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Development and Characterization of Advanced Mutants of Mungbean for Yield-Contributing Traits

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THIS STUDY aims to characterize sixteen advanced mungbean mutants (M_4 generation) for yield-related traits and biometrical analyses of the traits for efficient selection. The experiment was conducted employing a randomized complete block design with three replications. Data on ten key traits [days to first flowering (DFF), days to first harvest (DFH), days to last harvest (DLH), plant height (PH), number of branches per plant (NBP), number of pods per plant (NPP), pod length (PL), number of seeds per pod (NSP), 100-seed weight (100-SW), and yield per plant (YPP)] were recorded. Significant variability among genotypes was observed for all traits. Higher YPP was recorded in the mutants DEMS22 (28.55 g), DGR41 (24.14 g), BEMS62 (23.5 g), DGR51 (23.35 g), DEMS61 (23.3 g), and BEMS61 (23.15 g). High heritability and high genetic advance (%) was observed for YPP (97% and 54.23%, respectively), NPP (97% and 52.42%, respectively), NBP (90% and 42.06%, respectively), PH (97% and 36.51%, respectively), 100-SW (93% and 34.76%, respectively), and NSP (95% and 27.5%, respectively). YPP showed significant positive correlation with DLH, and NPP. Path coefficient analysis identified DLH, NSP, and NPP as the primary direct contributors to yield. Principal component analysis (PCA) indicated that the first three PC explained 79.32% of the total variation, with DLH, NPP, PL, 100-SW, and YPP significant contributors to divergence. Cluster analysis categorized the genotypes into four distinct clusters where cluster III identified as the most promising. Therefore, the promising mutants identified through this study can be used for developing high-yielding varieties.

Keywords: Mungbean; mutagenesis; yield; heritability; cluster analysis.

Introduction

Malnutrition has become a global concern, posing significant challenges to public health and well-being in developing countries (Siddiqui et al., 2020). Medical institutes are now recommending plant-based dietsto combat malnutrition and improve human health (Zafar et al., 2023). A substantial source of plant-based nutrition, pulses like mungbean are referred to as "rich man's vegetable" and "poor man's meat" due to their high protein content (Jahan et al., 2020). Mungbean (*Vigna radiata* L.), a self-pollinated diploid species (2n = 2x = 22), also known as green gram, moong, or golden gram, is a fast-growing, highly nutritious, and multipurpose crop (Pathak et al., 2023). Being a leguminous crop, it is an important source of amino acids, proteins, dietary fiber, and unsaturated fatty acids (Hou et al., 2019). Its grains contain 22-28% protein, 60-65% carbohydrates,1-1.5% fat, 3.5-4.5% fiber, and high levels of vitamins and minerals (Jahan et al., 2020). Its properties also help treat hepatitis and gastritis and its antihypertensive, antidiabetic, and anticancer effects (Kumar and Singhal, 2009). Additionally, mungbean contains a range of phytochemicals, including steroids, triterpenoids, glycosides, flavonoids, alkaloids, and polyphenols, which contribute to its antioxidant, antitumor, anti-inflammatory, and antimicrobial activities (Priya et al., 2012; Tang et al., 2014). Given its rich nutritional profile and versatility, mungbean is valuable as a food source and a cornerstone of sustainable agricultural systems.

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In Bangladesh, mono-cropping coupled with the imbalanced use of inorganic fertilizers, pesticides, and intensive land cultivation without organic fertilization has significantly deteriorated soil quality and fertility. Over 65% of agricultural land experiences declining fertility, while 85% of cultivable land has organic matter levels below the minimum requirement (Hossain, 1990). As nitrogen-fixing legume mungbean can play a significant role in addressing these issues by fixing atmospheric nitrogen, thereby enhancing soil fertility and reducing dependency on synthetic fertilizers (Sharma et al., 2010; Naik et al., 2020). Furthermore, it benefits from symbiosis with growth-promoting rhizobacteria, which colonize the plant roots and enhance growth (Hasanuzzaman et al., 2020). Additionally, given its short growth cycle, mungbean can be integrated into rice-based mono-cropping systems, boosting cropping intensity. Including short-duration, high-yielding mungbean in this cropping pattern can improve the nutrition of poor households and contribute to sustainable soil management. Globally, mungbean is cultivated on approximately 7.5 million hectares, with annual production exceeding 5 million metric tons (Obasi et al., 2024; Nair and Schreinemachers, 2020). Despite its importance as a nutritious and economically valuable crop, mungbean cultivation in Bangladesh faces numerous challenges, including biotic and abiotic stresses, climate variability, and declining soil fertility. In the 2020–2021 season, mungbean was grown on 112,709.60 acres, yielding 45,565.80 metric tons, with an average yield of just 0.404 metric tons per acre (BBS, 2023). This yield is significantly lower than those recorded in research plots and predicted by crop models, such as the Agricultural Production Systems Simulator (APSIM) (Geetika et al., 2022; Chauhan and Williams, 2018), highlighting the urgent need for developing high-yielding mungbean varieties through breeding programs.

For successful crop improvement, phenotypic and genotypic variability in the germplasm is very important. In mungbean, where natural variability is limited, enhancing genetic diversity has become a key focus for breeders and researchers. This can be achieved through hybridization and natural or induced mutation using physical and chemical mutagens (like gamma rays, ethyl methane sulfonate, and epichlorohydrin) (Kumar et al., 2021). Mutagens have proven effective in enhancing mungbean quality, increasing pod and seed numbers, 100-seed weight, seed yield, and tolerance to biotic and abiotic stresses (Wasif et al., 2023). Gamma rays have been successful in producing semi-dwarf plants and distinct mutant varieties with altered pod and leaf traits (Vanniarajan and Chandirakala, 2020; Sarma et al., 2022). For these features, mutation breeding has already led to the development of 34 commercial pulse crops and 97 variants globally (Sarma et al., 2022; Jamil et al., 2022). In breeding programs, giving considerable attention to selecting yield-contributing traits is mandatory since yield is a complex trait influenced by multiple factors (Jahan et al., 2020). Therefore, trait association studies, including correlation coefficients, principal component analysis, and clustering patterns, are crucial for developing selection indices and play a key role in identifying and prioritizing these desirable traits and in developing improved high-yielding varieties. Considering the above facts, we have developed a large set of advanced mutant lines (M4 generation) of mungbean from BARI Mung-6 and Durdona through physical and chemical mutagenesis. The current study aims (i) to evaluate the performance of these advanced mutants for yield-contributing traits (ii) to assess the relationships, variability, and heritability of the studied traits and (iii) to assess the diversity of the advanced lines for selecting better parents for maximizing genetic gain.

Materials and Methods

Experimental site and duration

The field experiment was conducted at the Farm Research Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, during the Kharif-I season of 2024 (March-July). The experimental site is situated in the Sonatota series of gray floodplain, within the Agro-ecological Zone (AEZ 9) of the Old Brahmaputra Floodplain. The region is predominantly medium-high land, with silt-textured soil that has low levels of organic matter and fertility. The soil pH ranges from 6.5 to 6.7. The weather report of the growing period of the experiment area is presented in Fig. 1.

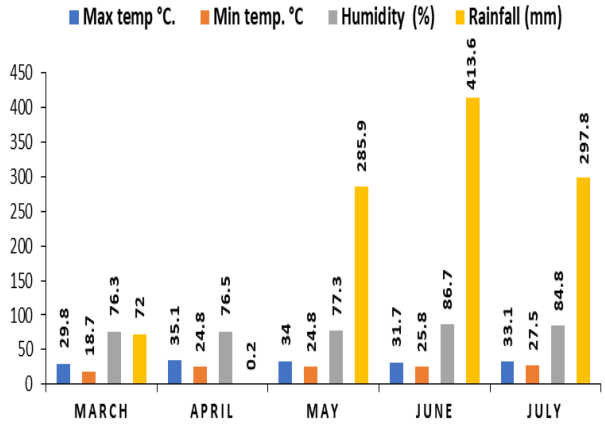

Fig. 1. Monthly average maximum and minimum temperature (℃), humidity (%), and rainfall (mm) of the experimental area from March 2024 to July 2024.

Development and Characterization of Mutants through Chemical Mutagenesis

Ethyl Methanesulfonate (EMS) was employed to induce mutations in the seeds of BARI Mung-6 and Durdona varieties. Fresh and healthy seeds were initially soaked in distilled water for 6 hours to enhance seed coat permeability. Following imbibition, the seeds were treated with a 0.3% EMS solution for an additional 6 hours. After the treatment period, seeds were thoroughly washed with running tap water for 2 hours to eliminate any residual EMS, thereby preventing phytotoxic effects during subsequent planting.

Development of Mutants through Gamma Irradiation

Seeds from the Durdona variety were subjected to gamma irradiation at the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The seeds were exposed to gamma radiation at doses of 200 Gy and 300 Gy. After treatment, the seeds were sown in the field to produce the M_1 generation. Subsequently, seeds from both the EMS-treated and gamma-irradiated M_1 plants were bulked and sown for the M_2 generation. This process continued through the M_3 generation, where phenotypic traits were assessed to identify plants with desirable characteristics. Sixteen superior lines from the M_3 population were selected and each line was sown individually for advancement to the M_4 generation. Along with these advanced mutants, two-parent varieties (BARI Mung-6 and Durdona) and a check variety (Binamoog-8) were included in the study. Detailed information on the materials is presented in Table 1.

Table 1. List of mungbean mutants/varieties used in the field experiment and their sources.

Serial No.	Genotype name	Genotype type	Stage	Source
1	BARI Mung-6	Variety		BARI
2	Binamoog-8	Variety		BINA
3	Durdona	Variety		Uzbekistan
4	BEMS61	EMS mutant of BARI Mung-6	M_4	BAU
5	BEMS62	”	M_4	BAU
6	BEMS63	”	M_4	BAU
7	DEMS21	EMS mutant of Durdona	M_4	BAU
8	DEMS22	”	M_4	BAU
9	DEMS51	”	M_4	BAU
10	DEMS52	”	M_4	BAU
11	DEMS61	”	M_4	BAU
12	DEMS62	”	M_4	BAU
13	DGR11	Gamma mutant developed from Durdona irradiated with 200 gray	M_4	BAU
14	DGR12	”	M_4	BAU
15	DGR21	”	M_4	BAU
16	DGR31	”	M_4	BAU
17	DGR41	Gamma mutant developed from Durdona irradiated with 300 gray	M_4	BAU
18	DGR51	”	M_4	BAU
19	DGR52	”	M_4	BAU

Land Preparation and Fertilizer Application

The experimental field was prepared using a power tiller for ploughing and cross-ploughing, followed by laddering to achieve proper tilth and leveling while removing weeds and debris. Fertilizers and manures, including cowdung, urea, triple super phosphate, muriate of potash, and gypsum at 2500-45-80-40-75 kg/ha, were applied during final land preparation. All fertilizers were applied at this stage except for urea, half of which was applied 25–30 days after sowing.

Experimental Design and Sowing

The experiment followed a randomized complete block design (RCBD) with three replications. Each block had 19 experimental units, with a plot size of 4 m² (2.5 m × 1.6 m) consisting of four rows. Rows were spaced 30 cm apart, and the distance between experimental units was 60 cm. Seeds were directly sown in the line sowing method to the experimental field on 16 March 2024.

Intercultural operation

Intercultural operations, including irrigation, weeding, and staking, were conducted to optimize plant growth. Early in the growing season, thinning was carried out to promote uniform growth, followed by weeding at 30 and 45 DAS to manage weed density. To control pests, Trichoderma (5 g/L) and Tido Plus (2 g/L) were applied twice to prevent infestations of mungbean hairy caterpillars.

Harvesting
Harvesting times varied among genotypes due to differences in maturity periods. Pods were collected from selected plants, sun-dried, and kept in separate harvesting bags. After drying, the seeds were carefully extracted by hand.

Data collection
Data on ten quantitative traits (Days to first flowering (DFF), Days to first harvest (DFH), Days to last harvest (DLH), Plant height (PH), Number of branches per plant (NBP), Number of pods per plant (NPP), Pod length (PL), Number of seeds per pod (NSP), 100-seed weight (100-SW), Yield per plant (YPP)) were recorded for the study. Five plants were randomly selected for each replication, and the mean values of these plants for each trait were used for statistical analysis.

Statistical analysis
The collected data were compiled and analyzed using R software (version 3.4.1). The analysis encompassed one-way ANOVA to evaluate variance among genotypes and estimates of genotypic and phenotypic variances, coefficients of variation, heritability, and genetic advance. Additionally, correlation studies, path analysis, principal component analysis (PCA), cluster analysis, and dendrogram construction were conducted to assess the relation among traits and genetic diversity and classify genotypes.

Estimation of genetic parameters
Following the procedures described by Allard 1960 and Johnson et al. 1955, genetic parameters such as genetic variance, broad-sense heritability (h^2b), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), genetic advance (GA), and genetic advance as a percentage of the mean (GA%) were computed. According to the standards put forth by Deshmukh et al. (1986), the PCV and GCV estimates were classified as low (<10%), moderate (10%–20%), and high (>20%). Similarly, Johnson et al. (1955) defined GA% as low (<10%), moderate (10%–20%), and high (>20%), and broad-sense heritability (h2b) as low (0%–30%), medium (31%–60%), and high (>60%).

Estimation of correlation coefficient
The phenotypic and genotypic correlation coefficient was estimated using the formula suggested by Miller et al. (1958).

Path coefficient analysis
Utilizing the following formula provided by Dewey and Lu (1959), path analysis was performed.

Cluster analysis

Cluster analysis was performed using Ward's method (Ward, 1963).

Results

Analysis of variance and Mean performances for yield contributing traits of the nineteen mungbean genotypes (mutants/ varieties) under field condition

The ANOVA showed highly significant variation among genotypes for DFF, DFH, DLH, PH, NBP, NPP, PL, NSP, 100-SW, and YPP at 0.1% level, while DFF exhibited significant variation at the 5% level (Supplementary Table 1). The mean performances of nineteen mungbean genotypes for yield and yield-contributing traits are shown in Table 2. Significant differences were observed among the studied mutants for the trait DFF. The minimum number of DFF (37.33 days) was recorded in the advanced mutants DEMS51, followed by BEMS63 (37.66 days), DEMS22 (37.66 days), DEMS21 (38.00 days), and DGR31 (38.00 days). The maximum number of days (41.00 days) for flowering was required for the genotypes DGR21, BEMS61 (40.00 days), DGR12 (39.33 days), and DEMS62 (39.33 days), respectively (Table 2). Regarding DFH, DEMS61 was the earliest maturing genotype, requiring 52.66 days, followed by DGR52 (53.00 days), BEMS63 (53.33 days), and DGR41 (53.33 days). In contrast, the mutant DEMS62 required the longest time (60.00 days) to harvest the first mature pod (Table 2). The most prolonged duration for the last pod harvest was observed in DEMS22 (114.00 days), followed by DGR51 (113.00 days) and DGR41 (112.66 days). The shortest duration was seen in DGR11 (103.66 days), followed by Binamoog-8 and DGR12 (105.00 days each) and BARI Mung-6 (106.00 days) (Table 2). The lowest PH was recorded in DEMS21 (53 cm), followed by DEMS22 (53.50 cm), DGR51 (57.60 cm), and DEMS61 (59.05 cm). The highest PH was found in DGR21 (95.50 cm), followed by BEMS63 (89.00 cm), BEMS61 (87.10 cm), and BEMS62 (83.55 cm) (Table 2). The highest number of NBP (8.50) was recorded in DGR21 followed by BEMS61 (6.35). Conversely, the lowest NBP was observed in BARI Mung-6 (3.50) and Binamoog-8 (3.85) (Table 2). The maximum NPP was recorded in the advanced mutant DEMS22 (59.90), followed by BEMS61 (59.60) and DEMS21 (54.50), whereas the minimum NPP was found in DGR21 (19.50) (Table 2). The highest PL was observed in DEMS61 (10.32 cm), followed by DGR31, DEMS21, DEMS62, and DEMS22 (10.29, 10.22, 10.21, and 10.06 cm, respectively). Conversely, the lowest PL was found in BEMS63 (7.90 cm), followed by Binamoog-8 (7.97 cm) (Table 2). The highest NSP was observed in BEMS63 with 11.20 seeds, followed by Binamoog-8 (10.50), and BEMS61 (10.35). In contrast, the lowest NSP was recorded in DEMS21 (5.25), followed by DEMS62 (7.65 seeds) (Table 2). The advanced mutant DEMS62 exhibited the highest 100-SW (6.92 g),

followed closely by DGR41, DGR51, DGR31, and DEMS61 (6.90, 6.69, 6.58, and 6.44 g, respectively). On the other hand, BEMS63 recorded the lowest 100-SW (3.79 g) (Table 2). There was a significant variation in YPP among the mungbean mutants. DEMS22 produced the highest YPP (28.55 g), followed by DGR41 (24.14 g), BEMS62 (23.50 g), DGR51 (23.35 g), DEMS61 (23.30 g), and BEMS61 (23.15 g). Conversely, DGR11 exhibited the lowest YPP (10.32 g), followed by DGR12 (10.40 g) and DGR21 (11.55 g) (Table 2).

Table 2. Mean performances of yield- traits of nineteen advanced mutants/varieties.

Genotypes	DFF	DFH	DLH	PH	NBP	NPP	PL	NSP	100-SW	YPP
BARI Mung-6	39.00b-d	56.66b-d	106.00g	69.90e	3.50i	46.50c	8.13gh	9.15ef	4.08hi	17.05g
Binamoog-8	38.33c-e	56.00c-e	105.00g	64.47f	3.85hi	41.10de	7.97h	10.50b	4.24gh	14.95h
Durdona	38.33c-e	55.66c-f	108.00f	74.87d	4.60e-g	42.00de	8.70ef	10.10bc	5.357f	19.02f
BEMS61	40.00ab	53.66h-k	112.66bc	87.10b	6.35b	59.60a	8.20gh	10.35b	4.52g	23.15bc
BEMS62	38.33c-e	54.66e-i	111.33d	83.55c	4.72d-g	42.80de	9.07d	9.40de	6.07de	23.50b
BEMS63	37.66de	53.33i-k	108.667f	89.00b	4.37f-h	43.20d	7.90h	11.20a	3.79i	19.50ef
DEMS21	38.00c-e	57.00bc	110.00e	53.00j	5.20cd	54.50b	10.22ab	5.25j	6.25c-e	17.07g
DEMS22	37.66de	55.33d-g	114.00a	53.50j	4.40f-h	59.90a	10.06a-c	8.55gh	5.97e	28.55a
DEMS51	37.33e	54.66e-i	108.66f	65.10f	5.75c	28.70g	8.37fg	9.40de	5.07f	16.58g
DEMS52	38.33c-e	53.66h-k	110.00e	62.50fg	4.20gh	42.20de	9.92bc	8.55gh	6.19c-e	20.74de
DEMS61	38.33c-e	52.66k	112.00b-d	59.05hi	5.27cd	42.50de	10.32a	9.15ef	6.44b-d	23.3b
DEMS62	39.33bc	60.00a	108.33f	60.60gh	4.80d-f	36.50f	10.21ab	7.65i	6.92a	14.16h
DGR11	38.66b-e	57.66b	103.66h	69.70e	5.00de	28.20g	9.11d	9.75cd	5.25f	10.32i
DGR12	39.33bc	55.33d-g	105.00g	64.80f	4.80def	26.50g	8.38fg	9.25ef	4.997f	10.40i
DGR21	41.00a	55e-h	108.00f	95.50a	8.50a	19.50h	8.84de	9.05ef	4.97f	11.55i
DGR31	38.00c-e	54.33f-j	111.67cd	62.30fg	4.45e-g	33.70f	10.29a	9.15ef	6.58a-c	19.02f
DGR41	38.00c-e	53.33i-k	112.66bc	59.85g-i	4.30 f-h	40.70 de	9.81 c	9.30 ef	6.90 a	24.14 b
DGR51	38.00 c-e	54.00 g-k	113.00 ab	57.60 i	5.00 de	43.00 d	8.43 fg	8.15 h	6.69 ab	23.35 b
DGR52	38.00 c-e	53.00 jk	111.00 de	64.10 f	5.75 c	40.00 e	8.61 ef	8.95 fg	6.30 b-e	21.85 cd
Mean	38.50	55.05	109.46	68.23	4.99	40.58	9.08	9.09	5.61	18.85
Range	37.33-41.00	52.66-60.00	103.66-114.00	53.00-95.50	3.50-8.50	19.50-59.90	7.90-10.32	5.25-11.20	3.79-6.92	10.32-28.55
SE	0.16	0.25	0.40	1.60	0.14	1.38	0.11	0.16	0.13	0.66
CV (%)	2.59	1.50	0.62	2.64	6.95	4.28	2.31	2.79	4.69	4.62
LSD Value	1.65	1.36	1.13	2.99	0.57	2.87	0.34	0.42	0.43	1.44

Here, DFF= Days to first flowering, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-seed weight (g), YPP =yield per plant (g)

Estimation of genetic variability, heritability, and genetic advance for yield contributing traits

The genetic parameters for the studied traits are presented in Table 3. High phenotypic variance (σ^2_g) and genotypic variance (σ^2_P) were observed for PH (152.75, 149.48), NPP (112.64, 109.62), and YPP (26.14, 25.38), while the lowest values were recorded for DFF (1.46, 0.46) and PL (0.80, 0.75) (Table 3). GCV and PCV values were highest for YPP (26.72%, 27.11%), NPP (25.79%, 26.15%), and NBP (21.46%, 22.56%), indicating substantial genetic variation, while traits like PL (9.58%, 9.85%), DFH (3.22%, 3.55%), and DFF (1.76%, 3.13%) had lower GCV and PCV values (Table 3). In this study, the majority of traits demonstrated high heritability ($h^2b > 60\%$), with YPP, NPP, and PH exhibiting the highest values (97% each), followed by NSP and DLH (95%). PL had a heritability of 94%, 100-SW (93%), NBP (90%), and DFH (82%) (Table 3). In contrast, DFF showed moderate heritability ($30\% \leq h^2b \leq 60\%$) with a value of 31% (Table 3). Genetic advance ranged from 0.78-24.91. GA was the highest for PH (24.91), followed by NPP, YPP, DLH, DFH, NSP, NBP, 100-SW, PL, and DFF (21.27, 10.22, 6, 3.31, 2.5, 2.09, 1.95, 1.74, and 0.78, respectively). The high GA% (>20%) values were estimated for YPP (54.23%), NPP (52.42%), NBP (42.06%), PH (36.51%), 100-SW (34.76%), and NSP (27.5%), respectively, whereas PL (19.19 %) showed moderate GA (10-20%) values. The lowest GA (<10%) was recorded for DFH (6.01 %), DLH (5.48%) and DFF (2.04 %), respectively (Table 3).

Table 3. Estimation of genetic parameters for morphological traits related to yield of nineteen advanced mutants/varieties of mungbean.

Characters	σ^2_g	σ^2_P	GCV (%)	PCV (%)	h^2b (%)	GA	GA (%)
DFF	0.46	1.46	1.76	3.13	31	0.78	2.04
DFH	3.14	3.82	3.22	3.55	82	3.31	6.01
DLH	8.93	9.39	2.73	2.80	95	6.00	5.48
PH	149.48	152.75	17.91	18.11	97	24.91	36.51
NBP	1.14	1.26	21.46	22.56	90	2.09	42.06
NPP	109.62	112.64	25.79	26.15	97	21.27	52.42
PL	0.75	0.80	9.58	9.85	94	1.74	19.19
NSP	1.53	1.60	13.62	13.91	95	2.50	27.50
100-SW	0.96	1.03	17.47	18.09	93	1.95	34.76
YPP	25.38	26.14	26.72	27.11	97	10.22	54.23

Here,σ^2_g = Genotypic variance, σ^2_P = Phenotypic Variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, h^2b = Heritability in a broad sense, GA = Genetic advance, GA (%) = Genetic advance as a percentage of mean; DFF= Days to first flowering, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-seed weight (g), YPP =yield per plant (g)

Estimation of genotypic and phenotypic correlation coefficient

In both genotypic and phenotypic correlations, YPP exhibited a significant positive association with DLH (rg = 0.88**, rp = 0.87**) and NPP (rg = 0.71**, rp = 0.71**), while showing a significant negative correlation with DFF (rg = -0.64**, rp = -0.31*) and DFH (rg = -0.59**, rp = -0.53**). YPP also showed a significant positive correlation with 100-SW (rp = 0.36**) at the phenotypic level (Table 4).

At both levels, DFF showed a significant positive correlation with PH (rg = 0.73**, rp = 0.38**) and NBP (rg = 0.81**, rp = 0.39**). A significant negative correlation between DFH and DLH was also observed (rg = -0.57*, rp = -0.50**). Moreover, DFH exhibited a significant negative association with NSP (rp = -0.34**) at the phenotypic level. DLH showed a significant positive correlation with NPP (rg = 0.54*, rp = 0.54*) and 100-SW (rg = 0.57**, rp = 0.56**) at both levels and a significant positive association with PL (rp = 0.40**) at the phenotypic level (Table 4). PH also showed a significant positive relation with NBP (rg= 0.48 *, rp= 0.47 **) and NSP (rg= 0.59**, rp= 0.56 **), whereas it was negative with PL (rg= -0.54 *, rp= -0.52 **) and 100-SW (rg= -0.60 **, rp= -0.58**) at both levels. NBP, at the phenotypic level, showed a significant negative correlation with NPP (rp= -0.34 **). PL exhibited a significant positive association with 100-SW (rg = 0.79**, rp = 0.76**) at both levels, whereas NSP displayed a significant negative correlation with PL (rg = -0.61**, rp = -0.57**) and 100-SW (rg = -0.62**, rp = -0.55**) (Table 4).

Table 4. Correlation coefficients of yield and yield contributing traits of nineteen mungbean genotypes.

Characters		DFF	DFH	DLH	PH	NBP	NPP	PL	NSP	100-SW
DFH	G	0.32								
	P	0.18								
DLH	G	-0.40	-0.57 *							
	P	-0.17	-0.50**							
PH	G	0.73**	-0.16	-0.20						
	P	0.38**	-0.15	-0.19						
NBP	G	0.81**	-0.15	0.10	0.48 *					
	P	0.39**	-0.13	0.08	0.47 **					
NPP	G	-0.40	-0.12	0.54 *	-0.22	-0.35				
	P	-0.18	-0.11	0.54**	-0.22	-0.34**				
PL	G	-0.19	0.14	0.43	-0.54 *	-0.08	0.12			
	P	-0.10	0.13	0.40**	-0.52**	-0.05	0.12			
NSP	G	0.08	-0.37	-0.23	0.59**	-0.07	-0.17	-0.61**		
	P	0.03	-0.34**	-0.21	0.56 **	-0.05	-0.17	-0.57**		
100-SW	G	-0.35	-0.02	0.57**	-0.60**	-0.03	0.03	0.79**	-0.62**	
	P	-0.16	-0.02	0.56**	-0.58**	-0.01	0.03	0.761**	-0.55**	
YPP	G	-.64**	-0.59**	0.88**	-0.20	-0.21	0.71**	0.24	-0.007	0.37
	P	-0.31 *	-0.53**	0.87**	-0.21	-0.21	0.71**	0.23	0.0007	0.36 **

Note: * and ** indicate significance at 5% and 1% probability levels, respectively. Here, G= Genotypic & P= Phenotypic correlation. DFF= Days to first flowering, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-seed weight (g), YPP =yield per plant (g)

Path-coefficient analysis

The genotypic and phenotypic direct and indirect effects of various traits on YPP in nineteen advanced mungbean genotypes are detailed in Table 5. In this study, DLH exhibited the highest genotypic positive direct effect on YPP (0.947), followed by DFF (0.249), NPP (0.144), and NSP (0.128). Conversely, NBP had a substantial negative direct effect (-0.423), followed by PH (-0.14), PL (-0.14), DFH (-0.135), and 100-SW (-0.007) on YPP (Table 5). Genotypic path analysis revealed positive indirect effects on YPP via DFF from NBP (0.204) and PH (0.184). Through DLH, traits such as 100-SW (0.547), NPP (0.519), PL (0.411), and NBP (0.102) exhibited high positive indirect effects, while DFH (-0.54), DFF (-0.38), NSP (-0.224), and PH (-0.191) displayed significant negative indirect effects. Furthermore, DFF showed high negative indirect effects on YPP via both PH (-0.103) and NBP (-0.347), while NPP demonstrated a high negative indirect effect through NBP (-0.149). Additionally, 100-SW had a high negative indirect effect on YPP via PL (-0.111) (Table 5).

At the phenotypic level, significant positive direct effects on YPP were observed from DLH (0.608), NPP (0.355), NSP (0.204), and 100-SW (0.14). In contrast, NBP (-0.135) and DFH (-0.122) exhibited strong negative direct effects on YPP. Additionally, DFF (-0.047), PL (-0.037), and PH (-0.004) also exerted moderate positive direct effects on YPP (.5). Regarding indirect effects, 100-SW (0.345), NPP (0.328), and PL (0.244) showed strong positive indirect impacts on YPP through DLH. Conversely, DFH (-0.309), NSP (-0.131), PH (-0.121), and DFF (-0.108) exhibited strong negative indirect effects on YPP through DLH (Table 4). Through NPP, DLH (0.192) demonstrated a high positive indirect effect on YPP, whereas NBP (-0.123) showed a high negative indirect effect. PH (0.116) also had a high positive indirect effect, while PL (-0.117) and 100-SW (-0.114) exerted high negative indirect effects on YPP via NSP. Additionally, PL (0.106) had a strong positive indirect effect on 100-SW concerning YPP at the phenotypic level (Table 5).

The residual factor values for genotypic and phenotypic path analysis were 0.052 0.057, indicating the proportion of unexplained variation in YPP that is not accounted for by the traits included in the analysis (Table 5).

Table 5. Partitioning of genotypic and phenotypic correlations into direct and indirect effects of ten traits related to yield by path analysis in mungbean genotypes (diagonally bold figures indicate the direct effect).

Characters		DFF	DFH	DLH	PH	NBP	NPP	PL	NSP	100-SW	YPP
DFF	**G**	**0.249**	-0.044	-0.380	-0.103	-0.347	-0.058	0.028	0.011	0.003	-0.64**
	P	**-0.047**	-0.022	-0.108	-0.001	-0.053	-0.066	0.004	0.006	-0.023	-0.31 *
DFH	**G**	0.080	**-0.135**	-0.540	0.023	0.065	-0.018	-0.020	-0.048	0.000	-0.59 **
	P	-0.009	**-0.122**	-0.309	0.001	0.018	-0.040	-0.005	-0.070	-0.003	-0.53 **
DLH	**G**	-0.100	0.077	**0.947**	0.028	-0.046	0.079	-0.061	-0.030	-0.004	0.88 **
	P	0.008	0.062	**0.608**	0.001	-0.012	0.192	-0.015	-0.044	0.079	0.87 **
PH	**G**	0.184	0.022	-0.191	**-0.140**	-0.207	-0.032	0.076	0.075	0.005	-0.2
	P	-0.018	0.019	-0.121	**-0.004**	-0.064	-0.079	0.020	0.116	-0.081	-0.21
NBP	**G**	0.204	0.021	0.102	-0.068	**-0.423**	-0.050	0.012	-0.010	0.000	-0.21
	P	-0.019	0.016	0.054	-0.002	**-0.135**	-0.123	0.002	-0.012	-0.001	-0.21
NPP	**G**	-0.100	0.017	0.519	0.031	0.149	**0.144**	-0.018	-0.023	0.000	0.71**
	P	0.009	0.014	0.328	0.001	0.046	**0.355**	-0.005	-0.035	0.005	0.71 **
PL	**G**	-0.050	-0.020	0.411	0.076	0.035	0.019	**-0.140**	-0.079	-0.006	0.24
	P	0.005	-0.016	0.244	0.002	0.008	0.043	**-0.037**	-0.117	0.106	0.23
NSP	**G**	0.021	0.051	-0.224	-0.082	0.034	-0.026	0.087	**0.128**	0.005	-0.007
	P	-0.001	0.042	-0.131	-0.002	0.008	-0.062	0.021	**0.204**	-0.078	0.0007
100-SW	**G**	-0.087	0.004	0.547	0.085	0.015	0.005	-0.111	-0.079	**-0.007**	0.37
	P	0.008	0.002	0.345	0.002	0.001	0.014	-0.029	-0.114	**0.140**	0.36 **
Residual effect for genotypic path-coefficient = **0.052**& phenotypic path-coefficient = **0.057**											

Note: * and ** indicate significance at 5% and 1% probability levels, respectively. Here, G= Genotypic, P= Phenotypic partitioning. DFF= Days to first flowering, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-seed weight (g), YPP =yield per plant (g)

Principal component analysis (PCA)

PCA was performed to evaluate which variables most significantly impacted the yield performance of the mungbean mutants **(Table 6, Fig. 2).** Variables with eigenvalues greater than one were considered the most influential contributors to the principal components. The first three PCs explained 79.32% of the total variation, whereas PC1 accounted for 38.71% of the total variance, with DLH (0.78), 100-SW (0.76), YPP (0.76), PL (0.68), and NPP (0.58) showing the highest positive coefficients. At the same time, PH (-0.71), DFF (-0.60), and NSP (-0.52) had high negative coefficients. PC2 explained 23.42% of the variation with high positive loadings from NSP (0.67), YPP (0.61), PH (0.45), and DLH (0.41), whereas high negative coefficients were noted for DFH (-0.77), PL (-0.49). PC3 contributed 17.19% of the variation, with NBP (0.87), DFF (0.57), and DLH (0.43) exhibiting high positive loadings.

In the constructed biplot, genotypes located in the top left quadrant, specifically 2 (BEMS62), 5 (DEMS22), 8 (DEMS61), 14 (DGR41), 15 (DGR51), and 16 (DGR52), exhibited high values for YPP, DLH, and NPP **(Fig. 2).** Genotypes in the top right quadrant, including 1 (BEMS61), 2 (BEMS62), 6 (DEMS51), 18 (Binamoog-8), and 19 (Durdona), were characterized by high values for NSP, NBP, and PH. In the bottom left quadrant, genotypes 4 (DEMS21), 7 (DEMS52), 9 (DEMS62), and 13 (DGR31) were distinguished by their longer PL and higher 100-SW. Lastly, the bottom right quadrant featured genotypes 6 (DEMS51), 10 (DGR11), 11 (DGR12), and 17 (BARI Mung-6), which were notable for their higher values of DFF and DFF **(Fig. 2).**

Table 6. Principal components (PCs) for ten traits of nineteen mungbean genotypes from PCA with eigenvectors (loadings) of the first three PCs.

Characters	PC1	PC2	PC3
DFF	-0.60	-0.18	0.57
DFH	-0.23	-0.77	-0.26
DLH	0.78	0.41	0.43
PH	-0.71	0.45	0.35
NBP	-0.34	0.001	0.87
NPP	0.58	0.38	-0.20
PL	0.68	-0.49	0.27
NSP	-0.52	0.67	-0.22
100-SW	0.76	-0.38	0.33
YPP	0.76	**0**.61	0.07
Eigenvalue	3.87	2.34	1.72
(%) Variance explained	38.71	23.42	17.19
Cumulative variance (%)	38.71	62.13	79.32

Here, DFF= Days to first flowering, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-seed weight (g), YPP =Yield per plant (g)

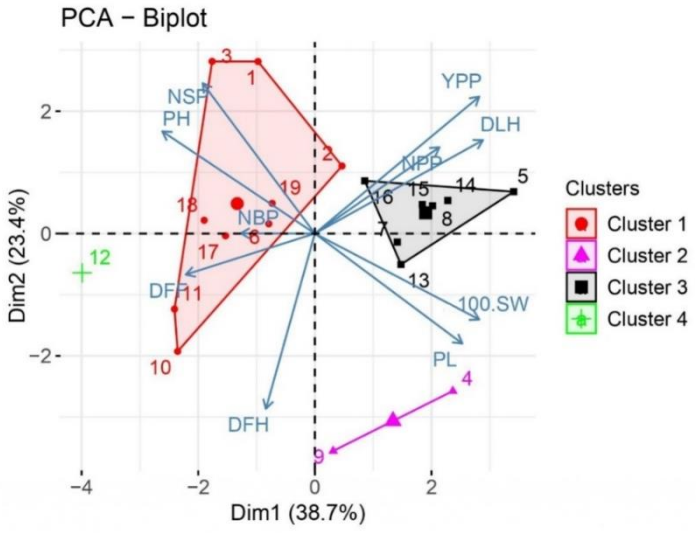

Fig. 2. PCA biplot showing mungbean genotype clusters in PC1 and PC2.

Genetic diversity studies

Grouping of genotypes into various clusters

From the D^2 analysis of the experimental population, four distinct clusters were identified, as illustrated in Fig. 3 and detailed in Table 7. The 19 genotypes were categorized into four clusters based on their distribution pattern (Fig. 3). Cluster I contained the most genotypes, with 9, followed by Cluster III, with 7; Cluster II, with 2; and Cluster IV, with a single genotype.

Average intra and inter-cluster distance

Regarding intra-cluster distances, Cluster II exhibited the most significant distance (3.62), followed by Cluster I (3.51) and Cluster III (2.27) (Fig. 4). The largest inter-cluster distances between Clusters II and IV (7.06) and the lowest for Clusters I and III (4.31) (Fig. 4).

Cluster mean values and trait contributions

The average performance of each cluster for each trait is presented in Table 8. Cluster 4 exhibited the highest DFF (41.00), PH (95.50), and NBP (8.50). Cluster 2 had the highest NPP (45.50), DFH (58.50), PL (10.22), and 100-SW (6.59). Cluster 3 recorded the longest DLH (112.05) and the highest YPP (22.99). Cluster 1 showed the highest NSP (9.90) and moderate values across other traits, including DFF (38.56), DFH (55.30), DLH (107.67), PH (74.28), NBP (4.77), NPP (39.84), PL (8.43), 100-SW (4.82), and YPP (17.17) (Table 8). The results D^2 showed that DLH contributed the most to total divergence at 33.00%, followed by PH (20.10%) and DFH (15.98%), while NBP (0.31%) had the lowest contribution (Figure 5).

Table 7. Clustering pattern of 19 Mungbean Mutants/Varieties Based on Euclidean Distance.

Cluster no.	Total no. of genotypes	Genotypes	Source
I	9	BEMS61	GPB, BAU
		BEMS62	GPB, BAU
		BEMS63	GPB, BAU
		DEMS51	GPB, BAU
		DGR11	GPB, BAU
		DGR12	GPB, BAU
		BARI Mung-6	(BARI)
		Binamoog-8	(BINA)
		Durdona	Uzbekistan
II	2	DEMS21	GPB, BAU
		DEMS62	GPB, BAU
III	7	DEMS22	GPB, BAU
		DEMS52	GPB, BAU
		DEMS61	GPB, BAU
		DGR31	GPB, BAU
		DGR41	GPB, BAU
		DGR51	GPB, BAU
		DGR52	GPB, BAU
IV	1	DGR21	GPB, BAU

Here, GPB, BAU= Department of genetics and Plant breeding, Bangladesh Agricultural University, BARI = Bangladesh Agricultural Research Institute, BINA = Bangladesh Institute of Nuclear Agriculture

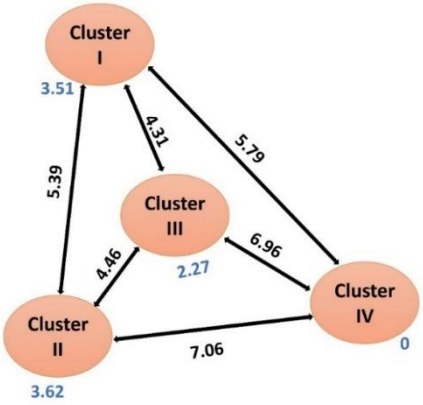

Fig. 3. Average inters and intra-cluster distances (D^2) among nineteen mungbean mutants/varieties in four clusters. Here, intra-cluster distance values are present in blue and inter-cluster distances in black.

Table 8. Cluster mean of ten quantitative traits of nineteen mutants/varieties of mungbean.

Characters	Cluster 1	Cluster 2	Cluster 3	Cluster 4
DFF	38.56	38.67	38.05	41.00
DFH	55.30	58.50	53.76	55.00
DLH	107.67	109.17	112.05	108.00
PH	74.28	56.80	59.84	95.50
NBP	4.77	5.00	4.77	8.50
NPP	39.84	45.50	43.14	19.50
PL	8.43	10.22	9.64	8.84
NSP	9.90	6.45	8.83	9.05
100-SW	4.82	6.59	6.44	4.97
YPP	17.17	15.62	22.99	11.55

Here, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-Seed weight (g), YPP =yield per plant (g)

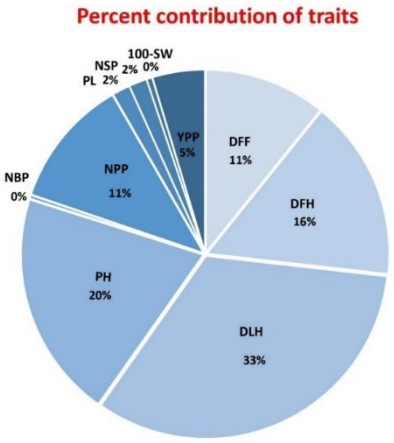

Fig. 4. Percent contribution of all ten characters in nineteen mutant/genotypes towards genetic divergence. Here, DFH= Days to first harvest, DLH= Days to last harvest, PH=Plant height (cm), NBP =No. of branches per plant, NPP =No. of pods per plant, PL =Pod length (cm), NSP = No. of seeds per plant, 100-SW= 100-Seed weight (g), YPP =yield per plant (g)

Discussion

Analysis of variance for yield and yield contributing traits of nineteen mungbean mutants/ varieties

Globally, the primary objective of mungbean breeding programs is to develop varieties with high-yield potential. The availability of breeding material with notable genetic variation is necessary to achieve this goal (Sarkar et al., 2018). Induced mutagenesis, a process of genetic modification through agents like EMS and gamma radiation, is a powerful tool for generating variability and improving yield traits, making it invaluable in genetic research and breeding programs (Pathak et al., 2023; Raina et al., 2016). In this study, ANOVA revealed significant variation for all the studied traits (Supplementary Table 1). Consistent with these findings, Rahevar et al. (2024) observed considerable variation in most traits during the evaluation of mungbean mutants, except for DFF. Ali et al. (2024) and Singh et al. (2011) also observed substantial variability in morphological traits during their assessments of mungbean mutants. This variability offers valuable potential for breeding high-yielding, resilient mungbean varieties suited to diverse environmental conditions.

Mutation breeding has been shown to induce significant genetic variability for flowering and maturity patterns in mungbean (Khattak et al., 2008; Singh, 2009). In this study, the mean performance DFF showed significant variation among the genotypes. DEMS51, BEMS63, and DEMS22 were among the earliest to flower compared to other mutants and their parents, indicating their potential for shorter crop duration (Table 2). In contrast, some genotypes required more time to reach DFF, reflecting variability in flowering periods. This variation is valuable for breeding programs to develop early-flowering varieties adapted to different environments. Yaqoob and Rashid (2001) and Khattak et al. (2008) similarly identified early-flowering mutants in their studies. Mutants such as DEMS61, DGR52, BEMS63, and DGR41 were identified as the earliest maturing genotypes, reaching the first pod harvest sooner than others. Previous research has also successfully identified early-maturing mutant lines through gamma

radiation, with certain varieties maturing in less than 50 days (Sarkar and Kundagrami, 2018). Early-maturing varieties are valuable as post-rice crops in water-limited environments, enhancing water-use efficiency and yield, thus supporting sustainable agriculture in resource-constrained areas (Samson et al., 2020). Significant differences were also observed for DLH, with DEMS22, DGR51, and DGR41 showing the most extended harvesting duration, while DGR11 and Binamoog-8 had the shorter harvesting duration. Prolonged durations to the last harvest, such as those observed in DEMS22 and others, may indicate that these mutants possess an extended flowering period, leading to asynchrony in pod maturity. This asynchrony can sometimes be beneficial, as studies have shown that multiple harvests lead to greater yields than single or double harvests (Bhowaland and Bhowmik, 2014). Conversely, early-maturing genotypes like DGR11, which complete harvesting quickly, are well-suited for regions with shorter growing seasons for boosting cropping intensity.

Mutagenic treatments induced significant variation in PH among the evaluated mungbean mutants, with several mutants exhibiting reduced height compared to their parent varieties. For instance, DEMS21 showed a notable reduction of approximately 20 cm compared to its parent, Durdona. Similarly, DEMS22, DGR51, and DEMS61 displayed semi-dwarf growth habits (Table 2). Interestingly, these shorter-statured mutants ranked among the highest yielders, indicating that reduced PH did not hinder productivity. Previous studies have reported similar findings, where mutagenic treatments led to altered morphological traits such as plant stature and growth habit in mungbean (Reddy, 2006; Ali et al., 2024). In this study, induced mutations led to significant variability in NBP, with values ranging from 8.5 to 4.3. Notably, the mutant DGR21 exhibited the highest number of branches, outperforming its parent, Durdona (Table 2). Previous studies show that gamma rays and EMS effectively increase NBP in mungbean (Khan and Wani, 2006; Dewanjee and Sarkar, 2018). These treatments enhance the number of fertile branches, which boosts light interception and photosynthetic efficiency, ultimately supporting better plant growth and development (Mounika, 2020). Breeding programs prioritize genotypes with higher NPP due to its direct contribution to yield improvement (Azam et al., 2023; Tran and Truong, 2023). This study observed significant variation in NPP, ranging from 19.5 to 59.9. Mutants such as DEMS22, BEMS61, and DEMS21 demonstrated substantial increases in NPP compared to their parent varieties (Table 2). These results underscore the effectiveness of induced mutations in enhancing key yield traits, positioning these mutants as promising candidates for breeding high-yielding mungbean varieties.PL also contributed significantly to the genetic diversity among the studied mutants. In this study, DEMS61 exhibited the longest PL at 10.32 cm, while BEMS63 had the shortest at 7.9 cm, which is in line with other researchers (Wani et al., 2018; Ali et al., 2024). Significant variation in NSP was observed among the mutants in this study, ranging from 8.55 to 11.2 seeds per pod. Mutant BEMS63 demonstrated the highest NSP, surpassing all other mutants (Table 2). This variability presents valuable opportunities for breeders to select genotypes with enhanced seed set potential, directly associated with yield improvement. Similar findings were reported by Dewanjee and Sarkar (2017) and Ali et al. (2024). The advanced mutant DEMS62 recorded the highest 100-SW, indicating its potential for enhanced yield, while BEMS63 showed the lowest value, highlighting the genetic diversity among the evaluated mutants (Table 2). Similar findings in previous studies underscore the critical role of 100-SW in determining yield potential (Ali et al., 2024).

YPP is the primary objective for mungbean breeders, and this study revealed a notable increase in YPP among the mungbean genotypes compared to their parental lines. Significant variation in YPP was observed, with high-yielding mutants such as DEMS22, DGR41, BEMS62, DGR51, DEMS61, and BEMS61 achieving yields ranging from 23.15 g to 28.55 g. In contrast, their parental varieties, including Dorduna (17.05 g), BARI Mung-6 (19.02 g), and the check variety Binamoog-8 (14.95 g), exhibited lower yield potentials compared to these advanced mutants (Table 2). Therefore, these mutants need further characterization in the subsequent generation to obtain high-yielding genotypes. This increase in YPP aligns with previous regional yield trials, where mutant lines consistently demonstrated superior performance over standard varieties (Dewanjee and Sarkar, 2017; Ali et al., 2024).

Estimation of genetic variability, heritability, and genetic advance for yield contributing traits

Genetic variability is essential for effective crop improvement through breeding. The success of breeding programs relies on the availability of genetic variation and the inheritance of desirable traits, enabling breeders to select superior individuals from diverse populations (Yoseph et al., 2022; Kumar et al., 2024). Therefore, estimating heritable components is essential for adopting appropriate breeding procedures. In this study, the observed mutants displayed significant variation, providing ample sources for selecting desirable traits (Table 3). As expected, the PCV was slightly higher than the GCV, a common finding in genetic studies. The narrow gap between PCV and GCV suggests minimal environmental influence on these traits (Meena and Bahadur, 2013; Bhardwaj et al., 2023). High PCV and GCV values for YPP, NPP, and NBP indicate a broad genetic base and considerable

potential for genetic improvement of these traits. (Abbas et al., 2018). Similar findings were also reported by Garg et al. (2017) and Yoseph et al. (2022).

Heritability is crucial for understanding trait transmission from parents to offspring, guiding plant breeders in selecting elite genotypes from diverse genetic pools (Ajaykumar et al., 2023; Jain and Lal, 2024). Broad-sense heritability includes additive and non-additive (dominant and epistatic) variances, providing a comprehensive view of trait inheritance (Nirmaladevi et al.,2015). Traits are categorized based on heritability into high (>60%), medium (30%-60%), and low (<30%) heritability (Robinson et al., 1949). This study noted high heritability for all traits except DFF, which aligns with Ajaykumar et al. (2023) and Jain and Lal (2024). However, heritability values are most informative when paired with genetic advances, offering a solid framework for selection strategies. GA is a crucial parameter reflecting genetic variability and heritability of traits, guiding breeders in prioritizing traits for selection (Wondimu and Bogale, 2017). Genetic advance ranged from 0.78 to 24.91, with PH showing the highest GA, followed by NPP and YPP. The GA (%) offers insight into the potential magnitude of improvement for each trait. PH, PL, NSP, 100-SW, YPP, NPP, and NBP demonstrated high GA (%), indicating significant genetic variability and promising opportunities for enhancement through selection (Table 3). High heritability coupled with GA (%) of the mean was observed for YPP, NPP, NBP, PH, 100-SW, and NSP, indicating strong potential for selection and genetic improvement through breeding. Comparable results have been reported by Salman et al. (2023) who noted high genetic advance in conjunction with high heritability for traits such as YPP, PH, and NPP. Manivelan et al. (2019) also emphasized the significance of traits with high heritability with elevated GA (%) in facilitating genetic gains within breeding programs. By prioritizing traits that show high genetic advance percentages, breeders can effectively refine selection strategies to maximize genetic improvement (Harini et al., 2022). Conversely, traits like DFH and DLH exhibited high heritability but low GA (%), indicating non-additive genetic effects and limited potential for advancement through simple selection processes (Chowdhury et al., 2023).

Correlation analysis

Correlation analysis is essential for understanding trait relationships and how individual traits contribute to a crop's genetic improvement (Zannat et al., 2023). In mungbean, yield is a complex polygenic trait influenced by various independent factors. Understanding these associations is crucial for developing effective selection criteria for yield improvement (Nandini, 2024). Genotypic correlation reflects the inherent genetic association between traits, while phenotypic correlation accounts for both genetic and environmental influences. In this study, most trait pairs exhibited higher genotypic correlations than phenotypic ones (Table 4), suggesting that environmental factors can mask or reduce the phenotypic expression of traits (Kamani et al., 2024). This highlights the importance of considering both types of correlations in breeding decisions. In both genotypic and phenotypic correlation, YPP exhibits a strong positive association with DLH and NPP. This suggests that greater yield is linked to extended growth periods and increased pod production. Additionally, YPP shows a significant positive correlation with 100-SW at the phenotypic level, indicating that heavier seeds contribute to higher overall yield. Conversely, significant negative correlations with DFF and DFH imply that earlier flowering (lower DFF) might be beneficial for yield, as it allows for a longer period for pod and seed development. These findings underscore the importance of optimizing flowering time, pod number, and seed weight to enhance YPP in mungbean. These findings align well with some contradictions to the results reported by Alom et al. (2014), Khan et al. (2024), and Kamani et al. (2024). At both the genotypic and phenotypic levels, DFF exhibited significant positive correlations with NBP and PH, suggesting that early-flowering plants tend to be taller and more branched. DFF also demonstrated a significant negative correlation with DLH at both the genotypic and phenotypic levels. Comparable finding was obtained by Alom et al. (2014) and Mundiyaraet al. (2024). This indicates that earlier harvesting may be associated with reduced harvesting duration (Alam et al., 2011). DFH showed a significant negative association with NSP at the phenotypic level, despite no significant relationship at the genotypic level. This highlights the influence of environmental factors on trait expression, indicating that while earlier harvests may correlate with fewer seeds per pod, the underlying genetic basis may not support this relationship (Kamani et al., 2024). DLH exhibited significant positive correlations with 100-SW and NPP at both levels, indicating that an extended harvesting duration allows for increased seed production and may enhance overall seed weight. Alom et al. (2014) and Mundiyara et al. (2024) also investigated that a higher maturity period can significantly increase the 100-SW and NPP. PH showed significant positive correlations with NSP and NBP at both levels, indicating that taller plants tend to produce more pods and branches. This relationship may result from improved photosynthetic capacity and light interception in taller plants, enhancing biomass and reproductive output (Wang et al., 2015). Conversely, significant negative correlations with 100-SW and PL suggest that increased height may reduce seed weight and

PL, highlighting a potential trade-off between vegetative growth and reproductive traits. Similar trends were reported by Kamani et al. (2024), while Alom et al. (2014) noted a negative association with PL but differing results for 100-SW. Phenotypically, NBP showed a significant negative correlation with NPP, while no significant relationship was observed at the genotypic level, indicating environmental influences on this association. Khan et al. (2024) reported negative correlations at both levels, suggesting that increased branching might intensify resource competition, reducing NPP.PL showed strong positive correlations with 100-SW at both genotypic and phenotypic levels, suggesting that longer pods contribute to greater seed weight, a key yield component consistent with Tabasum et al. (2010). However, the significant negative correlation with NSP indicates a trade-off, as increased PL may reduce NSP. Similarly, the negative correlations between NSP and 100-SW at both levels underscore a compromise between seed quantity and quality, critical for optimizing yield, aligning with Alom et al. (2014).

Path analysis

Given that yield is influenced by interrelated traits, changes in one component can affect the entire cause-and-effect network, altering the magnitude and direction of associations between yield components. While simple correlation studies reveal the nature and degree of trait associations, they do not fully capture the complex relationships influencing yield. Path coefficient analysis is essential for understanding the true associations of component traits in determining yield. Biradar et al. (2007) and Pathak et al. (2023) also highlighted the importance of partitioning correlations into direct and indirect effects for a more accurate understanding of trait relationships.

In this study, path coefficient analysis was conducted with YPP as the dependent variable to assess the contribution of various traits to mungbean yield. Both genotypic and phenotypic analyses revealed that DLH, NPP, and NSP had strong positive direct effects on YPP, indicating their key roles in enhancing yield potential. At the genotypic level, DFF also showed a positive direct effect, while 100-SW had a significant positive effect at the phenotypic level, highlighting the importance of seed weight. These findings suggest that direct selection for these traits could improve yield. DLH and NPP further showed significant correlations with YPP, confirming their importance as yield-determining traits, consistent with previous studies (Tabasum et al., 2010; Alom et al., 2014; Asari et al., 2019). In contrast, NBP, DFH, PL, and PH exhibited negative direct effects on YPP. PH and PL showed strong negative influences at the genotypic level, suggesting that these traits may reduce yield by diverting resources from reproductive growth. Additionally, DFF exhibited a negative direct effect on YPP at the phenotypic level, implying that delayed flowering may lower the yield. These results are in line with previous studies, though some discrepancies were also noted (Biradar et al., 2007; Asari et al., 2019; Nandini, 2024).

At the genotypic level, DFF showed negative indirect effects on YPP via PH and NBP, suggesting that early flowering could hinder vegetative growth. NPP also showed a negative indirect effect through NBP, highlighting that excessive branching might reduce yield. Conversely, at the phenotypic level, 100-SW, NPP, and PL had strong positive indirect effects on YPP through DLH, indicating that longer growth periods enhance yield potential. The positive indirect effects of DLH through NPP further underscore the importance of extended growth durations for maximizing yield. These findings align with previous studies by Mundiyara et al. (2024) and Srivastava et al. (2024). The genotypic path coefficient residual effect was 0.052, indicating that 94.8% of YPP variation is explained by the traits. Similarly, the phenotypic residual effect was 0.057, accounting for 94.3% of YPP variation, highlighting the robustness of the model used in this study.

Principal component analysis

PCA is a powerful statistical technique designed to distill a complex set of variables into a limited number of linear relationships that retain most of the original information (Azam et al., 2023). In this study, PCA identified three PCs with eigenvalues greater than one, accounting for 79.32% of the total variance. PC1 accounted for 38.71%, PC2 for 23.42%, and PC3 for 17.19%, consistent with the findings of Shyamalee et al. (2016). PC1 identified DLH as the most influential positive contributor, with 100-SW and YPP also showing strong positive contributions. On the other hand, PH and DFF had negative contributions, suggesting that these traits may not necessarily align with higher yields, consistent with the findings of Jadhav et al. (2021). PC2 highlighted NSP and YPP, while PC3 emphasized the positive effect of NBP. This observation is corroborated by Shyamalee et al. (2016), who noted similar trends regarding these traits. These results highlight the key characteristics influencing trait variability and provide a deeper understanding of the dynamics governing trait expression in the mutants.

Furthermore, PCA generated biplots that revealed significant genetic diversity among the mutants based on their distribution patterns. The top left quadrant, including mutants 2 (BEMS62), 5 (DEMS22), 8 (DEMS61), 14 (DGR41), 15 (DGR51), and 16 (DGR52), showed superior performance in YPP, DLH, and NPP, indicating the

potential for yield improvement. The top right quadrant, with mutants 1 (BEMS61), 2 (BEMS62), 6 (DEMS51), 18 (Binamoog-8), and 19 (Durdona), was characterized by high NSP, NBP, and PH, reflecting strong plant vigor and reproductive success. Mutants 6 (DEMS51), 10 (DGR11), 11 (DGR12), and 17 (BARI Mung-6) in the bottom right quadrant exhibited early flowering and maturity, while mutants 4 (DEMS21), 7 (DEMS52), 9 (DEMS62), and 13 (DGR31) in the bottom left quadrant showed longer PL and higher 100-SW, useful for improving seed size and productivity. This approach of grouping genotypes using variable biplots to classify a large number of mungbean genotypes based on multiple performance traits has been effectively employed in studies by Kanavi et al. (2020) and Azam et al. (2023).

Genetic diversity studies

D^2 analysis groups genotypes based on trait similarities and differences, offering valuable insights into genetic and phenotypic diversity, which aids decision-making in breeding and conservation programs. This study revealed four distinct clusters, indicating significant genetic diversity within the mungbean population. Cluster I, the largest, comprised nine genotypes, reflecting a broad genetic base, with a notable performance for NSP (Table 8), indicating consistent performance and stable yield potential across environments. Cluster II, with two genotypes, stood out for its highest values for NPP, DFH, PL, and 100-SW, suggesting strong potential for higher pod production and larger seed size, traits directly linked to yield improvement. Cluster III included seven genotypes and exhibited the longest DLH and highest YPP, highlighting its adaptability to extended harvesting periods and potential for higher yield, making it a valuable candidate for yield enhancement programs. Cluster IV, containing a solitary genotype, exhibited the highest values for DFF, PH, and NBP, suggesting delayed flowering, taller plants, and more branching, which may lead to resource competition and reduced yield. This presence of solitary clusters highlights a high degree of heterogeneity, which could be valuable for breeding programs (Mohanty et al., 2020). This clustering analysis emphasizes the genetic diversity within the population and highlights key traits for targeted breeding in yield improvement programs. Interestingly, the clustering was not based on common parentage but rather on morphological differences. Cluster I, for instance, included mutants from different parental lines along with other genotypes, suggesting that phenotypic variations largely drive genetic divergence. This observation aligns with the findings of Dixit and Swain (2000) and Gupta et al. (2001).

The intra- and inter-cluster D^2 values provide valuable insights into the genetic divergence within and between the clusters. For intra-cluster distances, Cluster I and II exhibited higher values, indicating considerable variability among their genotypes. The lower distance of Cluster III and IV suggests that their genotypes have similar genetic constitutions, i.e., homogeneous and less divergent. The largest inter-cluster distance was observed between Clusters II and IV, followed closely by Clusters III and IV. This suggests that the genotypes in these clusters are highly divergent, indicating that crossing genotypes from these clusters could potentially maximize genetic variation in hybridization programs (Mohanty et al., 2020). Comparable findings were also obtained by other researchers (Kingsly and Aravinth, 2023; Srivastava et al., 2024).

The divergence analysis revealed significant variation in trait contributions to genetic divergence among genotypes. DLH, PH, and DFH were the most influential traits, emphasizing their importance for genotype differentiation and targeted selection in breeding. YPP also showed moderate contribution, underscoring its role in improving reproductive success and seed production. These findings align with previous studies (Jadhav et al., 2021; Sridhar et al., 2022; Srivastava et al., 2024), though some studies report differing views on traits like NSP, reflecting the influence of environmental conditions or genetic backgrounds. Overall, the varied trait contributions offer valuable opportunities for targeted selection in mungbean breeding to enhance yield, plant architecture, and environmental adaptability.

Conclusions

This study identified high-performing mungbean mutants (DEMS22, DGR41, BEMS62, DGR51, DEMS61, and BEMS61) with superior seed yield compared to their parental lines and check variety. Variability studies indicated that the PCV was slightly higher than the GCV, suggesting minimal environmental influence on trait expression. High heritability, coupled with a high GA%, was observed for YPP, NPP, NBP, PH, 100-SW, and NSP, highlighting their strong potential for effective selection and genetic improvement. Correlation and path analyses revealed that DLH, NPP, and NSP had the most significant positive direct effects on YPP, while DFF and DFH negatively correlated with yield. In PCA, three components had a significant effect on total diversity. Cluster analysis grouped the mutants into four clusters, with genotypes from Cluster III demonstrating the highest yield potential. The lower distance between Clusters III and IV suggests that their genotypes share similar genetic constitutions, whereas the largest inter-cluster distance between Clusters II and IV indicates substantial genetic diversity, offering opportunities for increased heterosis through strategic crossing. These findings offer a robust framework for selecting and improving mungbean cultivars with enhanced yield potential for breeding programs.

Given their promising performance, top-performing mutants should undergo further field trials to validate their potential and evaluate their stability across different environmental conditions, contributing to the development of high-yielding mungbean varieties vital for combating malnutrition and promoting sustainable agriculture.

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