Quality Deterioration of Commercial Bean (*Phaseolus vulgaris*) Seeds Stored under Contrasting Environmental Conditions in Sri Lanka

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Abstract

Bean is a popular vegetable grown in most parts of Sri Lanka. Farmers and home gardeners purchase seeds in packeted form retail outlets. However, quality problems are frequently reported, especially on low field germination. Quality parameters namely, percentage germination, field emergence, seedling length, vigour index and chlorophyll fluorescence analysis were used to determine the effect of different storage environmental conditions on seed longevity of two bean varieties during a two-year storage period, 2016 to 2018. Seeds in triple-laminated packets stored in four contrasting conditions; low temperature storage conditions (Tem. 5°C, 65% RH); three geo-environmental conditions namely, Gannoruwa (mid country wet zone), Kundasale (mid country intermediate zone), and Mahailuppallama (low country dry zone) showed varied behavior in seed quality parameters respective of the variety. It was apparent that germination test alone does not represent the full potential of field emergence. Results of the present study revealed that when field emergence and vigour are also taken in to account, both varieties of bean seeds can be stored under ambient conditions at Gannoruwa, Kundasale or Mahailuppallama for a maximum period of 12 months, without compromising the seed quality. The seeds stored under low temperature conditions (i.e. 5 °C), maintained the seed germination over 75% for a period of 24 months.

Keywords: Field emergence; Germination; OJIP analysis; Storage condition; vigour index

Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crops in Sri Lanka. Total extent under cultivation in Sri Lanka in 2017 was 7723 ha and the total annual production was reported as 87,385 mt (Department of Census and Statistics, 2017). In Sri Lanka, certified and basic seeds of beans are produced in seed farms of the Department of Agriculture (DOA) and related contracted farmer fields. Following harvest and processing, seeds are stored in bulk form at a central location named Rikillagaskada (7.145˚ N: 80.782˚ W). Thereafter, seeds are transported to Kundasale (7.267˚ N: 80.683˚ W) government farm and kept under cold room conditions. Finally, these quality certified seeds are transported to the Vegetable Seed Centre in Gannoruwa (7.283˚ N: 80.583˚ W), packed in triple laminated Aluminum foil (TLA) and sent to sales outlets located in many locations including the Dry Zone of the country. Even though the germination percentage of these seeds under laboratory conditions is
satisfactory and meets certification standards, poor field performance has been observed by farmers, throughout the country. This situation puts the Department of Agriculture under enormous pressure as the farmers tend to question the reliability of the certified seeds. However, several previous researches indicate that storage conditions are the prime factors that determines the longevity and storability of many seeds (Adsul et al., 2018; Copland and McDonald, 1995; Pradhan et al., 2012; Shelar et al., 2008).

Therefore, the present study was conducted to elucidate the effect of contrasting storage environmental conditions on seed quality parameters and to determine the appropriate storage environmental conditions in Sri Lanka for bean seeds packed in triple laminated aluminum foil.

**Materials and Methods**

Certified seed lots of two pole bean varieties; Keppetipola Nil (KN) and Bandarawela Green (BG) were tested in the study. Initial germination and moisture percentage of KN (90%, 9.8%) and BG (93%, 10.1%) were recorded. Twenty-eight kilograms of Seed lots for each treatment were transported to the Vegetable Seed Center at Gannoruwa for triple laminated packing in 10 g packs as retail packets. Initial bulk seeds which were stored in polysacs and later packed in to TLA were stored at the following locations: (a) Gannoruwa Vegetable Seed Centre, (b) Kundasale Seed Sales Centre and (c) Mahailluppallama Seed Sales Centre of the DOA under ambient conditions which represent the climatic conditions on mid-country wet zone (MCWZ), Mid country intermediate zone (MCIZ) and low country dry zone (LCDZ), respectively. Retail packets from initial bulk seeds stored in polypropylene were kept in (d) Low temperature condition (Bottle cooler- 5 °C and 65% RH) at Kundasale Seed Sales Centre as a control. All seeds packs were sampled at monthly intervals over a period of 24 months and subjected to laboratory and field tests at Gannoruwa, Peradeniya. The experiment design was Completely Randomized Design with three factor factorial arrangement and each combination was replicated three times.

Agro-ecological regions in Sri Lanka are defined by mainly latitude and rainfall distribution pattern. Mid country wet zone (MCWZ) is typified by a latitude between 300-900 m and an annual rainfall of more than 2500 mL. Mid country intermediate zone (MCIZ) receives an annual rainfall of 1750-2500 mL, and areas with latitude and rainfall below 300 m and 1750 mL respectively, belong to low country dry zone (LCDZ) (Punyawardhana, 2008). Temperature and RH of the study locations was monitored using thermo – hygrometer and maximum and minimum daily temperature. The morning and evening Relative Humidity values were obtained from the Natural Resources Management Center (NRMC) of DOA.

Germination percentage, hypocotyl length at first count of germination and seed vigour index were recorded according to the guidelines of the International Seed Testing Association (ISTA, 2015). Prescribed sand media was used to plant 25 x 4 seeds per germination tray and allowed to germinate inside a germination chamber at 25±1 °C with 8 hours light. Normal seedling count was obtained on the 5th day of germination test and expressed as a percentage. Hypocotyl length of randomly selected 20 seedlings was measured at the 5th day of the germination using a ruler from base of the shoot to base of the cotyledons of the seedling. Seedling vigour index was determined by multiplying the percentage of germination and the hypocotyl length as prescribed by Abdul Baki and Anderson (1973). Germination tests were conducted at the Central Seed Testing Laboratory (CSTL) of the Department of Agriculture.

Field tests (seedling emergence) were conducted as Randomized Complete Block Design with three replicates at Post-control Field # 1 of Seed Certification Service, Gannoruwa. One-hundred seeds were sown in well-pulverized soil and normal seedlings which emerged at 10th day of the planting were calculated as percentage of field emergence. Shoot length of randomly selected 30 seedlings were measured using a ruler from base of the shoot to base of the first leaf of the seedling at the 10th day of the planting.

The polyphasic OJIP chlorophyll fluorescent transient and related parameters (OJIP analysis) were measured by using FluorPen 100 fluorometer (PSI, Czech Republic) at the time of 5th day of the seedlings germinated under laboratory conditions. OJIP chlorophyll fluorescent transients and its related parameters were analysed using procedures described by Strasser et al., 2000.

Mathematical models have been developed to predict the longevity of crop seeds in store under the controlled environmental conditions. The “basic viability equation” was the first attempt developed incorporating three constants to explain the dependence of seed survival on seed moisture content, temperature and an inherent property of species (Roberts 1973). After that, Ellis and Roberts (1980) introduced an improved viability equation including four viability constants (two for temperature, one for moisture content and one related to moisture that defined the species). Using this equation, it was able to explain variability in longevity between seed lots of the same and different species with the initial viability of the seed lot. σ represents the standard deviation of the distribution of deaths in time. Kσ and Kv are constants.

\[
\log\, \sigma = \log\, K_\sigma + K_v - C_1 m - C_2 t \quad \text{[1]}
\]

Equation [1] may be re-written as

\[
\log\, \sigma = K_l - C_1 m - C_2 t \quad \text{[2]}
\]

Where
KL = log Kσ + Kv  \[3\]

According to Finney (1977), seed survival curves are plotted as probit percentage viability with the time, and the straight lines of negative slope are produced which can be defined as 1/σ. Therefore, survival (viability remaining) can be predicted by following equation.

\[ v = K_i - (1/\sigma) p \]  \[4\]

\(v\) is viability (probit %) in \(p\) storage period and \(K_i\) is the initial viability (probit %) before storage. The relative differences in longevity between seed lots is maintained in all environments and it is not dependent on species. \(C_1\) and \(C_2\) are constants.

It can be rewritten as follows. \(v = K_i \frac{p}{10} KL - C_1m - C_2t\)  \[5\]

Viability \(V\) is converted in to probit values (probit%) at any time in storage and \(p\) is a combination of moisture content (m) and temperature (t). \(KL - C_1m - C_2t\) are species-specific constants. Initial viability \(K_i\), have a large effect on predictions of longevity.

Improved viability equation developed further as follows

\[ v = K_i \frac{p}{10} K_E - C_w \log m - C_H t - C_Q t \]  \[6\]

\(K_E, C_w, C_H, C_Q\) are the viability constants which apply to all genotypes and seed lots within a species. Percentage viability of any seed lot of a species can be estimated in relation to improved equation [6]. This enables calculation of the expected time for viability to fall from any initial percentage viability to any final percentage viability for any seed lot stored under any combination of temperature and moisture content over wide range of conditions.

However, these models are relatively accurate in constant environments (constant moisture and temperature).

Simplified viability equation Andreoli (2004) simplified the Ellis and Roberts equation and proposed a new model [7] that Can be applied to open storage as well.

\[ V = K_i (1/\sigma) p \]  \[7\]

\(V\) is the probit percent germination at the time \(p\) and for which \(K_i = v\) when \(p = 0\). Plate 1 demonstrate the \((Vp-V_i)/p\), where \(Vp\) is the probability at the time \(p\) and \(V_i\) is the initial germination in probit and is a measure of the slope of a seed survival curve.

\[ (Vp-V_i)/p = -t_g \beta \]  \[8\]

The slope or \(t_g \beta\) of equation (8) is a direct measure of the slope \((1/\sigma)\) of the seed survival curves. Therefore, the regression coefficient of the improved equation (6) \(\sigma (10 K_E - C_w \log m - C_H t - C_Q t)\) is equal to the coefficient \(t_g \beta\) of the simplified equation (8). Therefore, \(t_g \beta\) is the seed deterioration rate under any storage condition as expressed by the angular co-efficient of the survival curve and rewritten as follows:

\[ V_p = V_i - (t_g \beta) \cdot p \]  \[9\]

The slope or \(t_g \beta\) is reliant on the seed storage environmental conditions (Ellis and Roberts, 1980; Roberts, 1973).

Plate 1: Diagram illustrated simplified equation of seed survival that has a slope of \(t_g \beta\) developed by Andreoli, 2004)

Data were analyzed using the SAS (V9 for Windows) statistical analytical package. The germination data were transformed in to probit values using Finney’s table (Finney, 1952). The seed deterioration curves in two varieties under different storage conditions were analyzed by probit analysis and adjusted by equation 9 \((V_p = V_i - (t_g \beta) \cdot P)\) to predict the bean seed viability under ambient environmental conditions.

**Results and Discussion**

*Ambient temperature and relative humidity of storage environments during the storage period.*

Mean monthly maximum and minimum air temperature (T) and the relative humidity (RH) of the selected locations during the study period (March 2016 to March 2018) are shown in Fig. 1 and 2.
Table 1: mean annual temperature and mean annual RH and differences of different seed storage conditions during 2016March-2018March

| Storage Condition            | Mean Temperature (°C) | Mean difference between Max and Min | Mean RH(%) | Mean difference between Morning and Evening |
|------------------------------|-----------------------|------------------------------------|------------|------------------------------------------|
| Low temperature condition(Bottle cooler) | Max: 5 | Min: 5 | 0 | 65 | 65 | 0 |
| Gannoruwa(MCWZ)             | 29.7±1.3              | 21.4±1.4                           | 8.3±1.8    | 78.8±3.0 | 69.2±7.4 | 9.6±5.5 |
| Kundasale(MCIZ)             | 3.06±1.4              | 20.7±1.0                           | 9.9±1.8    | 77.3±4.2 | 63.8±6.3 | 13.6±6.3 |
| MahaiIlluppallama(LCDZ)     | 32.7±1.7              | 23.3±1.8                           | 9.4±1.5    | 83.1±4.7 | 60.8±8.2 | 22.4±6.8 |

Source: Database of NRMC, DOA

Seed Germination Under Low Temperature Conditions

During the study period, there was no influence of the storage environment and variety on seed germination. After 24 months, the seeds maintained high germination within the seed certification standard of greater than 75%, when packed in TLA and stored in the bottle cooler (5°C and 65 % RH) (Fig. 3).

Seed Germination Under Ambient Environmental Storage Conditions

The germination data were converted in to probit values using Finney’s table (Finney 1952) and adjusted by equation 9 (Vp = Vi - (tgβ). P) to predict the bean seed viability under ambient environmental conditions. tgβ is the seed deterioration rate of each variety which was not affected by the genotype or seed quality but affected by the storage environmental conditions (Ellis and Roberts,1980; Roberts,1973; Roberts and Abdulla,1968). Since Vi is the
probit of the percentage viability at the beginning of the storage period, the seed deterioration rate for each storage condition and variety was determined using the initial viability (at time=0) and that after 30 days of storage (Vt− V30). Then the simplified equation proposed by Andreoli (2004) was applied to predict the loss of storage time during each storage condition.

As shown in the Fig. 4, the deterioration of tgβ of the bean variety BG in Gannoruwa was 0.1028 (r² = .89), Kundasale 0.0776 (r² = .89) and Mahailuppallama 0.2203 (r² = .85). Although seed lots having same initial quality deterioration rate for the same variety at different storage conditions were varied, the deterioration rate of bean variety KN in Gannoruwa was 0.0977 (r² = .81), Kundasale 0.0997 (r² = .87) and Mahailuppallama 0.1775 (r² = .95). Highest deterioration rate for both varieties was observed in Mahailuppallama. Observed highest deterioration in the bean seeds after 24 months would be due to the temperature and relative humidity fluctuations at the high-end. Lowest deterioration rate for variety BG was observed in Kundasale and for variety KN in Gannoruwa. Although findings of behaviour on ambient environment conditions on commercial seeds are scarce, Marcondes et al., (2011), used this simplified model to estimate germination change and viability loss during the conventional storage of fungicide treated and untreated wheat seeds which gives high accuracy. Tgβ, that is seed deterioration rate, was high in the conventional storage conditions where temperature was in the higher ends. Similar results were obtained by Andreoli (2004) in soybean and corn seeds in Brasilia and also these results were in compliance with the viability equation of Ellis and Roberts (1980).

Table 2 presents the difference between estimated and observed values of storability time for both varieties which did not vary much under ambient storage conditions. Simplified model nearly accurately predicted the potential storability.

Fig. 3: Deterioration of bean variety (a) Bandarawela Green (BG) and (b) Keppetipola Nil (KN) stored in low temperature condition expressed as the probit of germination fitted by probit analysis using Finney’s table (Finney 1952) (5ºC and 65% RH) for 2 years (from March-2016 to March-2018)

Fig. 4: Deterioration of bean variety (a) Bandarawela Green (BG) and (b) Keppetipola Nil (KN) stored in Gannoruwa (G), Kundasale(K) and Mahailuppallama(MI) ambient storage conditions expressed as the probit of germination fitted by probit analysis using Finney’s table (Finney 1952) ) for 2 years(from March-2016 to March-2018).
### Table 2: Mean initial germination (Vi), and time taken to reach the minimum seed certification standard of 75% germination (p75), loss of storability (months) and coefficient of determination ($r^2$) observed in bean seeds, variety BG and KN, stored in three different environmental conditions

| Variety | Storage condition | Initial Germination (% | Estimated P75 (Months) | Observed P75 (Months) | Difference between estimated and observed storability time (Months) | $r^2$ |
|---------|-------------------|------------------------|------------------------|-----------------------|---------------------------------------------------------------|-------|
| BG      | Gannoruwa (MCWZ)  | 98.3                   | 16                     | 16                    | 0                                                             | .89   |
|         | Kundasale (MCIZ)  | 98.3                   | 22                     | 20                    | -2                                                            | .89   |
|         | Mahailuppallama (LCDZ) | 98.3               | 10                     | 13                    | +3                                                            | .85   |
| KN      | Gannoruwa (MCWZ)  | 99.0                   | 17                     | 17                    | 0                                                             | .81   |
|         | Kundasale (MCIZ)  | 99                     | 18                     | 17                    | +1                                                            | .87   |
|         | Mahailuppallama (LCDZ) | 99                 | 11                     | 12                    | -1                                                            | .94   |

### Table 3: Effect of four different storage conditions on two bean seed varieties on field emergence percent after storage periods ranging from 1 to 24 months.

| Stored Time (Months) | Low temperature (LT) | Storage condition | Mahailuppallama (MF) | Storage condition | Mahailuppallama (MF) |
|----------------------|-----------------------|-------------------|----------------------|-------------------|----------------------|
|                      | Gannoruwa (G) | Kundasale (K) |                      | Gannoruwa (G) | Kundasale (K) |                      |
| 1                    | 93.3^a            | 96.3^A             | 95.6^a               | 98.3^A            | 96.0^a             | 92.3^A               | 96.3^A            | 88.3^A            |
| 6                    | 90.0^a            | 90.0^B             | 87.6^b               | 81.0^B            | 93.0^a             | 88.0^a               | 91.6^ab           | 83.0^B            |
| 12                   | 89.3^a            | 83.3^C             | 76.6^c               | 60.0^c            | 90.0^ab           | 78.3^C               | 86.0^c            | 60.3^C            |
| 18                   | 78.5^b            | 48.0^D             | 63.0^d               | 0                 | 79.0^e             | 70.0^D               | 67.0^d            | 26.3^D            |
| 24                   | 72.0^e            | 28.3^E             | 45.3^e               | 0                 | 72.0^d             | 8.3^E                | 15.0^e            | 0.4^E             |
| %CV                  | 6.06               | 13                 | 9.94                 | 11.4              | 4.68               | 7.8                  | 8.16              | 15.3              |

*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 varieties to compare within columns.*

Seed deterioration is an irreversible degenerative natural process which usually expresses as the loss of quality, viability and vigour during storage or under adverse environmental conditions (Kapoor et al., 2011). High temperature and high relative humidity causing in alterations in genetic integrity leads to more rapid deterioration of seeds (Kapoor et al., 2011).

**Effect of Storage Time, Storage Condition and Variety on Field Emergence**

Storage conditions, variety and stored time interactively affected the field emergence percentage significantly ($P<0.0001$). Field emergence percentage of seed stored in different conditions decreased with the storage time. Table 3 presents the time taken to reach the field emergence in both varieties where seeds stored in low temperature condition showed the best performance. Even though seeds were stored in a low temperature conditions, field emergence percentage was lower than the laboratory germination values, however, above the seed certification standard values (70%) with the study period.

In addition, shoot length in emerged plants were significantly affected by the storage condition (Fig. 5). High quality seedlings need to avoid losses from mortality and slow growth after planting (Figueiredo et al., 2011). The results revealed that the shoot length of seedlings was significantly higher in seeds stored in low temperature condition than seeds stored in other three ambient environmental conditions. Shoot length of emerged seedlings of the seeds stored at Gannoruwa and Kundasale showed same behavior and the lowest was observed in Mahailuppallama. Vijay et al., (2015) also concluded that seed deterioration depends on chemical composition and storage environment by studying hybrid sunflower-RSFH-130 (*Helianthus annuus*).
Vigour Index

There was a significant interaction effect among the storage time, storage condition and variety \((p < 0.0001)\) with the vigour index. Effect of storage conditions and variety on vigour index is given in Table 4. Highest vigour index was observed in seeds stored under low temperature conditions and the lowest under the Mahailuppallama (LCDZ) environmental conditions.

Based on the Tables 2 and 3, the germination percentage is not a valid predictor of field emergence for bean seeds. Vigour tests such as accelerated aging, respiration rate and ATP content considerably correlated with forage yield and seed respiration rate, and can be used to predict the yield of first year of meadow brome grass (Reginal, 1987). Kazem Ghassemi and Bahareh (2014) also showed that low vigour seeds lose their grain yield at considerable amount in Maize. Present study also suggests that vigour index becomes a critical indicator than standard germination and field emergence. Use of percent germination to predict the field performance of a seed lot may be an over estimation.

Our previous study on okra (Ariyarathna et al., 2020) showed that germination test alone does not represent the full potential of field emergence. In addition, the same study revealed that, when field emergence and vigour are also taken in to account, varieties MI-5 and Haritha of okra seeds (initial germination and moisture of 94.93 and 10.0, 10.5) can be stored under ambient conditions at Gannoruwa, Kundasale or Mahailuppallama for a maximum period of 12 months, without compromising the seed quality. The seeds stored under low temperature conditions (i.e. 17 °C), maintained the seed germination over 75% for a period of 24 months.

**Polyphasic Chlorophyll Fluorescence Transients - OJIP Test**

OJIP test was carried out in PS II reaction centers in chlorophylls present in seedlings. In this method, step-wise chlorophyll fluorescence transients were measured in logarithmic time scale. O-J-I-P refers to fluorescence intensity at 50µs, 2 ms, 30 ms and maximum intensity respectively (Strasser et al., 2000). These values and related parameters are used to identify the physiological status of the plant cells in response to a stress (Strasser et al., 2000).

In this study, OJIP readings were taken at the time of commencement of the decline in germination and field emergence at Mahailuppallama in 8th month and 4th month respectively. (Fig. 6-8).

Among the specific fluxes in the PS II reaction centers, only the ABS/RC showed an increase with time of aging, irrespective of the storage location and variety (Fig. 7).
explained by Lawlor and Tezara (2009), the increase in specific fluxes is partly due to the small number of active RC in the PS II. According to Strasser and Srivastava (1995), increased ABS/RC on leaves with N deficiency indicates an increase of antenna size. In addition, inactivation of some PS II reaction centers can also be led to an increase ABS/RC (Lu et al, 2001). Therefore, it can be speculated that the increase of specific fluxes in seedlings in the present study may be due to the inactivation of some PS II RCs as seeds tend to deteriorate within the storage period under different stress conditions prevailing in ambient environments.

Fig. 6: Quantum efficiencies or flux ratios analyzed with OJIP fluorescent transients in bean seedlings of variety BG (a,b,c,d) and variety KN(A,B,C,D) stored in Low temperature condition(a. A), Gannoruwa (b,B), Kundasale (c,C) and Mahailuppallama (d,D) during T4 -forth month and T8-eighth month. Means of maximum quantum yield of primary PS II photochemistry (\(\Phi_{\text{Po}}\)), probability at \(t=0\) that a trapped excition moves on electron in to the electron transport chain beyond QA- (\(\Psi_o\)) and quantum yield of electron transport at \(t=0\) (\(\Phi_{\text{Eo}}\)) are given.
Fig. 7: Specific energy fluxes analyzed with OJIP fluorescent transients in bean seedlings variety BG (e,f,g,h) and variety KN(E,F,G,H) stored in Low temperature condition(e, E), Gannoruwa (f, F), Kundasale (g,G) and Mahaluppallama (h,H) during T4–forth month and T8-eighth month. Means of absorption (ABS), trapping (TR) and electron transport (ET) per reaction center (RC) are given.

The quantum efficiencies (flux ratios) were either declined or remained the same in seedlings of both varieties of beans produced with aged seeds (Fig. 6). However, in the seedlings produced with aged seeds in low temperature condition, increased flux ratios were noted (Fig. 6a, 6A). These discrepancies were observed as the timing of measurements was not uniform across the locations. The performance index (Pi_Abs), which is the indicator of overall performance of the PS II antenna complex, (Strasser et al., 2000) shows a clear decline with aging in the seedlings under all four storage conditions. (Fig. 8) The lower Pi_Abs values reflect the lower vitality in photosynthetic tissues (Bussotti et al., 2006, Beneragama et al, 2014). Similarly, in our previous study of bean, the Pi_Abs of seedlings of 7-month old seeds of common bean showed a decreased Pi_Abs values compared to those of fresh seeds (Bandulasena et al, 2017). Therefore, the Pi_Abs can effectively be used to evaluate the seedling performance as affected by the aging of seeds under different environment conditions. Similar explanations have been presented previously by Strasser and Strasser (1995) and Krüger et al., (1997).
Fig. 8: Performance Index analyzed with OJIP fluorescent transients in bean seedlings variety BG (A) and variety KN(B) stored in Low temperature condition (LT), Gannoruwa (G), Kundasale (K) and Mahailuppallama (MI) during T4 –forth month and T8-eighth month. Means are given.

Conclusions
Bean seeds varieties; Bandarawela Green and Keppetipola Nil packed in TLA packets and stored in low temperature conditions (bottle cooler-5° T and 65% RH) were the most suitable for commercial use under prolonged storage. Under ambient environmental conditions, seeds packed in TLA packets could be stored for a considerable time period in locations where environmental T is between 21°C -31°C and RH between 64%–77% at Kundasale (MCIZ). At Mahailuppallama (LCDZ) where the temperature is between 23°C -33°C and RH is between 61% -83%, was recognized as unsuitable for storage of bean seeds (less than 10 months) even though packed in TLA. Gannoruwa (MCWZ) was identified as an intermediary area to secure the seed quality of bean. The importance of seed storage temperature alone on storage life and seed quality parameters; germination and vigour in bean seed was prominent.

More studies are needed to validate the vigour tests to predict the level of field germination before issuing the seed lots specially for storage beyond one year after harvest. Fluorescence quenching analysis and JIP test derived from the polyphasic rise of fluorescence transients can be a useful tool in assessing the physiological status of the seedling by further studies.

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Author’s Contribution
Formal analysis, R.A.I.S.A.; Funding acquisition, R.A.I.S.A.; Designing and planning, R.A.I.S.A., S.L.W. and C.K.B.; Administration, S.L.W. and C.K.B.; Resources, R.A.I.S.A. and C.K.B.; Supervision, S.L.W. and C.K.B.; validation, C.K.B.; visualization, R.A.I.S.A. and C.K.B.; writing-original draft, R.A.I.S.A.; review and editing, S.L.W. and C.K.B. Final form of manuscript was approved by all authors.

Conflict of Interest
The authors declare that there is no conflict of interest with present publication appreciated.

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