271	0.9683	-0.0039	20.26	4.66	10.01	10.1±0.437	0.9581	-0.0028	29.76	6.19	13.63
	Haritha	10.9±0.579	0.9533	-0.0039	21.37	4.34	9.90	10.3±0.245	0.9627	-0.0024	34.72	7.39	16.07

Ki – Probit value of initial seed viability; $1/\sigma$ – measure of seed deterioration in storage; σ – standard deviation of seed death in storage; P75% – time in years for seed viability reduction with 75%; P50% – seed half-life or measure of time to 50% seed viability in storage.

In the present study, the seed longevity was measured on the basis of the σ value (standard deviation of mortality during storage), which defines the period of percentage viability that is reduced by one probit as described by Hong et al. (1998). According to Ellis and Roberts (1980), the life span of a seed-lot, the time until all the seeds have lost viability, depends on the value of σ and on the proportion of the seeds which are viable at the initial stage of the storage, Ki (in Probit). Although, in the current study, bean variety BG in both packing materials showed a higher initial value of Ki, seed deterioration displayed a lower value in poly sack bags. It resulted a long longevity in P₅₀ in poly sack bags compared to that in polypropylene. Seed deterioration was similar in okra varieties MI-5 and Haritha in polypropylene but their changes in initial viability made the differences in longevity. Seeds stored in poly sack bags showed a lower value of deterioration and moisture (Table 2).

Seed certification standards and procedures are adopted to make sure that the farmers are provided with high quality seed of superior varieties so grown and handled to ensure authenticity and genetic purity and a high standard of seed quality (Seed Certification Hand book part II, 1983). For all varieties of bean and okra, 75% is the seed certification standard. In this study, p₇₅ was maintained over minimum 3 years which is of great use to commercial seed production and selling. Based on these standards and results, it is clear that periodical checking the viability prior to issuing seed lots to the farmer is essential.

In tropics, storing seeds for a considerable length of time can be difficult without reliable electricity for cooling and drying. However, storing seeds under low temperature and RH is the existing practice which is a relatively inexpensive technology mostly used in prolonged storage of seeds in tropics (ECHO Staff, 2016).

4.2. Effect of storage time and packing materials on seed quality parameters

Seed quality parameters, i.e. seed germination, seed moisture, field emergence and vigour index significantly changed with the storage duration and reduced in varieties of bean (Table 3) and okra (Table 4).

64		Variety	BG	Variety KN				
Storage Time (Months)	Germination	Moisture	FE	VI	Germination	Moisture	FE	VI
1	98.3 ^A	13.66 ^a	97.16 ^A	1740.42 ^a	96.0 ^A	14.33 ^a	93.3 ^B	1679.48 ^a
6	97.8 ^A	13.38 ^a	93.66 ^{AB}	1623.88 ^b	96.5 ^A	13.21 ^b	96.1 ^A	1730.36 ^a
12	96.3 ^{AB}	12.63 ^b	92.0 ^B	1508.13 ^c	95.0 ^{AB}	13.06 ^c	91.83 ^B	1558.0 ^b
18	94.3 ^B	12.23 ^b	89.8 ^B	1370.72 ^d	92.6 ^B	12.75 ^d	96.0 ^C	1394.48°
24	89.5 ^C	12.56 ^b	83.5 ^C	1109.27 ^e	83.16 ^C	12.33 ^e	59.0 ^D	1109.61 ^d
acking material								
Polypropylene	95.3 ^A	13.72 ^a	90.46 ^A	1435.19 ^b	90.8 ^B	14.30 ^a	81.86 ^B	1474.76 ^b
Poly sack	95.0 ^A	12.06 ^b	92.0 ^A	1505.78 ^a	94.53 ^A	11.97 ^b	84.73 ^A	1514.01 ^a
CV(%)	2.01	2.89	4.32	3.77	2.26	0.66	2.43	3.48

Table 3 Effect of storage time and packing material on seed germination, moisture, field emergence (FE) and vigour index (VI) in bean varieties at the end of 1,6,12,18 and 24 months

Means with the same superscript letters within columns are statistically non-significant at 5% level of probability (DMR test). Please note the upper case and lower-case letters for two different parameters to compare within rows

In this study, the germination percentage of both of varieties of bean was observed above 83% while moisture content was also maintained below 14.3% up to 24 months. Results of field emergence of variety BG maintained 83.5% up to 24 months where variety KN showed 59% after 24 months of the storage. Seed germination, seed moisture, field emergence and vigour index of variety KN differed with packing material. Moisture content of seeds of variety KN stored in poly sack was within the limits of the seed certification standards. Although seeds stored in constant low temperature and RH conditions, seed qualities differed with the packing material. This appreciable decline of field emergence attributed to losses in germination during high moisture (13-14%) temporary storage regime for about two months immediately after harvest and actual processing by machine and drying. Seeds stored at polypropylene showed higher mean values of moisture compared to those stored in poly sack bags. Similarly, past literature reviewed that storage temperature and moisture content are the most important factors affecting seed longevity (Ellis and Hong, 2007).

According to Roberts (1999), the patterns of loss of viability are similar for all orthodox seeds and for any given set of storage conditions, however, different species may show considerable differences in longevity. Seed aging during storage over time is an inevitable phenomenon. Its degree and speed of decline in seed quality strongly depends on storage conditions, plant species and initial quality of seeds (Elias & Copeland 1994; Balešević-Tubić *et al*, 2005) and seed genetic traits (Malenčić *et al.*, 2003). Balešević-Tubić *et al.* (2010) concluded from their research that there was a significant difference of quality exists among cultivars of the same crops maintained during storage. Seed structure and climatic origin of seeds have also been identified as genetic factors associated with seed longevity (Probert et al. 2009). Excess moisture in seeds enhances seed deterioration, thereby reducing the value of germination in the field (Delouche 1980). Due to hygroscopic nature of a seed, it absorbs moisture from air and tends to reach an equilibrium with relative humidity if it is stored in an environment where relative humidity is lower than surrounding environment (Copland, 1976). Guptha (1976) also identified that the longevity of seeds in storage is influenced by four major factors; genetics, quality of the

seed at the time of storage, initial moisture content of seed and ambient relative humidity and temperature of storage environment.

Environments that surround seeds damage to their properties as a result of gases (particularly oxygen), water vapour, light, temperature, microorganism (bacteria, fungus, virus) macroorganisms (rodents, insects, birds) that are ubiquitous in many warehouses and outlets (Robertson, 2013). Polypropylene (PP) comprises of several qualities for packaging such as lower density (900 kg/m³), non-porous, low water vapour transmission, medium gas permeability, good resistance to gasses and chemicals, good abrasion resistance, high temperature stability, good gloss and high clarity than polythene (Roberston, 2013). Poly sacks have a good moisture barrier and high gas permeability (Ramesh, 2017). Moreover, permeability results found for low density polythene (LDPE) were 5.21 g.m⁻².day⁻¹ (refrigeration temperature) and 5.20 g.m⁻².day⁻¹ (28 °C). For PP, it was 6.95 g.m⁻².day⁻¹ (refrigeration temperature) and 8.3 g.m⁻².day⁻¹ (28 °C). LDPE and PP have 5.21 and 8.3 (g.m⁻².day⁻¹) water vapour transmission rates, respectively. This result shows that PP and LDPE are more suitable as packaging material (Ramesh, 2017).

Table 4 Effect of storage time and packing material on seed germination, moisture, field emergence (FE) and vigour index (VI) in okra varieties at the end of 1,6,12,18 and 24 months

Storage Time	8 <u></u>	Variety 1	MI-5	Vareity Haritha				
(Months)	Germination	Moisture	FE	VI	Germination	Moisture	FE	VI
1	95.3ª	10.3 ^A	87.5 ^b	1178.96 ^A	94.8 ^a	10.8 ^A	88.1 ^a	1186.05 ^A
6	94.6a ^b	10.4 ^{AB}	91.3 ^a	1098.43 ^B	94.3 ^a	10.8 ^A	81.6 ^c	1128.8 ^B
12	94.3 ^{ab}	10.1 ^{AB}	90.3 ^a	1041.85 ^C	94.8 ^a	10.6 ^B	86.3 ^b	1043.87 ^C
18	89.6 ^c	9.9 ^C	82.1 ^c	969.28 ^D	92.5 ^b	10.4 ^C	72.3 ^d	957.00 ^D
24	88.1 ^c	9.1 ^D	74.5 ^d	848.0 ^E	88.6°	9.5 ^D	67.5 ^e	917.37 ^E
acking material								
Polypropylene	92.01 ^a	10.15 ^A	89.4 ^a	1026.98 ^B	90.71 ^b	10.8 ^A	82.8 ^a	1041.13 ^B
oly sack	92.89 ^a	10.12 ^A	86.4 ^b	1061.76 ^A	93.93 ^a	10.2 ^B	81.2 ^a	1080.73 ^A
CV (%)	3.04	1.04	5.43	5.2	2.9	1.17	6.21	5.13

with the same superscript letters within columns are statistically non-significant at 5% level of probability (DMR test). Please note the upper case and lower-case letters for two different parameters to compare within rows

However, germination percentage of both of varieties of okra was observed above 88% and moisture content was also maintained below 10.8% up to 24 months. Results of field emergence of variety MI-5 maintained 74.5 % up to 24 months where variety Haritha showed 70% up to 24 months of the storage. Although seed germination and seed moisture of variety Haritha differed with packing material, they were within the limits of the seed certification standards. These quality parameters were maintained in acceptable level in seeds stored in both packing materials of both of varieties without any inconsistencies.

As well understood, germination test results do not provide sufficient information to the farmers and do not guarantee the field performances of seeds, especially when the seeds are stored for a longer duration even under constant environmental conditions inside a sealed packet.

Although the packaging material has a strong effect on seed deterioration, especially in relation to moisture absorption (Sultana et al, 2016), in this study, the packaging materials significantly affected the moisture absorption. Therefore, the observed deterioration in okra and bean seeds after 24 months was merely due to moisture fluctuations.

In previous studies, seedling emergence in the field was controlled by an array of interacting factors, including genetic constitutions, seed dormancy, seed vigour, depth of planting, soil aeration, temperature and water supply (Forcella *et al.*, 2000; Samarah and Al-Kofani, 2008). The present study shows that, both varieties of beans maintained their field emergence potential above 50% until the end of two years, which is appreciable under tropical environments in Sri Lanka. The evaluation of vigour test for predicting field performances in future resultant crop is very important for commercial cultivation. Although many researchers reported that there is a significant correlation between standard laboratory germination and field emergence, many inconsistencies and difficulties with the accurate prediction of field emergence have often been reported (Kolsinska *et al.*, 2000; Samarah and Al-Kofahi, 2008; Wang *et al.*, 2004). Germination tests are used to estimate normal seedling production under optimal germination conditions (Atwater, 1978; Geneva, 2008).Though, standard germination test is not considered as a good indicator of field emergence (Alillo, 2011) and this test always does not have ability to reflect the field emergence potential of the seed lots (Morad, 2013).

Basic objective of vigour testing is to be responsible for a consistent identification of variances in physiological potential among seed lots and this implies a more sensitive parameter than the germination test alone (Marcos, 2015). Seeds with high vigour better able to withstand storage conditions with any form of stress (e.g. changes in temperature or relative humidity in ambient storage conditions) and decline in quality at a slower rate than seeds with lower vigour. Post-storage performance, even under controlled storage conditions, depends on the vigour of the seed lots (ISTA, 2009).Vigour index decreased continuously over the two years of period irrespective of the packing material in varieties of bean and okra.

The current study reveals that the potential germination of a seed lot is sufficiently represented by the standard laboratory germination test and longevity of the seed lot can be predicted using probit analysis. The difference between potential germination and actual germination in the field is interrelated with the packing material, storage time and moisture content. The underlying mechanisms of these responses warrant further investigations.

CONCLUSION

The results obtained from prediction of P₇₅ values indicated that they are above standard level of germination and no difference in loss of viability during the two years of storage period under the low temperature and RH conditions irrespective of the packing material compared with the initial viability. Significant differences in moisture, field emergence and vigour index are attributed due to the packing material of all varieties except MI-5. However, seed qualities considered in the present study (germination, moisture, field emergence and vigour index) showed wide variation between varieties within the same species even under the same storage condition. Both poly sack and polypropylene were suitable as packing material to maintain viability up to the seed certification standard over two years of storage period. Poly sack was more appropriate than polypropylene to keep viability more than two years of storage under low temperature and RH condition.

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