Seed Res. 53 (2): 115-120, December, 2025
doi: https://doi.org/10.56093/SR.v53i2.3

Storage Duration Impacted Hardseededness and Seed Coat Physico-chemical Parameters in Mung bean (*Vigna radiata* L.)

DEBASHIS PAUL[1,2], SK CHAKRABARTY[1*], LATA NAIN[3] AND PRATIK DHAKADE[2]

[1]Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India
[2]ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra-441108, India
[3]Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India
*deba.13.paul@gmail.com

(Received September 2025; Revised October 2025; Accepted October 2025)

ABSTRACT: Mung bean (*Vigna radiata* (L.) Wilczek) is an important pulse crop of India; however, hardseededness poses a major constraint to its germination and grain quality. The present study investigated the kinetics of hardseededness and associated biochemical changes in six hard-seeded mung bean genotypes (TM 96-25, LGG 460, PUSA 9072, MH 421, V 61-73, PUSA 9531) stored for 6, 12, and 18 months under ambient conditions. Key parameters measured included per cent hard seed, hardness breaking force (N), Acid Insoluble Lignin% (AIL%), Acid Soluble Lignin% (ASL%), and total phenol content (mg/g). Results revealed a significant reduction in hardseededness with increased storage duration. The mean hard seed percentage decreased from 31.73% in fresh seeds to 11.61% after 18 months. Concurrently, the parameters such as Acid Insoluble Lignin (%), Acid Soluble Lignin (%), hardness breaking force (N) and total phenol content (mg/g) significantly declined over the storage period, indicating a weakening of the seed coat. This change is likely attributed to alteration in membrane integrity and loosening of hilum cells, which enhances water permeability. While 18-month storage reduced germination below Indian minimum seed certification standards, 12-month stored seeds maintained acceptable quality. Therefore, utilizing 12-month stored seeds of hard-seeded mung bean genotypes is recommended for improved field emergence, uniform plant growth, and higher yields in seed production programs.

Keywords: Hardseededness, Lignin content, Hardness breaking force, Storage duration, Green gram

INTRODUCTION

Mung bean (2n=22) (*Vigna radiata* (L.) Wilczek) or green gram is a globally important pulse crop, especially in the south-east Asia including India [1]. Originating from India and Central Asia, green gram has rapidly spread to several countries around the world where it is consumed as a whole grain, dal, and sprouted grain in a variety of ways [2]. Green gram is successfully grown under a variety of climatic and soil conditions in the South and South-East Asia, Africa, South America, and Australia, leading to its recognition as the hardiest of all pulse crops.

Harseededness, a condition that renders the seeds impermeable to water and gases, is an inherent problem in more than 50%of the Indian green gram varieties that show hardseededness ranging from 10 to 70% [3]. Hardseededness involves a seed coat that mechanically constrains the embryo, restricting the seeds to germinate even under favorable environmental conditions. A larger proportion of hard seeds at harvest lowers the grain quality and impairs proper cooking and digestion, while posing risk of reduced yield, if used as a seed, due to uneven germination and poor stand establishment [4, 5]. Scanning electron microscope analysis revealed that hard seeds typically have closely packed hilum cells and closed strophioles, whereas non-hard seeds tend to have rougher surfaces, opened strophioles, and loosely packed hilum cells, facilitating water entry [6].

Previous studies reported that hardseededness is influenced by genotype, scarification treatments and growing conditions. Studies on mung bean revealed that seeds produced in the *Kharif* season exhibited a higher degree of hardseededness compared to those from the summer season. This is attributed to factors like higher atmospheric temperature during seed maturity in summer, and lower levels of lignin, calcium, phenol, and structural carbohydrates in summer-produced seeds [7]. Non-hard seeds and those produced in the summer season showed faster water uptake and earlier radical protrusion compared to seeds produced during *Kharif* season [8].

Storage time may also influence hardseededness in legumes. Hardseededness greatly delays germination of seed coat and some seeds never germinate until they receive scarifying treatment. Previous researcher [9] reported the phenomenon of hard-to-cook nature in beans and structural changes during storage and imbibition. Dry beans when stored at high temperature (41°C) and humidity (75%, 100%) for short time periods showed alterations in attachments between plasmalemma and cell wall as well as disintegration of organelles and inclusions of the cytoplasm. Electron micrographs clearly revealed structural differences between fresh and aged beans during imbibition. A significant deterioration in flavor and texture of beans after 12 months storage at 25 °C and 13% moisture content has been reported [10].

Although, it was believed that the hard-to-cook defect of beans was due to structural changes in the cotyledons, but the chemical reactions responsible are not completely understood. [11, 12] found that hardseededness of 17 lines in *Trifolium subterraneum* stored under stimulated high diurnal fluctuating temperature was influenced by both production year and plant genotype.

Earlier researchers [13] observed the effects of water content during storage on physiological activity of cucumber seeds. During storage, percentage germination declined steadily and ageing rate increased with increasing water content. Activity of the antioxidant enzymes, catalase, peroxidase and superoxide dismutase declined and leakage of electrolytes increased from stored seeds with greater changes observed in seeds stored at progressively higher water contents. Ageing resulted in a general deterioration of seed cells and could be slowed down by drying seeds to moisture content as low as 2.4%. [14] found that mung bean seed deterioration is linked to sugar hydrolysis and lipid peroxidation. During storage, glucose and lipid peroxidation products increased significantly. Amadori products correlated with lipid peroxidation, peaked in early aging, while Maillard products correlated with sugar hydrolysis, increased steadily throughout storage and correlated with reduced seed vigor.

While existing research highlights the impact of storage on hardseededness and deterioration in legumes but interplay between these seed coat structural lignin and the development of hardseededness in mung bean genotypes during storage is still limited. The present research aims to bridge this gap by investigating the relationship between specific biochemical parameters and the kinetics of hardseededness development among mung bean genotypes under storage conditions.

MATERIALS AND METHODS

The seeds of six hard seeded (HS) genotypes *viz.*, TM 96-25, LGG 460, PUSA 9072, MH 421, V 61-73, and PUSA 9531 of mung bean were used for this study. The genotypes were stored for 6, 12, and 18 months after harvesting, at Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi under ambient conditions and the physico-chemical parameters were recorded.

To assess the per cent hard seed, three replicates of 100 seeds from each genotype were placed equidistantly between moist germination papers as per the Between Paper (BP) method recommended by International Seed Testing Association. The rolled towels were placed in a seed germinator maintained at 25^{o}C constant temperature, 90% relative humidity, and 12h photoperiod with regular water supply for 8 days. At the final count, the non-germinated seeds that failed to imbibe any moisture and found intact upon tapping with forceps and gentle pressing between fingertips were identified as hard seed and expressed in percentage. Hard seeds were separated and used afterwards for recording hardness breaking force and biochemical parameters like lignin content and total phenol content.

The lignin content was estimated in two fractions as Acid Insoluble Lignin (AIL%) and Acid Soluble Lignin (ASL%) from selected genotypes. The estimation was done as per National Renewable Energy Laboratory Technical report no. NERL/TP-510/42618: Determination of structural carbohydrates and lignin [15].

Hardness breaking force was measured with texture analyzer machine (TA-HDi texture analyzer, Stable Micro-system, UK) following a modified method of [16]. To measure the force required to break the seed coat, intact seeds were tested using the instrument. The instrument operated at a test speed of 0.50 mm/s, with a pre-speed of 1.00 mm/s and a post-speed of 2.00 mm/s. A load cell count of 500 was used. During the test, seeds were consistently oriented in the same direction. The force in Newtons (N) needed to break the seed coat was recorded as the first peak on the output graph. The samples were loaded sequentially, and the average force values from ten seeds in three replicates were recorded, as previously described by [17].

Total phenol content from the selected genotypes was estimated in three replications. The total phenolic content was determined using Folin–Ciocalteu Assay[18]. 100 mg of dry extract was dissolved in 10 mL of 80% methanol, then further diluted to 100 mL with the same solvent to achieve a final concentration of 1 mg/mL. Then, 1 mL of this methanolic extract solution was transferred to a 25 mL volumetric flask, followed by the addition of 9 mL of distilled water. Next, 1 mL of Folin-Ciocalteu phenol reagent was added, and the mixture was shaken. After 5 minutes, 10 mL of 7% sodium carbonate solution was introduced. The solution was then diluted to the 25 mL mark with distilled water and thoroughly mixed. After incubating the mixture for 90 minutes at room temperature, its absorbance was measured at 765 nm using a spectrophotometer.

The data recorded from field and laboratory experiments were checked for normality and analyzed in a Randomized Block Design (RBD) using SAS statistical Software (SAS Institute Inc., Cary, NC). Means of the treatments were separated based on the Tukey's test with 95% level of significance (α=0.05).

RESULTS AND DISCUSSION

Six predominately hard seeded genotypes produced in *Kharif,* 2018 were kept in storage for 6 months, 12 months and 18 months respectively and the change in physico-chemical parameters were compared with fresh seed. Earlier studies showed that after 18 months of storage the germination percentage decreased below Indian Minimum Seed Certification Standards for mung bean genotypes, so the highest storage period kept was 18 months in the present study. The hard seed percentage was 31.73 in fresh seeds of hard-seeded genotypes, and which declined gradually as the storage period increased. The mean per cent of hard seed was 21.06, 17.0 and 11.61 after 6, 12 and 18 months of storage, respectively. The present hard seed values showed significantly higher value in fresh seeds compared to stored seed. It was statistically at par for 6 and 12 months of storage but significantly higher in comparison to that in 18 month stored seeds (Figure 1).

In terms of AIL (%) the fresh seed of hard seeded genotype contained more acid insoluble lignin and was significantly higher in comparison to that of the stored seeds. In fresh seed the AIL (%) was 14.76 (%) and decreased to 14.03 (%), 12.35(%) and 10.82 (%) after 6, 12 and 18 months of storage, respectively. The AIL (%) after 6 months of storage was statistically at par with fresh seed though the value was lower than the fresh seed. The 18 months stored seeds showed significantly lower values in comparison with 6 and 12 months- stored seed. In case of Acid Insoluble Lignin (AIL%) the genotypes had higher ASL (%) content in fresh seed which declined gradually as the storage period increased. In fresh seeds of hard seeded genotypes produced in *Kharif,* 2018, the ASL (%) was 1.52 and decreased to 1.34, 1.21 and 1.17 after 6, 12 and 18 months of storage respectively. The ASL (%) was statistically non-significant between 12 and 18 months stored seeds but was significantly lower as compared to that in fresh and 12 months stored seeds (Figure 1).

The hardness breaking force (N) was higher in freshly harvested seeds of hard seeded genotypes grown in *Kharif,* 2018 and decreased to 43.03,40.39 and 36.83N after 6,12 and 18 months of storage, respectively. The force required to break the seed coat of hard seeded genotypes that were freshly harvested in *Kharif,* 2018 was 58.20 N. There was non-significant difference

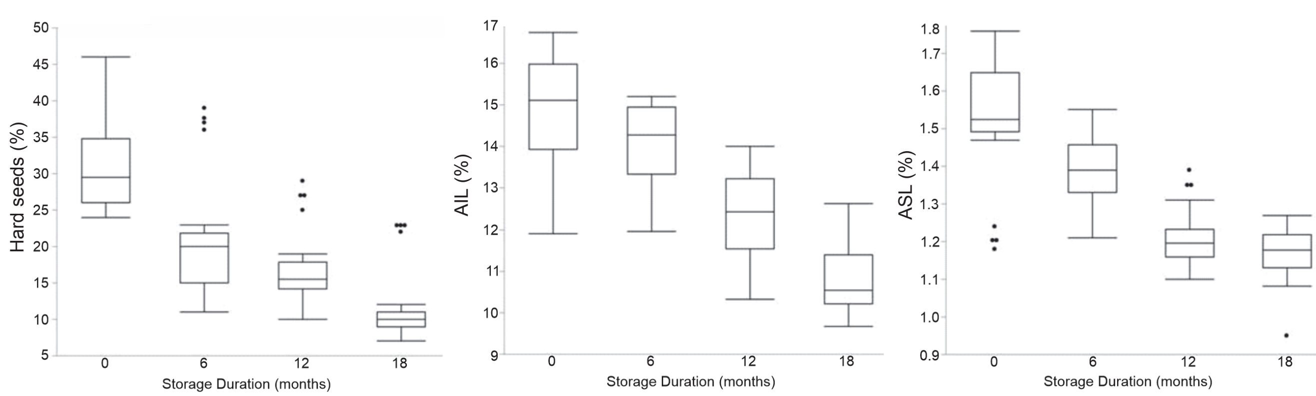

Figure 1. Box plot representing genotypic variation on impact of storage duration on hard seeds (%), Acid Insoluble Lignin (%) and Acid Soluble Lignin (%)

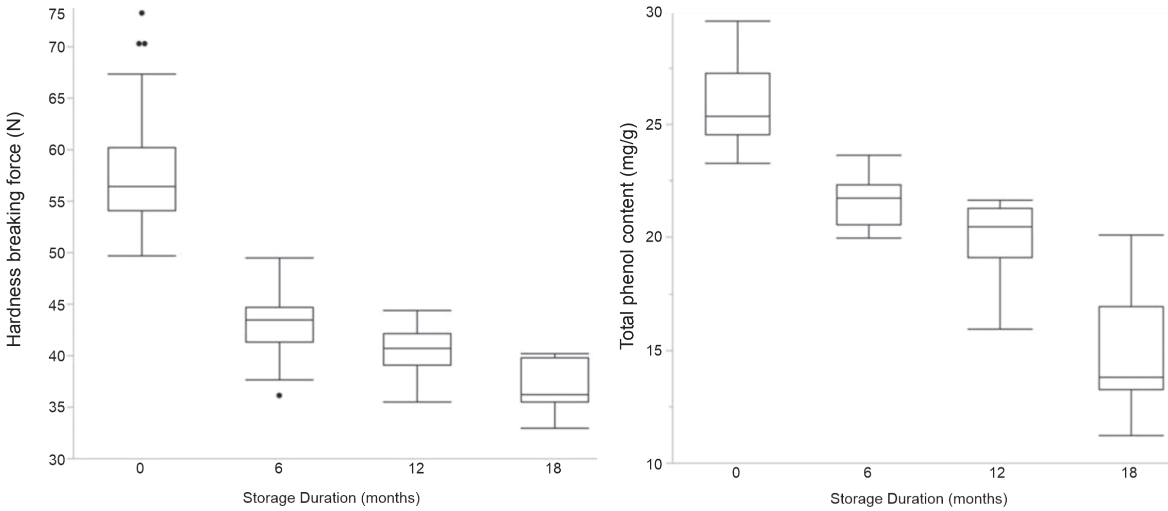

Figure 2. Box plot representing genotypic variation on impact of storage duration on Hardness breaking force (N) and total phenol content (mg/g)

between hardness breaking force of 6 and 12 months - stored seeds but was statistically higher compared to force required to break the seed coat of 18 months stored seed. In case of total phenol content, the freshly harvested seeds of hard-seeded genotypes showed significantly higher phenol content compared to stored seeds. The phenol content (mg/g as GAE) was 25.83 in fresh seeds and decreased gradually as the storage period increased. It declined to 21.59 (mg/g as GAE) (Figure 2), 19.84 and 14.90 after 6, 12 and 18 months of storage, respectively. The 6 months-stored seeds showed significantly higher phenol content in comparison with 12 and 18 months - stored seeds and 12 months- stored seeds also contained significantly higher phenol content compared to 18 months- stored seeds. Except ASL (%), all other physico-chemical parameters were significantly lower in 18 months-stored seeds compared to 12 months-stored seeds. The ASL (%) was statistically at par in 12 months and 18 months-stored seeds (Table 1).

During storage, there was decrease in per cent hard seeds as the storage period increased. Similarly, with increase in storage duration there was gradual decrease in acid soluble and acid insoluble lignin content. Similar trend was there for total phenol content also. During storage there was lower activity of polyphenol oxidase, which hinder the polymerization of phenols to lignin. Under ambient condition the exchange of humidity between seed and surrounding environment made the hilum cells loosened and the seeds became permeable to water. The results were confirmed using scanning electron microscopy and also through estimation of hardness breaking force that the stored seeds required lower force to break the seed coat and were characterized by loose hilum cells and comparatively opened strophiole in comparison with freshly harvested seeds [6]. The stored seeds required lower time for radical protrusion compared to fresh seeds which also confirmed that the stored seeds possessed loose hilum cells and the seeds were permeable to water. The storage experiment was conducted only for up to 18 months since beyond 18 months, the final germination percentage was reduced below the Indian minimum seed certification standards

Table 1. Influence of storage duration on hardseededness and physic-chemical parameters

Storage duration	% hard seeds	AIL (%)	ASL (%)	Hardness breaking force (N)	Total phenol (mg/g)
0 months	31.73^{a}	14.76^{a}	1.52^{a}	58.20^{a}	25.83^{a}
6 months	21.06^{b}	14.03^{a}	1.39^{b}	43.01^{b}	21.59^{b}
12 months	17.0^{b}	12.35^{b}	1.21^{c}	40.39^{b}	19.84^{c}
18 months	11.61^{c}	10.82^{c}	1.17^{c}	36.83^{c}	14.90^{d}

*Different letter within a particular column indicates significant difference ($p<0.05$)

recommended for mung bean varieties. Previous researchers [19] have reported that storage of seed lots that have high proportion of hard seeds, under ambient condition, can improve the germination and seedling emergence by reducing impermeability of the hard seeds. Moreover, if storage temperature is enhanced, hardseededness is often overcome due to the easy permeability of seed coat to water.

Changes in the membrane of testa cells might be the primary reason for seed hardness deterioration. It has been reported by [20] that white beans stored in tropical and temperate conditions had reduced hard seed numbers. The possible reason that was attributed was a lignification type mechanism that loosened the testa cells making it water permeable. Earlier research [21] reported that soluble carbohydrates generally decline with seed ageing and this decline might result in limited availability of respiratory substrates for germination. The decrease in total soluble sugars in other legumes during storage at ambient conditions has been reported in literature [22, 23]. Tannin concentration was found to be associated with the rate of water absorption, percentage of hard seed and incidence of testa cracking [24] in *Vicia faba*. Earlier studies [25] reported seed coat colour changes during storage, according to prevailing environmental conditions; in these cases, changes occurred concomitantly with seed physiological deterioration and increase in seed coat permeability. It is reported that the changes in biochemical composition of the membrane might be the primary reason for seed hardness reduction.

During storage, there could be a reduction in bound water that facilitates water absorption [20]. A characteristic of many species with physical dormancy or hardseededness is the presence of a 'water-gap complex' [26]. This is defined as a morpho-anatomically complex structure, characterized by the presence of a strophiole (/lens/lid) [27]. The strophiole forms part of the palisade/ impermeable layer of the seed coat. In the process of dormancy-breaking during storage, the strophiole becomes separated from the palisade layer, allowing water into the seed and facilitating imbibition to occur. [28] recently reported a structure called the pleurogram that makes up a large part of the seed coat in some *Fabaceae* species and acts as a hygroscopic valve that functions during seed maturation to germination. The pleurogram acts as a pathway for entry of water into the seed, serves as a water gap in seeds with physical dormancy, thereby regulating dormancy-break/ germination during storage.

CONCLUSION

Storage duration significantly reduces hardseededness in mung bean genotypes. Over 18 months, all the parameters viz., hard seed percentage, Acid Insoluble Lignin (AIL), Acid Soluble Lignin (ASL), hardness breaking force, and total phenol content decreased, indicating a weakening of the seed coat and increased permeability. This deterioration, likely due to changes in membrane integrity and loosening of hilum cells, improves water uptake and germination time. While 18-month storage reduced germination below the recommended standards, 12-month stored seeds maintained acceptable quality. Hence, it may be concluded that 12 months-stored seeds of predominantly hard seeded genotypes may be used for sowing under seed production programme to achieve higher field emergence, uniform plant growth and higher seed yield.

REFERENCES

1. KANG YJ, SK KIM, MY KIM, P LESTARI, KH KIM, BK HA, TH JUN, WJ HWANG, T LEE, J LEE, AND S SHIM (2014). Genome sequence of mungbean and insights into evolution within *Vigna* species. *Nature Communications*, **5**: 3696. https://doi.org/10.1038/ncomms6443
2. ARYASHAD M, A SADEGHI, M NOURI, M EBRAHIMI, M KASHANINEJAD, AND M AALAMI (2023). Use of fermented sprouted mung bean (Vigna radiata) containing protective starter culture LAB to produce clean-label fortified wheat bread. *Int. J. Food Sci. Technol.,* **58(6)**: 3310-3320. https://doi.org/10.1111/ijfs.16236.
3. HUMPHRY ME, CJ LAMBRIDES, SC CHAPMAN, EA AITKEN, BC IMRIE, RJ LAWN, CL MCINTYRE, AND CJ LIU (2005). Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. *Plant Breeding*, **124**: 292-298. https://doi.org/10.1111/j.1439-0523.2005.01084.x
4. PAUL D, SK CHAKRABARTY, HK DIKSHIT, AND Y SINGH (2018). Variation for hardseededness and related seed physical parameters in mungbean [*Vigna radiata* (L.) Wilczek]. *Indian Journal of Genetics and Plant Breeding* **78**: 333-341. https://doi.org/10.31742/IJGPB.78.3.7
5. ERMIS S, E ÖZDEN, AND E YILDIRIM (2024). Seeds of resilience: physiology and mechanisms of hardseededness. *In Seed Biology-New Advances*. Intech Open, https://doi.org/10.5772/intechopen.1003847
6. PAUL D, SK CHAKRABARTY, G CHAWLA, A KUNDU AND A MAITY (2024). Assessment of Hard seededness and Its Seasonal Dynamics Through Scanning Electron Microscopy in Green Gram (Vigna radiata) Genotypes. *Agricultural Research*, 1-8. https://doi.org/10.1007/s40003-024-00751-5
7. PAUL D, SK CHAKRABARTY, L NAIN AND A MAITY (2025). Studies on key seed physico-chemical factors and climatic variables regulating hardseededness in green gram (Vigna radiata L.) genotypes. *Crop and Pasture Science*, **76(1)**.
8. PAUL D, SK CHAKRABARTY AND S DUTTA (2021). Understanding the water absorption dynamics in relation to hardseededness in mung bean (Vigna radiata L.) genotypes. *Seed Research*, **49(2)**: pp. 92-97.

9. MARSTON E AND GM JACKSON (1981). Hard-to-cook phenomenon in beans: Structural changes during storage and imbibition. *Journal of Food Science*, **46**: 1379-85.

10. MORRIS HJ AND ER WOOD (1956). Influence of moisture content on keeping quality of dry beans. *Food Technology*, **10**: 225-229.

11. HOHLBERG AI AND DW STANLEY (1987). Hard-to-cook defect in black beans: protein and starch considerations. *Journal of Agricultural and Food Chemistry*, **35:** 571-576.

12. SMITH GR (1988). Screening of *Subteraneam clover* for persistent hard seed. *Crop Science*, **28**: 998-1000.

13. ZENG XY, JR FU AND XW ZHANG (1998). The effects of water content during storage on physiological activity of cucumber seeds. *Seed Science Research (United Kingdom)*, **8**: 65-68.

14. NARAYANA MURTHY UM AND WQ SUN (2000). Protein modification by Amadori and Maillard reactions during seed storage: Roles of sugar hydrolysis and lipid peroxidation. *Journal of Experimental Botany*, **51**: 1221-1228.

15. SLUITER A, B HAMES, R RUIZ, C SCARLATA, J SLUITER, D TEMPLETON AND D CROCKER (2012). NREL/TP-510-42618 analytical procedure-Determination of structural carbohydrates and lignin in Biomass. *Laboratory of Analyrical. Procedures*, **17**.

16. SASIKALA VB, R RAVI, AND HV NARASIMHA (2011). Textural changes of green gram (*Phaseolus aureus*) and horse gram (*Dolichos biflorus*) as affected by soaking and cooking. *Journal of Texture Studies,* **42(1)**: 10-19. https://doi.org/10.1111/j.1745-4603.2010.00263.x

17. PAUL D, SK CHAKRABARTY, HK DIKSHIT, AND SK JHA (2019). Hardness breaking force: an alternative method to evaluate hardseededness in mung bean (*Vigna radiata*). *Seed Science and Technology* **47(2)**: 155-160. https://doi.org/10.15258/sst.2019.47.2.04

18. MAHMOOD A, N NGAH, AND MN OMAR (2011). Phytochemicals constituent and antioxidant activities in Musa x Paradisiaca flower. *European Journal of Scientific Research*, **66**: 311-318. http://irep.iium.edu.my/12879/.

19. AFZAL MA, MM BASHIR, NK LUNA, MM HAQUE, MA BAKER AND MS AKHTAR (2003). Picking times and storage duration effects on hardseededness in mungbean [Vigna radiata (L.) Wilczek]. *Asian Journal of Plant Sciences*, **2**: 566-68.

20. STANLEY DW, TE MICHAELS, LC PLHAK AND KB CALDWELL (1990). Storage Induced hardening in 20 common bean cultivars. *Journal of Food Quality*, **13**: 233-247.

21. PETRUZELLI L AND G TARANTO (1989). Wheat ageing: The contribution of embryonic and non-embryonic lesions to loss of seed viability. *Physiolia Plantarum*, **76**: 289-294.

22. SHARMA S, A KAUR, A BANSAL AND BS GILL (2011). Positional effect on soybean seed composition during storage. *Journal of Food Science and Technology*, (DOI 10.1007/s13197-011-03 41-0.)

23. NAIR RM, AD CRAIG, TD ROWE, SR BIGGINS AND CH HUNT (2004). Genetic variability and heritability estimates for hardseededness and flowering in balansa clover (Trifolium michelianum Savi) populations. *Euphytica*, **138**: 197-203.

24. KANTAR F (1994). Seed hardedness in grain legumes. *Journal of the Faculty of Agriculture*, **25**: 100-106.

25. SILVA AA, SM CARMELLO AND J NAKAGAWA (1988). Germinacao e vigor sementes de Crotalaria lanceolata E. Mey. I. Influencia da cor do tegumento e da posicao dos frutos na infrutescencia. *Revista Brasileira de Sementes,* **10:** 67-73.

26. BASKIN JM AND CC BASKIN (2004). A classification system for seed dormancy. *Seed Science Research*, **14**: 1-16.

27. GAMA-ARACHCHIGE NS, JM BASKIN, RL GENEVE AND CC BASKIN (2013). Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany*, **112**: 69-84.

28. RODRIGUES-JUNIOR AG, CC BASKIN, JM BASKIN AND OC DE-PAULA (2021). The pleurogram, an under-investigated functional trait in seeds. *Annals of Botany*, **127**: 167-174.