

Understanding the Water Absorption Dynamics in Relation to Hardseededness in Mung bean (*Vigna radiata* L.) Genotypes

DEBASHIS PAUL^{1,2*}, S K CHAKRABARTY¹, NIDHI² AND SUMAN DUTTA³

¹Division of Seed Science and Technology, ICAR-IARI, New Delhi, India,

²ICAR-Central Institute for Cotton Research, Regional Station, Sirsa, Haryana, India

³Ramakrishna Mission Vivekananda Educational & Research Institute, Kolkata, West Bengal, India

*debashis.paul@icar.gov.in

(Received April 2021; Revised September 2021; Accepted November 2021)

ABSTRACT: Hardseededness, common in majority of pulse crops, is considered an undesirable character which adversely affects their culinary use. Hardseededness results from reduced permeability of the seed coat to water, hence also impacts germination. Addressing a knowledge gap in this area, the current investigation was undertaken on three hardseeded and three non-hardseeded mung bean genotypes in an attempt to understand the impact of genotype, storage period, and scarification treatments on water absorption dynamics in mung bean. The water uptake patterns of three hardseeded and three non-hardseeded genotypes produced during the *Kharif* and summer seasons of 2019, were studied in fresh and 18-month stored control and scarified seeds. The degree of hardseededness, judged by imbibition time, was higher in seeds produced during the *Kharif* season compared to the summer season in all genotypes. Notably, non-hard seeds displayed a higher increase in weight per unit time, affirming a more rapid water uptake compared to hard seeds produced during the *Kharif* season. Conversely, genotypes grown in the summer season showed a lower lag phase and early radical protrusion. Stored seeds exhibited a pattern similar to those produced in the summer season with a lower lag phase duration. Scarified seeds demonstrated the shortest lag phase, with the second rise in water absorption occurring within 8-10 hours. It was concluded that mung bean may be cultivated in summer season for the purpose of seed production, and the study emphasized the effectiveness of scarification as a proven method to alleviate coat-imposed dormancy, facilitating easier water entry into the seeds and promoting early radical protrusion.

Keywords: Imbibition, Hard seeds, *Kharif*, summer, Mung bean

Mung bean (*Vigna radiata* L.), also known as green gram, is a major pulse crop of India originated in India and Central Asia, with chromosome number $2n=22$ [1, 2]. Green gram, can be consumed as whole grain dal, as sprouts or processed in a variety of ways. It is an excellent source of high quality protein (25%) with easy digestibility having lysine (4600 mg/g N) and tryptophan (60 mg/g N), 0.86-0.96% crude fat, 3.37-4.05(%) ash, 4.13-5.01(%) crude fiber and 62-65(%) carbohydrates on dry weight basis. The sprouted seeds of green gram are rich in ascorbic acid (vitamin C), riboflavin and thiamine [3]. Mature seeds from various plant species, particularly legumes including exhibit impermeability to water and gases, or possess a seed coat that mechanically restricts entry of water to the embryo, an essential step for germination. As a result, these seeds do not readily germinate even under favorable environmental conditions. This characteristic is referred to as hardseededness, hard coatedness, hard seed coat

dormancy, or seed coat impermeability. [1]. It is also attributed to the obstruction of water absorption by the seed coat and hindrance in gaseous exchange, consequently restricting the germination process [1]. Hardseededness is also a serious problem in a majority of pulse crops and affects the quality of cooked dishes. Though hardseededness could be beneficial for long term seed survival, by protecting against seed decay and improve agronomic qualities under certain conditions [3,4], it is not desirable for sowing immediately after harvest and for the food processing industry. The presence of hard seeds in seed lots of agricultural crops is undesirable because they contribute to heterogeneous seedling emergence, potentially reducing yields, delaying harvest [5, 6] and diminishing the ability to compete with weeds. Previous studies on *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* reported hardseededness through imbibition, germination, and ageing testing [7]. Imbibition percentage increased with prolonging the

soaking duration, rising of soaking temperature, decreasing of seed moisture content, and darkening of seed coat color. The seeds of both these plants tended to become hard at maturity with long storability, which could be considered as an adaptation to the dry and extremely cold environments. Impermeability of seed coat is one of the main reasons of dormancy in legumes which deters the absorption of water by the embryo resulting in delayed germination [8,9]. The waxy layer of cuticle on the outermost region of the testa most critical in causing hindrance of water absorption in legume seeds. High callose levels in parenchyma layer might be related to low permeability in clover seeds [10]. The relative contribution of five independent variables in water imbibitions in cowpeas was studied by [11] using regression analysis which revealed that during the first three hours of water uptake, the seed coat thickness was the most important variable; from 3 to 12 h, the size of the hilum became the most important variable; and from 12 to 24 h, the percentage of protein in the cotyledon was the most important variable. Various explanations were proposed to account for the different permeability of the seed coats to water in hard and soft seeds in soybean which has tightly bound palisade cells [12], thickened seed coat tissues [13, 14], lack of pits [15], presence of endocarp deposits [16], dark colour [17], closed hilum and/or micropyle [18-20], modifications of the outer tangential walls of palisade cells [21]. The present investigation aims to address the knowledge gap regarding the impact of genotype, storage period, and scarification treatment on water absorption dynamics in mung beans.

MATERIALS AND METHODS

Three hard seeded (HS) genotypes (TM 96/25, PUSA 9531 and PUSA RATNA) and three non hard seeded (NHS) genotypes (Pant-M-5, IPM 02/3 and SM 11/75) were collected from the Division of Seed Science of Technology, ICAR-Indian Agricultural research institute, New Delhi. The water absorption patterns were observed in all genotypes produced in *Kharif 2019*, summer 2019, as well as 18 months stored and scarified seeds. Before conducting experiments to examine the impact of storage duration and scarification treatment on water imbibition patterns, the seeds underwent storage under ambient environmental conditions. Additionally, the seeds were subjected to scarification for 50 seconds between two sandpapers (0 grit). The weight (g) of 25 seeds for each genotype were taken and placed in petri plates in three replications. The increase in seed weight (g) of 25 seeds

was taken after every two hours interval up-to 20 hours for all the six genotypes. After every two hours the increase in weight was recorded. This procedure was done similarly for the genotypes produced in summer season, stored seeds and scarified seeds. The water uptake pattern of the genotypes produced in *Kharif 2019*, summer 2019, stored and scarified seeds were presented in the figure no. 2, 3, 4 and 5, respectively.

STATISTICAL ANALYSIS

The data recorded from laboratory experiments were analyzed in triplicate adopting SAS statistical Software (SAS Institute Inc., Cary, NC). Mean differences, descriptive statistics and regression equations of the permeability pattern were performed using appropriate statistical methods. Means of the treatments were separated based on the Tukey's Honestly Significant Difference test with $\alpha=0.05$.

RESULTS AND DISCUSSION

The percentage of hard seeds in *Kharif* produce ranged from 31.67 to 49.67 in HS genotypes, while it varied from 5.0 to 6.33 in NHS genotypes. In contrast, the percentage of hard seeds in summer produce ranged from 5.33 to 9.0 in HS genotypes, and varied from 2.33 to 3.67 in NHS genotypes.

The HS genotypes exhibited significantly higher percentages of hard seeds ($p < 0.05$) compared to NHS genotypes during *Kharif 2019*. However, during Summer 2019, except for TM 96/25, the other genotypes were statistically comparable in terms of hard seed percentage. Genotype TM 96/25 displayed the highest percentage of hard seeds in both *Kharif* (49.67) and summer (9.0) seasons. For NHS genotypes, a similar trend in hard seed occurrence was observed between *Kharif* and summer seasons. (Table 1). Similar trends were also observed

Table 1. Variation for percent hard seeds in *Kharif 2019* and summer 2019 produced mung bean genotypes

Genotypes	% hard seeds (K-2019)	% hard seeds (S-19)
Hard seeded		
TM 96/25	49.67 ^a	9.0 ^a
PUSA 9531	31.67 ^b	6.67 ^{ab}
PUSA RATNA	32.0 ^b	5.33 ^{ab}
Non-hard seeded		
IPM 02-3	4.67 ^c	2.33 ^b
SM 11/75	5.0 ^c	3.67 ^{ab}
PANT M 5	6.33 ^c	3.0 ^b

*Different letters within a column indicate significant difference ($p < 0.05$). K -2019: *Kharif 2019*, S-2019: summer 2019.

by the previous researchers in mung bean [28-29]. The occurrence of hard un-germinated seeds in leguminous crops during germination test has been reported in different legumes viz. soybean [22-26], and common bean [27]. Different levels of hardseededness in green gram has been reported [28-29]. Transient hardseededness is common in green gram cultivars which could vary in the range of 0-70% [30].

Water Imbibitions pattern of mung bean seeds produced in Kharif and summer seasons, stored seeds and scarified

It was recorded that there was a higher rate of water uptake in case of seeds of non-hard compared to hard

seeded genotypes (Figure 1). The second rise in water uptake was observed after 16 hours of imbibitions having the lag phase of almost 6 hours. The higher increase in weight per unit time in case of non hard seeds confirmed the higher rate of water uptake. On the other hand the same genotypes produced in summer (2019) season showed a shorter duration of lag phase (4 hours) and reached the second peak of water uptake just after 12 hours of imbibitions only. There was a higher rate of water uptake by the seeds when produced in summer season compared to Kharif season (Figure 2). Researchers [32] reported that the seed coat controls water absorption up to a certain level of moisture. The differences in water

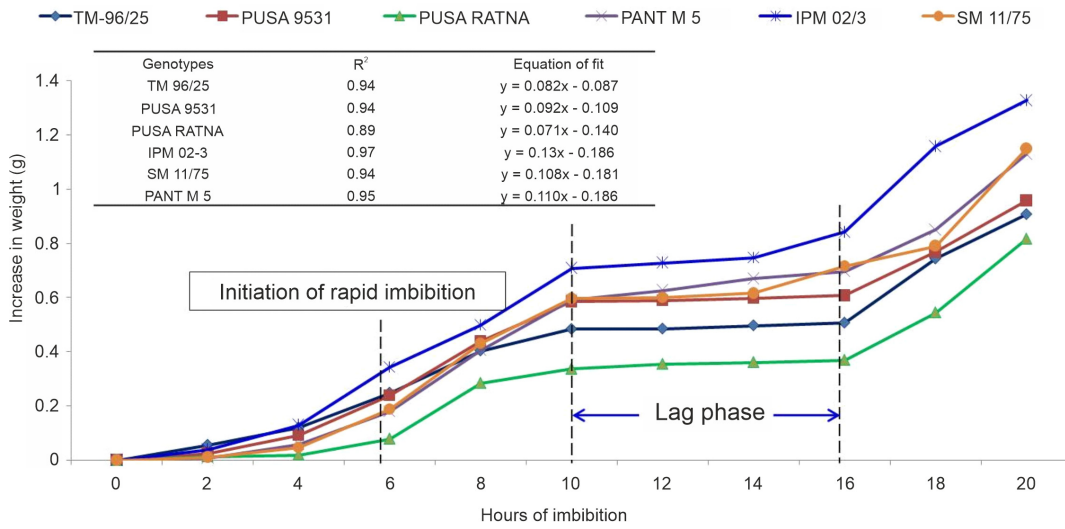


Figure 1. Water imbibition pattern of mung bean genotypes produced in Kharif 2019

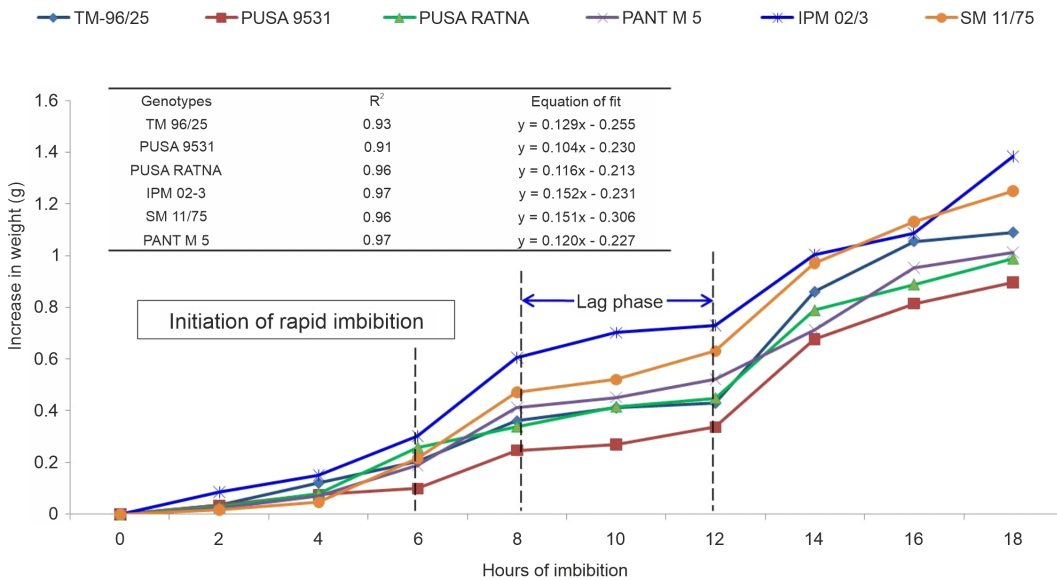


Figure 2. Water imbibition pattern of mung bean genotypes produced in summer 2019

absorption might be due to the differential solubilization of the starch during soaking which is caused mainly by differences in starch structure, seed size and membrane permeability [31, 32]. Different substances such as tannins, waxes, phenols, pigments, and germination inhibitors, may also be found in seed coats of different species affecting permeability [33]. When the genotypes were stored for 18 months before water uptake was recorded; the water uptake pattern showed a similar trend as summer season produced seeds having a lower

duration of lag phase (4 hour) of water uptake to reach to the second increase in weight after 12 hours of imbibitions (Figure 3). During storage a gradual reduction in percent hard seeds is seen, which are confirmed the scanning electron microscopy [36]. In case of the scarified seeds our study revealed that the lag phase was notably shorter, lasting only for 2 to 3 hours, and the second peak of water uptake was attained after 8 hours of imbibition. There was a rapid uptake of water irrespective of the genotypes (Figure 4). The results confirmed that

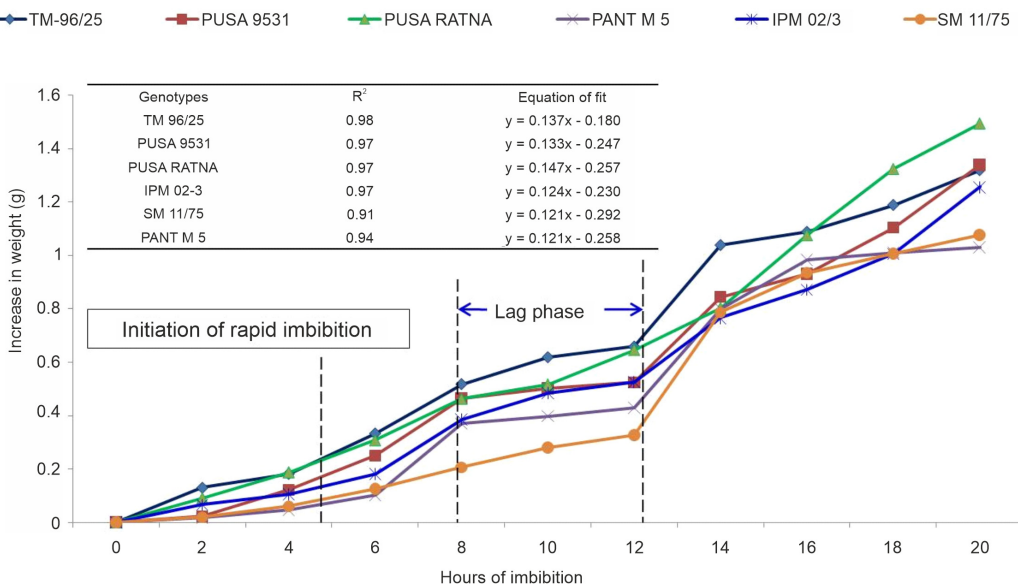


Figure 3. Water imbibition pattern of mung bean genotypes stored for 18 months

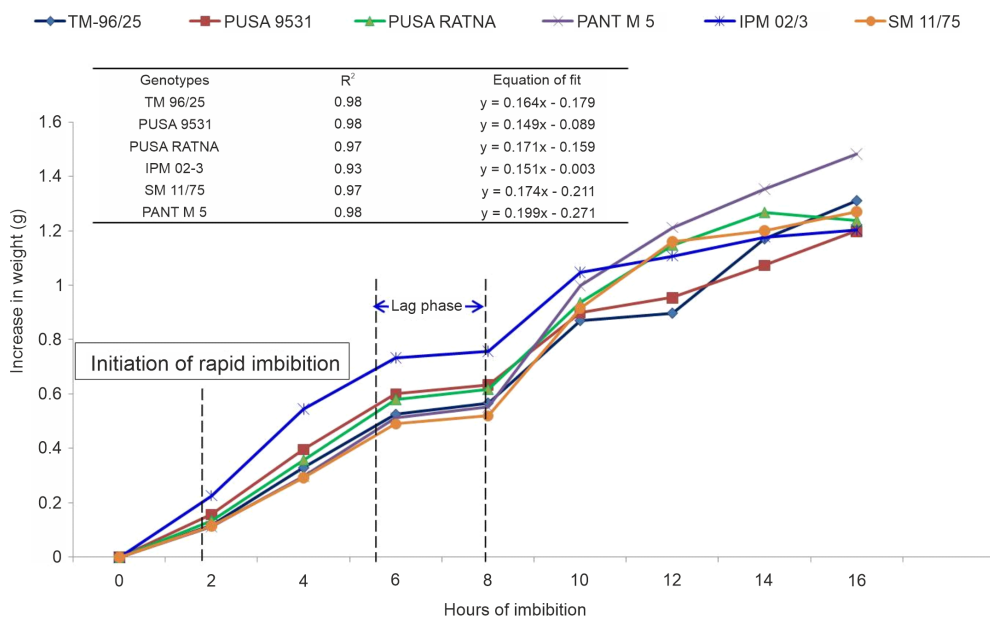


Figure 4. Water imbibition pattern of scarified seeds of mung bean genotypes

scarification is a good proven option to remove coat imposed dormancy that leads to ease of water entry to the seeds for early radical protrusion. Previous study noted [11] that during the first 3 h to 12 h, the hilum size was the most important controller of imbibition in cowpea (*V. unguiculata*) seeds while the percentage of proteins in cotyledon was important between 12 h and 24 h of imbibition. Porous seed coats are usually permeable and non porous ones are impermeable [34]. Acid scarification of fenugreek seeds with concentrated H₂SO₄ for 10 min reduced hardseededness and improved seed quality characteristics such as seedling fresh weight, vigor and electrolyte leakage from treated seeds [35].

CONCLUSION

The imbibition time for both hard and non-hard genotypes produced during the *Kharif* season was extended, reaching 16 hours of imbibition with an almost 6-hour lag phase. Conversely, genotypes cultivated in the summer season exhibited a shorter lag phase (4 hours) and achieved the second peak of water uptake after just 12 hours of imbibition. For scarified seeds, the results indicated a significantly shorter lag phase of 2 to 3 hours, and the second peak of water uptake was observed after 8 hours of imbibitions. Storage of seeds and scarification reduced the imbibitions time and promoted early radical protrusion through alleviation coat-imposed dormancy.

ACKNOWLEDGEMENT

The first author gratefully acknowledges the Indian Council of Agricultural Research (ICAR) and Indian Agricultural Research Institute (IARI) for providing the financial and infrastructure facilities for conducting the experiments.

REFERENCES

- KARIVARATHARAJU TV, V RAMAKRISHNAN AND DD SUNDARARAJ (1974). Effect of hard seed-coat on germination of green-gram. *Indian Journal of Agricultural Sciences*, **44(8)**: 525-7.
- BASKIN JM AND CC BASKIN (2004). A classification system for seed dormancy. *Seed Science Research*, **14**: 1-16.
- CHAUDHARY RP, SHARMA, SK AND DAHAMA AK (2003). Yield components of mungbean [*Vigna radiata* (L.) Wilczek] as influenced by phosphorus and thiourea. *Ann. Agric. Res.*, **24**: 203-204.
- Ma PX (2004). Scaffolds for tissue fabrication. *Materials Today*, **7(5)**: 30-40.
- BACIU-MICLAUS D (1970). Contribution to study of hard seed coat structure properties of soybean. *Proceedings of the International Seed Testing Association*, **35**: 599-617.
- HSU KC, K ETEMADI AND E PFENDER (1983). Study of the free burning high intensity argon arc. *Journal of Applied Physics*, **54(3)**: 1293-301.
- YANG Y, MH RITZWOLLER, FC LIN, MP MOSCHETTI AND NM SHAPIRO (2008). Structure of the crust and uppermost mantle beneath the western United States revealed by ambient noise and earthquake tomography. *Journal of Geophysical Research: Solid Earth*, **113(B12)**.
- CALERO E, SH WEST AND K HINSON (1981). Water absorption of soybean seeds and associated causal factors. *Crop Science*, **21(6)**: 926-33.
- WAREING PF AND PF SAUNDERS (1971). Hormones and dormancy. *Annual Review of Plant Physiology*, **22(1)**: 261-88.
- BHALLA PL AND HD SLATTERY (1984). Callose deposits make clover seeds impermeable to water. *Annals of Botany*, **65**: 125-128.
- SEFA-DEDEH S AND DW STANLEY (1979). The relationship of microstructure of cowpeas to water absorption and dehulling properties. *Cereal Chemistry*, **56**: 379-86.
- BALLARD LAT (1973). Physical barriers to germination. *Seed Science and Technology*, **23**: 205-230.
- WYATT JE (1977). Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat color. *Journal of the American Society for Horticultural Science*, **102**: 478-480.
- MIAO H, BR WEI, DM PEEHL, Q LI, T ALEXANDROU, JR SCHELLING, JS RHIM, JR SEDOR, E BURNETT AND B WANG (2001). Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway. *Nature Cell Biology*, **3(5)**: 527-30.
- CHACHALIS D AND M L SMITH (2001). Imbibition behavior of soybean (*Glycine max* (L.) Merrill) accessions with different testa characteristics. *Seed Science and Technology*, **28**: 321-31.
- YAKLICH RW, WP WERGIN, AND EL VIGIL (1986). Special secretory cells in the soybean seed coat. *Protoplasma*, **134**: 78-87.
- WYATT JE (1977). Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat color. *Journal of the American Society for Horticultural Science*, **102**: 478-480.
- HYDE EOC (1954). The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. *Annals of Botany*, **18**: 241-256.
- BALLARD LAT (1973). Physical barriers to germination. *Seed Science and Technology*, **23**: 205-230.
- HU HH, J KANDAMPULLY AND TD JUWAHEER (2009). Relationships and impacts of service quality, perceived value, customer satisfaction, and image: an empirical study. *The Service Industries Journal*, **29(2)**: 111-25.
- WERKER E, I MARBACH AND AM MAYER (1979). Relation between the anatomy of the testa, water permeability and the presence of the phenolics in the genus *Pisum*. *Ann Bot*, **43**: 765-71.
- ZHANG B, P CHEN, A SHI, A HOU, T ISHIBASHI AND D WANG (2008). Putative quantitative trait loci associated with calcium content in soybean seed. *Journal of Heredity*, **100**: 263-269.

23. SUN L, Z MIAO, C CAI, D ZHANG, M ZHAO, Y WU, X ZHANG, SA SWARM, L ZHOU, ZJ ZHANG AND RL NELSON (2015). GmHs1-1, encoding a calcineurin-like protein, controls hardseededness in soybean. *Nature Genetics*, **47**: 939-943.
24. BELLALOU N, JR SMITH, A MENGISTU, JD RAY AND AM GILLEN (2017). Evaluation of exotically-derived soybean breeding lines for seed yield, germination, damage, and composition under dryland production in the midsouthern USA. *Frontiers in Plant Science*, **8**: 176.
25. ZHANG X, J ZHAO, Y BU, D XUE, Z LIU, X LI, J HUANG, N GUO, H WANG, H XING AND L QIU (2018). Genome-wide association studies of soybean seed hardness in the Chinese mini core collection. *Plant Molecular Biology Reporter*, **36**: 605–617.
26. BU Y, X ZHANG, C WANG, J GUO, X ZHANG, X LI, Q YAN, J ZHAO AND H XING (2018). Conditional and unconditional QTL analyses of seed hardness in vegetable soybean (*Glycine max* L. Merr.). *Euphytica*, **214**: 1-21.
27. SOLTANI A, WALTER KA, WIERSMA AT, SANTIAGO JP, QUIQLEY M, CHITWOOD D, PORCH TG, MIKLAS P, MCCLEAN PE, OSORNO JM, LOWRY DB (2021). The genetics and physiology of seed dormancy, a crucial trait in common bean domestication. *BMC Plant Biology*, **21**: 1-7.
28. PAUL D, SK CHAKRABARTY, HK DIKSHIT AND Y SINGH (2018). Variation for hardseededness and related seed physical parameters in green gram [*Vigna radiata* (L.) Wilczek]. *Indian Journal of Genetics*, **78**: 333-341.
29. PAUL D, SK CHAKRABARTY, HK DIKSHIT, SK JHA, G CHAWLAANDY SINGH (2019). Heritability of hardseededness in green gram [*Vigna radiata* (L.) Wilczek] under varying environments. *Indian Journal of Genetics*, **79**: 197-203.
30. LAWN RJ AND GJ REBETZKE (2006). Variation among Australian accessions of the wild green gram (*Vigna radiata* ssp. *sublobata*) for traits of agronomic, adaptive, or taxonomic interest. *Australian Journal of Agricultural Research*, **57**: 119-32.
31. ABU-GHANNAM N AND B MCKENNA (1997). The application of Peleg's equation to model water absorption during the soaking of red kidney beans (*Phaseolus vulgaris* L.). *Journal of Food Engineering*, **32(4)**: 391–401.
32. FRIAS J, C VIDAL-VALVERDE, S SOTOMAYOR, C DIAZ-POLLAN AND G URBANO (2000). Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *The Journal European Food Research and Technology*, **210(5)**: 340–45.
33. WOODSTOCK LW (1988). Seed imbibition: a critical period for successful germination. *Journal of Seed Technology*, **12**: 1-15.
34. YAKLICH RW, EL VIGIL AND W WERGIN (1984). Scanning electron microscopy of soybean coat. *Scanning Electron Microscopy*, **2**: 991-1000.
35. PANDITA VK, S NAGARAJAN AND D SHARMA (1999). Reducing hardseededness in fenugreek by scarification technique. *Seed Science and Technology*, **27**: 627-31.
36. PAUL D AND SK CHAKRABARTY (2022). Assessment of Genetic Divergence for Hardseededness with Field Emergence and Storage Duration in Mungbean Genotypes [*Vigna radiata* (L.) Wilczek]. *International Journal of Plant & Soil Science*, **34(8)**: 56-64.