Original Research Article

Assessment of genetic divergence for hardseededness with field emergence and storage duration in Mungbean genotypes [*Vigna radiata*(L.)Wilczek]

ABSTRACT

Hardseededness in legume crops induces uneven germination or non-synchronous seedling emergence leading to poor and un-uniform plant establishment and uneven maturity, which ultimately reduces the seed yield. An experiment was conducted with 51 mungbean genotypes to assess the influence of hard seeds on field emergence and also to know the effect of storage period on the occurrence of hard seeds. The standard germination test was performed with freshly harvested seeds and also with the seeds after 18 months of ambient storage. The results indicated that percent hard seeds varied significantly across the genotypes. The percent hard seeds showed significant negative correlation with percent normal seedlings and field emergence. More interestingly, the average *per cent* hard seeds decreased to 5.5% in stored seeds from 18.1% in the fresh seeds. A reduction in seed hardness following storage of 18 months under ambient conditions was recorded in the identified hardseeded genotypes indicating the possibility of increased field emergence using stored seeds for sowing.

Key Words: Germination, Normal seedling, Dead seed, Seed storage, Physical dormancy, Correlation, Cluster analysis

1. INTRODUCTION

Mungbean (Vigna radiata (L.) Wilczek) is the third important pulse crop of India in terms of total production (annonymous, 2019). Green gram, also known as mung bean, originated in India and Central Asia with chromosome number 2n=22 (Kang et al., 2014). Physical dormancy, generally referred to as hardseededness, also affects the germination and plantability of legumes crops such as mung beans. (Souza *et al*., 2001). Hardseededness leads to uneven germination, decreases the percentage of seedling growth in the field and causes problems with proper cooking and digestion. Hardseededness is distinguished by an impermeable seed coat due to the presence of suberin layered phenolics and palisade cells. (Marbach and Mayer, 1974). The seed coat provides a protective layer between the embryo and the environment (Quinlivan and Nicol, 1971). Many researchers have shown that the key site of impermeability is the cuticle and the thickness of the cuticle is positively associated with the degree of impermeability (Kolattukudy, 1981). Depending on the chemical composition and the seed coat structure of the varieties bred under different atmospheres, the degree of hardseedeeness may vary (Baskin and Baskin, 2001). Storage of seeds 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 changed, hardseededness is often overcome due to the easy permeability of seed coat to water. Further, storage period of seeds may also influence the occurrence of hardseededness in mung bean genotypes (Afzal *et al*, 2003). Development of genotypes with lower occurrence of hard seeds need a thorough understanding on the variation for hardseededness across the genotypes. In mung bean utilization of genetic variation for higher field emergence along with lowered hard seeds for future breeding program is the need of the hour. To the breeders an understanding of the nature and magnitude of variability among the genetic stocks is of prime importance. Genetic diversity is one of the important tools to

qualify genetic variability in both cross- and self-pollinated crops (Murty and Arunachalam 1966, Gaur *et al.*, 1978). Such a study also permits to select the genetic divergent parent to obtain the desirable recombinant in the segregating generations. Therefore, the present study was conducted to evaluate the genetic divergence for hardseededness with field emergence across mung bean genotypes and also to assess the influence of storage period on the proportion of hard seeds.

2. MATERIALS AND METHODS

The seeds of 51 genotypes of mung bean were obtained from Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. These genotypes were sown in July, 2017 and harvested in October, 2017 followed by germination test of the progeny seeds in December, 2017. A portion of seeds designated for sowing the next crop in April, 2018 and the remaining seeds were stored at ambient conditions in the laboratory at an average temperature varying from 9^0C to 39^0C (Min. temp: 5^0C-21^0C, Max temp: 13^0C-48^0C) and relative humidity varying from 67% to 83%. The seeds were tested for germination in the month of May, 2019 after a storage period of about 18 months.

2.1 Germination test: Seed germination test was conducted using Between Paper (BP) method as per ISTA rules, 2017. Four replications of 100 seeds from each genotype were placed equidistantly on moist germination paper. The rolled towels were placed in seed germinator at 25^oC for 7 days. The first and final count was recorded at fifth and seventh day after putting, respectively. The data was recorded on the basis of number of normal seedlings, abnormal seedlings, number of hard seeds and dead seeds at final count and expressed in percentage. Ungerminated seeds that were intact on tapping were considered as hard seeds, whereas, seeds that were rotten or disintegrated on tapping were considered as dead.

2.2 Field emergence test: The number of seeds emerged in the form of seedling out of 100 seeds sown was recorded in each genotype starting at 3rd day after sowing and this was continued up to 10 days at an interval of 2 days and the data was expressed in percentage.

2.3 Cluster analysis: Cluster analysis of 51 mung bean genotypes in fresh and stored seed lots were done using SAS software (SAS Institute Inc., Cary, NC) using squared Euclidian distance method. *Per cent* normal seedlings, abnormal seedlings, dead seeds, hard seeds, and field emergence were used for the cluster analysis in fresh and stored seed lots.

3. RESULTS AND DISCUSSION

3.1 Germination test

Result showed that, the presence of hard seeds ranged from 3 to 52% among the genotypes with a mean value of 18.10% (Table 1). Genotype TM 96/25 showed highest percentage of hard seeds (52%), whereas, genotypes HUM-12, GM-4, IPM 205/7 and SM 11/75 showed the lowest percentage of hard seeds (3%). Presence of normal seedlings ranged from 28 to 84% with a mean value of 59.18%, whereas, abnormal seedlings ranged from 3 to 28% among the genotypes tested (Table 1). The dead seeds ranged from 0 to 24%. Field emergence (from the progeny seeds) ranged from 15 to 67% among the genotypes and the genotypes with lowered occurrence of hard seeds remarkably showed higher field emergence (Table1). Our results were in confirmation with observations made in soybean (Arechavaleta-Medina and Suyder, 2001) and mungbean (Marwanto, 2007; Jitender *et al*., 2017).

3.2 Correlation matrix

The occurrence of percent hard seeds showed significant negative correlation with percent normal seedlings($r = -0.7$, $p<0.0001$) and percent field emergence($r = -0.4$, $p<0.01$) (Table 2). It was evident that the higher occurrence of percent hard seeds resulted into lower number of

normal seedlings which ultimately led to reduced field emergence. Moreover, the percent hard seed showed negative correlation with abnormal seedlings and dead seeds, though the correlation was statistically non-significant. On the other hand, percent normal seedlings was positively correlated with field emergence percentage ($r = 0.5$, $p<0.001$). The similar observation that the hardseededness was strongly correlated with emergence was recorded by Chachalis et al., (2008) in *Hibiscus trionum*. So, higher proportion of normal seedlings in germination test led to improved field emergence in mung bean (Table 2).

3.3 Cluster analysis

3.3.1 Fresh Seed Lot: Cluster analysis is an effective tool to access the genetic diversity among genotypes. Jahan et al., (2020) performed cluster analysis using mung bean genotypes for yield and yield attributing characters against salt stress. In cluster analysis for fresh seeds, the dendrogram prepared using squared Euclidian distance method showed six clusters consisted of 14, 11, 6, 6, 11 and 3 genotypes, respectively (Table 3). Cluster 2 consisted of 11 genotypes that showed highest percent hard seeds, lowest percent normal seedlings and lowest percent field emergence. On the other hand, cluster 6 consisted of three genotypes that showed lowest percent hard seeds (8.7%), which resulted in higher percent normal seedlings (67.3%) ultimately leading to highest percent field emergence (62.7%). It was clear that recurrence of parent hard seeds showed a strong, negative correlation with field emergence percentage (Table 3 and Figure1a).

3.3.2 Stored seed lot: In cluster analysis of stored genotypes, showed four clusters consisted of 12, 7, 11 and 21 genotypes, respectively (Table 4). After 18 months of storage period, cluster four consisted of 21 genotypes showed lowest average percent hard seeds (1.71%), highest average normal seedling percentage (81.43%) and lowest percent dead seeds (13.62%). Among the 21 genotypes, 8 genotypes showed percent hard seeds varied from 21 to 32 % in fresh seeds lots

(Table. 4 & Fig. 1b). Result indicated there was a strong environmental influence on the occurrence of hard seeds in mung bean genotypes. Quinlivan and Nicol (1971) reported that hardseededness controlled by both genetic and environmental factors. Paul *et al*. (2019) also indicated similar kind of results that the trait hardseededness influenced by varying environmental situations in mung bean. Interestingly, the cluster two showed highest percent hard seeds (20.29%), lowest normal seedling percentage (52.85%), lowest percent abnormal seedlings (9.71%) and lowered dead seeds (17.14%) also. These genotypes were true hard seeded genotypes and controlled by specific genetic factor. Singh *et al* (2005) confirmed that hardseededness in mung bean is controlled by single dominant gene. Paul *et al*. (2018) observed that the trait hardseededness in mung bean having a broad sense heritability of 0.96 when sown during the period between July to October. Ladizinsky (1984) also suggested that the hardseededness of wild lentil species is due to a hard seed coat and controlled by a single gene. Singh *et al* (1983) reported a single dominant gene controlling hard seeds using a cross between *Vigna radiata* and *sublobata*. However, analysis of a RIL population following a cross between hard and non-hard varieties in mungbean suggested four QTLs associated with hardseededness (Humprey *et al.*, 2005). For the first time the gene associated to hardseededness has been identified in soybean seed pearmeability, used a map-based approach and identified the gene as *GmHs1*-1that governs hardseededness in soybean (Sun et al., 2015). It was very evident that in case of the hard seeded genotypes the rate of deterioration during ambient storage was slower resulted in lowered number of abnormal seedlings and dead seeds. A higher percentage of normal seedlings in stored seed lots showed the possibility of higher field emergence if used for sowing purpose as the percent normal seedlings showed a strong positive correlation with field emergence percentage (Table. 2). A balance between the occurrence of hard seeds and the period of seed storage might be a way out to reduce the problem of lowered field emergence and uniform germination due to hard seeds in mungbean.

3.4 Storage study

Result indicated that, in general, the number of hard seeds and abnormal seedlings reduced significantly while normal seedlings and dead seeds increased significantly in the stored seeds of 18 months (data not presented). As per the Indian Minimum Seed Certification Standards (IMSCS) the minimum germination percentage for seed certification of mung bean is 75%. From the previous studies it was observed that beyond 18 months of storage the germination percentage comes below 75% due to increase of number of dead seeds. For the above said reason the period of storage study was kept 18 months. Result indicated that the average *per cent* hard seeds decreased to 5.5% in stored seeds from 18.1% in the fresh seeds. Only 15.5% of genotypes showed more than 10% hard seeds in the 18 months stored seeds while 74.51% of genotypes showed more than 10% hard seeds in the fresh seed lots (Figure 2). Notably, the hard seeds in genotype PUSA 9531 reduced drastically from 31% in fresh to 6% in stored seeds.

It is reported that the membrane of testa deteriorates during storage due to seed ageing that might lose solid and water holding capacity of seeds. Changing 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 (Stanley *et al*., 1990). A characteristic of many species with physical dormancy or harseededness is the presence of a 'water-gap complex' (Baskin *et al*., 2000; Gama-Arachchige *et al*., 2010, 2011, 2013; Baskin and Baskin, 2014). This is defined as a morpho-anatomically complex structure, characterized by the presence of a strophiole (/lens/lid) (Gama-Arachchige *et al*., 2013). 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 imbibition to occur (Baskin *et al*., 2000; Baskin and Baskin, 2014). Rodrigues *et al*., (2020) recently reported a

structure called the pleurogram that makes up a large part of the seed coat insome *Fabaceae* species and acts as a hygroscopic valve during seed maturation to germination. As a pathway for water entry into the seed, the pleurogram acts as a water gap in seeds with physical dormancy, thereby regulating dormancy-break/germination during storage. In our study, the reduced hard seeds after 18 months storage might be due to these facts.

Stanley *et al*.(1990) reported that white beans stored in tropical and temperate condition had reduced hard seed numbers, because of breakdown of lignin, which loosened the testa cells making water permeable inside the seed. In our study, there was fluctuation in diurnal temperature under ambient storage (Min. temp: 5^0C-21^0C, Max. Temp.: 13^0C-48^0C) and relative humidity that might lead to unplugging of seed coat openings making the water available to the embryo (Baskin, 2004). Evers (1991) also reported that high constant temperature and temperature fluctuations are major factors in overcoming hardseededness in most of the legumes.

4. CONCLUSION

Significant variation was observed for hardseededness across the mung bean genotypes. Greater proportion of hard seeds indicated lower percent normal seedling in germination and filed emergence tests, leading to grouping of genotypes of lowest percent hard seeds and highest field emergence together in the cluster analysis. The genotypes with lowered hard seeds with high field emergence percentage may be used for the development of desirable cultivars in future breeding program. On the other hand, during storage, the proportion of hard seeds and abnormal seedlings reduced but also, due to increase in storage life of seeds there was a higher natural death of the seeds. A balance between these two phenomena i.e., occurrence of hard seeds and the period of seed storage might be a strategy to solve the problem of poor seed germination and field emergence due to presence of hard seeds.

Declaration

The authors declare no conflict of interest.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. The research is part of a Ph. D. thesis of the first author to be submitted to the Post-graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, India. The first author gratefully acknowledges the Indian Council of Agricultural Research (ICAR) for providing financial assistance for the study.

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Table 1. Seed germination traits and field emergence of mungbean genotypes produced in July, 2017

Genotypes	Hard seed (%)	Normal seedling (%)	Abnormal seedling (%)	Dead seed (%)	Field Emergence(%)
PUSA 0831	16	64	10	10	50
HUM 1	26	40	14	20	34
TM 96/25	52	28	14	6	37
IPM 288	16	72	4	8	44
PUSA VISHAL	17	80	3	0	51
LGG 460	22	56	12	10	42
EC398891	36	42	14	8	47
MH 521	31	46	10	13	33
OMG 1031	29	40	18	13	28
IPM 02-15	6	64	14	16	53
PORBANDER MUNG	15	64	9	12	44
IPM 409-4	25	52	13	10	46
HUM 12	3	84	4	9	64
IPM 02/3	4	68	12	16	62
PUSA 1033	7	68	19	6	57
IPM 205/7	3	70	13	14	40
GANGA 1	13	56	17	14	37
SM 11-75	3	74	16	7	64
PUSA 0672	29	62	5	4	47
IPM 02/14	23	50	14	13	40
PUSA 1333	13	70	5	12	28
GM 4	3	78	8	11	47
COGG 912	7	64	12	17	48
RMG991	13	58	12	17	30
KM 11-26	10	56	18	16	32
PUSA 9072	37	48	9	6	38
MH 421	22	62	8	8	45
V 61-73	28	44	10	18	30
PUSA 0871	9	82	7	2	47
IPM 409-1	13	54	9	24	39
AKM 9904	5	58	25	12	15
PDM 139	13	72	5	10	62
PS 16	4	60	12	24	53
PARKESH NEPHAL	15	60	12	13	38
IPM 205-4	31	50	8	11	37
IPM 02-19	20	44	18	18	51
EC398885	15	70	4	11	38
TM 96-2	20	56	8	16	35
HUM 16	27	46	13	14	25
HUM 2	16	60	14	10	67
MH 2-15	24	54	12	10	43
GANGA 8	26	52	10	12	24
PUSA BAISAKHI	21	48	7	24	44
PANT M-5	4	80	10	6	51
PUSA 9531	31	52	8	9	36
IPM 02-16	12	70	7	11	53
HUM 6	14	60	20	6	42

PUSA 105	25	66	6	3	42
ML 1451	16	40	28	16	44
PUSA 1031	24	64	10	2	49
PUSA RATNA	29	60	10	1	46
Mean	18.10	59.18	11.37	11.35	43.12
SE (m)	1.35	1.44	1.49	1.36	2.26
CV	10.54	3.5	18.57	16.89	7.41
CD (p=0.05)	3.84	4.1	4.25	3.87	6.44

Table 2. Correlation among the seed germination traits and field emergence of mungbean genotypes

Traits	**Hard seed (%)**	**Normal seedling (%)**	**Abnormal seedling (%)**	**Dead seed (%)**	**Field Emergence(%)**
Hard (%)	1.0				
Normal (%)	-0.7**	1.0			
Abnormal (%)	-0.1	-0.5**	1.0		
Dead (%)	-0.2	-0.4**	0.2	1.0	
Field Emergence	-0.4**	0.5**	-0.2	-0.2	1.0

**, p<0.01;

Values with no asterisks were not significantly correlated with any of the traits

Table 3. Cluster analysis of the mungbean genotypes based on seed germination traits and field emergence in fresh seed lot

Cluster number	Number of genotypes	Hard (%)	Normal (%)	Abnormal (%)	Dead (%)	Field Emergence (%)
1	14	21.6	59.1	10.1	9.2	42.6
2	11	32.2	44.4	11.6	11.8	33.5
3	6	9.2	59.7	11.0	20.2	49.8
4	6	13.0	52.3	21.0	13.7	36.8
5	11	9.8	75.3	6.4	8.5	47.7
6	3	8.7	67.3	16.3	7.7	62.7

Table 4. Cluster analysis of the mungbean genotypes based on seed germination traits and field emergence in stored seed lot

Cluster number	Numbe r of Genoty pes	Norm al (%)	Abnormal (%)	Hard (%)	Dead (%)
1	12	63.08	12.16	5.67	19.08
2	7	52.85	9.71	20.29	17.14
3	11	57.27	4.18	3.27	35.27
4	21	81.43	3.43	1.71	13.62

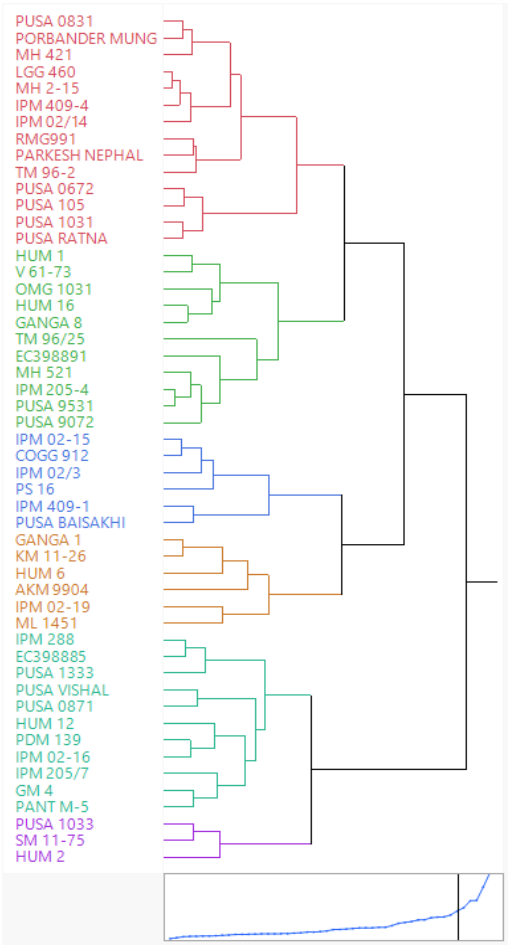

Fig. 1(a)

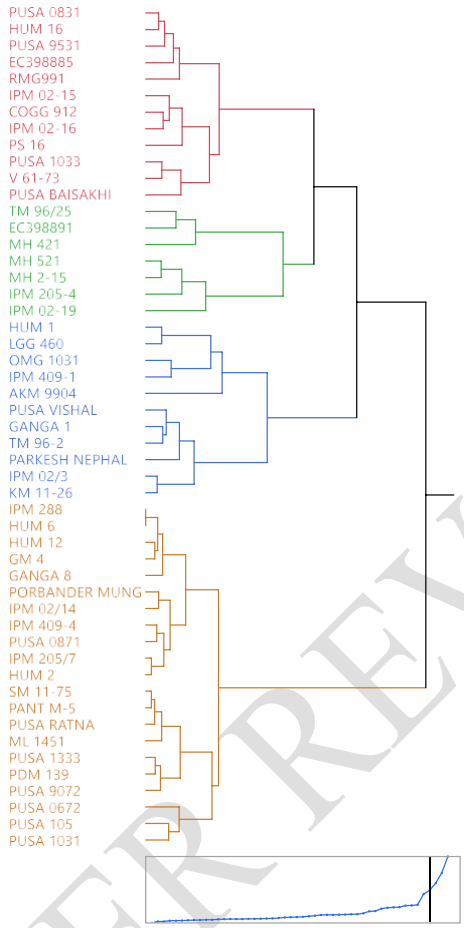

Fig. 1(b)

Figure 1. a) Dendrogram of the mungbean genotypes based on seed germination traits and field emergence in fresh seed lot

b) Dendrogram of the mungbean genotypes based on seed germination traits in stored seed lot

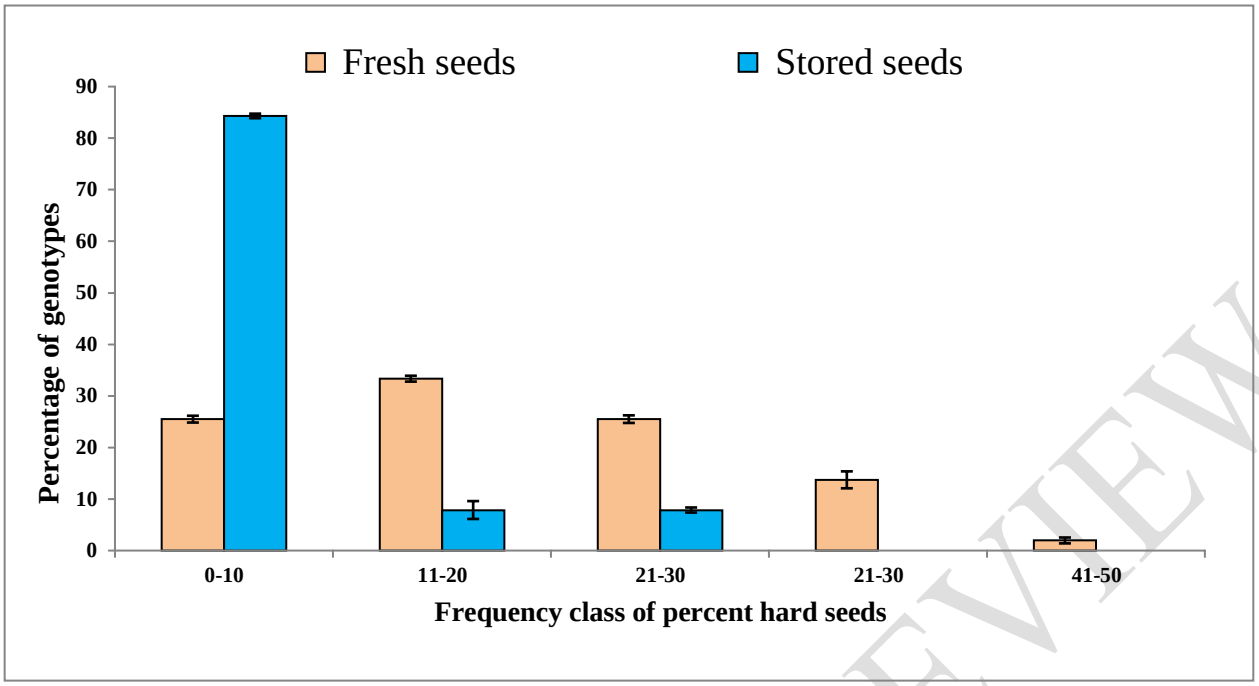

Figure 2: Effect of storage period on reduction in hard seeds