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Kitomar, Doring

Research Suare

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Doring J. Kitomari
doringjulius@gmail.com

The Nelson Mandela African Institution of Science and Technology
Pavithravani B. Venkataramana
The Nelson Mandela African Institution of Science and Technology
Angela G. Mkindi
The Nelson Mandela African Institution of Science and Technology
Steven R. Belmain
University of Greenwich
Philip C. Stevenson
Royal Botanic Garden

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Abstract

Background: Exploring genetic variation and screening for disease resistance is an important step in crop breeding initiatives but is lacking for many bean varieties including mung bean. The present study evaluated the diversity of 42 morpho-agronomic traits and screened mung bean genotypes for resistance to powdery mildew disease. A total of 132 mung bean and rice bean (R200) genotypes (as checks) were evaluated in an augmented incomplete block design across two cropping seasons. Pivot tables were used to analyse qualitative data, whereas the variation of 13 quantitative traits was examined using the generalized linear model (PROC GLM), agglomerative hierarchical clustering (AHC), and principal component analysis (PCA).

Result: The genotypes displayed a wide variation for the majority of traits evaluated and significant differences were observed among genotypes, block effect, and between seasons. Similarly, the effects due to checks, genotypes, and genotypes and controls were significant. One mung bean (G32) genotype and one rice bean (R200) exhibited resistance to powdery mildew under field conditions. Principal component analysis revealed that the first four PCs explained 59.77% of the total variation among the genotypes studied. In addition, cluster analysis grouped all the genotypes into four major clusters.

Conclusion: The trait variation recorded and resistance to powdery mildew disease provide valuable insight for developing breeding strategies especially with respect to reducing losses in mung bean and rice bean to powdery mildew.

Introduction

Mung bean [*Vigna radiata* (L.) Wilczek] also known as green gram is an annual pulse crop that is cultivated widely in tropical and subtropical regions [1]. The crop is of importance in economics, food, fodder and in improving soil organic matter [2]. It is rich in nutrients such as digestible protein,[3] iron [4], and Zinc [5, 6]. The main producers of this crop are India, Myanmar, China, Indonesia, Thailand, Kenya, and Tanzania [7]. The average global yield of mung bean is 0.73 tons/ha, [8] whereas in Tanzania the yield is much lower at 0.4 tons/ha [9], indicating a yield gap for this crop. Tanzania's area for mung bean production is 91,063 ha, primarily by smallholder farmers, with just 53ha cultivated by large-scale farmers under rainfed conditions [9] .

Mung bean is affected by several biotic factors such as powdery mildew, yellow mosaic virus, Cercospora leaf spot (CLS), anthracnose, pod borer, bruchid, aphids, thrips and whitefly[10–12]. Additionally, abiotic stress including drought, salinity, high temperature, and waterlogging [13, 14] cause reduced yields [11]. Among biotic stresses powdery mildew (PM) caused by two fungal pathogens *Erysiphe polygoni* DC[15] and *Podosphaera xanthi* [16], are the most destructive diseases in mung bean. It is estimated that a yield loss at the seedling stage can be 100%[17] and 40–60% at both the vegetative and reproductive stages [16, 18]. Generally, employing host plant resistance stands out as a preferred control method due to its environmental, economic and social advantage. s [11]. Therefore, the identification of PM resistance in mung bean genotypes would provide an important trait for breeding programmes.

For effective control using resistant cultivars in mung bean, genetic diversity is key to developing new varieties with improved desirable traits [19]. These include both farmer-preferred traits and breeder-preferred traits [20]. Several studies have reported that genetic diversity in mung bean is limited, presenting a significant challenge for breeders [21, 22]. Thus, the characterisation of trait diversity in mung bean genotypes provides a key step in improvement for traits including disease resistance [23]. Addressing grower's needs through the deployment of resistant cultivars can meet the growing global demand for food [24] and requires an evaluation of all trait diversity in mung bean collections [24, 25].

Rice bean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] is a neglected crop with limited production [26]. It is regarded as a minor food and fodder crop and is often grown as intercrop or mixed crop with maize, sorghum or cowpea [26]. Like the other Asiatic *Vigna* species, rice bean is a fairly short-lived warm-season annual. Grown mainly as a dried pulse, it is also important as a fodder, a green manure and a vegetable [26]. In the past it was widely grown as lowland crop on residual soil water after the harvest of long-season rice, highlighting the crop is drought tolerant. The main limitation to rice bean production is its tendency to shatter, making it harvest problematic [27]. There is little information in the published literature on rice bean which was used as check regarding agro-morphological traits and genotypes which are resistant to powdery mildew. This gap underscores the importance of our study in addressing this knowledge gap and enlighten on importance aspects of rice bean cultivation. In this study we evaluated the trait variation and identified sources of powdery mildew resistance. This information is valuable for breeders in pre-breeding and breeding programs aimed at developing new varieties.

Materials and methods

Study site description

Field experiments were conducted in two growing seasons (March - June 2021) and (January-April 2022), at Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania. NM-AIST-Arusha lies at a latitude of 3° 24' 3.1284" S and longitude of 36° 47' 41.1576" E, and an elevation of 1188 m.a.s.l. Figure 1.

Plant materials

The study material consisted of 132 genotypes (Table S1) collected from the Australian Grains Gene Bank, National Bureau of Plant Genetic Resources (NBPGR), and Tari-Selian, Arusha. Two mung bean genotypes Mum-2 and Sweta and one rice bean (R200) [28] was used as a check.

Field experiment

The experimental plot was first cleared, tilled, and then harrowed until a precise tilth was attained using a hand hoe. Thereafter, the experiment was laid out using an augmented incomplete block design that was developed by a statistical tool on the website of the Indian Agricultural Statistical Research

Institute (IASRI) [29]. The three check and the experimental materials (132) were assigned in the ten blocks, with each block having 16 genotypes including the checks which were replicated once. Each genotype was planted in 4 rows of 1 m long with 45cm inter-row spacing and 10 cm intra-row spacing. Rainfall was the main source of water to the plants with supplemental irrigation supplied from borehole tap water when the soil was found dry. PM infection and development depended on natural inoculum in the vicinity, mung bean was grown without spraying of fungicides for PM disease screening. The repeat experiment during the summer season followed similar procedures to those used in the initial experiments during the long rain season.

Data collection of morpho-agronomic traits and powdery mildew evaluation

The descriptor of mung bean developed by the International Board for Plant Genetic Resources

[30] was followed for data collection Table S2. A total of 29 qualitative traits recorded from each genotype includes recorded hypocotyl colour, seedling vigour, pod colour at immature stage, colour of ventral suture of immature pod, pod colour at mature stage, shape of the ripe pod, attachment of mature pod peduncle, pod pubescence, constriction of pod between the seeds, length of the peduncle, raceme position, calyx colour, corolla colour, leafiness, terminal leaflet shape, terminal leaflet length, leaf colour, petiole colour, petiole length, leaf senescence, growth habit, lodging, shattering in the field, growth pattern, seed colour, mottling on seed, lustre on seed surface, seed shape and hilum of seed. Quantitative data on 13 traits including length of the branch, plant height, number of branches per plant, number of clusters, number of pods per plant, number of seed per pods, pod weight, seed weight, weight 1000 seeds, pod length, days to flowering, days to maturity and shelling percent were recorded as described by IBPGR[30]

Powdery mildew screening

After PM disease appearance, identification of PM disease-causing pathogen was done by assessing the spore micro-morphology using a florescent microscope at a magnification of 40x as described by Braun [31]. Data on PM incidence was recorded by counting the diseased plants and % incidence was calculated according to the formula described by Mulbrhan [32]. Disease severity of powdery mildew disease was then collected according to a scale of 0 to 5 as described by Gawande [33] Table S3. All the data on disease severity were collected from ten randomly selected plants per genotype.

Statistical data analysis

For continuous traits, due to the unusual performance of 23 genotypes, the data were analysed from 109 genotypes. The collected qualitative traits were subjected to statistical analysis for frequency and mean distribution using a pivot table in Microsoft Excel (MS Excel) [34], to evaluate the genotype's qualitative traits distribution. The data recorded for 13 quantitative traits were first analysed separately for each season to obtain a mean square error that was used to obtain F-probability. After this a combined analysis of variance was performed using the general linear model (PROC GLM) of the RStudio version 4.2.3 [35], to evaluate the effect of season on trait performance. To examine the differences in genotype performance between the two seasons, restricted maximum likelihood was performed as described by Lubadde [36].To understand the trait variation among genotypes, quantitative data were used to calculate genetic parameters such as phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), and genetic advance as per cent of the mean (GAM) as described by Yimram [37]. Broad sense heritability (H^2) for quantitative traits was estimated as per the method described by Fehr [38].The quantitative traits were also analysed using descriptive statistics, mean, principal component analysis (PCA), multivariate biplot, and agglomerative hierarchical clustering (AHC) with the help of XLSTAT software [39] to ascertain differences in quantitative traits performance. Data on powdery mildew disease were used to calculate the per cent disease index (PDI) to determine the level of disease in the genotypes as described by Mulbrhan [32] Eq. 1.

$$Plantdiseaseindex\,(PDI) = \frac{\text{Numberofindividualratings}}{\text{Numberofplantsassessed}} \times \frac{100}{\text{Maximumscale}} \ldots\ldots (\text{Eq. 1})$$

Results

Variation in qualitative traits among the genotypes at the germination stage

Figure 2 presents the frequency distributions of variations in qualitative traits examined during the germination stage. Hypocotyl colour at 10 days of germination was greenish purple (59.63%) followed by green (23.85%) and purple16.51%. For seedling vigour, medium was the most frequently observed phenotype class (69.72%), followed by vigorous 25.69% and poor 4.59%.

Variation in the inflorescence traits

During this stage, mung bean genotypes under study were observed and documented for the following qualitative traits: Length of the peduncle, raceme position, calyx colour, corolla colour, leafiness (Fig. 3). Length of the peduncle was long with the mean frequency of (42.20%) while short peduncle (22.94%) was the least observed trait on the studied mung bean genotypes. For raceme position, intermediate raceme position was observed in 77.98%, followed by above the canopy type (16.51%) and pods not visible (5.50%). Most genotypes (77.98%) showed the greenish-purple colour of calyx on the flower at the flowering stage. The corolla colour was greenish yellow, light yellow, and deep yellow, and the maximum genotypes 64.22% had greenish yellow. Leafiness during the flowering period for the majority of genotypes was sparse (55.05%), while other genotypes were medium (26.61%) and abundant (18.35%).

Variation of vegetative traits

In the vegetative stage, traits such as terminal leaflet shape, terminal leaf length, leaf colour, petiole colour, petiole length, leaf senescence and growth habit were recorded (Fig. 4). Terminal leaflet shape for most of the genotypes had cuneate shape (93.02%) and ovate (6.98%). For terminal leaflet length, large was observed in 66.67%, followed by medium (29.46%) and small (3.88%). Leaf colour was dominated by green (72.09%) followed by dark green (27.91%) among studied genotypes. For petiole colour, greenish purple was the most frequently observed phenotype class (74.42%), followed by green 20.16% and purple 5.43%. The medium (58.91%) and short (34.88%) petiole length were prominent whereas 6.20% were long among studied genotypes. Variation was also noticed for leaf senescence, intermediate (65.89%) and not visibly senescent (30.23%) were prominent, while few (3.88%) were conspicuously concurrent. Erect growth type was predominant (51.16%) over semierect (41.86%) and spreading (6.98%).

Variation in the pod traits

At this stage, all the genotypes showed variations in pod traits such as pod colour, colour of ventral suture of immature pod, shape of the ripe pod, attachment of mature pod peduncle, pod pubescence, and constriction of pod between seeds (Fig. 5). Light green immature pod (63.30%) was the most frequent trait, followed by deep green (36.70%). There was variation in the colour of ventral suture of immature pod with a maximum purple colour (77.06%). The genotypes produced pods of several colours at the maturity stage, including straw, tan, and brown, and there was a greater frequency of those with black pods (51.38%). Attachment of mature pod peduncle to the angle of around 90° was observed at pod filling in 72.48% of genotypes, whereas few (3.67%) genotypes showed pendant type of attachment. The heavily (66.03%) and intermediate pubescence (32.11%) were prominent, whereas few (1.83%) were glabrous. Around 61.47% of the genotypes showed pod constriction at the pod-filling stage, which is the critical attribute of the legume crop.

Variation of maturity traits

Lodging, shattering in the field and growth patterns were the qualitative traits monitored at this growth stage. Figure 6 shows the frequency distribution of genotypes for each trait variation. Lodging of 109 genotypes was intermediate, none and heavy. Intermediate (42.64%) and none lodging (30.23%) were more frequent in most genotypes. 1% of the genotypes under study exhibited field shattering, whereas the remaining (99%) percent showed no shattering ability. More than 78.29% of genotypes had determinate growth pattern whereas 21.71% were indeterminate.

Variation of seed traits

Freshly harvested seeds had wide range of colours such as yellow, greenish yellow, light green, dark green and mixed in Fig. 7. However, light green (50.46%) and dark green (33.03%) were predominant colour. The phenotypic class mottling on the seed surface was not observed on 61.47% of mung bean genotypes, while light and medium phenotypic classes were observed on around 37.78% and 2.75% of mung bean genotypes under study. Genotypes with dull lustre on seed surface were more frequent (94.50%) compared with shiny (5.50%) lustre. Variation was also noticed for seed shape, drum and oval shaped seeds were more prominent, while 18.35% were round seed shape. Genotypes with non-concave hilum were relatively more frequent (83.49%) than those with concave (16.51%).

Powdery mildew disease incidence and severity

A total of 108 mung bean genotypes including three checks were screened for powdery mildew resistance. The susceptibility of the genotypes to powdery mildew differed, 78 genotypes were categorized as moderately susceptible, 17 were susceptible, 13 moderately resistant, one highly susceptible, and two were highly resistant to the powdery mildew in Fig. 8.

Descriptive analysis and genetic parameters of quantitative traits of mung bean genotypes

The evaluation encompassed 13 quantitative traits in mung bean genotypes. Descriptive statistics unveiled considerable variations in mung bean genotypes (Table 1). Plant height was a highly variable trait ranging from 23.46 to 94.94 cm for G16 and C3 genotypes, respectively, with a mean of 43.02 cm. The number of branches per plant exhibited the least variability, ranging from 0.73 (G9) to 5.55 (C3), with an average of 2.36 branches. Days to flowering ranged from 33 (G68) to 42 (G32) days, with an average of 38 days. Some of the genotypes matured after 62 days (minimum days to maturity by G9), while some genotypes were found to be maturing late (74 days by G32). The weight of 1000 seeds ranged from 25.23 to 82.21 g for G33 and G3 genotypes, respectively, with an average of 45.12 g. Pod length also varied from 4.21 (G28) to 9.11 (G62) cm, with an average of 6.1 cm. A considerable variation was observed for the length of the branch, number of clusters, pods weight, number of seeds per pod, seed weight and shelling percent (Table 1).

The statistical parameters related to breeding values were investigated and the outcomes are detailed in Table 1. Genetic variability studies were computed using the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense (H^2) and genetic advance as percentage of mean (GAM) were estimated for 13 traits. The PCV exceeded the GCV for all the traits with minor differences in the case of days to maturity, pod length, weight of 1000 seeds and days to flowering. All traits had high PCV (> 20%) except for length of the branch, pod length, and number of seed per pods where PCV was intermediate (10–20%). Low PCV (0–10%) was also observed for days to 50% flowering and days to maturity. The estimation of GCV

in relation to PCV potentially help in determining the degree of genetic variation. From the study, the estimate for GCV ranged from 3% for days to maturity to 52.05% number of pods per plant. The value for PCV ranged from 3.05% for days to maturity to 57.95% seed weight.

All the studied traits reflected high heritability while lowest value of heritability (82.6%) was observed for a number of clusters and the highest value of heritability value (97.35%) for weight of 1000 seeds (Table 1). In terms of genetic advance as a percentage of the mean (GAM), most of the traits were observed with high GAM (> 20) except for days to 50% flowering and Days to maturity which showed intermediate (12.81%) and low (6.07%), respectively in Table 1.

Taking into account H^2 estimates with GAM is essential for discerning gene action, as breeders rely on both values for trait improvement. High H^2 associated with GAM was evident for in all traits except days to maturity and days to flowering, which displayed low and medium GAM, respectively.

Table 1
Descriptive analysis, genetic variability and heritability values of quantitative traits of mung bean genotypes across two cropping seasons in Tanzania

Trait	Mean ± SE	Range		GCV	PCV	H^2	GAM
		Min	Max				
Length of the branch (cm)	28.72 ± 0.46	14.43(G25)	39.63(G9)	14.35	16.31	77.89	26.41
Number of branches per plant	02.36 ± 0.11	0.73(G9)	05.55(C3)	37.11	41.69	79.93	68.64
Number of clusters	10.13 ± 0.41	06.90(G83)	76.57(C3)	34.06	37.50	82.60	64.07
Number of pods per plant	30.08 ± 1.49	03.13(G99)	23.85(G73)	52.05	53.31	95.27	104.83
Pod length (cm)	06.10 ± 0.09	04.21(G28)	9.11(G62)	15.21	15.54	95.78	30.70
Plant height (cm)	43.02 ± 1.20	23.46(G16)	94.94(C3)	25.31	26.99	88.18	48.97
Pod weight (g)	18.94 ± 1.02	03.29(G103)	46.80(C3)	50.61	57.11	79.37	93.60
Number of seeds per pod	09.62 ± 0.15	04.99(G73)	12.63(G92)	15.00	16.40	83.50	28.37
Seed weight (g)	11.35 ± 0.66	01.43(G50)	36.73(C3)	47.77	57.95	68.80	82.24
Weight of 1000 seeds (g)	45.12 ± 1.14	25.23(G33)	82.21(G3)	25.44	25.78	97.35	51.80
Days to 50% flowering	38.00 ± 0.25	33.03(G68)	42.20(G32)	06.55	06.93	88.39	12.81
Days to maturity	65.53 ± 0.19	62.00(G9)	74.00(G32)	03.00	03.05	96.46	06.07
Shelling percent	61.99 ± 1.51	31.25(G118)	93.11(G59)	22.22	24.21	83.49	42.12

GCV; Genotypic Coefficient of Variation. PCV; Phenotypic coefficient of variation: GAM Genetic advance over percent mean H^2: Heritability in a broad sense

Analysis of variance of the quantitative traits across the two cropping seasons

The combined analysis of variance of the quantitative traits revealed significant differences ($p < 0.001$) in all the traits (Table 2). All the traits, except for the number of branches per plant, number of pods per plant and weight of 1000 seed, showed substantial cropping season effects. Additionally, length of the branch and the number of branches per plant were significantly impacted by block effects. Significant differences among the genotypes were observed as shown in Table 2 for all traits. Exceptionally, the number of clusters and days to flowering showed no significant difference among the genotypes ($p > 0.05$). It was figured out that there are significant differences ($p < 0.001$) between checks for all the analysed traits (Table 3), with the exception of number of seeds per pod and shelling percent. The selected characteristics also significantly differed between checks and genotypes, except for number of seed per pods, day to flowering and days to maturity.

Table 2
Combined analysis of variance and sum of squares for the selected quantitative traits across two cropping seasons

Traits	Model (ANOVA)					Block effect					Seasonal effect				
	DF	sum squares	mean squares	F	Pr > F	DF	sum squares	mean squares	F	Pr(> F)	DF	sum squares	mean squares	F	*p*
Length of the branch (cm)	110	4735.6	43.05	16.48	< 0.001	9	73.7	8.19	3.58	< 0.001	1	39.9	39.9	15.29	< 0.001
Number of branches per plant	110	452.75	4.12	36.75	< 0.001	9	2.07	0.23	2.18	< 0.001	1	0.13	0.13	1.16	0.28
Number of clusters	110	5516	50.15	3.32	< 0.001	9	31.3	3.47	0.22	0.99	1	1433.5	1433.5	94.93	< 0.001
Number of pods per plant	110	42878	389.8	3.07	< 0.001	9	71	7.89	0.06	1	1	14.53	14.53	0.11	0.74
Pod length (cm)	110	191.43	1.74	20.15	< 0.001	9	0.521	0.06	0.66	0.75	1	4.14	4.14	47.96	< 0.001
Plant height (cm)	110	71673	651.57	7.3	< 0.001	9	153	17	0.18	1	1	1651.6	1651.6	18.51	< 0.001
Pod weight (g)	110	314545	285.95	4.2	< 0.001	9	337.7	37.52	0.54	0.85	1	1256.6	1256.6	18.43	< 0.001
Number of seeds per pod	110	387.6	3.52	2.91	< 0.001	9	2.54	0.28	0.22	0.99	1	110.2	110.2	91.11	< 0.001
Seed weight (g)	110	183964	167.24	4.59	< 0.001	9	194.2	21.58	0.58	0.81	1	138.03	138.03	3.79	0.05
Weight of 1000 seeds (g)	110	51820	471.09	11.83	< 0.001	9	78	8.69	0.21	0.99	1	131.1	131.1	3.29	0.07
Days to flowering	110	1167.74	10.62	2.81	< 0.001	9	7.93	0.88	0.22	0.99	1	800.36	800.36	211.9	< 0.001
Days to maturity	110	956.6	8.7	12.42	< 0.001	9	2	0.22	0.31	0.97	1	378	378	540	< 0.001
Shelling percent	110	53375	485.23	2.85	< 0.001	9	235	26.15	0.15	1	1	2870.1	2870.1	16.87	< 0.001
Significantly different among genotypes at $p < 0.001$															

Table 3

Combining analysis of the interactions and differences between genotypes and checks for the selected quantitative traits across two cropping seasons

Contrast (Differences)												
Traits	Among Genotypes (108)				Among Checks (2)				Checks vs Genotypes (1)			
	sum squares	mean squares	F	Pr > F	sum squares	mean squares	F	Pr(> F)	sum squares	mean squares	F	*p*
Length of branch (cm)	4508	41.71	16.49	< 0.001	20	10.22	4.04	0.0195	54	54.4	21.5	< 0.001
Number of branches per plant	202.9	1.88	17.83	< 0.001	242.2	121.12	1149.75	< 0.001	7.5	7.51	71.32	< 0.001
Number of clusters	2301	21.3	0.857	0.8	3030	1515	60.94	< 0.001	660	660	26.55	< 0.001
Number of pods per plant	34849	322.7	2.422	< 0.001	3834	1917.2	14.39	< 0.001	736	736	5.52	0.02
Pod length (cm)	161.91	1.499	13.15	< 0.001	8.65	4.323	37.92	< 0.001	8.55	8.55	75.03	< 0.001
Plant height (cm)	2246	208	2.011	< 0.001	47956	23978	231.84	< 0.001	7410	7410	71.65	< 0.001
Pod weight (g)	16962	157	2.026	< 0.001	11775	5888	75.94	< 0.001	3052	3052	39.3	< 0.001
Number of seeds per pod	329.7	3.053	1.556	0.01	11.7	5.84	2.98	0.054	3.2	3.18	1.62	0.21
Seed weight (g)	11259	104.3	2.75	< 0.001	6191	3095.6	81.65	< 0.001	5832	5832	153.8	< 0.001
Weight of 1000 seeds (g)	24836	230	5.45	< 0.001	25983	12992	307.9	< 0.001	2028	2028	48.06	< 0.001
Days to flowering	497.7	4.61	0.509	1	164	82.02	9.06	< 0.001	0.7	0.7	0.08	0.78
Days to maturity	734.7	6.8	2.162	< 0.001	125	62.52	19.87	< 0.001	5.1	5.07	1.61	0.21
Shelling percent	41199	381.5	1.949	< 0.001	752	376.1	1.922	0.15	22572	22572	115.3	< 0.001
Significantly different among genotypes at $p < 0.001$												

Principal component analysis of the quantitative traits of mung bean genotypes

The quantitative traits of the genotypes of mung beans were grouped into four main components using principal component analysis (PCA). The first four components with eigenvalues greater than one (> 1), collectively explained 59.77% of the variation. While eigenvalues (1) were observed for PCs 5–13. The PC1 contributed 27.45% variation, was predominantly associated with yield and its related traits (seed weight, pods weight, pods per plants and number of branches) (Table 4). In contrast, the PC2 was significantly influenced by traits related to seed quality (weight of 1000 seeds) and yield quality (shelling percent), with a negative influence on PC1. The length of the branch highly influenced the PC3 component, while days to maturity positively contributed to PC4.

Principal components, PC1 and PC2 jointly explained 40.75% of the biplot in Fig. 9. The biplot illustrated negative correlations of the days to maturity and days to 50% flowering with yield attributes, including seed weight, pod weight, number of pods per plant and number of clusters. Positive correlations were observed between seed weight, number of pods per plant, and pod weight. Shelling percent and weight of 1000 seeds were positively correlated, and selecting these traits will be influential in determining seed yield.

Table 4
Eigen values, proportion variability and quantitative traits that accounted for the four principal component of mung bean genotypes

Traits	PC1	PC2	PC3	PC4
Length of the branch (cm)	0.197	0.033	0.744	0.199
Number of branches per plant	0.654	0.079	0.040	0.045
Number of clusters	0.515	0.273	0.408	-0.164
Number of pods per plant	0.747	-0.467	-0.122	0.067
Pod length (cm)	0.147	0.495	-0.141	0.397
Plant height (cm)	0.651	0.268	0.364	0.141
Pod weight (g)	0.862	-0.339	-0.163	0.133
Number of seeds per pod	0.388	0.195	-0.128	-0.106
Seed weight (g)	0.901	-0.143	-0.268	-0.011
Weight of 1000 seeds (g)	0.018	0.625	-0.267	0.368
Days to 50% flowering	-0.002	0.319	-0.033	0.465
Days to maturity	-0.215	-0.436	-0.208	0.609
Shelling percent	0.284	0.505	-0.407	-0.431
Eigenvalue	3.569	1.729	1.286	1.188
Variability (%)	27.450	13.298	9.891	9.135
Cumulative (%	27.450	40.748	50.639	59.774

Cluster analysis of selected quantitative traits of mung bean genotypes

In the current study, the dendrogram was clustered using complete linkage, showing the hierarchical clustering of mung bean genotypes. Figure 10 showed numerous clusters portraying associations among genotypes. Cluster analysis categorized genotypes into four major clusters based on their 13 quantitative traits. Cluster III had the highest number of genotypes (42), followed by Cluster IV (28), Cluster II (27) and finally cluster I (14) genotypes.

The mean values of 13 quantitative traits are detailed in Table 5, showcasing differences between clusters for all traits. Genotypes with a higher number of pods per plant, pod weight, number of seeds per pods, seed weight were found in cluster I. While a higher 1000 seed weight was observed in cluster II. Cluster III exhibited maximum mean value for the number of branches per plant, number of clusters and plant height. The highest mean value of the length of the branch was recorded for cluster IV. On the other hand, early flowering and maturity were recorded for cluster I and cluster III, respectively.

Table 5
Estimates of traits means of the mung bean genotypes grouped into clusters using complete linkage method based on quantitative traits

Traits	Clusters				Grand mean
	I (14)	II (27)	III (42)	IV (28)	
Length of the Branch (cm)	26.74	27.32	27.29	33.21	28.64
Number of branches per plant	01.82	02.05	03.00	01.94	02.20
Number of clusters	07.50	08.35	12.35	09.80	09.50
Number of pods per plant	37.53	20.98	35.98	26.26	30.19
Pod length (cm)	05.97	06.30	05.95	05.78	06.00
Plant height (cm)	41.98	38.22	45.75	44.06	42.50
Pod weight (g)	24.38	12.66	22.93	16.31	19.07
Number of seeds per pod	10.16	08.97	09.73	09.80	09.67
Seed weight (g)	15.16	0.8.04	14.07	08.56	11.46
Weight of 1000 seeds (g)	41.80	53.98	41.88	43.08	45.19
Days to 50% flowering	37.03	38.17	37.73	38.72	37.91
Days to maturity	66.71	65.81	64.66	65.96	65.79
Shelling percent	64.21	67.91	63.09	53.49	62.18

Discussion

Qualitative traits

This study reported wide variability in the phenotypic traits of mung bean, and morphological variations as shown in Plate S1. Breeders consider the identification of qualitative traits to differentiate germplasm collections in satisfying farmers' preferences. Most of these traits are genetically regulated, simple to determine, and provide a distinctive description of the given germplasm [40]. The frequency distribution obtained for the observed traits showed the presence of a maximum possible range of variability. Each of the other traits was predominated by a single attribute that appeared more than the others. The current findings are consistent with other scientists who have reported wide variation in mung bean genotypes [25, 37, 41].

It is interesting to note that the leaf pubescence showed polymorphism, with the majority of the genotypes having high pubescence. This suggests that pubescence on the leaf surface is evident to reduce plant water stress in areas of arid environments and help in reducing drying rate of harvested forage [42]. Besides a plants' pubescence also serves as a defence mechanism against insects that feed on the leaf [43].

With respect to seed traits, most of the genotypes exhibited variation such as seed surface colour, seed shape lustre, and mottling of the seed. Given that seed traits were essential determinant of consumers' preference, [44] Gayacharan, found seed traits were heavily influenced by traits such as seed shape, seed surface colour, and lustre on seed surface with shiny green seed coats are generally preferred to those with dull seed coats. Among different seed colours, yellow has been reported to be a preferred colour for oviposition and bruchid development compared to green and black seed colours [12] while [37] Yimram, argue persuasively that bruchid resistance is determined by seed colour. Similarly, [45] Schafleitner, suggest that variability of seed coat colour influences bruchid resistance.

Variations were noted for pod traits such as pod colour, shape of the ripe pod, pod pubescence, pod constriction, pod attachment. Genotypes with black pod colour were relatively more frequent than those with brown colour. It is notable that black-coloured mature pods help prevent seeds inside the pod from discoloration [44]. Our results demonstrated that most of the genotypes were heavily pubescent, which plays a crucial role in plant defence against insect damage [46]. The density and length of trichomes or hairs influences the preference of specific insect pests such as thrips [47, 48]. It is interesting to note, constricted pods were more common than those with non-constricted pods. The results of this study provide support for the view that constricted pods shatter less frequently compare to non-constricted pods [49]. The current findings confirm the view that constricted pods are the crucial traits of mung bean genotypes.

Plant growth habit varied among the genotypes from erect, semi-erect and spreading. Interestingly, the findings suggest that the majority of genotypes exhibited an erect and semi-erect growth habit. The current findings strengthen the view that plant growth habit is related to the cropping system as well as ecological adaptation [50]. More importantly, disease tolerance or high yielding traits may be connected to the gene that determines erect and semi-erect growth habit [25].

Response of mung bean genotypes to powdery mildew

Variation in resistance to powdery mildew was observed in the genotypes used in our study, but only one mung bean genotype and check (R200) were recorded as resistant. Previously, several workers reported that there was variation in resistance among the genotypes of mung bean against powdery mildew[51–53]. Environmental factors such as photoperiod, humidity, and temperature influence development of disease [54]. The genotype (G32) was highly resistant to the disease; however, it had poor yield-related traits and late flowering period so might be a useful genotype for breeding with a better yielding one. These results support previous findings on mung bean which was found resistant not only for powdery mildew but also for mung bean yellow mosaic virus (MYMV) and Cercospora leaf spot but found to have less yield-related traits and late flowering [54]. Additionally, the check, rice bean (C3) (*V. umbellata*) demonstrated resistance to powdery mildew and exhibited favourable yield traits (number of branches per plants, number of clusters, plant height, pods weight, and seed weight) compared to mung bean genotypes. Despite of its susceptibility to pod shattering [55], rice bean is evidently a valuable crop with significant potential for breeding and it can be used as valuable multipurpose crop (green manure, fodder and grain).

Quantitative traits

The success in crop improvement programs is principally dependent on heritable variation available in the breeding materials. Evaluating and understanding genetic variability present in the genotypes is crucial and results in efficient use and selection. The extent of variation, particularly in the yield and yield-related traits, determine which genotypes are more important in breeding programs [20]. Genotype effect for the selected traits was found for all the traits except for number of clusters and days to flowering. This implies that the various genotypes used in the study have diverse phenotypic and possibly genetic traits. One possible explanation for the block effect that was observed during the study was due to the heterogeneity of the soil that affected the checks. The present study confirms previous findings in which soil properties influence physiological and morphological traits of plants [56] .

The estimation of GCV in relation to PCV potentially help in determining the degree of genetic variation. The difference between the two was not significant which suggests that the expression of traits and observed variation primarily resulted from genetic factors while greater differences show the impact of the environment. In general, all traits in the current study showed lower GCV values than PCV values, a crucial consideration while selecting traits of interest. These results support previous findings on mung bean revealing higher estimations for PCV than GCV which showed similar effects of the environment on expression of the traits[57, 58]. From our study, the estimate for GCV ranged from 3% for days to maturity to 52.05% number of pods per plants. The value for PCV ranged from 3.05% for days to maturity to 57.95% seed weight. This result is in agreement with previous findings by Jangra [59] who observed that traits with wide differences in PCV and GCV were more prone to environmental fluctuation than those with narrow differences in traits such as days to maturity and the number of branches per plant. Maximum differences between GCV and PCV values were recorded for seed weight, indicating that the environments had a greater influence on how these traits were expressed. While, the minimum differences between GCV and PCV estimates were noted in days to maturity, indicating that the expression of these traits was influenced by genotypic factors or fixable genes with low environmental influence. This finding was consistent with earlier reports [60–62].

The heritability highlights how quantitative traits are inherited which is crucial when making selections. Higher heritability of the traits combined with high GCV are anticipated to be important for selection decisions based on phenotypic performance[50]. In the present study, high heritability was observed for all the traits. This suggests that these morpho-agronomic traits are regulated by additive gene activity and are appropriate for continual selection throughout mung bean improvement programmes. This is in line with previous studies by[58, 63] which reported higher magnitude of heritability on mung bean genotypes. This suggests that if these traits are utilised as selection criteria in mung bean crop improvement, expected gains from selection will be high [64]. A heritability estimates with GAM taken into account can help to determine the nature of gene action because selection based on the two values is important to breeders for improvement of traits. High heritability which associated with GAM was observed for all the traits with the exception of days to maturity and days to flowering, which had low and medium GAM, respectively. This suggests that phenotypic selection is an efficient method for improving these traits. According to previous studies, similar results were observed for plant height, pod length, number of seeds per pod, number of pods per plant and number of branches per plants [65, 66]. Therefore, the high heritability and genetic advance linked to these traits indicate that they can efficiently be passed down to subsequent generations.

The goal of a plant breeding scheme is to create high-yielding cultivars that are appropriate for the growing environment, breeding techniques like selection can only be used when desired genetic variability is widely available. Analysis of variance is the foremost method to reveal the variability of traits. With regard to quantitative traits analysis of variance revealed high significant mean squares, a sign of high genetic diversity in genotypes. Results from combined analysis variance for two cropping seasons revealed that variability in mung bean genotypes was identified to be significant for all the traits being studied. This result agreed with previous findings of Belay [67] which revealed that analysis of variance of six mung bean genotypes exhibited differences in varietal traits such as, plant height, days to 50% flowering, seed yield, weight of 1000 seeds and number of seeds per pod ($p < 0.05$) with the exception of number of pods per plant. Based on the results in Table 3, length of the branch, number of clusters, pod length, number of seeds per pods, Days to 50% flowering, days to maturity and shelling percent were affected by the cropping season. These effects could be explained by the agroclimatic characteristic of the season. The effect of season on yield and yield related traits has been reported in mung bean [68, 69] which is consistent with finding of several researchers [70] on common bean and [71] on cowpea [72].

During selection, traits that significantly contribute to PCA variation indicates the genotypes collection's variability[71]. Previous study by Zubair, [73] found that the first four PCs with eigenvalues > 1 contributed 85.49% of the variability among 40 mung bean genotypes. In another study by Jeberson [74], the first PCs with eigenvalues > 1 contributed 88.4% of the variability among 24 mung bean genotypes. In our data, the first PC1 reflected yield potential genotypes; hence, the genotypes contributing to this component can undergo direct selection [75].The PC2 gathered seed quality and yield quality genotypes. The current findings contradict the previous study by Das [76], which found PC2 to have late maturing genotypes with low yielding and was

more related to vegetative growth. The current study was performed for two crop seasons; Hence, multi-location trials conducted over a period of years are required to collect more detailed information on the performance of the genotypes.

The origin of the biplot in a PCA represents the average value of all traits [77]. Our results shows negative correlation between days to maturity and days to 50% flowering which is consistent with, Tahir [75] who found that days to 50% flowering and days to maturity were negatively correlated with yield attributes such as 100 seed weight, pod length, pod per plant, harvest index and seed per pod. Our study's results support the view that the selection of short-duration mung bean genotypes will offset yield potential. The number of branches and number of clusters showed a strong association with pod weight. In contrast, Tahir [75] found that the number of branches and pods per plant was associated with yield per plant. Shelling percent and weight of 1000 seeds were positively correlated, and selecting these traits will be influential in determining seed yield. As explained by scatter plot, the degree of variations and relationships between various traits demonstrated the strength of the connection between the quantitative traits.

Cluster analysis assists in identifying genotypes that contrast for various qualities, which can be used to inform mung bean improvement. The information generated by clustering also improves the effectiveness of exploring different genes, polymorphic markers, and marker trait associations and physiological mechanism [44].Variations that already exist may be a result of genotype, environment, or a combination of the two. Based on the results cluster analysis of genotypes based on average linkage grouped into four major clusters and cluster I had genotype with good traits that can be used in breeding programs. The results of the cluster analysis are closely supported by previous reports on mung bean [78–81]. In these studies, beneficial traits related to yield in mung bean were scattered in different clusters and offered a good opportunity for the selection of parents for mung bean improvement programs.

The evaluation of the qualitative and quantitative traits of mung bean genotypes is an important primary step for crop improvement. In order to effectively utilise the germplasm, it is important to evaluate and understand the extent of the trait variation to utilise them in crop improvement. This study demonstrated that there is a more beneficial variation in agro-morphological traits in the mung bean gene pool than previously reported [21, 81].

Conclusions

Analysis of agro-morphological traits revealed significant variability both in qualitative traits and quantitative traits assessed through genetic parameters. This variability offers opportunities to exploit in breeding programs. The genotypes showed useful traits that can be exploited in improving yield, life cycle, and plant type and resistance to the disease. The rice bean which was used as check (C3) owing to observations of resistance demonstrated resistance to powdery mildew in a controlled field experiment and exhibited favourable yield related traits. However, the species which is also reportedly drought tolerant has a shattering tendency of pods which presents a challenge during harvesting. Additionally, powdery mildew resistance varied across genotypes with just one mung bean genotype exhibiting resistance to the disease. However, its trait limitations included poor yield and late flowering period. The result produced from the present study forms further research and breeding initiatives to utilize observed variability in development of mung bean varieties with improvement in disease resistance and agronomic performance.

Declarations

Ethics approval and consent to participate.

Not applicable

Consent for publication.

Not applicable

Competing of interests.

The authors declare no conflicts of interest

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Author Contribution

Conceptualization: DJK, PBV, PCS, SRB, AGM. Methodology: DJK, PBV, SRB, PCS, AGM. Field: DJK. Analysis: DJK. Data curation: SRB. Writing original draft preparation: DJK. Reviewing: DJK, SRB, PBV, PCS, AGM. Project administration: AGM, SRB. Funding acquisition: SRB. All authors contributed to the article and approved the submitted version

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Data Availability

The data that support the findings of this study are not openly available and are available from the corresponding author upon reasonable request.

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Figures

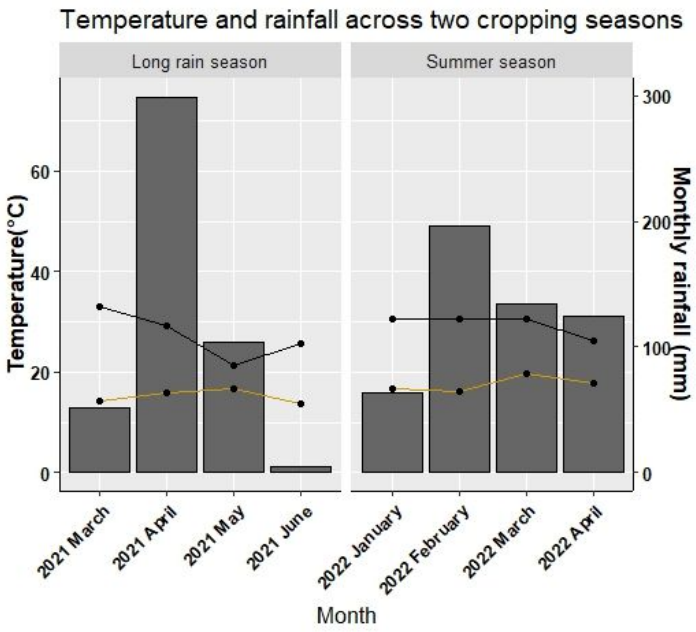

Figure 1

Climograph for study area based on monthly average maximum, minimum temperature and total rainfall during the study period. In the graph, bars represent rainfall, while lines depict minimum and maximum temperature over the course of the study period.

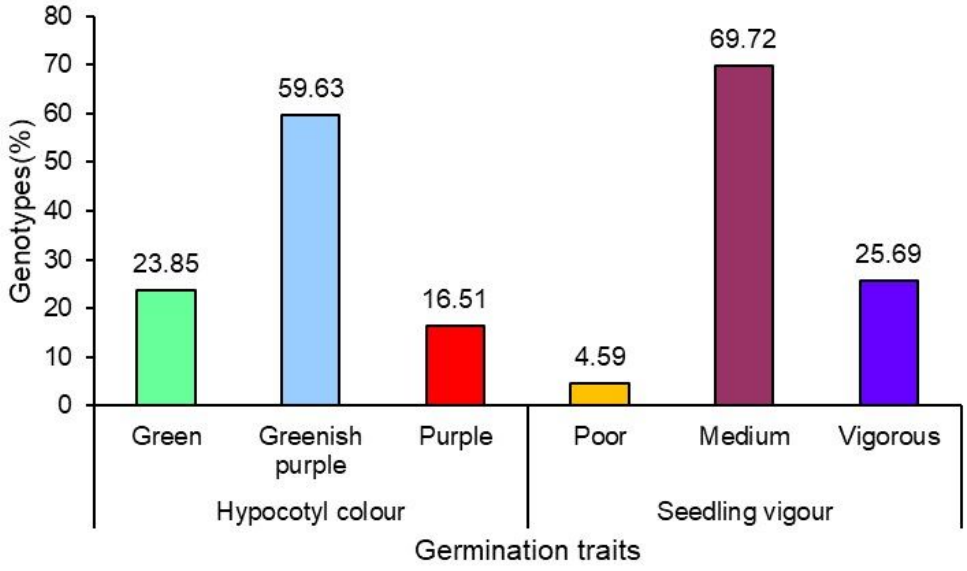

Figure 2

Variations in selected germination traits of mung bean genotypes

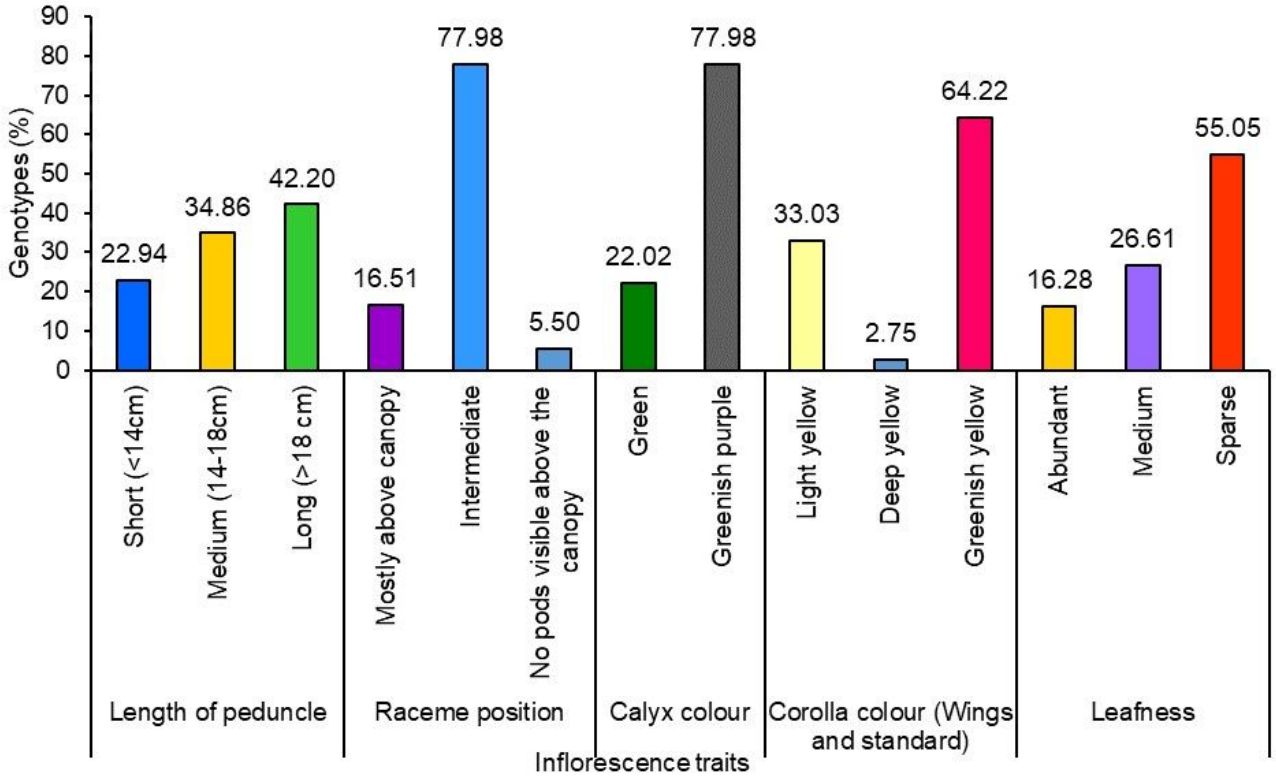

Figure 3

Variation in selected inflorescence traits of mung bean genotypes

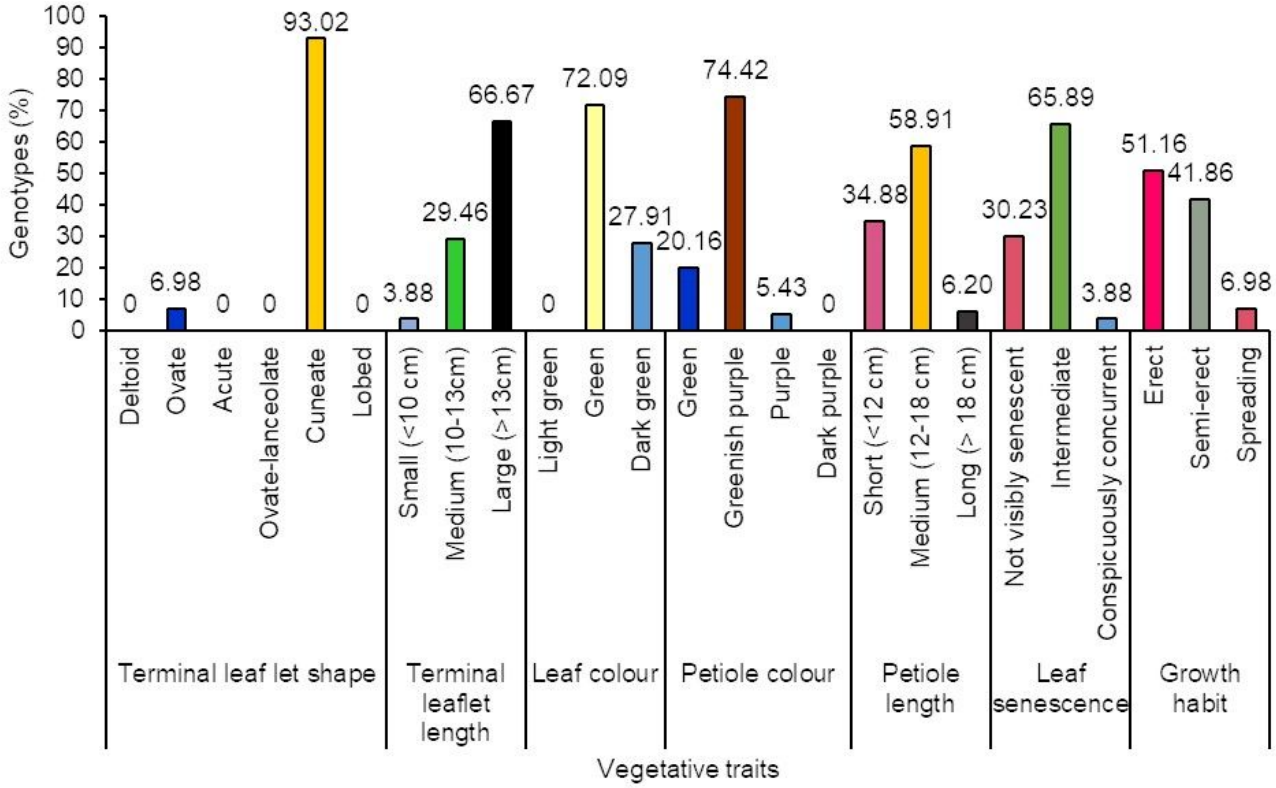

Figure 4

Variation in selected vegetative traits of mung bean genotypes

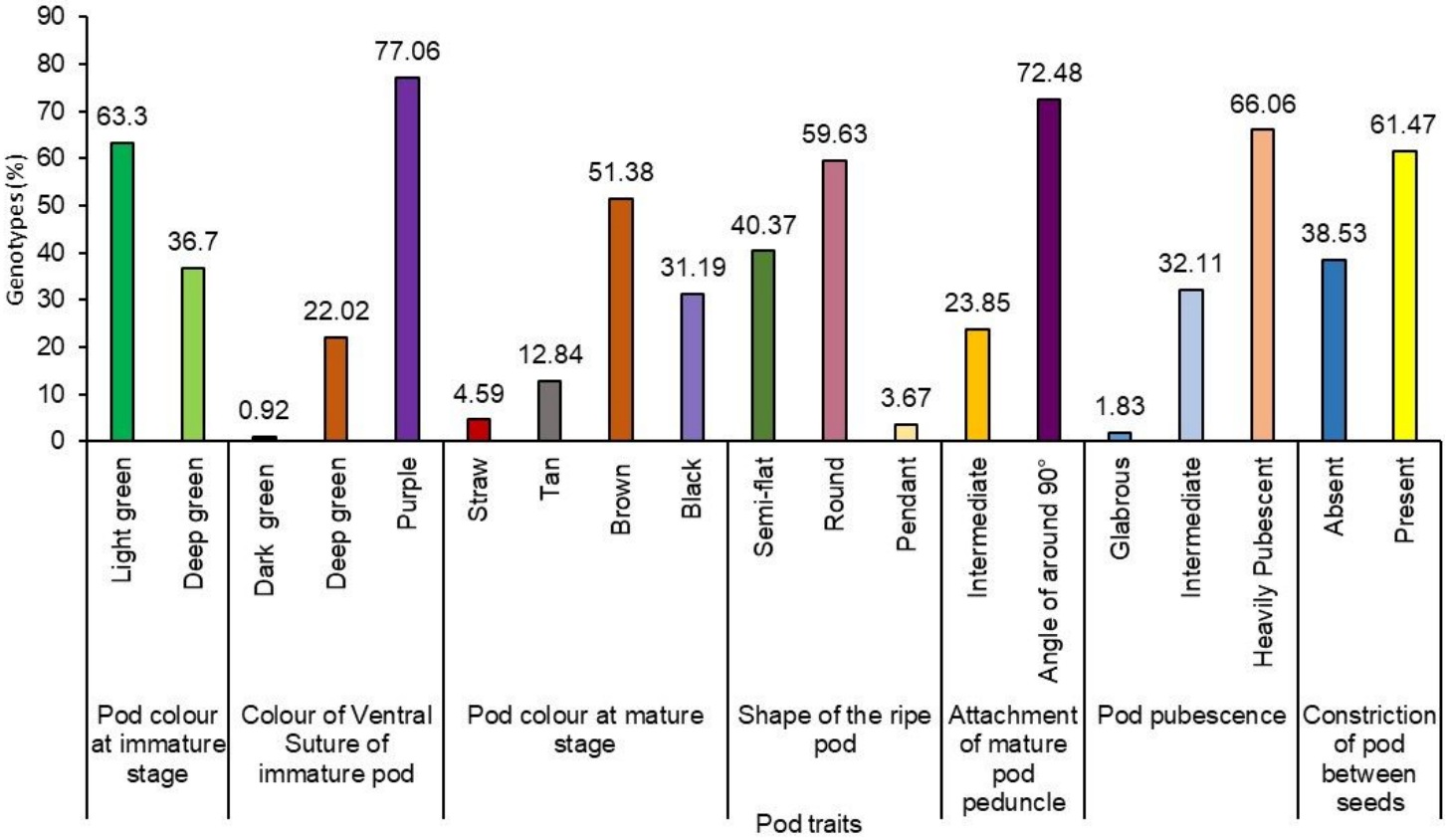

Figure 5

Variation in selected pod traits of mung bean genotypes

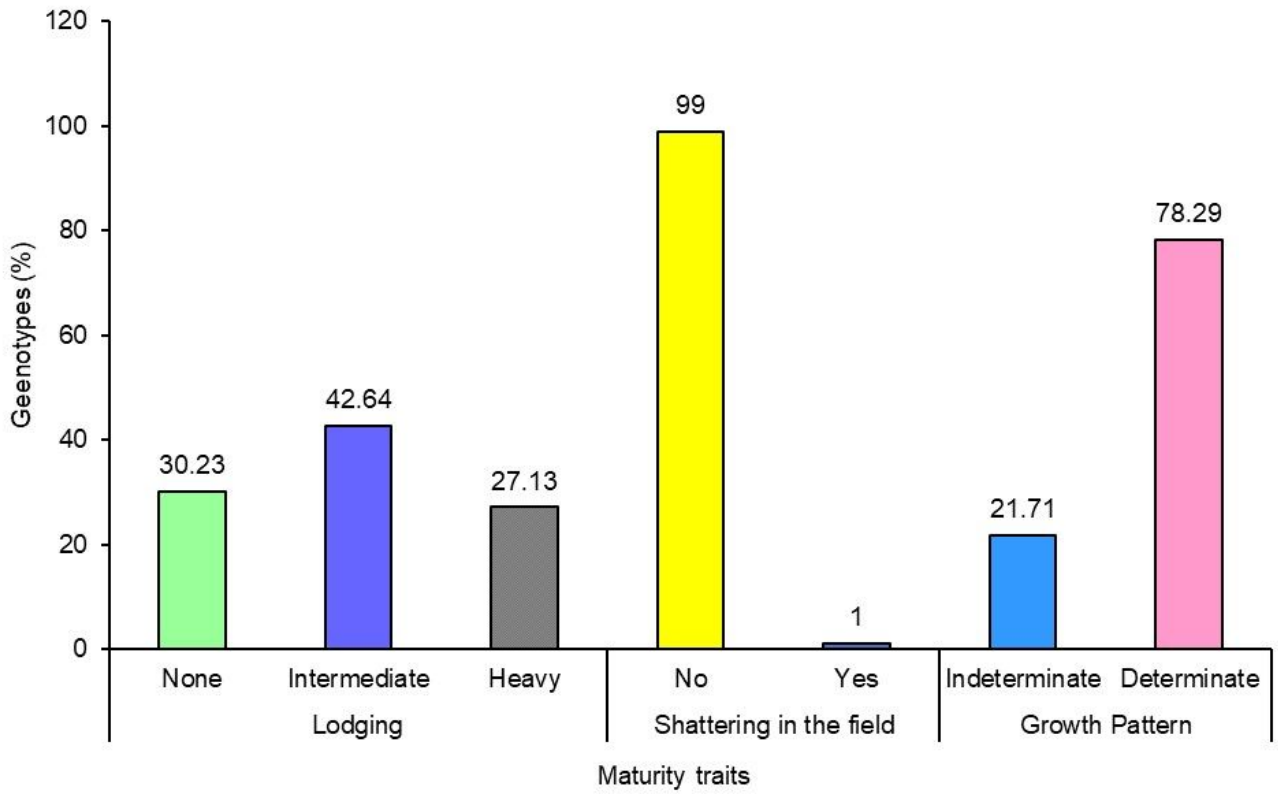

Figure 6

Variation in selected maturity traits of mung bean genotypes

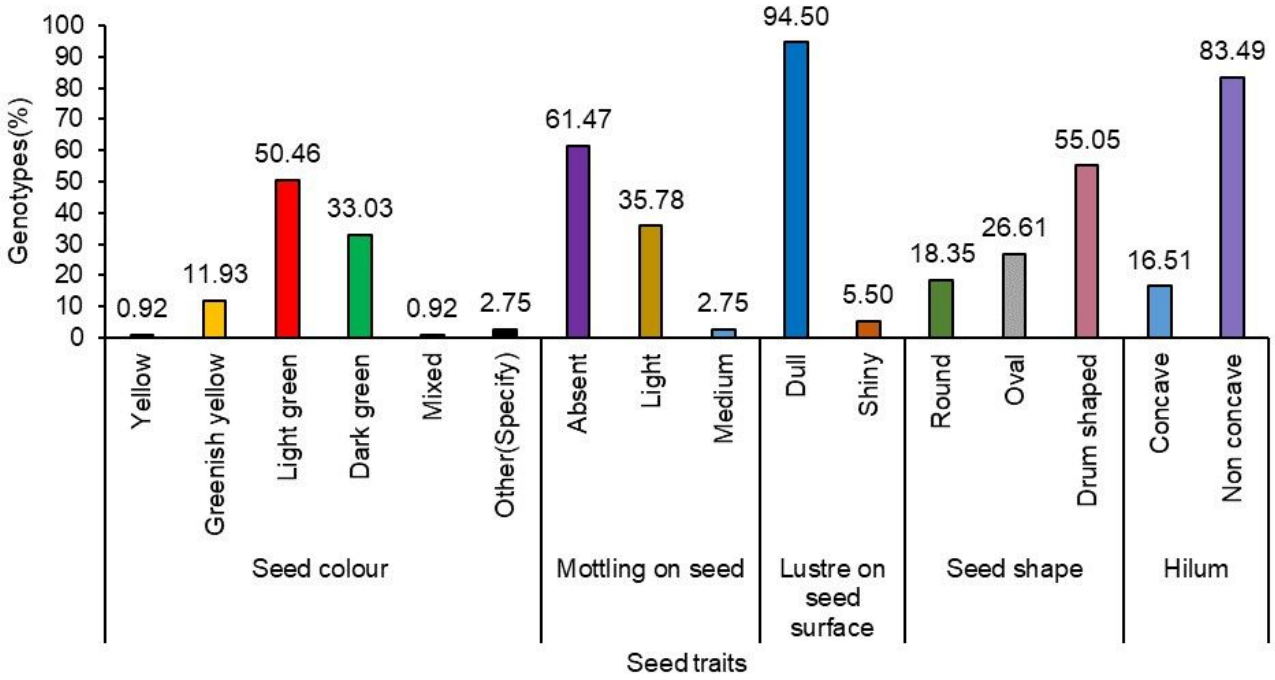

Figure 7

Variations in seed morphology traits of mung bean genotypes

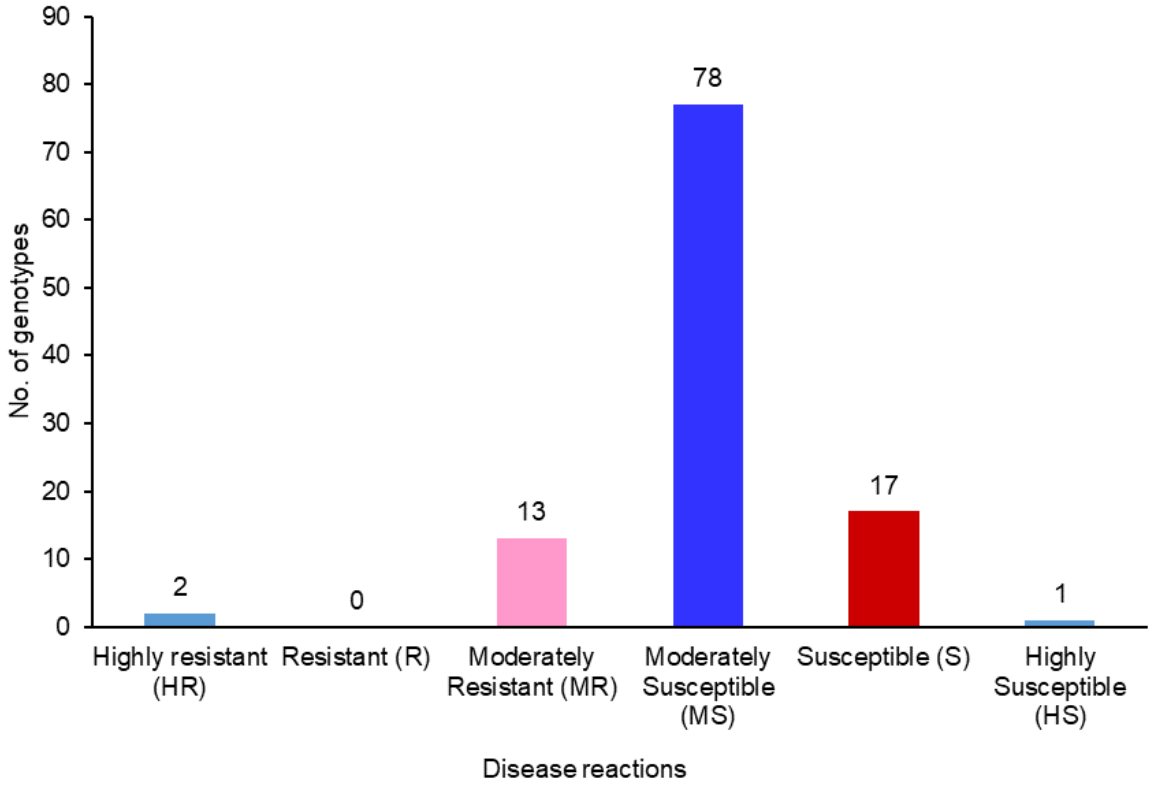

Figure 8

Response of mung bean genotypes to powdery mildew diseases

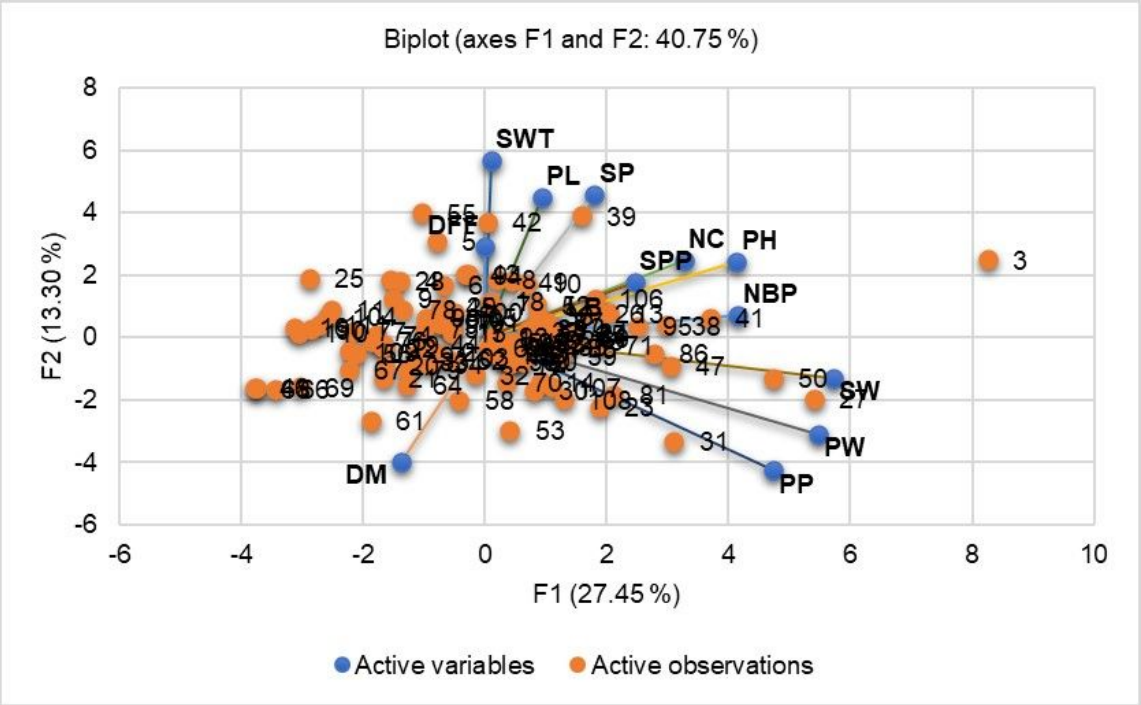

Figure 9

Scatter plot diagram of principal component analysis of quantitative traits of mung bean: length of the branch (LB), plant height (PH), no. of branches per plant (NBP), number of clusters (NC), number of pods per plant (PP), number of seeds per pod (SPP), pod weight (PW), seed weight (SW), weight of 1000 seeds (SWT), pod length (PL), days to flowering (DFF), days to maturity (DM) and shelling percent (SP)

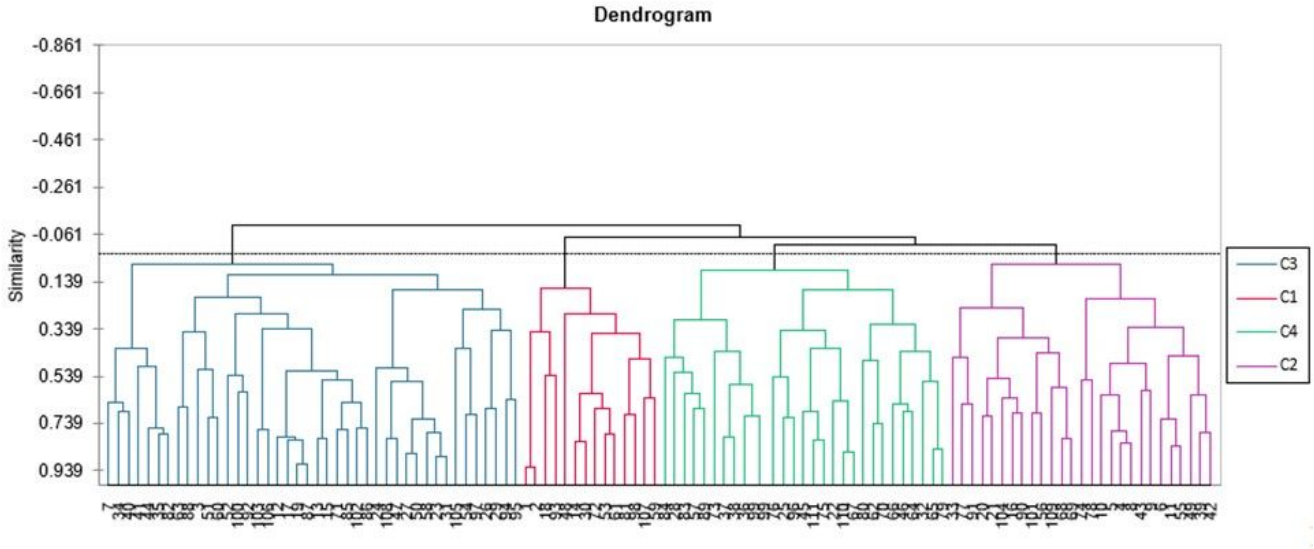

Figure 10

Genetic similarity dendrogram (complete linkage) representing the relationship among mung bean genotypes based on Pearson's similarity co-efficient using quantitative traits

Supplementary Files

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- Additionalfile1.docx