Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author: Yosep Seran Mau

Assignment title: Agroteknologi

Submission title: Inheritance of seed coat color and heritability of agronomic ...

File name: 2_populations_of_Fore_Belu_and_Local_Sabu_Reciprocal_Cro...

File size: 1.26M

Page count: 10

Word count: 8,275

Character count: 41,652

Submission date: 25-Jun-2023 10:17AM (UTC+0700)

Submission ID: 2122073449

Inheritance of seed coat color and heritability of agronomic characters of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties

by Yosep Seran Mau

Inheritance of seed coat color and heritability of agronomic characters of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties

YOSEP SERAN MAU♥, ANTONIUS S. S. NDIWA, WIDASARI BUNGA, ZAINAL ABIDIN,
TITIK S. HARINI, SHIRLY S. OEMATAN, EFFY ROEFAIDA, ARISTARKHUS TALOIM,
ADRIANA GADJI, MARIA RISNAWATI, RIGOBERTUS ASA NANA

Department of Agrotechnology, Faculty of Agriculture, Universitas Nusa Cendana. Jl. Adisucipto, Kupang 850001, East Nusa Tenggara, Indonesia.
Tel.: +62-380-881580, ♥email: yosepmau@staf.udana.ac.id

Abstract. *Mau YS, Ndiwa ASS, Bunga W, Abidin Z, Harini TS, Oematan SS, Roefaida E, Taloim A, Gadji A, Risnawati M, Nana RA. 2023. Inheritance of seed coat color and heritability of agronomic characters of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties. Biodiversitas 24: 2647-2656.* Mungbean (*Vigna radiata* (L.) R.Wilczek) is an important pulse crop well adapted to the agro-climatic conditions of East Nusa Tenggara Province, Indonesia. However, the productivity of mungbean in East Nusa Tenggara is low (~0.5 t ha⁻¹). Plant breeding programs can overcome this problem by assembling superior varieties. This study aimed to determine the genetic control of seed coat color and heritability of agronomic traits of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties. The observed data included seed coat color and agronomic traits. Seed coat color was subjected to chi-square analysis while agronomic traits were subjected to a simple statistical analysis. The results showed that seed coat color was controlled by one co-dominant gene. The plant heights of F2 fell between the two parental means while the number of pods and seed weight fell below the two parental means. The F2 values range was wide and the maximum F2 values were above the parental means. The phenotypic and genetic variability was wide while either the phenotypic diversity or genetic diversity was moderate to high. The heritability values of the six observed traits were high. The highest was observed on the harvesting date (0.92 and 0.94 for reciprocal crosses), whereas the lowest was on seed weight per plant (0.50 and 0.58 for reciprocal crosses).

Keywords: Fore Belu, gene action, heritability, Local Sabu, reciprocal crosses

INTRODUCTION

Mungbean (*Vigna radiata* (L.) R. Wilczek) is a widely cultivated pulse crop well adapted to the semi-arid agro-climatic conditions of East Nusa Tenggara (ENT) Province, Indonesia. This crop is usually planted as a second-season crop after maize and rice. Mungbean grain is known to contain high nutritional values, especially protein, vitamin B, carbohydrates, folic acid, iron, and phenolic compounds (Xie et al. 2019). Mungbean is also known as a legume/pulse crop that can enrich soil fertility through the fixation of atmospheric nitrogen (N_2) in symbiosis with rhizobium bacteria in the soil (Yimram et al. 2009). Thus, mungbean is one of the most important legume crops due to its contribution to crop diversification and sustainable agriculture, most specifically in the semi-arid dryland agriculture regions in the tropics.

The mean productivity of mungbean in ENT at the farmer level is still low (~0.5 t ha⁻¹), while that at the national level is ~1.18 t ha⁻¹ (BPS 2019). As a comparison, mungbean mean productivity at the experimental farm level in ENT Province could reach about 1.24 t ha⁻¹ (Mau et al. 2017) while the potential yield of superior national varieties can reach up to 2.5 t ha⁻¹ (Balitkabi 2016). The low yielding ability of local cultivars is caused by poor cultivation techniques, drought stress, low yielding ability,

and pest and pathogen infestations. Except for poor cultivation techniques, the other mentioned problems can be overcome through plant breeding programs. The assembly of superior varieties can be done through various ways, such as crosses/hybridizations utilizing parental varieties with superior traits, including those of local varieties (Fehr 1993; Simmonds and Smartt 1999).

About 25 superior mungbean varieties have been released by The Indonesian Ministry of Agriculture from 1945 to 2016. Meanwhile, cultivated varieties of mungbean in ENT Province are mostly local cultivars. Fore Belu is a local mungbean variety of ENT Province that has been released as a national variety (Muge et al. 2005), which is known for its soft seed texture and high mean yields but the weakness is its long duration (70-90 days) (Mau and Bunga 2021), while most national superior varieties are early maturing with harvesting date of around 58-70 days after planting (Balitkabi 2016). The Local Sabu variety is another local mungbean variety of ENT Province, known for its black seed coat color. The black color of the seed coat of legumes indicates the presence of polyphenolic compounds such as flavonoids and anthocyanin which are known to be highly beneficial to human health as an antioxidant, anti-tumor, anti-cancer, anti-hypertension, and can protect the liver, anti-diabetic, anti-cholesterol, etc. (Chávez-Santoscoy et al. 2013; Tsamo et al. 2019; Lim et

al. 2021; Damián-Medina et al. 2022).

Hou et al. (2019) proposed the black seed coat mungbean as a superior variety with high antioxidant content as a source of healthy functional food, which shows the importance of black seed coat mungbean for human health. Besides its black seed coat color, the Local Sabu mungbean cultivar also possesses desirable traits such as short duration (45-50 days) (Mau and Bunga 2021), although the mean yield is still low. Thus, Fore Belu and Local Sabu varieties were hybridized to produce a superior variety that combines the desirable traits of the two varieties. Information on genetic control and the heritability of a desirable trait is useful for the breeders to choose the most appropriate method and time for selecting the desirable trait (Fehr 1993; Simmonds and Smartt 1999).

The superior traits selected in the mungbean segregating populations of Fore Belu and Local Sabu reciprocal crosses included qualitative traits. Effective selection requires high genetic diversity of the traits under selection. Thus, assessment of a trait's genotypic and phenotypic variability in a segregating population is essential to effectively select the genotypes that harbor desirables trait (Ha and Lee 2019; Mogali and Hedge 2020). This study aimed to determine the genetic control of seed coat color and to determine the heritability of agronomic characters of F2 populations of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties.

MATERIALS AND METHODS

Research site
This study was carried out in the Integrative Archipelagic Dryland Field Laboratory of Universitas Nusa Cendana, Indonesia (10.15452° S and 123.66993° E, 110 m asl.) from May to August 2022. The soil type of the study site was Entisol with a clay-loam texture (Mau et al. 2019).

Plant materials
The plant material used in this study included two parental lines (Fore Belu and Local Sabu), F1 plants and F2 families of reciprocal crosses between Fore Belu and Local Sabu varieties (Table 1).

Research design
The experiment was carried out using a Randomized Block Design consisting of the mungbean genotype as treatment. The treatments included two parental materials (Fore Belu and Local Sabu), F1.1 plants of Fore Belu (♀) x Local Sabu (♂) cross, F1.2 plants of Local Sabu (♀) x Fore Belu (♂) cross, 10 F2.1 families of Fore Belu (♀) x Local Sabu (♂) cross, and 10 F2.2 families of Local Sabu (♀) x Fore Belu (♂) cross (Table 1). Each F2 family was derived from a single F1 plant of the reciprocal crosses. In total, 24 mungbean genotypes/populations were evaluated, and each genotype was grown in two plots as replicates, thus, total of 48 experimental units were evaluated. The planting area was cleared of weeds and debris and plowed using a hand tractor. The experimental field was divided into two blocks as replicates, each consisted of 24 plots of 1.5 m x 1.5 m size. Within-block distance was 2 m while within plot distance was 0.5 m.

Table 1. Plant materials employed in the present study

Genotype code	Origin	Source
FB (Fore Belu)	Parental line / Belu District	Belu District, ENT Province
LS (Local Sabu)	Parental line / Sabu-Raijua District	Sabu-Raijua District, ENT Province
F1.1.	F1 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
F1.2.	F1 seeds of Local Sabu (♂) x Fore Belu (♀)	Universitas Nusa Cendana
FBLS.2.1	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.2	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.3	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.4	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.5	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.6	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.7	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.8	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.9	F2 seeds of Fore Belu (♀) x Local Sabu (♂)	Universitas Nusa Cendana
FBLS.2.10	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.1	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.2	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.3	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.4	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.5	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.6	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.7	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.8	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.9	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana
LSFB.2.10	F2 seeds of Local Sabu (♀) x Fore Belu (♂)	Universitas Nusa Cendana

Cultivation and observed parameters

Each mungbean genotype was planted with two seeds per planting hole, but only one plant was retained from one week after planting until harvesting. The planting distance was 30 cm x 30 cm, so each plot consisted of 25 plants. An NPK (16:16:16) compound fertilizer was applied at planting time with a rate of 200 kg ha^{-1}. Irrigation was provided daily during crop growth and development. Weeding was carried out manually, pests were controlled using the insecticide spray using Decis (25 EC) at two weeks and three weeks after planting to control *Aphis craccivora* Koch 1854, while the fungicide Trivia (73 WP) was sprayed once at four weeks after planting to control the *Cercospora* leaf spot disease caused by *Cercospora canescens* Ellis & G. Martin. Pod harvesting was done when about 80% of the pods in each plant within the plot turned black and easy to explode.

Observed variables included plant height at flowering (cm) and plant height at harvesting (cm), harvesting date (days after planting/DAP), number of pods per plant (pod), seed weight per plant (g), and seed coat color. Observations were made on 20 plants of each parental line, 10 plants of F1.1., and 10 plants of F1.2. In the F2 generations of Fore Belu x Local Sabu cross, 150 plants producing seeds were observed, while in the Local Sabu x Fore Belu cross, 170 plants were observed. In total, 320 F2 plants were observed.

Data analysis

Data on the seed coat color of the parental lines, F1 plants, and F2 plants were classified into three classes, i.e., black, green, and black-green mottle. They were subjected to chi-square analysis to determine the genetic control/inheritance of the trait. The observed data of quantitative characters of the F2 population were used to calculate genetic parameters, such as mean, variance, standard deviation, range, and phenotypic variability. In addition, the agronomical traits data were subjected to statistical analysis to determine the mean, range, minimum and maximum values, phenotypic variances, genotypic variance, genetic diversity coefficient, phenotypic diversity coefficient, and heritability values of the traits.

Broad sense heritability was calculated following the formula described by Stansfield (1991) as follows:

$h^2 = \delta^2_G / \delta^2_P$

$\delta^2_P = \delta^2_G + \delta^2_E$

$\delta^2_E = (\delta^2 \text{ Parent } 1 + \delta^2 \text{ Parent } 2)/2$

$\delta^2_P = \delta^2_{F2}$

Where δ^2_G: genotypic variance, δ^2_P: phenotypic variance, δ^2_E: environmental variance, and the heritability was then classified into high ($h^2 \geq 0.5$), moderate ($0.2 < h^2 < 0.5$), and low ($h^2 \leq 0.2$).

The genetic Coefficient of variation and phenotypic coefficient of variation were calculated according to Moedjiono and Mejaya (1994) as follows:

$GDC = (\delta^2_G / \bar{x}) \times 100\%$

$PDC = (\delta^2_P / \bar{x}) \times 100\%$

Where GDC: genetic diversity coefficient, δ^2_G: genotypic variance, $\bar{x}$: population mean, PDC: phenotypic diversity coefficient, and δ^2_P: phenotypic variance. GDC and PDC were classified into low (GDC/PDC$\leq$25%), moderately low (25 <GDC/PDC$\leq$ 50%), moderately high (50 <GDC/PDC$\leq$ 75%), and high (75 <GDC/PDC$\leq$ 100%) (Moedjiono and Mejaya 1994). Principal Component Analysis (PCA) was also performed employing the observed quantitative characters to see the genetic divergence of the F2 population and reveal the characters mostly responsible for the observed variation. All data analysis was performed using a Microsoft Excel program version 2016.

RESULTS AND DISCUSSION

Inheritance of seed coat color

The qualitative trait observed in this study was seed coat color, as the two parents used in the crosses had different seed coat colors, green in Fore Belu and black in Local Sabu (Figure 1). Therefore, reciprocal crosses between Fore Belu and Local Sabu were carried out to determine the genetic control of the trait. As a result, the seed coat color of all F1 plants was green and black mottled, while that of the F2 population fell into three classes, namely green, green and black mottle, and black (Figure 1). The results showed three phenotypic classes of seed coat color in the F2 population. Thus, two hypotheses of inheritance of seed coat color traits were proposed, i.e., one gene pair with codominant gene action or two non-allelic genes with epistatic gene action. The one gene pair hypothesis was 1:2:1 for seed color segregation of green, green and black mottle, and black, while the two gene pairs hypothesis followed the segregation ratios of 9:4:3, 12:3:1, 9:6:1 and 7:6:3 for green, green and black mottle and black seed coat colors. The hypothesis was tested using the chi-square test (χ^2) (Table 2).
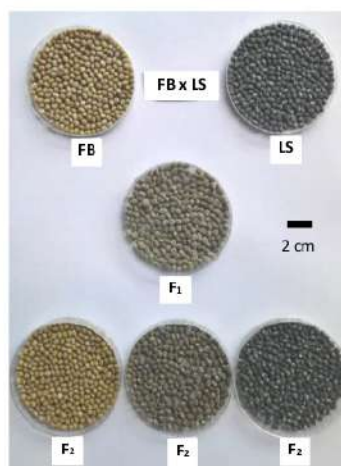

Figure 1. The seed coat color of Fore Belu (FB: green), Local Sabu (LS-black), F1 (green-black mottle), and F2 (left to right: green, green-black mottle, and black)

Table 2. Chi-square (χ^2) analysis of seed coat color trait in the F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties

Hypotheses	Observed			Expected			χ^2 (calculated)	P (0.05)[1]
	Green	Green & black mottle	Black	Green	Green & black mottle	Black		
Fore Belu ($\female$) x Local Sabu ($\male$)								
One gene								
1:2:1	41.0	80.0	29.0	37.50	75.00	37.50	1.33^{ns}	0.51
Two genes								
9:4:3	41.0	80.0	29.0	84.38	37.50	28.13	30.61*	<0.0001
12:3:1	41.0	80.0	29.0	112.50	28.13	9.38	68.51*	<0.0001
9:6:1	41.0	80.0	29.0	84.38	56.25	9.38	29.55*	<0.0001
7:6:3	41.0	80.0	29.0	65.63	56.25	28.13	9.68*	0.087
Local Sabu ($\female$) x Fore Belu ($\male$)								
One gene								
1:2:1	35.0	87.0	48.0	42.5	85.0	42.5	1.067^{ns}	0.59
Two genes								
9:4:3	35.0	87.0	48.0	127.5	31.9	10.6	103.7*	<0.0001
12:3:1	35.0	87.0	48.0	95.6	42.5	31.9	47.04*	<0.0001
9:6:1	35.0	87.0	48.0	95.6	63.8	10.6	56.42*	<0.0001
7:6:3	35.0	87.0	48.0	75.3	64.5	32.3	21.25*	<0.0001

Note:[1] χ^2 (0.05,1) = 3.84 and χ^2 (0.05, 2) = 5.99, ns: not significant at 5% significance level (P>0.05), *Significant at 5% significance level (P<0.05)

The χ^2 analysis results showed that for the Fore Belu x Local Sabu cross, the calculated χ^2 was 1.33 (P=0.51), which is not significantly different (P>0.05) from the 1:2:1 segregation ratio. In contrast, the calculated χ^2 for the two-gene pairs segregation ratios, i.e., 30.61, 68.51, 29.55, and 9.68, respectively, were significantly different (P<0.05) from the seed coat color segregation pattern hypothesis of 9:4:3, 12:3:1, 9:6:1 and 7:6:3. Thus, the results revealed that the seed coat color segregation pattern in the F2 population of Fore Belu cross ($\female$) x Local Sabu ($\male$) cross followed the segregation pattern of one gene pair 1:2:1, which indicates that the seed coat color trait in this cross is controlled by mono-gene with codominant gene action. A similar segregation ratio was observed on F2 population of the Local Sabu ($\female$) x Fore Belu ($\male$), suggesting monogenic inheritance with codominant gene action as observed in its reciprocal cross. As the results of reciprocal crosses (Fore Belu ($\female$) x Local Sabu ($\male$) and Local Sabu ($\female$) x Fore Belu ($\male$)) were similar, there was no cytoplasmic effect in the inheritance pattern of this trait. Hence, the trait is controlled by a nuclear gene. In other words, the direction of the cross does not affect the phenotypic expression of seed coat color traits; thus, the individual F2 plants from reciprocal crosses can be pooled/combined and used for selection in the next generations.

Population parameters of quantitative characters

The quantitative characters observed in this study included plant height at flowering (PHF), plant height at harvesting (PHH), harvesting date (HD), number of pods per plant (NP/P), and seed weight per plant (SW/P). The population parameters of the observed quantitative characters of the F2 population included mean, variance, standard deviation, range, and phenotypic variability (Table 3). The result shows that for the Fore Belu ($\female$) x Local Sabu ($\male$) cross, the means of the three parameters, namely PHF,

PHH and HD, ranged between the mean of the two parental lines. In contrast, the means of NP/P and SW/P were lower than those of the two parents, but in general, the five characters had a wide range of means, with the maximum mean values above that of the two parents. This implies the segregation of these characters in the F2 population. A similar situation did occur in the reciprocal cross, Local Sabu ($\male$) x Fore Belu ($\female$), indicating that the direction of the crosses did not affect the expression of the observed quantitative characters.

The means of plant height of F2 plants at flowering (PHF) of the reciprocal crosses (35.15 cm and 34.73 cm) were shorter than that of the parental line Fore Belu (40.0 cm), but almost similar to that of Local Sabu (34.0 cm). Similarly, the plant height at harvesting (PHH) of the F2 plants (56.41 cm and 52.93 cm) was shorter than that of Fore Belu (67.0 cm), but close to that of Local Sabu (55.0 cm). Meanwhile, the means of harvesting date of F2 plants of the reciprocal crosses (58.14 DAP and 57.27 DAP) was close to that of Local Sabu (59.0 DAP), but shorter than that of Fore Belu (64.0 DAP) (Table 3).

The means of the number of pods per plant (NP/P) of F2 reciprocal crosses (25.97 pods and 24.16 pods) were lower than that of Fore Belu (43.8 pods) and Local Sabu (33.8 pods). Furthermore, the means of seed weight per plant (SW/P) of the F2 populations (17.22 g and 15.16 g) were also lower than that of Fore Belu (26 g) and Local Sabu (20.5 g). Nevertheless, the maximum values of these characters of the F2 populations were higher than those of the two parental lines. Thus, there is an opportunity for these maximum values to be selected in the next generation to produce lines with desired superior traits. The phenotypic variability values of the six quantitative characters fell into a wide variability category. This wide variability category indicates a high level of diversity, which may occur because the F2 populations are segregating populations

with the maximum level of heterozygosity. This high degree of variability provides an opportunity to select the desired traits.

Genetic parameters of quantitative characters

The genetic parameters of the F2 populations were calculated using the observed quantitative/agronomic characters/traits. The estimated genetic parameters of the observed quantitative characters included phenotypic variance and environmental variance are presented in Table 4. The results revealed that the broad sense heritability values ranged from 0.58 to 0.94 for Fore Belu (♀) x Local Sabu (♂) cross, and 0.50 to 0.92 for Local Sabu (♀) x Fore Belu (♂) cross. The heritability range is classified as high, indicating that the observed variability in the studied F2 population is mostly due to genetic factors. Thus, the variability that existed in each trait is highly likely to be passed on to the next generation. Furthermore, as the heritability values of the reciprocal crosses fell into the same range, then, the ability to pass on the trait to the offspring does not depend on the direction of the crosses.

The flowering date had the highest broad sense heritability values of 0.92 and 0.94 for reciprocal crosses, which indicated that the variation in flowering date in F2 populations had a high chance of being inherited by the next generation (F3). The same situation applies to the plant height at flowering (0.70 and 0.74), plant height at harvesting (0.71 and 0.79), and the number of pods per plant (0.83 and 0.87).

Heritability values of seed weight per plant (SW/P) of the reciprocal crosses were 0.5 and 0.58, which indicate a medium to high ability to pass on the trait to the offspring (Stansfield 1991). High seed weight heritability means the variation in seed weight is highly likely to be inherited by the next generation, which can be selected to obtain genotypes with high seed yield. However, as a quantitative character, seed yield character is also influenced by the expression of yield-related components such as the number of productive branches per plant, pod number per plant, the weight of 100 seeds, etc. Thus, the seed yield character can also be indirectly selected by selecting yield-contributing characteristics tightly correlated with seed weight.

Table 3. Population parameter values of segregating F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties

Trait	$\bar{x}$	Range	$\sigma^2\rho$	$2\sqrt{\sigma^2\rho}$	Criteria
Fore Belu (♀) x Local Sabu (♂)					
PHF	35.15	17-46	56.07	14.70	Wide
PHH	56.41	29-75	48.89	13.94	Wide
HD	58.14	53-71	18.30	8.44	Wide
NP/P	25.97	6-89	181.42	26.93	Wide
SW/P	17.22	2-39	59.79	15.49	Wide
Local Sabu (♀) x Fore Belu (♂)					
PHF	34.73	11-49	49.14	14.02	Wide
PHH	52.98	24-65	68.55	16.56	Wide
HD	57.27	52-75	14.23	7.53	Wide
NP/P	24.16	4-56	140.16	23.68	Wide
SW/P	15.16	3-32	49.92	14.13	Wide

Note: PHF: Plant height at flowering, PHH: Plant height at harvesting, HD: Harvesting date, NP/P: Number of pods per plant, SW/P: Seed weight per plant. $\bar{x}$: Grand mean, $\sigma^2\rho$: Phenotypic variance, $2\sqrt{\sigma^2\rho}$: Phenotypic variability

Table 4. Genetic parameters of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties

Trait	$\sigma^2\rho$	σ^2e	σ^2g	$2\sqrt{\sigma^2g}$	Category	h^2_{bs}	Category	GDC	Category	PDC	Category
Fore Belu (♀) x Local Sabu (♂)											
PHF	56.07	14.51	41.56	12.89	Wide	0.74	High	18.34	High	21.30	High
PHH	48.89	14.13	34.76	11.79	Wide	0.71	High	10.45	Moderate	12.39	Moderate
HD	18.30	1.08	17.23	8.30	Wide	0.94	High	7.14	Moderate	7.36	Moderate
NP/P	181.42	24.00	157.42	25.09	Wide	0.87	High	48.31	High	51.87	High
SW/P	59.79	25.20	34.59	11.76	Wide	0.58	High	34.16	High	44.91	High
Local Sabu (♀) x Fore Belu (♂)											
PHF	49.14	14.51	34.63	11.77	Wide	0.70	High	16.94	High	20.18	High
PHH	68.55	14.13	54.42	14.75	Wide	0.79	High	13.92	Moderate	15.63	High
HD	14.23	1.08	13.16	7.25	Wide	0.92	High	6.33	Low	6.59	Moderate
NP/P	140.16	24.00	116.16	21.56	Wide	0.83	High	44.61	High	49.01	High
SW/P	49.92	25.2	24.72	9.94	Wide	0.50	High	32.80	High	46.61	High

Note: $\sigma^2\rho$: Phenotypic variance, σ^2e: Environmental variance, σ^2g: Genotypic variance, $2\sqrt{\sigma^2g}$: Genetic variability, h^2_{bs}: Broad sense heritability, GDC: Genetic diversity coefficient (%), PDC: Phenotypic diversity coefficient (%)

Estimated genetic diversity coefficients of the F2 population showed medium to high categories in Fore Belu (♀) x Local Sabu (♂) cross and low to high categories in its reciprocal (Local Sabu (♀) x Fore Belu (♂)) (Table 4). The highest coefficient of genetic diversity in reciprocal crosses was found in the number of pods per plant (48.31% and 44.61%), followed by dry seed weight per plant (34.16% and 32.8%), and plant height at flowering (18.34% and 16.94%), all of which are classified high according to Stansfield (1991). Meanwhile, the plant height at harvesting and harvesting date had low to moderate genetic diversity coefficients.

In the Fore Belu x Local Sabu cross, four observed traits were classified as high in both genetic diversity coefficient (GDC) and phenotypic diversity coefficient (PDC), except for plant height and harvesting and harvesting date classified as moderate in both GDC and PDC. Similar results were observed in the F2 reciprocals except for PHH, which had high PDC and HD, which had low GDC (Table 3). Meanwhile, PDC values of all observed traits were higher than those of the GDC, but the difference between the two was small except for seed weight per plant (SW/P) which had above 10% differences between DGC and PDC values.

Three observed traits/characters exhibited high heritability, wide genetic variability, and a high coefficient of genetic diversity, namely plant height at flowering, number of pods per plant, and seed weight per plant (Table 4). Furthermore, plant height at harvesting had wide genetic diversity and high heritability values but moderate genetic diversity values. In contrast, the harvesting date was wide in genetic variability, high in heritability but low in genetic variability coefficient. A high heritability value is not always followed by a high coefficient of genetic variability (expected genetic gain) value, as the latter depends on the selection intensity (k) and the additive variance.

Genetic diversity of F2 populations

A biplot of F2 populations based on Euclidean distance is presented in Figure 2. The cross and reciprocal cross of Fore Belu and Local Sabu produced F2 populations that shared almost the same pattern of scatter plots. Individual F2 plants of both the reciprocal cross of the two parental lines were scattered almost evenly along the four quadrants, indicating a high and wide variability level of the population (Figure 2). In the Fore Belu x Local Sabu cross (Figure 2A), six components were responsible for the total variation observed in the F2 population. PC 1 (61.34%) and PC 2 (18.89%) contributed 80.23% for the total observed variation, while other components contributed only around 0.2-10.5% for the variation in the data set. In PC 1, the traits that had positive loading factors included the number of pods per plant (NP/P) (0.84), seed weight per plant (SW/P) (0.45), and plant height at harvesting (PHH) (0.21), while in the PC2, the plant height at flowering (PHF), plant height at harvesting (PHH), and harvesting date (HD) had positive loading scores, respectively, 0.82 and 0.51, and 0.21. Similarly, in the F2 reciprocal cross of Fore Belu and Local Sabu (Figure 2B),

six components were also found to be responsible for the total observed variation, with around 80.23% of the total variation contributed by PC 1 (51.73%) and PC 2 (21.65%). Meanwhile, other components contributed only around 0.2-9.8% to the observed variation in the F2 population. In PC 1, the number of pods per plant (NP/P) had positive and the highest loading score (0.89), followed by seed weight (SW/P) (0.39), and plant height at harvesting (PHH) (0.19). Meanwhile, the plant height at flowering (PHF) and at harvesting (PHH) had positive loading scores in PC 2, respectively, 0.65 and 0.73, and either harvesting date (HD) or seed weight per plant (SW/P) contributed almost negligible loading score (0.04).

Discussion

The present study revealed a novel finding in the inheritances of seed coat color of mungbean in the parental lines employed. The seed coat color of the cross between the green (Fore Belu) and black (Local Sabu) seed coat colors of the parental mungbean varieties was controlled by one gene pair with a codominant action between the alleles. A codominant gene action refers to a type of inheritance in which two alleles of the same gene are expressed separately to yield different traits in a heterozygote individual. As a result, the phenotype of the individual is a combination of the parent's phenotype (Pierce 2017; NIH 2023; Biology Online Dictionary 2023). In the present study, both the green and black seed coat colors were expressed in the form of green-black mottled seed coat color in the F1 seeds (heterozygous) and also in the, presumably, heterozygous F2 individuals (Figure 1). The 1:2:1 ratio of green:green-black mottled:black seed coat colors supports the hypothesis of monogenetic and codominant inheritance of the seed coat color in the studied mungbean cross. The codominant gene action of seed coat color in mungbean has not been reported yet. In comparison, Lixia et al. (2013) demonstrated in their study that seed coat color in mungbean was controlled by mono-gene, and black color was dominant over green while green was dominant over yellow seed color. Similarly, anthocyanin coloration on different parts of the plant was also controlled by mono-gene, and the author suggested that anthocyanin coloration on the seedlings and leaf base might be controlled by that same gene or by tightly linked genes.

Furthermore, in a diallel cross involving four parental mungbean lines with different seed colors, Khattak et al. (1998) reported that black seed coat color was dominant over green, lemon yellow, and pale red, while green was dominant over lemon yellow, and pale red, lemon yellow was dominant over pale red. Additionally, Egbadzor et al. (2014) suspected that many genes are likely involved in the inheritance of seed coat color in cowpea (*Vigna unguiculata* (L.) Walp.), while García-Fernández et al. (2021) found that the genetic control of seed coat color in a recombinant inbred line population of common bean (*Phaseolus vulgaris* L.) are controlled by three independent genes, i.e., one gene is controlling white color and two epistatic genes are controlling the black color. The two genes controlling the black color were confirmed to involve in the anthocyanin biosynthesis pathway.

The results revealed no differences between the cross and reciprocals of the two parental lines in terms of the F1 and F2 segregation of seed coat color, indicating the absence of a cytoplasmic effect on the inheritance of seed coat color and hence the trait is controlled by a nuclear gene. Similar results were reported by Khattak et al. (1998) who found no cytoplasmic effect in the inheritance of seed coat color in mungbean. On the contrary, Laurentin and Benítez (2014) reported that maternal/cytoplasmic effect is responsible for seed coat color inheritance in sesame (*Sesamum indicum* L.). Cytoplasmic/maternal inheritance occurs when the expression of a trait in F1 and F2 generations between a cross and its reciprocals are deferent in terms of the phenotype and segregation ratio of the phenotypes, indicating that the genes controlling the trait is located in the cytoplasm of the cell (Pierce 2017).

In contrast to seed coat color that was classified into green, black, and green-black mottle, the quantitative characters of the F_2 populations evaluated different significantly among families. This would have occurred because quantitative characters are usually controlled by many genes (Stansfield 1991), and their phenotypes are expressed continuously. Therefore, the expression of a quantitative trait is strongly influenced by environmental factors and measures of population and genetic parameters, such as the mean, variance, and coefficient of variation are needed to describe the characteristics of a trait/character. Prajapati et al. (2022) evaluated 39 mungbean genotypes and found high variability in the genetic parameters for yield and their associated attributes. Similarly, Anita et al. (2022) evaluated 11 characters on 38 mungbean genotypes and found significant differences among genotypes for all the characters.

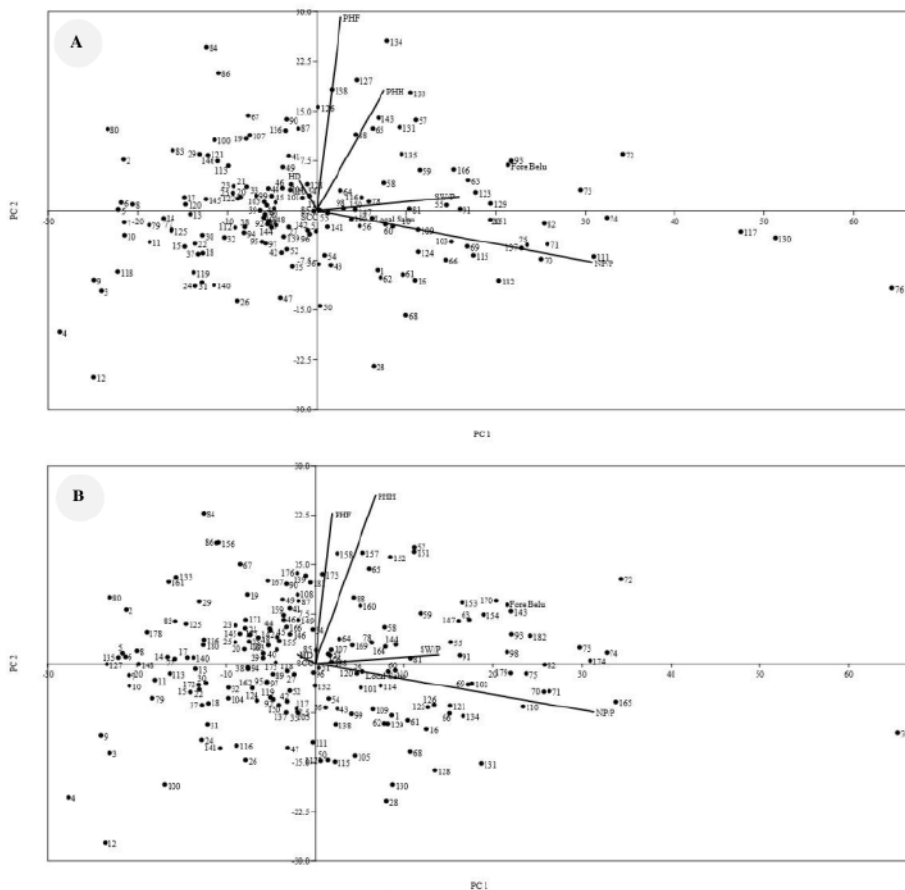**Figure 2.** PCA biplots. A. F2 population from the cross. B. Reciprocal cross of Fore Belu and Local Sabu parental lines. The biplot was constructed based on six traits, i.e., PHF (plant height at flowering), PHH (plant height at harvesting), HD (harvesting date), NP/P (number of pods per plant), SW/P (seed weight per plant) and SCC (seed coat color). Note: SCC was classified into 1 (green), 2 (black), and 3 (green and black mottle)

Population and genetic parameters are important aspects in plant improvement efforts through plant breeding, as information on the genetic parameters of a population will allow the breeders to choose the most efficient selection methods to obtain plant genotypes carrying the desired traits (Trustinah et al. 2021). According to Falconer and Mackay (1996), estimating genetic parameters is a major component of improving plant characteristics that meet the breeding objectives. Population parameters, such as means of observed characters of the F2 families mostly fell below those of the parental lines, indicating a large range of the character's values due to the maximum segregation at F2 generation. However, the maximum values of the F2 population for yield and yield contributing traits fell above that of the two parental lines, indicating heterotic effect and providing a high opportunity to select for genotypes with superior yield and yield contributing traits. In addition, other population parameters, such as phenotypic variability and genotypic variability of the observed traits were classified as wide, so selection can be effective in obtaining the desirable traits.

Heritability is a genetic parameter that describes the ability of an individual/line to inherit a certain character from their offspring. A high heritability value indicates that genetic factors have a bigger role than environmental factors in the expression of a trait/character (Barmawi et al. 2013; Lestari 2016). Characters that have a high heritability value possess a high genetic diversity. Thus, the selection of the desirable traits will be effective and can begin in the early generations.

According to Sanghera et al. (2013) and Rai et al. (2016), high heritability estimates with a high coefficient of genetic variability values are usually used to select superior lines. In this study, high heritability and genetic variability were found in the plant height at flowering, number of pods per plant, and seed weight per plant. The genetic parameters with high values suggest that the observed characters were more influenced by genetic factors as compared to environmental factors. This result is similar to Prajapati et al. (2022) that high heritability, as well as high genetic advance for traits such as plant height, pods/plant, 100-seed weight, seed yield/plant, and number of branches/plant suggesting the expression of these characters, were influenced by additive gene action. Therefore, these characters may serve as effective selection criteria for improvement of the seed yield in mungbean. Additionally, Sharma et al. (2018) evaluated 64 mungbean genotypes for phonological, morpho-physiological, and yield related traits, and found high heritability estimates for most of the traits. The high heritability of the observed traits suggests that direct selection may be carried out for improvement of these traits.

The results also revealed moderate to high genetic diversity coefficient (GDC) and phenotypic diversity coefficient (PDC) values of the F2 population. The PDC values of plant height at flowering, plant height at harvesting, harvesting date, number of pods per plant and seed weight per plant were higher than those of the GDC values. However, there were only small differences between GDC and PDC values except for seed weight per

plant. Therefore, due to the smaller differences between PDC and GDC values, selection based on phenotypic performance for plant height at flowering, plant height at harvesting, harvesting date, and the number of pods per plant would be effective in generating considerable genetic improvement on the tested mungbean population. On the other hand, seed weight per plant that showed a larger difference between GCV and PCV values indicated that the growing environment influences this trait. Thus, the selection is not effective on this trait at F2 generation, and selection of this trait would be more effective in later generations (Adhikari et al. 2018). High GDC and PDC in mungbean had also been reported by Prajapati et al. (2022) in 39 studied genotypes for all observed traits. Similarly, Anita et al. (2022) observed high GDC and PDC for seed yield per plant, pods/ plant, harvest index, plant height, and branches/ plant in 38 mungbean genotypes evaluated. In contrast, Sharma et al. (2018) evaluated 64 mungbean genotypes and found no high estimate for GDC and PDC in the studied genotypes, instead, the authors found only moderate estimates of GDC and PDC for number of clusters/plant, number of pods/cluster, biomass/plant, and number of pods/plant. Our results suggest that the level of genetic diversity coefficient and phenotypic diversity coefficient is very much dependent on the germplasm being evaluated, thus each mungbean germplasm collection must be evaluated to get information on the levels of GDC and PDC that are necessary for selection of desirable traits and improvement of mungbean.

Principal Component Analyses (PCA) is a statistical technique that is used to identify and discard duplicate genotypes with similar characteristics (Singh et al. 2016). In addition, Holme et al. (2019) stated that PCA is also used to classify a large number of variables into important components and determine their contribution to the total observed variation. Our results showed that PCA revealed wide genetic diversity of the studied F2 populations, as shown by the scatter plots of the individual F2 families along the four quadrants (Figure 2). This wide genetic diversity will be very useful for efficiently selecting the traits of interest. The results also revealed that traits that mostly contribute to the observed variability included the number of pods per plant, seed weight per plant, and plant heights. The high genetic variability, as demonstrated by the biplot in Figure 2, is in line with the calculated broad sense heritability (Table 4), thus, these traits are most likely to be effectively selected for the next generations. According to Gayacharan et al. (2020), the first five principal components were responsible for 91.4% of total observed variation, and the biplot of PC1 and PC2 revealed a wide distribution of accessions, which may prove useful in future mungbean breeding programs. Additionally, Mwangi et al. (2021) evaluated a mungbean gene pool and found that the first three principal components (PC) accounted for 83.4% of the total observed phenotypic variation, and pod length, plant height, and seeds per pod were the variables most responsible for the variation. Overall, the present study results revealed high variation among the F2 population on the studied traits, and the traits mostly responsible for the variation, such as number of pods per plant, seed weight

per plant, and plant heights, need to be paid attention in future selection programs.

In conclusion, the seed coat color in the reciprocal crosses between Fore Belu and Local Sabu mungbean varieties was controlled by one codominant gene. Segregation of seed coat color in the reciprocal crosses between the two parental lines was similar, indicating the absence of a cytoplasmic effect on the trait. The heritability values of the six observed traits were high; the highest was observed on the harvesting date (0.92 and 0.94 for reciprocal crosses), and the lowest was on seed weight per plant (0.50 and 0.58 for reciprocal crosses).

ACKNOWLEDGMENTS

REFERENCES

Adhikari BN, Bhatta NR, Shrestha J, Dhakal B, Joshi BP. 2018. Agronomic performance and genotypic diversity for morphological traits among early maize genotypes. Intl J Appl Biol 2 (2): 33-43. DOI: 10.20956/ijab.v2i2.5633.

Anita, Kumhar SR, Kumar A, Gaur GK. 2022. Estimation of genetic variability for seed yield and yield related traits in mungbean (*Vigna radiata* (L.) Wilczek). Biol Forum 14 (2): 503-507.

Balitkabi [Balai Penelitian Tanaman Aneka Kacang dan Umbi]. 2016. Descriptions of Superior Mungbean Varieties 1945-2014. Indonesians Legume and Tuber Crops Research Institute, Malang. [Indonesian]

Biology Online Dictionary. 2023. Codominance. www.biologyonline.com/dictionary/codominance.

BPS [Badan Pusat Statistik]. 2019. Food Crops Production in 2019. Central Statistical Bureau, Jakarta. [Indonesian]

Barmawi M, Yushardi A, Sa'diyah N. 2013. Heritability and genetic advance selection of agronomic characters of soybean F2 population derived from crosses between Yeloow Bean and Taichung. Jurnal Agroteknologi Tropika 1 (1): 20-24. DOI: 10.23960/jat.v1i1.1882. [Indonesian]

Chávez-Santoscoy RA, Gutiérrez-Uribe JA, Serna-Saldívar SR. 2013. Effect of flavonoids and saponins extracted from black bean (*Phaseolus vulgaris* L.) seed coats as cholesterol micelle disruptors. Plant Foods Hum Nutr 68: 416-423. DOI: 10.1007/s11130-013-0384-7.

Damián-Medina K, Milenkovic D, Salinas-Moreno Y, Corral-Jara KF, Figueroa-Yáñez L, Marino-Marmolejo EN, Lugo-Cervantes E. 2022. Anthocyanin-rich extract from black beans exerts anti-diabetic effects in rats through a multi-genomic mode of action in adipose tissue. Front Nutr 9: 1019259. DOI: 10.3389/fnut.2022.1019259.

Egbadzor KF, Yeboah M, Gamedoagbao DK, Offei SK, Danquah EY, Ofori K. 2014. Inheritance of seed coat colour in cowpea (*Vigna unguiculata* (L.) Walp). Intl J Plant Breed Genet 8: 35-43. DOI: 10.3923/ijpbg.2014.35.43.

Fehr WR. 1993. Principles of Cultivar Development-Theory and Technique. Vol. 1. Macmillan Publishing Company, New York.

Falconer DS, Mackay TFC. 1996. Introduction to Quantitative Genetics. 4th Ed. Addison Wesley Longman, Harlow.

García-Fernández C, Campa A, Ferreira AJ. 2021. Dissecting the genetic control of seed coat color in a RIL population of common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 134 (11): 3687-3698. DOI: 10.1007/s00122-021-03922-y.

Gayacharan, Tripathi K, Meena SK, Panwar BS, Lal H, Rana JC, Singh K. 2020. Understanding genetic variability in the mungbean (*Vigna*

radiata* L.) genepool. Ann Appl Biol 177 (3): 346-357. DOI: DOI: 10.1111/aab.12624.

Ha J, Lee SH. 2019. Mung Bean (*Vigna radiata* (L.) R. Wilczek) Breeding. In: Al-Khayri JM, Jain SM, Johnson DV (eds). Advances in Plant Breeding Strategies: Legumes. Springer Nature, Cham. DOI: 10.1007/978-3-030-23400-3_10.

Holme IB, Gregersen PL, Brinch-Pedersen H. 2019. Induced genetic variation in crop plants by random or targeted mutagenesis: Convergence and differences. Front Plant Sci 10: 1468. DOI: 10.3389/fpls.2019.01468.

Hou D, Feng N, Yousaf L, Xue Y, Hu J, Wu J, Hu X, Shen Q. 2019. Mung bean (*Vigna radiata* L.): Bioactive polyphenols, polysaccharides, peptides, and health benefits. Nutrients 11: 1238. DOI: 10.3390/nu11061238.

Khattak GSS, Haq MA, Rana SA, Ashraf M. 1998. Inheritance of seed coat colour in mungbean (*Vigna radiata* (L.) Wilczek). Warasan Technol Suranaree 5 (3): 135-137.

Laurentin H, Benítez T. 2014. Inheritance of seed coat color in sesame. Pesquisa Agropecuária Brasileira 49 (4): 290-295. DOI: 10.1590/S0100-204X2014000400007.

Lestari AP. 2016. Effectiveness of the Selection Method and Environmental Condition for Production of Advanced Rice Lines Under a Sub-optimum Nitrogen Content. [Dissertation]. Post Graduate School of Institut Pertanian Bogor, Bogor. [Indonesian]

Lixia W, Cheng X-Z C, Wang S-H, Liu Y. 2013. Inheritance of several traits in mungbean (*Vigna radiata*). Acta Agron Sin 39 (7): 1172-1178. DOI: 10.3724/SP.J.1006.2013.01172.

Lim YJ, Kwon SJ, Qu S, Kim DG, Eom SH. 2021. Antioxidant contributors in seed, seed coat, and cotyledon of γ-ray-induced soybean mutant lines with different seed coat colors. Antioxidants 10: 353. DOI: 10.3390/antiox10030353.

Mau YS, Ndiwa ASS, Adar D, Gandut YRY, Madu VFY. 2017. Yield performance of eight mungbean (*Phaseolus radiatus*) genotypes in two locations in Manggarai District, East Nusa Tenggara, Indonesia. Intl J Trop Drylands 1: 24-31. DOI: 10.13057/tropdrylands/t010104.

Mau YS, Ndiwa ASS, Markus JER, Arsa IGBA. 2019. Agronomic performance and drought tolerance level of sweet potato hybrids grown in Kupang, East Nusa Tenggara, Indonesia. Biodiversitas 20 (8): 2187-2196. DOI: 10.13057/biodiv/d200812.

Mau YS, Bunga A. 2021. The effect of *Trichoderma* sp. application in several dosages of liquid formula on the growth and yields of Fore Belu and Local Sabu mungbean varieties. Research Report. Faculty of Agriculture, Universita Nusa Cendana, Kupang. [Indonesian]

Moedjiono, Mejaya MJ. 1994. Genetic variability of several characters of maize germplasm collection of Balitkabi (ILETRI) Malang. Zuriat 5 (2): 27-32. [Indonesian]

Mogali SC, Hegde GM. 2020. Recent Advances in Mungbean Breeding: A Perspective. In: Gosal SS, Wani SH (eds). Accelerated Plant Breeding Vol. 3. Springer, Cham. DOI: 10.1007/978-3-030-47306-8_9.

Muge P, Seran YL, Hosang EY, Nulic Y. 2005. The Release of Fore Belu Mungbean Variety. Agriculture Department, East Nusa Tenggara Province, Kupang. [Indonesian].

Mwangi JW, Okoth OR, Kariuki MP, Piero NM. 2021. Genetic and phenotypic diversity of selected Kenyan mung bean (*Vigna radiata* L. Wilczek) genotypes. J Gen Eng Biotechnol 19 (142): 1-14. DOI: 10.1186/s43141-021-00245-9.

NIH [National Institutes of Health]. 2023. Codominance. National Human Genome Research Institute. https://www.genome.gov/genetics-glossary/Codominance.

Pierce BA. 2017. Genetics a Conceptual Approach. 6th Edition. W.H. Freeman, MacMillan Learning, New York.

Prajapati SS, Singh SK, Shrivastava MK, Singh Y, Kumar P, Rahangdale S, Behera K. 2022. Assessment of genetic parameters for greengram (*Vigna radiata* (L.) Wilczek). Intl J Environ Clim Chang 12 (12): 840-848. DOI: 10.9734/IJECC/2022/v12i121522.

Rai PK, Sarker UK, Islam AKS, Rahman MA, Hasan M. 2016. Genetic study and selection in F4 generation of rice (*Oryza sativa* L.). J Biosci Agric Res 9: 768-774. DOI: 10.18801/jbar.090116.92.

Sanghera GS, Kashyap SC, Parray GA. 2013. Genetic variation for grain yield and related traits in temperate red rice (*Oryza sativa* L.) ecotypes. Not Sci Biol 5 (3): 400-406. DOI: 10.15835/nsb539088.

Sharma SR, Khedar OP, Lal C, Sharma V, Varshney N. 2018. Estimation of variability parameters in mungbean (*Vigna radiata* L. Wilczek) genotypes. Intl J Agric Sci 10 (14): 6646-6648.

Simmonds NA, Smartt J. 1999. Principles of Crop Improvement 2nd Edition. Blackwell Science Ltd., Oxford.

Singh S, Prakash A, Chakraborty NR, Wheeler C, Agarwal PK, Ghosh A. 2016. Trait selection by path and principal component analysis in *Jatropha curcas* for enhanced oil yield. Ind Crops Prod 86: 173-179. DOI: 10.1016/j.indcrop.2016.03.047.

Stansfield WD. 1991. Theory and Problem of Genetics. 3rd Edition. Schaum's Outline Series. Mc Graw-Hill Inc., Singapore.

Trustinah, Iswanto R, Hapsari RT, Nugrahaeni N, Soehendi R, Sundari T, Suhartina, Indriani FC, Mejaya MJ. 2021. Genetic parameters for determining useful parents in mungbean (*Vigna radiata* (L.) Wilczek) breeding for early maturity, small seed size, and high seed yield. Scientifica 21 (3): 1-7. DOI: 10.1155/2021/3910073.

Tsamo AT, Mohammed M, Ndibewu PP, Dakora FD. 2019. Identification and quantification of anthocyanins in seeds of Kersting's groundnut (*Macrotyloma geocarpum* (Harms) Marechal & Baudet) landraces of varying seed coat pigmentation. Food Meas 13: 2310-2317. DOI: 10.1007/s11694-019-00150-3.

Yimram T, Somta P, Srinives P. 2009. Genetic variation in cultivated mungbean germplasm and its implication in breeding for high yield. Field Crops Res 112: 260-266. DOI: 10.1016/j.fcr.2009.03.013.

Xie J, Du M, Shen M, Wu T, Lin L. 2019. Physico-chemical properties, antioxidant activities and angiotensin-I converting enzyme inhibitory of protein hydrolysates from mung bean (*Vigna radiata*). Food Chem 270: 243-250. DOI: 10.1016/j.foodchem.2018.07.103.

Inheritance of seed coat color and heritability of agronomic characters of F2 population of reciprocal crosses between Fore Belu and Local Sabu mungbean varieties