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Heritability of hardseededness in mungbean [*Vigna radiata* (L.) Wilczek] under varying environments

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Abstract

Presence of hard seeds in seed lots reduces the seedling emergence percentage in field leading to non-uniform, lowered plant population and uneven maturity. Seed hardness, characterized by no water imbibition, is controlled by both genetic and environmental conditions. To estimate the broad sense heritability (H^2) of hardseededness, 20 mungbean genotypes with >22% hard seeds were grown in four different environments (Env.) *viz.,* high average temperature and high soil moisture (Env. 1), mild temperature and low soil moisture (Env.2), mild temperature and high soil moisture (Env. 3) and low average temperature and low soil moisture (Env.4). The average per cent hard seed in seed lots was 5.42, 28.7, 19.4 and 33.9 under Env.1, Env.2, Env.3 and Env.4, respectively. The H^2 estimate of hardseededness under Env.1, Env.2, Env.3 and Env.4 was 0.67, 0.97, 0.96 and 0.98, respectively. Decreasing soil moisture, low temperature and delayed harvest increased occurrence of per cent hard seed. The force required in Texture Analysis Machine to break seeds produced under Env.1 ranged from 9.23 to 33.31 Newton while the same ranged from 39.51 to 71.53 Newton in seeds produced under Env.4. The Scanning Electron Microscope images indicated that the seeds produced under low average temperature and soil moisture had a presence of compact outer cell layer with low surface deposition and depression on the seed coat of seeds produced in Env.1 and there is existence of loose cells and cracks in the seed coat with high depression and surface deposition in the seeds produced under Env.4. A lower heritability of 0.67 for hardseededness in high temperature and soil moisture condition suggested that the character is influenced by stress conditions. Growing of genotypes under low temperature and moisture condition is suggested for screening of genotypes for hardseededness in mungbean.

Key words: Mungbean, hardseededness, heritability, hardness breaking force, Scanning Electron Microscope

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] belongs to the family Fabaceae and subfamily Papilionaceae. It is considered to be the hardiest among all pulse crops since it is grown extensively under varying climatic conditions. Mature seeds of many legume species, including mungbean, do not germinate readily under favorable environmental conditions because they are impermeable to water and gases or they have seed coat that mechanically constraints the embryo. This attribute has been called as hardseededness, hardcoatedness, hard seed coat dormancy, seed coat impermeability and exogenous dormancy. Therefore, the presence of hard seeds affects the grain quality and yield. Hardseededness is heritable and has been found in all commercial legume species. Its expression is strongly related to prevailing climatic factors during plant growth and seed germination. The cuticle of the palisade layer is the key factor that determines the permeability property of a seed coat (Ma et al. 2004). The impermeability of this layer has been chemically associated with the deposits of callose (*Trifolium subterraneum*), phenolics (*Pisum* spp.) and lipids (Annual *Trifolium* spp.) (Werker et al. 1979; Slattery et al. 1982; Zeng et al. 2005). The development of impermeability has also been associated with the metabolism of phenolic compounds and activity of peroxidase in the palisade layer, during the final stages of seed development (Marbach and Mayer 1974; Egley et al. 1983). The hard seeds were higher in *kharif*-sown crops as compared to summer-sown crops (Tomer and Kumari, 1991). Rainfall, temperature and relative humidity are common phenomenon in the development of hard seeds. Soil fertility, even

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application of lime also influences the hard seed formation in mungbean (Hazarika and Barua 2002). Seed texture is an important factor for the evaluation of hardseededness in green gram. Harder seeds generally decrease consumer acceptance of the food product. Although seed hardness provides evolutionary benefits, such as resistance to seed coat inhabiting pathogens and to seed spoilage when storing conditions are not optimum, it also causes impermeability in which seeds do not absorb enough water to germinate. Zhang et al. (2009) proposed high calcium concentrations to be one of the many causes of hard seed formation in soybean. Small seeds tend to have higher calcium content and, consequently, have a harder texture, while larger seeds have lower calcium content and are softer when cooked (Chen et al. 2001). Taira (1990) also reported that seed size was negatively correlated with seed water absorption and cooked seed hardness in soybean. Physical dormancy or hardseededness is usually considered to be a heritable trait, controlled by the testa or endocarp, which are derived from integuments of the ovule and the inner epidermal layer of the ovary wall, respectively (Evenari et al. 1966; Pe´rez-Garcý´a and Escudero 1997; Li et al. 1999a). To date, numerous natural mechanisms of physical dormancy breakdown have been described in different species of family *fabaceae*, particularly relating to various combinations of temperature and moisture changes, including high temperatures from summer insulation or fire, temperature fluctuations (Va´zquez-Yanes and Orozco-Segovia 1982) and wet heat (van Klinken and Flack 2005). As hardseededness is believed, in many cases, to have evolved to enable plants in harsh environments to survive (Baskin et al. 2000) it may be hypothesized that plants with physical dormancy would prosper under the more variable and warmer climate that is projected for the future (IPCC 2014). However, a number of questions need to be considered before such a conclusion can be drawn. Therefore, this research seeks to ask, first, what is the extent of hardseededness that varies under different growth conditions and second, is this variation genetic or environmental in origin, and to what degree is it heritable? In view of the above, a research programme has been undertaken with the objective to assess the variation for hardseededness with respect to different abiotic stress conditions, stage of harvesting, hardness breaking force and seed surface characteristics among mung bean genotypes.

Materials and methods

Experimentation

Field experiments were conducted under four different conditions (high average temperature and high soil moisture, mild temperature and low soil moisture, mild temperature and high soil moisture, low average temperature and low soil moisture) during anthesis to seed maturity with 20 genotypes having more than 22% hard seeds. The material was planted in the field of the Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi in randomized block design (RBD) with three replications having plot size of 2 rows of 4m length to record hardseededness following standard seed germination test (in *per cent*) at different abiotic stress conditions and to estimate heritability in broad sense. Seed germination test was conducted using Between Paper (BP) method as per ISTA rules, 2015. A second experiment was conducted with genotypes having more than 22% hard seeds under decreasing soil moisture post flowering till harvesting at different time to assess the effect of soil moisture level on hardseededness. Under this condition, ten plants in each genotype were tagged. The anthesis date of labelled flowers in the inflorescences was recorded. The seeds were harvested from 10 randomly tagged plants at 28, 35 and 42 days after anthesis. Number of hard seed was recorded following standard seed germination test and expressed in percentage. A third experiment was conducted under high soil moisture content. In this experiment an additional irrigation was provided after 21 days of flowering to maintain high soil moisture. The seeds were harvested from 10 randomly tagged plants at 14, 21 and 28 days after flowering to assess the effect of high moisture at different harvest time on hardseededness.

Soil moisture content and weather

Soil moisture was recorded at different harvesting time. Ten representative samples were taken from the plot and by proper mixing and hand halving method the soil sample brought to 500g. Then soil moisture was recorded by taking 10g of soil from each soil sample in aluminium boxes. The boxes were placed in hot air oven at 105°C for 72 hours and percent moisture content was calculated by following formula.

Soil moisture content (%) = $[(M_2-M_3)/(M_2-M_1)] \times 100$

Where M_1 = weight of empty aluminium box, M_2 = weight of aluminium box + sample before drying, M_3 = weight of aluminium box + sample after drying

Weather data during the crop growing periods under different conditions were collected from the Institutes' observatory (Division of Agricultural Physics).

Seed hardness breaking force

The force to break a seed has been measured with texture analyzer machine (TA-HDi texture analyzer). The instrument was used with the test speed: 0.50 mm/s, pre-speed:1.00 mm/s, post speed: 2.00 mm/s and load cell: 500. Ten seeds were taken from each selected genotype in three replications (in low average temperature and low soil moisture; and high average temperature and high soil moisture condition) to record the hardness breaking point. The force used to break the seed coat was recorded in Newton.

Seed surface characteristics

The study of surface characteristics of seed was done using Scanning Electron Microscope (SEM). For SEM studies, dry seeds were mounted on stubs and placed directly into a TUSCAN WEGA 3 scanning electron microscope at 10kv and at a magnification varied from 34X to 1.27kX for viewing and photography.

The data recorded from field and laboratory experiments were analysed following appropriate statistical methods (Sukhatme and Panse, 1954). The components of variance were estimated using a random-effect model and broad-sense heritability (H^2) for hardseededness across the four environments was estimated as $H^2 = Vg/Vp$, where H is the heritability estimate, Vg the variation in genotype, and Vp the variation in phenotype (Knapp et al. 1985).

Results and discussion

Heritability of hard seeds under abiotic stress conditions

The average *per cent* hard seeds were significantly different under high average temperature and high soil moisture (5.42), mild temperature and low soil moisture (28.7), mild temperature and high soil moisture (19.4), low average temperature and low soil moisture conditions (33.9) (Table 1). The average temperature during this period ranged from 25.4-32.3^{o}C and soil moisture content varied from 16.9-23.6% (Fig. 1). Seeds produced under high temperature and soil moisture condition showed very lesser extent of hardseeds suggesting that these conditions made hard seeds permeable to water. This may be due to larger seed size and lower seed density of seeds (Paul et al. 2018). A higher temperature during seed maturity could have played a role in faster seed development and disrupting the physiological processes in seed coat development. At high temperature and moisture a decrease in lignin content in the seed coat increases the capacity and velocity of absorption of water throughout the seed coat. (Marwanto et al. 2007) Similar observations have been recorded earlier in soybean (Arechava leta-Medina and Synder 1981) and mungbean (Marwanto et al. 2007). Under low or mild temperature situation it failed to break the physical dormancy resulting in higher occurrence of hard seeds. The broad sense heritability estimate of the mung bean genotypes in high average temperature and high soil moisture condition was 0.67, and in mild temperature and low soil moisture, mild temperature and high soil moisture, low average temperature and low soil moisture conditions were 0.97, 0.96 and 0.98, respectively suggesting that the character hardseededness was much stable under low temperature and irrespective of soil moisture condition (Table 1). A lower heritability of 0.67 for hardseededness in high temperature and soil moisture condition suggested that the character is influenced by abiotic stress conditions. Growing of genotypes under low

Table 1. *Per cent* hard seeds and heritability under different abiotic stress conditions

Condition	*Per cent* hard seeds				Heritability
	Mean	Max.	Min.	SD	
High temp. and high soil moisture	5.42	11	2	2.39	0.67
Mild temp. and low soil moisture	28.7	51	12	2.68	0.97
Mild temp. and high soil moisture	19.4	33	5	9.52	0.96
Low temp. and low soil moisture	33.9	52	20	7.12	0.98

CD (p=0.05) = 4.17, SD = Standard deviation; Min. = Minimum, Max. = Maximum

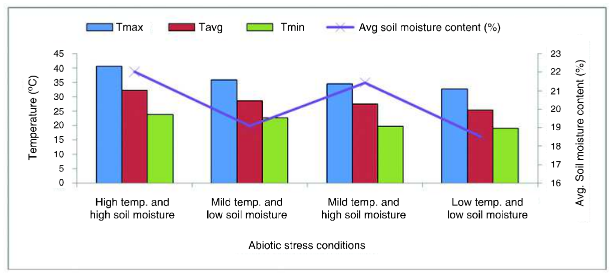

Fig. 1. Temperature and soil moisture content at different abiotic stress conditions

temperature and moisture condition is suggested for screening the genotypes for hardseededness in mung bean.

Depleting soil moisture condition on the occurrence of hard seeds

To know the effect of depleting soil moisture and stage of harvesting on the occurrence of hard seeds the 20 genotypes of mung bean were sown in depleting soil moisture condition and pods were harvested at 28, 35 and 42 days after anthesis. The *per cent* hard seeds were 24.8, 30.4, 35.9 at 28, 35 and 42 days after anthesis, respectively (Table 2). Lack of soil moisture (15%) limits the minerals and nutrients to the plants, in general and to the developing seeds, in particular. The effect of moisture stress during seed development on subsequent germinability appears to depend upon the mechanism of dormancy in the relevant species. Drought usually increases seed coat thickness or hardness, reducing its permeability and hence reducing germinability at least in short-term tests in case of mechanically imposed dormancy by a thick seed coat (Baskin et al. 2000). Hill et al. (1986) found that soybean seeds harvested from drought-stressed plants were 15-33% smaller in size but had a higher percentage of seed weight in the seed coat and 6-37% more impermeable after 72 hours of soaking. Marwanto et al. (2007) reported the role of lignin content in hardening the seeds. Higher lignin content protects the seeds from germination under unfavourable conditions. Due delayed harvest, lignin contents are likely to get accumulated in the seed coat, which hinder capacity and velocity of absorption of water throughout the seed coat. In soybean, under low soil moisture conditions, thicker and low permeable seed coat developed due to shortage of cytokinin and minerals (Nooden et al. 1985). The results in present study indicated that delay in harvesting under depleting soil moisture condition resulted into higher occurrence of hard seeds.

Table 2. Occurrence of hard seeds under depleting soil moisture condition at different harvesting time after anthesis

DAA	Soil moisture content (%)	Hard seed (%)				
		Mean	Max.	Min.	SD	CD(p=0.05)
28	21.9 (19-24.2)	24.8	44.5	11.6	9.8	2.6
35	18.2 (17-19.3)	30.4	58.3	12	11.9	1.93
42	17.3 (16.9-18.1)	35.9	56.4	16.6	10.5	1.85

DAA = Days after anthesis

High soil moisture condition on the occurrence of hard seeds

To know the effect of timely harvest under high soil moisture condition on the occurrence of hard seeds the 20 genotypes of mungbean were sown and pods were harvested at 14, 21 and 28 days after anthesis. The per cent hard seeds recorded were 21.5, 24.2, 15.3 at 14, 21 and 28 days after anthesis, respectively (Table 3). Result showed that the *per cent* hard seeds increased up to 21 days after anthesis when the soil moisture content decreased to 19.63% from 20.17%. The soil moisture content in the range of 21.2-25.8% during 21 days to 28 days showed a decreased *per cent* hard seeds. This decrease in hard seeds could be due to increase in soil moisture that might have reversed the process of seed hardening (Nooden et al. 1985). The physiological maturity in mungbean has been reported as 25 days after anthesis in which the *per cent* hard seeds was the minimum among all the stages of harvesting. The study did not mention the soil moisture level at various stage of harvesting (Anurag et al. 2009). However, it needs to be further investigated the reason of getting lower hard seeds in higher soil moisture at harvest maturity.

Table 3. Occurrence of hard seeds under high soil moisture condition at different harvesting time after anthesis

DAA	Soil moisture content (%)	Hard seed (%)				
		Mean	Max.	Min.	SD	CD(p=0.05)
14	20.17 (18.4-22.4)	21.5	38	6	9.5	2.53
21	19.63 (18.1-21.7)	24.2	46	7	11.9	1.83
28	23.07 (21.2-25.8)	15.3	32	5	8.3	2.63

DAA = Days after anthesis

Hardness breaking force

The seed hardness was examined using a seed texture analysis machine of seeds produced under high temp and high soil moisture, and low temp and low soil moisture condition. The data indicated that the force required to break the seeds produced under high temperature and moisture ranged from 9.23 to 33.31 Newton (N) with an average of 24.3N in comparison to 39.51 to 71.53 N with a mean value of 56.4N in seeds produced under low temperature and low soil moisture conditions (Table 4). Zhang et al. (2009) proposed high calcium concentrations to be one of the many causes of hard seed formation in soybean. Small seeds tend to have higher calcium content and, consequently, have a harder texture, while larger seeds have lower calcium content and are softer when cooked (Zhang et al. 2009; Chen et al. 2001). Similar kind of observation was also made in soybean (Orazaly et al. 2015). The temperature variables differed to a greater extent at the time of maturity in different abiotic stress conditions. Average maximum temperature in high temperature and soil moisture condition was 39-40°C with an average temperature of 30-32.3°C while the same in low temperature and soil moisture condition was 19 to 32°C with an average of 25.4°C. The fluctuation in temperatures resulted into initiation of softening process of seed which may be interpreted as a physical expansion and contraction effect that caused the splitting of the elongated palisade cells in the lens, which ultimately resulted into ease of water entry (Taylor 2004). In *Stellaria media*, seed collected from the field throughout the year germinated more readily if matured in summer than that in winter (Van der Vegte, 1978), and experiments under controlled conditions confirmed the differential imbibitions to be due to the temperature during development. A higher temperature during seed maturity could have played a role in faster seed development and disrupting the physiological processes in seed coat development. At high temperature and moisture a decrease in lignin content in the seed coat increases the capacity and velocity of absorption of water throughout the seed coat. On the other hand, at low soil moisture condition lack of nutrients and minerals causes a thicker, less permeable seed coat to develop. In our study, higher temperature during seed maturity promoted an early harvest thereby avoiding the seed hardening process.

Table 4. Hardness breaking force under abiotic stress conditions

Condition	Hardness breaking force(N)				CD (P=0.05)
	Mean	Max.	Min.	SD	
High temp. high soil moisture	24.3	33.3	9.23	6.12	1.97
Low temp. and low soil moisture	56.4	71.53	39.51	8.42	4.71

Scanning Electron Microscopy (SEM)

The seeds produced under high temperature and soil moisture, and low temperature and low moisture condition were observed for the seed coat characteristics using SEM. The SEM images indicated that the seeds produced under low temperature and low moisture condition (hard seeds) had a compact outer cell layer with low surface deposits and

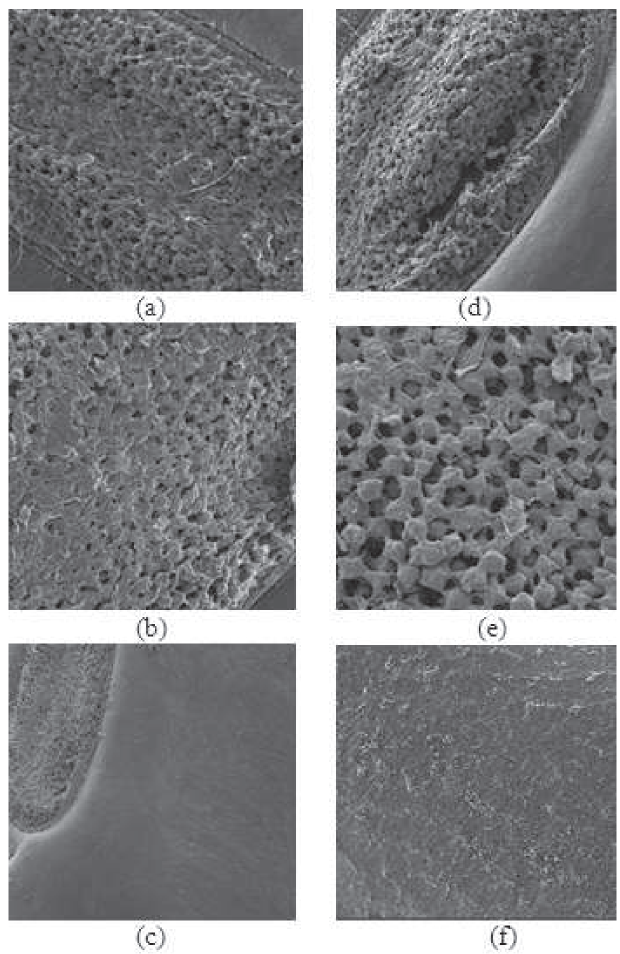

Fig. 2. Scanning Electron Microscopy of selected hard (a, b, c) and normal seeds (d, e, f). (a): closed hilum (249X) ;(b): compact outer cell layer (434X); (c): smooth outer surface (70X); (d): crack in hilum (258X); (e): loose outer cell layer (418X); (f): rough surface due to high surface deposition (429X)

depression on the seed coat. The cells are more compacted at hilum region of hard seeds and the inter-cellular spaces were also very less at hilum region of hard seeded genotypes while the seeds produced under high temperature and soil moisture (non-hard) seeds had loose outer cell layer and cracks in the seed coat with surface deposits (Fig. 2). The cells at hilum region of non-hard seeds are loosely arranged and inter-cellular spaces were also more which helped in easy imbibitions of water in case of non hard seeds. Smýkal et al. (2014) also reported that several cracks were found in permeable genotypes of soybean but in impermeable genotypes those cracks were not found and the surface deposition on seed coat seems to be responsible for water imbibitions in soybean genotypes. Ma et al. (2004) stated that the cuticle of permeable soybean genotypes are mechanically weak and develops cracks through which water can enter into the seed but cuticle of impermeable genotypes are mechanically strong and does not develop cracks under normal conditions.

Authors' contribution

Conceptualization of research (SKC); Designing of experiments (SKC, DP); Contribution of experimental materials (HKD); Execution of field/lab experiments and data collection (DP, YS); Analysis of data and interpretation (DP, SKC); Preparation of the manuscript (DP, SKC).

Declaration

The authors declare no conflict of interest.

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