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Dissection of morphological diverse breeding traits in greengram [*Vigna radiata* (L.) Wilczek] genotypes by means of DUS guidelines

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Abstract
A total of 39 genotypes of mungbean were taken for analysis in the present study for statistical analysis related quantitative traits. To investigate the morphological characterization among these mungbean genotypes using quantitative traits. The present study was carried out at the BSP Soybean unit, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh. The genotypes were sown in six rows of with 30 X 10 cm spacing under RCBD design with three replications. The seed sowing was made in the month of November 2021. Morphological characterization was done for ten characters and it was noted that for anthocyanin pigmentation thirty-five genotypes showed presence of pigmentation and in remaining four genotypes were absent for this trait, stem colour, one genotype of mungbean (PKVAM 4) showed purple colour stem, while remaining 38 genotypes showed green stem colour, stem pubescence, two genotypes (TJM 155 and TJM 143) showed pubescence on stem and the remaining 37 genotypes absence for this trait, two genotypes (MH 421and TJM 137) of mungbean showed deltoid leaf shape while remaining thirty-seven genotypes showed ovate leaf shape for this character, five genotype of mungbean showed green colour leaves, while remaining thirty-four genotypes showed dark green colour of leaves, thirty-seven genotypes of mungbean showed green colour leaf vein, while remaining two genotypes (Pusa-Vishal and GAM 5) were greenish purple leaf vein, thirty-eight mungbean genotypes showed green colour of petioles whereas, only one genotype (GAM 5) was green with purple splashes in petioles, out of total, thirty-eight genotypes of mungbean showed green colour of premature pod, while only one genotype (Yellow mung) was green with pigmented nature of premature pod colour, pubescence on pod was recorded in this investigation; it was noted that thirty-eight genotypes showed pubescence on the pod and the rest one genotype (TJM-235) was free of pubescence on pod and thirty-eight genotypes showed pod position intermediate and the remaining one-genotype (Pusa-Vishal) pod position was above the plant canopy. These identified diverse morphological breeding traits is present in very few of the genotypes included in this investigation. The identified diverse breeding traits may be employed in hybridization programme in developing diverse morphology based genotype/varieties so, that the genotypes with unique morphological identity will easily be identified in between the group of varieties. Maintenance of the varietal purity has been one of the major challenges in the research area and it is very important to check whether the genotypes or treatments or population under study are satisfying the requirements of morphological characterization protocols which includes distinctness, uniformity and stability. These unique identities will be helpful as an important morphological trait to distinguish one variety from the other in seed production programme. By the diverse identity of the varieties, genetic purity maintenance will be very easy and may stay in seed production chain for a very long time.

Keywords: Mungbean, principal component analysis, PC score

Introduction

Greengram [*Vigna radiata* (L.) Wilczek] being a self- pollinated, short duration leguminous crop that comes under family Fabaceae, subgenus *Ceratotropis* and genus *Vigna* with 2n=22 number of chromosomes. Its genome size is 579 Mb/1C (Sabatina *et al.*, 2021) [13]. This crop is of Indian origin and has two wild species and only one cultivated species. It is well suited to dry areas, mainly under irrigated conditions. This crop gives good response when subjected to numerous cropping systems contributing in protein an important element of human diet. During 2019-20, the total coverage under mungbean has been about 40.20 million ha with the production of 1.42 million tons (Anonymous, 2019-20) [1]. The protein available in this crop is palatable, simple to cook and absent in flatulence factors contrary to other legumes. Mungbean seeds contain protein (22.88to 24.65%), carbohydrate (62.6%), crude fibre (4.30 to 4.80%) and lipid (1.53 to 2.63%).

Apart from this, this particular crop adds a good range of micronutrients to the Indian vegan diet (Ramakrishnan *et al.*, 2013) [11]. Like other pulses, the protein of greengram has abundant lysine, which is an essential amino acid that is absent in cereals (Saleem *et al.,* 1998) [12]. PPV & FRA, 2001 was enacted by the India Government to ensure the protection of plant varieties which provides legal protection to the newly developed varieties, breeder's, researcher's, and farmer's right to bring motivation in the developmental growth of seed industry and provide the new varieties with economic importance which are used for breeding programme. PPV & FRA implements DUS testing that was initiated in 2007 with twelve crops. The criteria of novelty, distinctiveness, uniformity and stability is essential for the registration of new variety. A DUS test is generally taken up for two successive planting seasons either in field or glasshouse condition. Evaluation period primarily comprises characterization of various morphological traits of the concerned crop on the basis of the internationally agreed protocols (Rashtriyakrishi, 2015). Morphological characterization is considered as an important phenomenon for classification, visual identification and differentiation of the germplasm. Characterization is considerably beneficial to plant breeder in utilizing these germplasms in area of research programmes. Morphological markers are simple, inexpensive rapid to score and are highly heritable traits which are employed for the study of morphological characterization (Kumawat *et al.,* 2020), Germplasm characterization aids in grouping specific traits to obtain idea about traits that distinguish one genotype from the other (Lee *et al*., 2004) [7]. Piyada *et al*., (2010) [9] also emphasized on morphological characterization to check the variability and differentiate the crop germplasm. Some of the agro-morphological traits may be used as morphological breeding traits in crop improvement programme. These diverse traits will be utilized in mungbean breeding programme for improving the seed physical quality. It also helps in assessment of genetic variability and diversity present in available mungbean germplasm. (Singh *et al*., 2008) [4].

Materials and Methods

The experimental material comprised 39 diverse genotypes of mungbean received from the project entitled "Field Evaluation of Trombay Mutant Selections and Research Activities in Agriculture" and also from the pulse improvement programme, Department of Plant Breeding and Genetics, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India. The experiment was conducted in a randomized block design with three replications during *rabi* 2021. The genotypes were sown in six rows of with 30 X 10 cm spacing under RCBD design with three replications. The seed sowing was made in the month of November 2021. The observations were recorded at specific stages of crop growth period as per DUS descriptor. The anthocyanin pigmentation was noted at cotyledonary stage. Stem colour, stem pubescence, leaf shape, leaf colour, leaf colour and leaf vein colour were observed at 50 percent flowering stage. The petiole colour, colour of pre mature pod, pod pubescence and pod position were observed at the stage of fully developed green pods.

Results and Discussions

The primary step in describing and classifying the material under investigation is morphological characterization. Characteristics are used to evaluate the requirements for distinctness, homogeneity, and stability. Understanding the morphological features aids for the identification, selection, and transfer of favourable genes as well as the design of new populations is primary requisite of the genetic improvement programme. The use of standard descriptors to describe the features of a crop species is useful for better germplasm utilisation and conservation (Diederichsen and Richards, 2003). A study was conducted to classify 39 mungbean genotypes using mungbean characteristics. Because of the wide variation in qualitative features, an attempt was made to classify the mungbean genotypes and identify those using descriptors (Table 1). The 39 genotypes may well be distinguished from each other based on morphological differences.

Anthocyanin pigmentation: Thirty-five (35) genotypes showed anthocyanin pigmentation and in remaining four genotypes were absent for anthocyanin pigmentation.

Colour of stem: Colour of stem is one of the most important diagnostic and easily observable traits. It is widely used as a marker gene in genetic studies and breeding work. One genotype of mungbean showed green colour stem, while remaining 38 genotypes showed purple stem colour. Stem pubescence, pubescence on stem; two genotypes showing pubescence on stem and the remaining 37 genotypes absence of pubescence on stem. Plant growth habit is a distinguishing feature in plant characterization. indicating similarity in all of the genotypes for this trait (Jain *et al*., 2002) [3]; (Katiyar *et al*., 2008) [4]; (Singh *et al*., 2008); (Kaur *et al*., 2017) [4, 5] in mungbean.

Shape of leaf: Shape of leaf is one of the most important diagnostic and easily observable traits. It is widely used as a marker gene in genetic studies and breeding work. Two genotype of mungbean showed deltoid leaf shape stem, while remaining 37 genotypes showed ovate leaf shape. Similar findings (Kaur *et al*., 2017 in mungbean) [5]; Chakrabarthy and Agarwal (1989) [2] in blackgram.

Leaf colour: Leaf colour is one of the most important diagnostic and easily observable traits. It is widely used as a marker gene in genetic studies and breeding work. Five genotype of mungbean showed green colour leaves, while remaining 34 genotypes showed dark green colour of leaves. Similar findings (Kaur *et al*., 2017 in mungbean) [5]; Chakrabarthy and Agarwal (1989) [2] in blackgram.

Leaf vein colour: Colour of leaf vein is one of the most important diagnostic and easily observable traits. It is widely used as a marker gene in genetic studies and breeding work. Thirty 37 genotypes of mungbean showed green colour leaf vein, while remaining two genotypes were greenish purple leaf vein. Similar findings (Kaur *et al*., 2017 in mungbean); [5] Chakrabarthy and Agarwal (1989) [2] in blackgram.

Petiole colour: Petiole colour is one of the most important diagnostic and easily observable traits. It is widely used as a marker gene in genetic studies and breeding work. Thirty-eight genotypes mungbean showed green colour of petioles, while remaining one genotype was green with purple splashes in petioles.

Pod colour: Pod colour of premature pod is one of the most important diagnostic and easily observable traits during the pod development. It is widely used as a marker gene in genetic studies and breeding work. Out of total, thirty-eight genotypes of mungbean showed green colour of premature pod, while remaining one genotype was green with pigmented nature of premature pod. Similar observations recorded by the (Pratap *et al*., 2013)

Pod pubescence: Pod pubescence is one of the most important diagnostic and easily observable traits during the time of pod development. Thirty-eight genotypes showed pubescence on the pod and the remaining one genotype was free of pubescence on pod.

Pod position: Thirty-eight genotypes showing pod position above the plant canopy and the remaining one genotype pod position was intermediate.

In the present investigation, anthocyanin colouration, plant habit, stem pubescence, pre mature pod colour and leaflet lobes were same in all the lines and were not useful for discrimination but rest of the traits had lots of variability which can be exploited for the elite lines identification and utilization as reported by (Patel et al., 2019) and facilitate the easy registration with these distich characters present in the genotypes with PPV&FRA.

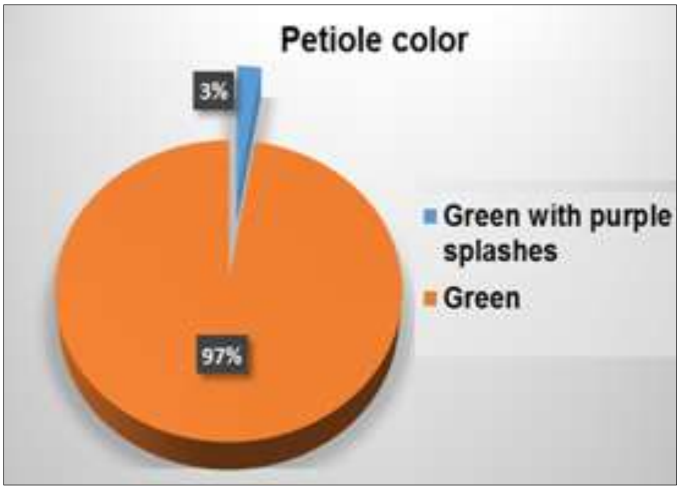

Fig 1: Petiole: Colour

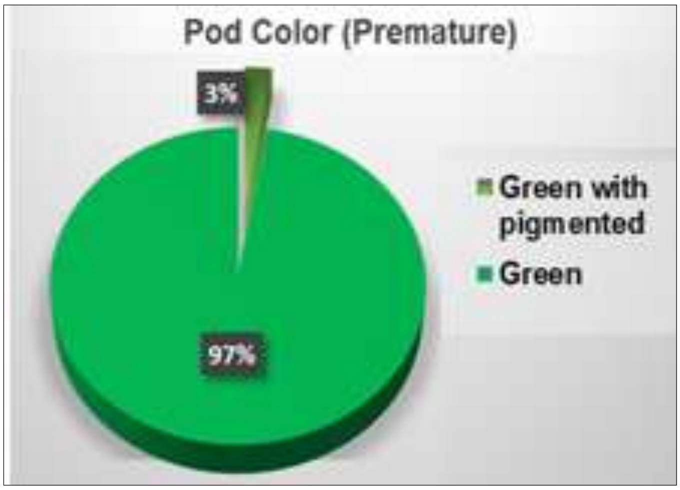

Fig 2: Pod: Colour of premature pod

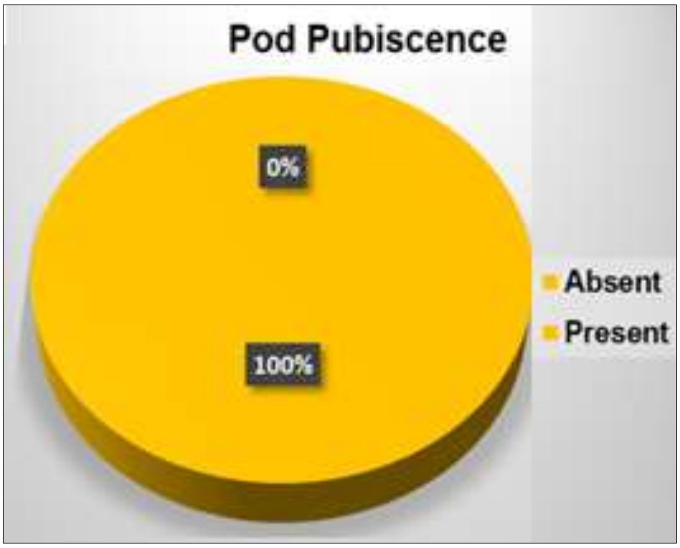

Fig 3: Pod: Pubiscence

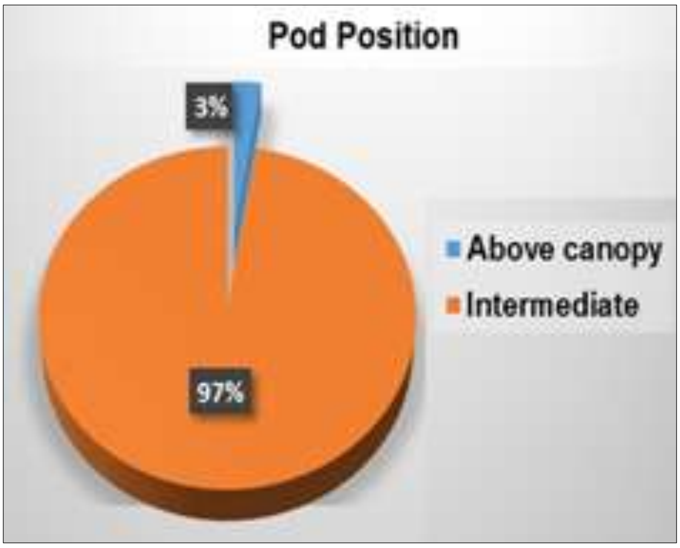

Fig 4: Pod: Position

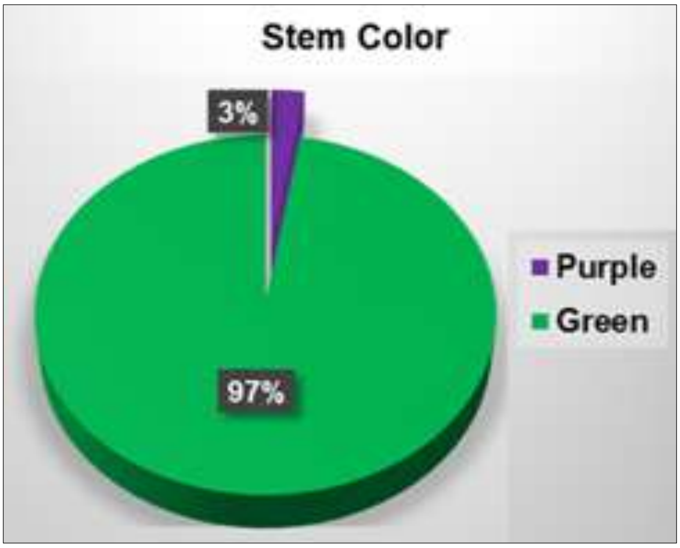

Fig 5: Stem: Colour

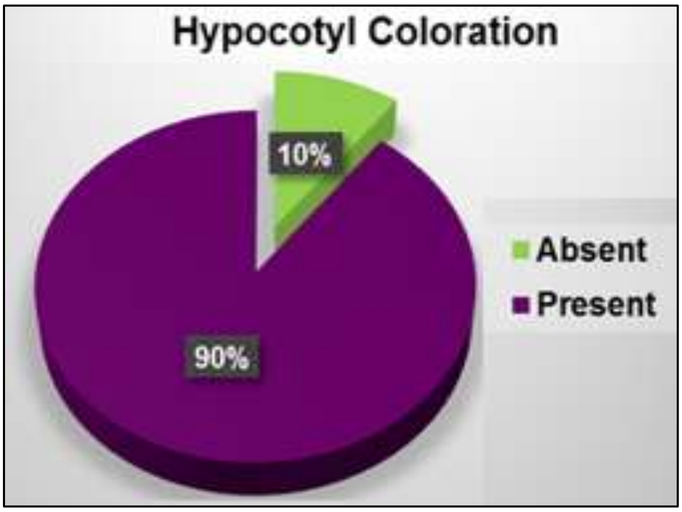

Fig 6: Hypocotyl Coloration

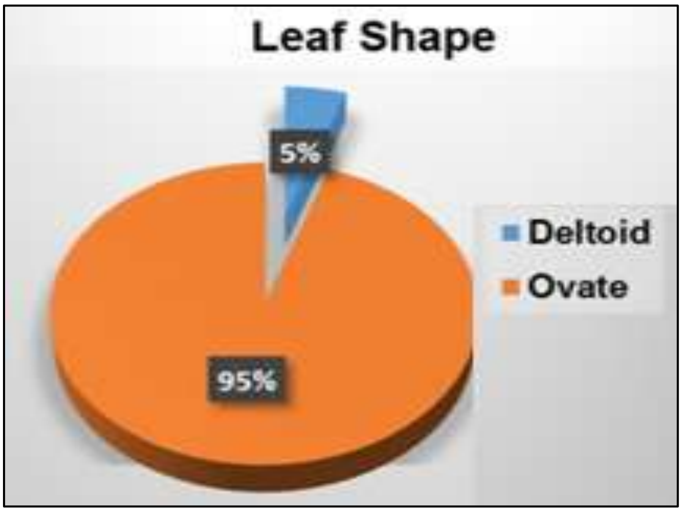

Fig 8: Leaf: Shape (terminal)

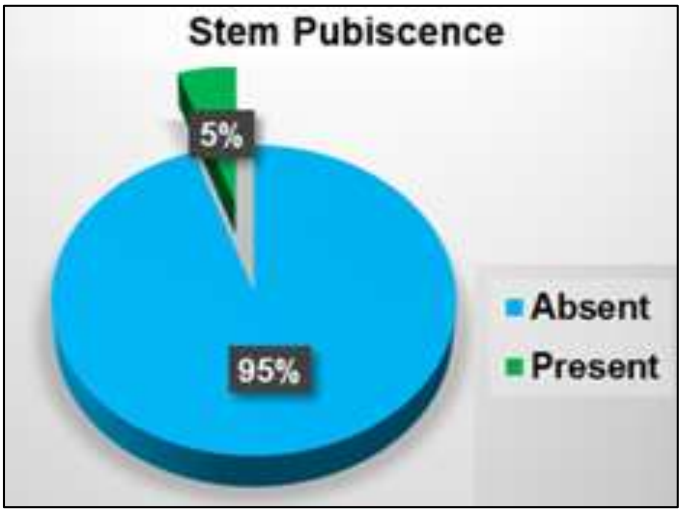

Fig 7: Stem: Pubiscence

Fig 9: Leaf: Vein colour

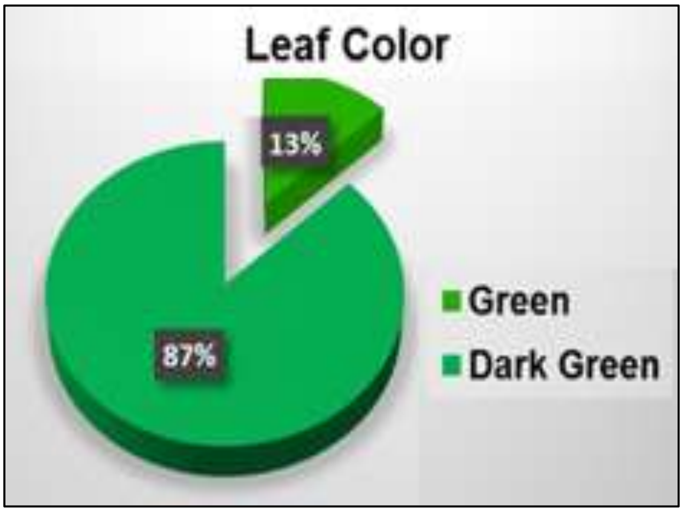

Fig 10: Leaf: Colour

Table 1: Morphological characterization as per DUS guideline

S. No.	Characteristics	Stage of observation	States	Score	Genotype Frequency	Percentage Contribution	Name of Genotypes
1	Stem: Anthocyanin coloration	Cotyledons unfolded	Absent	1	4	10%	Shikha, Virat, Hum-1 and PM-1623
			Present	9	35	90%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, PKVAM-4, TJM-196, TJM-137, GAM-5, TM-96-15, TJM-155 and LGG-460
2	Stem: Colour	50 % flowering	Green	1	1	3%	PKVAM-4
			Purple	3	38	97%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
3	Stem: Pubescence	50 % flowering	Absent	1	37	95%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, TM-96-15, PKVAM-4 Shikha, Virat, Hum-1 and PM-1623 and LGG-460
			Present	9	2	5%	TJM-143 and TJM-155
4	Leaf: Shape (terminal)	50 % flowering	Deltoid	3	2	5%	MH-421 and TJM-137
			Ovate	5	37	95%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, TM-96-15, TJM-143 and TJM-155, PKVAM-4, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
5	Leaf: Colour	50% flowering	Green	1	5	13%	PDM-111, Yellow mung, MH-421, PDM-139 and PUSA-13-51
			Dark green	2	34	87%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, TJM-37, TJM-124, TJM-231, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, ML-1299, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, TM-96-15, PKVAM-4, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
6	Leaf: Vein colour	50 % flowering	Green	1	37	95%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, PKVAM-4, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
			Greenish purple	2	2	5%	Pusa-Vishal and GAM-5
7	Petiole: Colour	50% flowering	Green	1	38	97%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, Pusa-Vishal, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, PKVAM-4, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
			Green with purple splashes	2	1	3%	GAM-5
8	Pod: Colour of premature pod	Fully developed green pods	Green	1	38	97%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, Pusa-Vishal, TJM-37, TJM-124, TJM-231, GAM-5, TJM-115, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, PKVAM-4, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
			Green with pigmented suture	2	1	3%	Yellow mung
9	Pod: Pubescence	Fully developed green pods	Absent	1	1	3%	TJM-235
			Present	9	38	97%	TJM-140, Ganga-8, Pusa-Vishal, PDM-11, Pusa-Vishal, TJM-37, TJM-124, TJM-231, GAM-5, TJM-115, TJM-111, GAM-5, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, PKVAM-4, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460
10	Pod: Position	Fully developed green pods	Above canopy	1	1	3%	Pusa-Vishal
			Intermediate	2	38	97%	TJM-235, TJM-140, Ganga-8, Pusa-Vishal, PDM-11, Pusa-Vishal, TJM-37, TJM-124, TJM-231, Yellow mung, TJM-115, GAM-5, TJM-111, TJM-134, TJM-236, TMB-37, Kanika, MH-421, ML-1299, PDM-139, PUSA-13-51, TJM-145, TJM-136, TJM-143, TJM-160, TJM-141, IPM-430-1, SL-668, MH-903, TJM-3, TJM-196, TJM-137, GAM-5, PKVAM-4, TM-96-15, TJM-155, Shikha, Virat, Hum-1 and PM-1623 and LGG-460

Conclusion

In morphological characterization it was recorded that the traits *viz*., stem colour, anthocyanin pigmentation on stem, stem pubescence, shape of leaf, Leaf colour, premature pod colour and pubescence on pod found to be most promising traits to differentiate the genotypes on morphological basis. These traits must be utilized for the development of Mungbean varieties contributing specific varietal traits. The study highlighted the importance of introducing new material in the breeding programmes to broaden the genetic base of the crop. Thus, characterization of elite improved lines holds an important significance in the identification of lines and their registration with PPV&FRA and maintenance of line having the information of genetic base. Lines found with unique traits and present only in few of the genotypes will be of great importance for the development of morphologically diverse breeding populations. These lines with unique morphological identity will be considered as a varietal marker in the seed production chain to maintain the genetic purity of the variety. These traits may also be useful when varieties may mix and pure-lines can easily be isolated very easily by normal selection method.

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