Indian Journal of Agricultural Sciences **93** (3): 269–273, March 2023/Article
https://doi.org/10.56093/ijas.v93i3.128735

Morpho-physiological trait variation of pre-harvest sprouting tolerance to simulated rain in mungbean (*Vigna radiata*)

P S RAO[1]*, T Y MADHULETY[1], R ANKAIAH[1] and S R VOLETI[2]

Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana 500 030, India

Received: 05 October 2022; Accepted: 27 February 2023

ABSTRACT

Preharvest sprouting (PHS) is one of the most important factors for lower productivity in mungbean [*Vigna radiata* (L.) R. Wilczek]. The morpho-physiological characters, ultra-structural variations in pods and seeds, changes and regulatory water absorption pathway of 30 mungbean genotypes in relation to pre-harvest sprouting behaviour was studied during *kharif* 2017–19 at Seed Research Technology Center, Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana. Mungbean genotypes subjected to PHS imposed by simulated rainfall were evaluated. Rainfall simulator generates a rainfall spectrum that was similar to natural rainfall. Genotypes LGG 450 and K 851 with low score (%), while ML 267 and MGG 295 with high score (%) were screened as tolerant and susceptible to PHS. Accordingly, higher seed yield was recorded in LGG 450 (4.94 g/plant) followed by K 851 (4.20 g/plant) while lower seed yield was recorded in ML 267 (0.26 g/plant) followed by MGG 295 (0.79 g/plant). The findings were further corroborated with SEM studies by presence of sparse, wiry, short, twist or shriveled trichomes; thick cuticular pod wall, wide locular gap between seed coat (seed) and pod wall helping for the slow diffusion of moisture from endosperm to embryo. Further, the presence of lea-protein-insulated starch grains of endosperm merits lipophilic nature which might hindered dissipation of water to embryo via endosperm. The SEM studies have established ultra-structural features that determine the resistance to pre-harvest sprouting of mungbean and development of future resistant lines identification.

Keywords: FSD, FSG, Mungbean, PMS, PHS, PHST, PHSS, SEM

Mungbean [*Vigna radiata* (L.) R. Wilczek] genotypes for pre-harvest resistance depend on architecture of the pod wall structure, and bimolecular alignment decides absorption and stimulates sprouting. Seed coat, the physical barrier protects the seed from adverse environmental conditions, maintains quality and disease-causing organisms. Warm, humid conditions at maturity in tropical and temperate regions are conducive to pre-harvest sprouting, resulting in rupture of seed coat and pod wall leading to reduction in seed quality and quantity. Genetic improvement has been made in developing disease resistance but not for abiotic factors like pre-harvest sprouting.

The yield gap in many high yielding varieties of mungbean could be mainly due to lack of resistance to wetting and sprouting due to rains at the time of physiological maturity (Rao *et al.* 2007) or just before harvesting. G×E interactions have major importance for plant breeders in developing improved varieties (Kumar *et al.* 2021 and Sanjeev *et al.* 2022). In our studies, we have selected 30 mungbean genotypes and evaluated for their variation in morpho-physiological traits due to preharvest sprouting (PHS) induced by simulated rain.

MATERIALS AND METHODS

A field experiment was conducted at Seed Research and Technology Centre, Rajendranagar, Hyderabad during *kharif* 2017–19 to evaluate the mungbean genotypes (30 accessions) against PHS damage. At pod maturity stage of the crop, the plants were exposed to alternate wetting and drying with rainfall simulator using overhead sprinklers. The plants were exposed to a diurnal regime of 6 h period with 9 mm per hour rainfall followed by 80 to 90% RH for 6 days. Ten randomly selected plants were harvested, threshed and weather damage was assessed by collecting data on pod and seed characteristics. Based on the response of mungbean genotypes, the pods of 2 genotypes LGG 450 (tolerant) and ML 267 (susceptible) subjected to Scanning Electron Microscope (SEM) at RUSKA Lab, College of Veterinary Sciences, ANGRAU Campus to study the thickness of pod wall, seed coat, their external surface and internal structures of seed. The fresh seed samples were

---

[1] Acharya N. G. Ranga Agricultural University, Guntur, Andhra Pradesh; [2] Indian Institute of Rice Research, Rajendranagar, Hyderabad. *Corresponding author email: sampalrao@gmail.com

transferred into glass vials and fixed in 3% gluteraldehyde in 0.05 M phosphate buffer (*p*H 7.2) for 24 h at 4°C. The treated samples were then post-fixed in 2% aqueous osmium tetroxide for 4 h and later in the same buffer for 2 h. The samples dehydrated in series of graded alcohol and dried to a critical point of drying, for SEM. The dried samples mounted over the stubs with double sided carbon tape. Finally, a thin layer of platinum (palladium) coat was applied over the samples using an automated sputter coater (JEOL JFC-1600) for about 3 min. All samples were coated with gold and examined with a HitachiS 570 SEM at 15 kV. The samples were then scanned using SEM (Model: JOEL- JSM 5600) with magnification from x50 to x1400.

RESULTS AND DISCUSSION

Evaluation of mungbean genotypes for PHS tolerance: High-low score evaluation method was conducted to screen the genotypes into susceptible and tolerant to PHS, based on mean performance of morphological and physiological parameters (Arunachalam and Bandyopadhay 1979). The criteria for selecting genotypes for PHS tolerance included morphological features, physiological parameters and pod characteristics. The number of genotypes falling under each category of high, medium and low varied and thus identification of PHS susceptibility or tolerance feature was determined (Table 1). Hence, 4 important physiological parameters directly related to sprouting damage, viz. (i) Rate of moisture absorption (%), pods with *in situ* sprouting (%) (Fig 1), (ii) sprouted seeds/pod (%) and (iii) Yield of healthy seed (%) were considered. Based on these selection protocols, our results indicated two varieties each, LGG 450, K 851 and ML 267, MGG 295 as tolerant and susceptible to sprouting. These entries were advanced for comparative assessment for their genotypic differences regarding pod wall, seed coat and seed proper studies.

PHS susceptibility or resistance showed variation among mungbean genotypes with regard to morpho-physiological characters. The yield reduction was very high (80–90%) in ML 267, MGG 295, MGG 336 and LGG 407 compared to others. Such yield reduction could be due to high moisture absorption by their pod walls (56–77%) or early seed sprouting. As a result of unseasonal rainfall before harvest, causing severe PHS and yield losses were also reported in cereals and pulses (Nagarajan and Radder 1983).

In the present study, mungbean varieties LGG 450, K 851, LGG 460, PIMS 4 and LGG 505 showed lower moisture absorption by pods (24 to 29%), resulting in a smaller number of sprouted seeds/pod (17–22%). PHS occurred in seeds following their exposure to periods of rainfall or high humidity on mother plant (Andrews 1982). Damage to testa due to weathering resulted in a loss of membrane integrity that potentially reduced the ability of a seed to resist excessive desiccation enhanced absorption of water at times of pre-harvest rainfall, and resulted in PHS and thereby spoilage of seed (Michel Peel 2000).

Further, the pod beak length (2.1–4.3 mm) and pod beak angle (21–33°) varied significantly among the genotypes. Pod characteristics such as pod wall thickness, pod wall wax (epicuticular) and hard seed (%) exhibited distinct relationship with yield reduction due to PHS among mungbean genotypes. Genotype LGG 450, K 851, PIMS 4 and LGG 460 exhibited distinctly higher values of

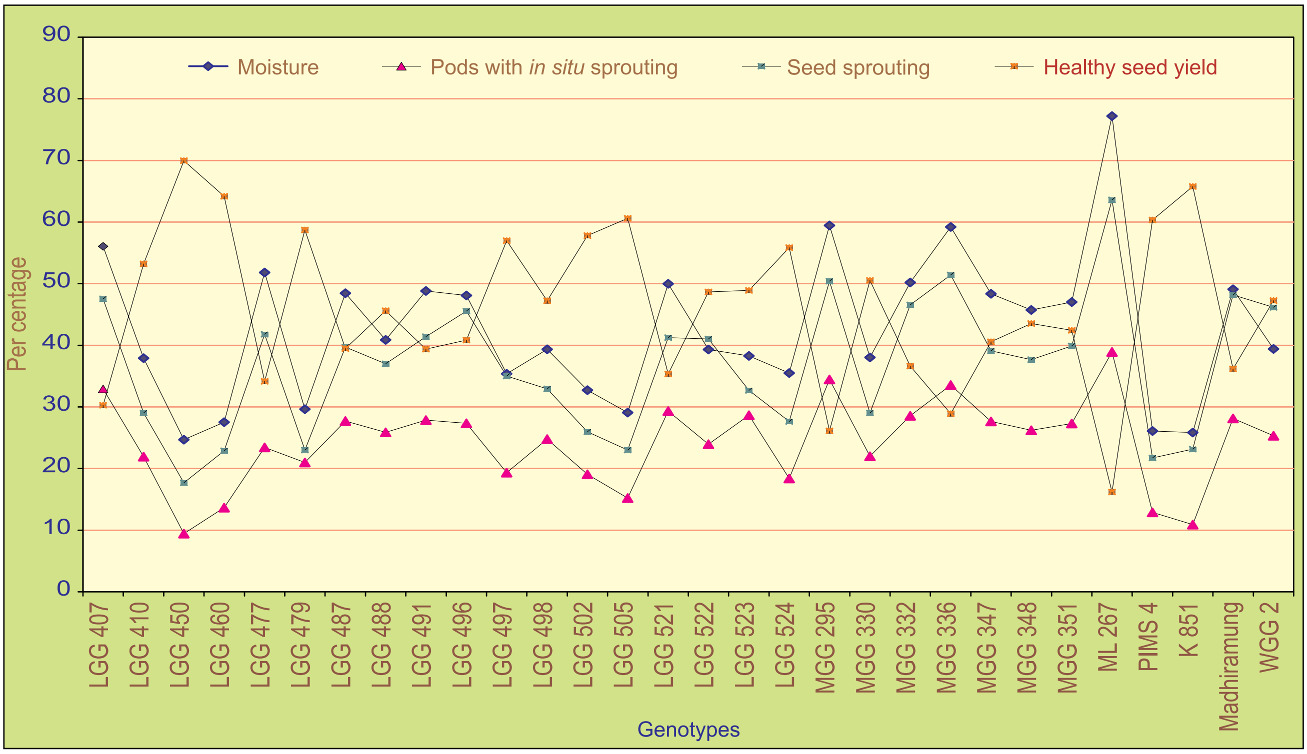

Fig 1 Relationship between Per cent moisture absorption, sprouting of pods (%), seeds (%) and yield in mungbean.

Table 1 Selection and evaluation of PHS of mungbean pod and seed characters affected by PHD under simulated rainfall

Genotype	Pods with *in-situ* sprouting	Split pods with *in situ* sprouting	Split pods with un sprouted seeds	Mould affected pods	Discolored pods	Healthy pods	Sprouted seeds	Mould affected seeds	Discolored seeds	Wrinkled seeds	Healthy seeds	Score
LGG 407	+1	+1	0	+1	+1	-1	+1	+1	+1	+1	-1	+6
LGG 410	0	0	0	0	0	0	0	0	0	0	0	0
LGG 450	-1	-1	-1	-1	-1	+1	-1	-1	-1	-1	+1	-7
LGG 460	-1	-1	0	-1	-1	+1	-1	-1	0	-1	+1	-5
LGG 477	0	0	0	+1	+1	0	+1	0	0	0	0	+3
LGG 479	0	0	0	0	0	+1	0	0	0	0	+1	+2
LGG 487	+1	+1	0	0	0	0	0	+1	+1	0	0	+4
LGG 488	0	0	0	0	0	0	0	0	0	0	0	0
LGG 491	0	0	0	0	0	0	0	+1	0	0	0	+1
LGG 496	0	0	+1	0	0	0	0	+1	0	0	0	+2
LGG 497	0	0	0	0	0	+1	0	0	0	0	0	+1
LGG 498	0	0	0	0	0	0	0	0	0	0	0	0
LGG 502	0	0	0	0	0	+1	0	0	0	0	+1	+2
LGG 505	0	0	0	-1	-1	+1	0	-1	0	-1	+1	-2
LGG 521	0	0	+1	0	0	0	+1	+1	0	0	0	+3
LGG 522	0	0	+1	0	0	0	0	0	0	0	0	0
LGG 523	0	0	0	0	0	0	0	0	0	0	0	0
LGG 524	0	0	0	0	0	+1	0	0	0	0	0	+1
MGG 295	+1	+1	+1	+1	+1	-1	+1	+1	+1	+1	-1	+8
MGG 330	0	0	0	0	0	0	0	0	0	0	0	0
MGG 332	0	0	0	0	0	0	+1	0	0	0	0	+1
MGG 336	0	+1	+1	+1	+1	-1	+1	+1	+1	0	-1	+5
MGG 347	0	0	+1	0	0	0	0	+1	0	0	0	+2
MGG 348	0	0	0	0	0	0	0	0	0	0	0	0
MGG 351	0	0	0	0	0	0	0	+1	0	0	0	+1
ML 267	+1	+1	0	+1	+1	-1	+1	+1	+1	+1	-1	+6
PIMS 4	0	-1	0	0	0	+1	-1	0	0	0	+1	0
K 851	0	-1	-1	-1	0	+1	-1	-1	0	-1	+1	-4
Madhiramung	0	0	0	0	0	0	+1	+1	+1	0	0	+3
WGG 2	0	0	+1	0	0	0	0	+1	0	0	0	+2
GM		5.00	1.46	0.58	1.62	0.67	13.39	3.55	1.27	0.27	0.39	4.75
SEm ±		0.29	0.01	0.0 1	0.01	0.01	0.62	0.34	0.01	0.01	0.06	0.27
CD (0.05)		0.82	0.02	0.02	0.03	0.02	1.08	0.97	0.04	0.02	0.16	0.77
CV (%)		10.02	0.80	1.69	0.98	1.93	8.34	16.67	1.83	5.33	24.90	9.93
SD		1.48	0.31	0.20	0.31	0.24	3.56	0.85	0.10	0.05	0.10	1.46
G M + S D = (+1)		6.31	1.73	0.75	1.87	0.88	15.96	4.20	1.33	0.32	0.48	6.04
G M – S D = (-1)		3.35	1.11	0.35	1.25	0.40	8.84	2.50	1.13	0.22	0.28	3.12

epicuticular wax (8.68–13.67 μg/cm^2) and higher percentage of hard seed (48–55). The genotypes, ML 267, MGG 336, MGG 295 and LGG 407 recorded lesser amounts of epicuticular wax and hard seed (27 to 29%). The hard seed coat prevented imbibition of water by the seed in soybean (Dougherty and Boerma 1984). The genotypes with higher podwall thickness (2.8 to 3.2 μm) showed less reduction in yield compared to other. It clearly indicates that possible genetic variation with respect to pod wall and water absorption characteristics are responsible for PHS and yield reduction. These results are in conformity with Williams *et al.* (1995). Hence these traits might be useful to screen PHS in mungbean. In soybean it was recommended a thick and/or dense pod walls as selection criteria and the presence of pubescence on the pod surface. Similar results in mungbean were reported earlier (Lawn *et al.* 1987, Naidu *et al.* 1994). LGG 450, K 851, LGG 460 and PIMS 4 consistently recorded lower damage and yield reduction. Similar type of damage by weather was progressed with discolouration, wrinkling and cracking of testa to precocious germination of the seeds in mungbean when pods were exposed to alternate cycles of wetting and drying (Williams *et al.* 1984 and Lassim *et al.* 1984). This situation probably results in increased water content that lead to testa expansion and increased seed respiration rate. Subsequent drying during intermittent rainfall also causes testa shrinkage and seed restoration to a physiologically inactive stage. Adverse weather conditions before harvesting cause damage to the seed ranging from pre-mature enzyme activity to an extent of complete sprouting (Williams *et al.* 1995) through loss in seed weight.

It can be inferred from our studies that the seed, pod traits and pre-harvest damage caused by weather, the genotypes, LGG 450, K 851, LGG 460 and PIMS 4 could be considered as PHS tolerant (PHST) while ML 267, MGG 295, MGG 336 and LGG 407 as PHS susceptible (PHSS). The SEM studies on pod structure and seed coats of the susceptible varieties ML 267 and MGG 295 against PHS showed that, pod wall experienced deep cracks and witnessed a greater number of pores on its surface. The pod wall thickness of MGG 295 was 423 μm (Supplementary Fig 1). The thickness of pod wall was lesser than K 851 and LGG 450 (Supplementary Fig 2). The fairly large elongated and a greater number of functional and turgid trichomes with high density on pod wall surface made easy access for movement of water when it was wet or subjected to simulated rainfall. Water droplets adhered to trichomes helped to absorb water could also enhance due to the more trichome density on the outer surface of the podwall. Thus, wetting of seed coat or absorption of water could also be enhanced due to the high density of trichomes on the outer surface of the pod wall (Rao *et al.* 2022).

Further, the mesocarp and endocarp seen in perforated tissues exhibited large longitudinal cracks in MGG 295 as well as in ML 267. This is immediately followed by large locular space (0.48 mm) in MGG 295 while 1.06 mm in ML 267 formed a reservoir around the seed proper for incoming water. In crops like soyabean, wheat (King and Richards 1984) mungbean (Singh *et al.* 2017) revealed that pod morphological characters are made congenial for wetting, rapid movement and water absorption of podwall and kept it moist which leads PHS under adverse climatic conditions due to thin podwall ultrastructural features like deep cracks with more pores, thin unicellular cuticular layer, high density, size and shape of the trichomes on the pod wall, thin cuticular layer. K 851 and LGG 450 have exhibited PHS resistance due to attribution. LGG 450 and K 851 pod wall surface had no cracks with very few numbers of pores (slight and slits). Trichomes sparsely with short, wiry twisted few number along the margin seen as it dried and less turgid nature of trichomes and thick pod wall. A very few short, flattened, less turgid, wiry and twisted trichomes in these genotypes indicate their dysfunction for absorption of water. This further emphasizes less scope for trapping of water droplets when they are wet. These morphological characters exhibited resistance against PHS, were reported earlier also (Singh *et al.* 2017).

The seed coat of ML 267 and MGG 295 (Supplementary Fig 1) are relatively thinner 77.3 μm (MGG 295) than that of K 851. The cotyledons of ML 267 had smaller starch granules embedded within protein bodies. The layers of pod wall, locular space and seed coat of ML 267 and MGG 295 indicated access to water movement across the seed layers (Supplementary Fig 3). Aforesaid, structural features culminating movement of imbibed water might prone the seed to pre-mature sprouting, when they were subjected to simulated or unseasonal rainfall.

The thickness of the mesocarp and endocarp of LGG 450 together measured 195 μm. The locular space was reduced conspicuously in LGG 450 while it was very small compared to that of K 851. Both these genotypes had three times lower locular space compared to that of ML 267 (Supplementary Fig 4). The locular space at placental region or at other places ranges from 34–39 μm in LGG 450. The seed coat of LGG 450 and K 851 was thicker (Supplementary Fig 5) compared to ML 267 and MGG 295 (77.3 μm). The cotyledons contained few but larger starch granules, partially covered with protein deposits. The embryo was seen interlocked within cotyledons measuring 2 × 3 and 558 × 275 μm. The embryonic space around the embryo was relatively more at mid width. This indicated that the embryo is unready to take up imbibitional growth for sprouting unlike that of ML 267 (Supplementary Fig 6). The longitudinal embryo was seen surrounded by a large space; embryonal space also indicated a space for accumulation of water helped at the time of imbibition (Supplementary Fig 7).

The thicker mesocarp almost tight with endocarp together with narrow locular space (no gap in case of LGG 450) formed a barrier for impeding free movement of water. The cotyledons contain almost naked starch granules with splashed protein bodies indicate less prone for absorption of water; also, the median width of the embryo indicates its un readiness for sprouting (Harris 1987). The variation in ultra-structural features of pods and seeds of mungbean varieties of ML 267, MGG 295, LGG 450 and K 851 examined under the SEM revealed more number of pores with few deep cracks on podwall surface, large elongated and a greater number of turgid and functional trichomes, thin unicellular cuticular layer with one-celled epidermis, a large locular space and protein bodies embedded between many smaller starch granules in cotyledonary area. SEM studies in the lines of biochemical enzymes and metabolites such as α-amylase, ABA in rice and wheat were reported but are scanty in mungbean.

SEM studies in wheat, revealed, starch granules in sprouted seed samples were partially hydrolyzed. Overall, α-amylase activity caused changes to the physicochemical properties of the PHS damaged wheat (Simsek *et al.* 2014). Earlier also, Lamichaney *et al.* (2017) fresh seed dormancy of 10-15 days is a desirable trait in mungbean.

The genotypes with higher reduction in seed yield, namely ML 267, MGG 295, MGG 336 and LGG 407 had lower amounts of epicuticular wax and lower values of hard seed. The pod and seed traits, and pre-harvest damage parameters caused by weather culminated these genotypes as pre-harvest sprouting susceptible (PHSS) while LGG 450, K 851, PIMS 4 and LGG 460 as pre-harvest sprouting tolerant (PHST).

The SEM studies have established ultra-structural features that determine the resistance to pre-harvest sprouting of mungbean hence, development of future resistant lines should be identified for these ultrastructural features by adapting field level screening of existing mungbean genotypes. Overall, our results suggest the multi-faceted approach in establishing the resistance/tolerance of mungbean genotypes with respect to pre-harvest sprouting.

REFERENCES

Andrews C H.1982. Pre-harvest Environment: Weathering in Soybean Seed Quality and Stand Establishment (INTSOY), pp. 19–25.

Arunachalam V and Bandyopadhyay A. 1979. Are "multiple cross multiple pollen hybrids an answer for productive populations in *Brassica campestris* var brown sarson? i. Methods for studying Mucomorphs". *Theoretical Applied Genetics* **54**: 203–07.

Dougherty RW and Boerma H R. 1984. Genotypic variation for resistance to pre-harvest sprouting in soybean crop. *Crop Science* **24**: 683–86.

Harris W M. 1987. Comparative ultrastructure of developing seed coats of 'hard-seeded' and 'soft-seeded' cultivars of soybean. *Glycine max* (L.) Merr. *Botanical Gazette* 148: 324–31.

King R W and Richards R A. 1984. Water uptake in relation to pre-harvest sproutingin wheat: Ear characteristics. *Australian Journal of Agricultural Research* **35**(3): 327–36.

Kumar A, Chand P, Thapa R S and Singh T. 2021. Assessment of stability performance and GXE interaction for yield and its attributing characters in bread wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding* **12**: 235–41.

Lamichaney A, Katiyar P K, Laxmi V and Pratap A. 2017. Variation in pre-harvest sprouting tolerance and fresh seed germination in mungbean (*Vigna radiata* L.) genotypes. *Plant Genetic Resources* **16**(5): 1–9.

Lawn R J, Williams R W and Imrie B C. 1987. Food legume improvement for asian farming systems. Wallis E S and Byth D E (Eds). Australian Centre for International Agricultural Research, Canberra, pp. 317.

Michael D Peel. 2000. News from the Minnesota Association of Wheat Growers. Wheat Growers Laud Senate Passage of PNTR, 2000.

Lassim M B, Chin H F and Abdullah WD. 1984. The effects of weathering in mungbean (*Vignar radiata* (L.) Wilczek). *Seed Quality Partanika* **7**: 77–81.

Nagarajan P and Radder G D. 1983. Studies on induction of seed dormancy in bunch type groundnut. *Seed Research* **11**(1): 24–31.

Naidu N V, Seenaiah P and Satyanarayana A.1994. Mechanism of resistance to pre-harvest sprouting in mungbean. *(In) Proceeding of International Symposium on Pulses Research,* New Delhi, April 2–6th, pp. 140–41.

Rao K L N, Rao C M and Rao Y K. 2007. Evaluation of greengram germplasm for tolerance to pre-harvest sprouting. *Environmental Protection,* pp. 51–54. Kumar A and Nehar S Daya (Eds). Publishing House, Delhi.

Rao P S, Madhullety T Y and Ankaiah R. 2022. Scanning electron microscopy and morpho-physiological features imparting differential tolerance to pre-harvest sprouting (PHS) in mungbean. *Legume Research* 10.18805/LR-4834 pp: 1–8.

Sanjeev K, Choudhary H K and A K Singh. 2022. Identification of stable genotypes for grain yield and its components in mungbean (*Vigna radiata*). *Indian Journal of Agricultural Sciences* **92**(7): 847–52.

Simsek S, Ohm J B, Lu H, Rugg M, Berzonsky W, Alamri M S and Mergoum M. 2014. Effect of pre-harvest sprouting on physicochemical properties of starch in wheat. *Foods* **3**(2): 194–207.

Singh P, Chourasiya V K and Verma P. 2017. Screening of mungbean (*Vigna radiata*) germplasm against precocious germination susceptibility. *International Journal of Pure Applied Biosciences* **5**(6): 1010–14.

Williams R W, Lawn R J, Imrie B C and Bytes D E. 1984. Weathering resistance in mungbean (*Vigna radiata* and *Vigna mungo*): Occurrences causes inheritance and screening procedures. *Australian Seeds Research Conference* Lawes pp. 191–97.

Williams R W, Lawn R J, Imrie B C and Bytes D E. 1995. Studies on weather damage in mungbean I. Effect of weathering on seed quality and viability. *Australian Journal of Agricultural Research* **46**: 887–900.