



SELECTION AND VALIDATION OF M4 SOYBEAN MUTANT LINES THROUGH MULTI-CHARACTERS APPROACH

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SUMMARY

Mutation breeding is more effective than conventional breeding when the existing breeding material has a narrow genetic base and the scarcity of germplasm to specific desired characters. Assembling superior soybean cultivars through mutation breeding requires an effective selection method since the selection for productivity only proved less effective. Therefore, the selection base needs to be broad and use multi-characters. The latest study aimed to analyze the effectiveness of the multi-characters approach in the selection and validation process of M4 soybean mutant lines. This study evaluated M4 and M5 mutant lines for various traits at the Citayam and Muara Experimental Garden, Bogor, Indonesia. The M4 and M5 mutant evaluations used an augmented design with 121 experimental units (172 lines and four check cultivars) and a randomized complete block design with 99 experimental units (29 lines and four check cultivars), respectively. Measured variables included the number of branches, filled pods, empty pods, and productive internodes, the weight of 100 seeds, and the seed weight per sampling plot. The result showed that the correlated multi-characters were effective for selection in the soybean mutant lines. The most effective secondary character resulted from the number of filled pods. Measuring the selection effectiveness continued up to the fifth generation (M5) to further validate the selected material. In total, nine mutant lines, i.e., A182-13, A179-1, A220-156, A196-57, A186-27, A190-38, A180-6, A195-50, and A188-32, attained selection with significant genetic potential for further study through the yield test.

Keywords: Soybean (*Glycine max* L.), genetic diversity, multivariate analysis, mutation breeding, secondary characters

Key findings: Assembling superior soybean cultivars through mutation breeding requires an effective method of selection. The multi-traits selection method showed efficiency in selecting the promising mutant lines. Obtaining the final 10 mutant lines proceeded based on the greater genetic potential, with further study of these lines through yield testing recommended.

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INTRODUCTION

Soybean (*Glycine max* L. Merrill), a crop considered brilliant gift from nature to humans, is one of the rich oil and protein sources (Fried *et al.*, 2018). Soybean is an economically important legume serving as one of the most used food materials in the global food industry (Timotiwu *et al.*, 2020). Soybean proved a vital commodity for being the raw material of 'tempe,' a highly popular and demanded daily food in Indonesia (Manurung *et al.*, 2018). Generally made from soybean, 'tempe' is a traditional fermented food that undergoes fermentation by *Rhizopus* spp.

Food security, in terms of soybean, is defined as a condition when all people always have sufficient access to soybean and their derivatives to fulfill their needs or requirements. In Indonesia, demand for soybean still gets fulfilled through its import of around 2.48 million t (86.4%) (Bayu, 2021). However, Indonesia profits less with this policy due to the rapid growth in population, i.e., 1.25% from 2010 to 2020, where increase in population also increases food demand (Statistic Indonesia, 2021). In contrast, the present situation faces an increase in agricultural land conversion, worsening the problem, and a challenge in increasing crop plant production, including soybean (Rondhi *et al.*, 2018; Rumanti *et al.*, 2018).

The formulation of advanced agricultural technology to boost soybean production, such as, assembling superior soybean cultivars, needs immediate action. In Indonesia, the soybean productivity is still considered low, ranging from 0.6–2.0 t ha⁻¹ (Mantau, 2015). Soybean importation mainly comes from the USA, which has higher soybean productivity of about 3 t ha⁻¹ (Richards *et al.*, 2015). The assembling of superior soybean cultivars can progress through conventional hybridization and biotechnology techniques (Acquaah, 2012; Syukur *et al.*, 2015). In biotechnology, mutation breeding becomes a favorite and desirable, especially in crop plants with small flowers, such as, soybean (Espina *et al.*, 2018; Zhang *et al.*, 2020; Kim *et al.*, 2021).

In plant breeding, the mutation techniques generally employ irradiation and chemical mutagens. However, both techniques resulted in a varied potential for biodiversity formation (Oladosu *et al.*, 2016; Chaudhary *et al.*, 2019). Although, the use of irradiation, especially with gamma rays, is more popular than chemical mutations (Sikora *et al.*, 2011; Tabti *et al.*, 2018; Djarot *et al.*, 2021). Gamma

rays irradiation can induce abrasions in chromosomal structures so that plants experience physiological and biochemical changes (Datta and Bose, 2012; Sianipar *et al.*, 2015). Yet, mutation through gamma rays successfully produced new cultivars in various crops, including soybean (Oladosu *et al.*, 2016; Yunita *et al.*, 2020). In a previous study, obtaining the M4 soybean populations through gamma rays mutation ensued, which needs further screening for effective selection (Lestari *et al.*, 2019).

In plant breeding, the effectiveness of selection partners with the biometric concept. In quantitative genetics, the production traits, especially productivity, are highly influenced by many polygenic genes (Kassahun *et al.*, 2013; Fellahi *et al.*, 2018). The more polygenic a trait is, the more susceptible a trait is influenced by the surrounding environment and genotype by environment interactions, more so if the character has a low heritability (Acquaah, 2012; Fellahi *et al.*, 2018). This situation confirmed that using only the productivity character cannot directly carry out effective selection for promising populations but requires multiple characters (Anshori *et al.*, 2021; Farid *et al.*, 2021a; Padjung *et al.*, 2021).

Earlier studies frequently reported the use of multiple characters in the selection processes of plant breeding on soybean (Woyann *et al.*, 2019), rice (Alsabab *et al.*, 2019; Akbar *et al.*, 2021), corn (Fadhli *et al.*, 2020), and wheat (Farid *et al.*, 2020). However, authenticating the effectiveness of that selection through an accurate validation can use multivariate analysis. Previous studies reported the use of validation in testing the secondary characters carried out on various crops like maize (Fadhli *et al.*, 2020), wheat (Farid *et al.*, 2021a), and rice (Okasa *et al.*, 2021). Therefore, research evaluating and selecting the M4 soybean mutant lines through the multi-characters approach and multivariate analysis needs implementation. The latest study aimed to analyze the effectiveness of the multi-characters approach in selecting and validating M4 soybean mutant lines for greater genetic potential.

MATERIALS AND METHODS

Breeding material and procedure

The recent research proceeded in February–May 2020 on the latosol soil of the Citayam Experimental Garden, Bogor, Indonesia (-

6.439756756693992, 106.79632802612095) at an altitude of 120 masl. The genetic material for this study used soybean seeds obtained from the M4 - mutant lines of the four cultivars Anjasmoro, Dering, Wilis, and Grobogan. The mutants derived from irradiation treatments used gamma rays at 4 Gy on explants of young embryos and calluses. This study used an augmented design divided into four blocks. In this design, the treatment level of the mutant lines was planted non-repetitively in the block, while repeated varietal treatment took place in each block. Meanwhile, the mutant lines tested consisted of 105 mutants of cultivar Anjasmoro, 12 mutants from cultivar Dering, 43 mutants from cultivar Wilis, 12 mutants from cultivar Grobogan, and four wild types, with a total of 188 experimental units.

The land preparation used the tractor to dig, stir, and overturn the soil. The land design accommodated the irrigation tunnel and planting plots. Seeds planted at a depth of 3–5 cm proceeded with a spacing of 40 cm × 15 cm and one seed per planting hole. Each line followed planting in two rows, and one row consisted of 30 plants. Fertilization progressed two weeks after planting, using NPK Phonska fertilizer (containing 15% N, 15% P₂O₅, and 15% K₂O) at a dose equivalent to 250 kg ha⁻¹. Additionally, the applied manure had a rate of 2 t ha⁻¹. Plants' maintenance took place at least once a week by weeding and pest control using chemical pesticides. Observations ensued on five plants per plot selected because of their healthy morphological appearance.

Data recording occurred on several variables at the generative phase, i.e., the number of branches (units), the number of filled pods (units), the number of empty pods (units), the weight of 100 seeds (g), the seed weight per sampling plot (g), and the number of productive internodes (units). The obtained data underwent analysis of variance in STAR 2.0.1 software, principal component analysis, and correlation analysis in Rstudio software with factoextra package. The use of three types of analyses aimed to assess the character in the selection of M4 soybean mutant lines.

Validation of the selection made in M5 Anjasmoro mutant

This experiment took place in February-May 2021 on the latosol soil of the Muara Experimental Garden, Bogor, Indonesia (-6.61193413118, 106.791114679 at an altitude of 264 masl). The genetic material used in the

latest experiment included 90 lines of the fifth generation of Anjasmoro soybean mutant (M5). This validation happened in a randomized complete block design with a single factor and three replications. The only factor was the soybean genotypes that consisted of 29 genotypes of cultivar Anjasmoro mutant derived from the previous experiment (the M4 experiment) and four check cultivars for comparison. The cultural practice applied in the subsequent experiment followed the previous one. The observations in this experiment focused on the selection of characters from selected samples of the previous experiment (the number of branches, the number of filled pods, and the seed weight per sampling plot). The data analyses on validation used a selection index based on principal component analysis using STAR 2.0.1 software (Anshori *et al.*, 2021) and validation with regression analysis using Excel software (Fadhli *et al.*, 2020).

Principal component analysis

Applying the principal component analysis (PCA) in the present experiment will reduce the complex dimensions into simple ones by using eigenvalues. In general, the PCA algorithm was stated as follows (Jolliffe and Cadima, 2016):

$$\alpha'_k x = \alpha_{k1} x_1 + \alpha_{k2} x_2 + \alpha_{k3} x_3 + \dots + \alpha_{kp} x_p = \sum_{j=1}^p \alpha_{1p} x_p$$

Notes: $\alpha'_k x$ = linear function of the maximum variance element x to k of the main component
 α_{11} = vector of the p constant = transpose of vector matrix.

Determination of the number of main components produced in PCA analysis took place by considering several principal components (PC) that have reached a total variance of more than 80% (Mattjik and Sumertajaya, 2011).

RESULTS

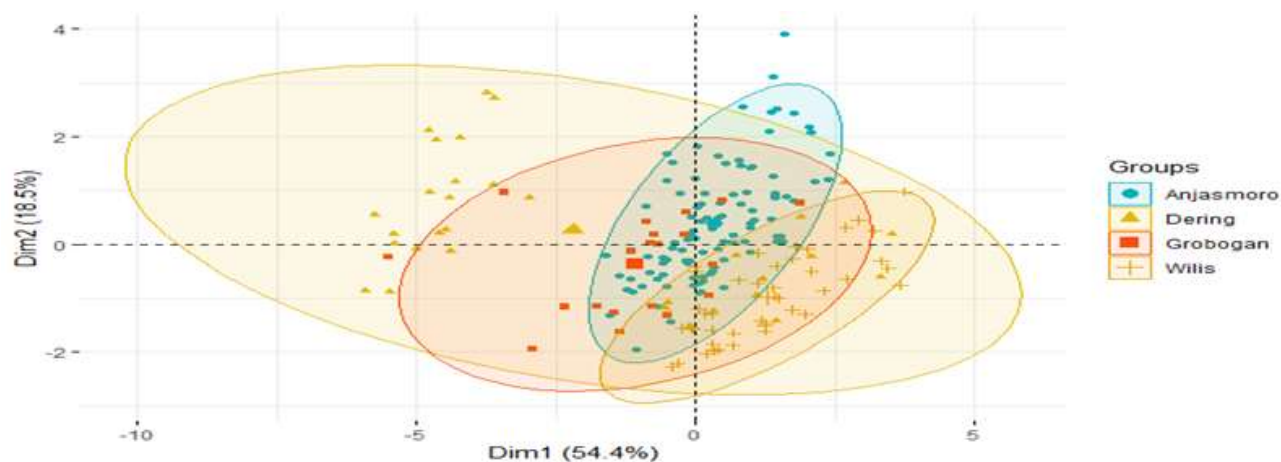
Genetic variance and heritability in M4 mutant lines

The analysis of variance and heritability of M4 soybean mutant lines based on seven agronomical characters appear in Table 1. The genotype diversity had a significant effect on the 100-seed weight (W100S), the number of branches (NB), and the number of filled pods

Table 1. Results of the analysis of variance and heritability of M4 soybean mutant based on seven agronomical characters.

Characters	Line (check)	Check	Line × Check	Error	Vg	Vp	H	CV
W100S	65.70**	83.90**	1.42	0.27	21.79	21.88	99.59	3.65
SWSP	122.69	1578.79*	432.76*	47.27	25.14	40.90	61.47	17.76
NPI	1.83	14.08**	1.15	0.93	0.36	0.67	53.94	11.70
NB	1.02**	1.34**	2.27**	0.12	0.3	0.34	88.24	11.06
NEP	26.23	55.70	169.96	20.41	2.33	9.13	25.51	37.68
NFP	187.98*	666.56**	141.71	16.35	57.21	62.56	91.30	10.63
PH	81.25	524.52**	21.50	30.30	19.12	29.22	65.44	12.36

Notes: ** significant at $p(\alpha)$ 0.01; * significant at $p(\alpha)$ 0.05; W100S = the weight of 100 seeds (g); SWSP = the seed weight per sampling plot; NPI = the number of productive internodes; NB = the number of branches; NEP = number of empty pods; NFP = the number of filled pod; PH = the plant height; Vg = genetic variance; Vp = phenotype variance; H = broad sense heritability; CV = coefficient of variance.

**Figure 1.** The principal component analysis among M4 soybean gamma ray-induced mutant lines.

(NFP). Wild-type cultivars had a relevant effect on all the growth characters except for the number of empty pods (NEP). The interactions between mutant lines and their wild types positively affected the seed weight per sub-plot and the number of branches. All the characters showed moderate heritability at above 50%, categorized as highly heritable except for the number of empty pods (NEP), with the heritability value at only 25.51%.

The biplot analysis was the first in-depth analysis based on principal component analysis (PCA) (Figure 1). The PCA analysis showed that all the cultivars and the mutant line groups relatively have overlapping variance. The widest mutant group of the variants showed in cultivar Dering. However, the mutant groups of the cultivars Anjasmoro and Willis have narrow and centered variants. Although, there were two outlier mutants in the Anjasmoro group. The Grobogan mutant group showed variance that did not center compared with the cultivars Anjasmoro and Willis mutant groups. However, its variants

were not wider than cultivar Dering mutant groups.

Using the heatmap correlation analysis showed that the seed weight per sampling plot (SWSP) significantly revealed positively correlated to the traits, i.e., the number of branches (0.52), plant height (0.5), the number of productive internodes (0.38), and the number of filled pods (0.52) (Figure 2). The number of branches also revealed a significant positive correlation with plant height (0.54), the number of productive internodes (0.66), and the number of filled pods (0.63). Plant height has confirmed a significant positive correlation with the number of productive internodes (0.67) and filled pods (0.72). The number of productive internodes also has a significant positive association with the number of filled pods (0.82).

The results of the boxplot analysis of the three main characters appear in Figure 3. The seed weight of the cultivar Anjasmoro mutants has wide and positive skewness properties (Figure 3A). Mutants of the soybean

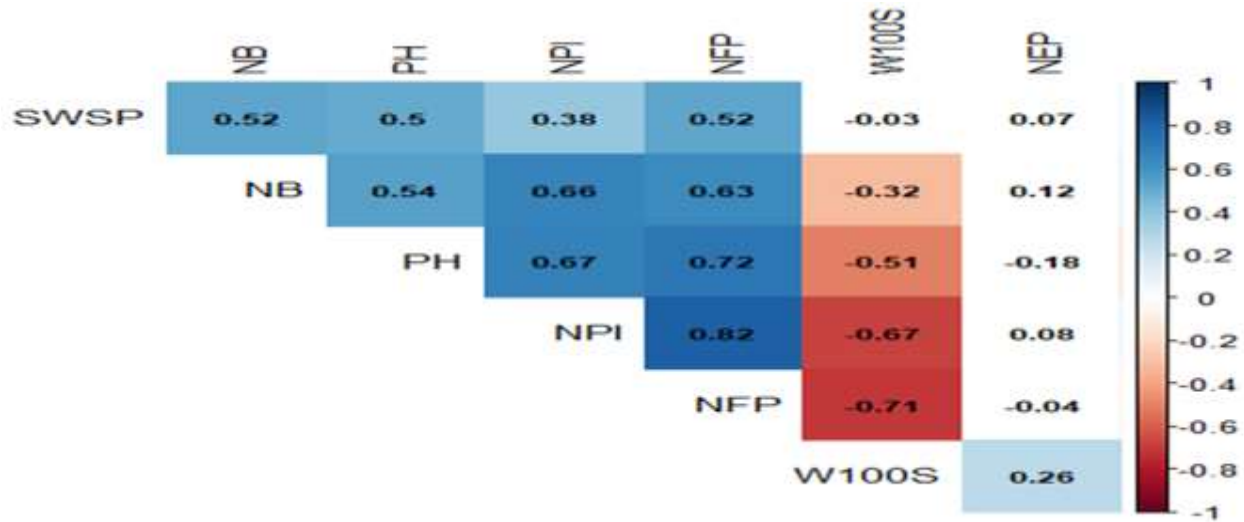


Figure 2. Heatmap correlation among growth characters of soybean mutant (W100S = the weight of 100 seeds [g]; SWSP = the seed weight per sampling plot; NPI = the number of productive internodes; NB = the number of branches; NEP = number of empty pods; NFP = the number of filled pods; PH = the plant height).

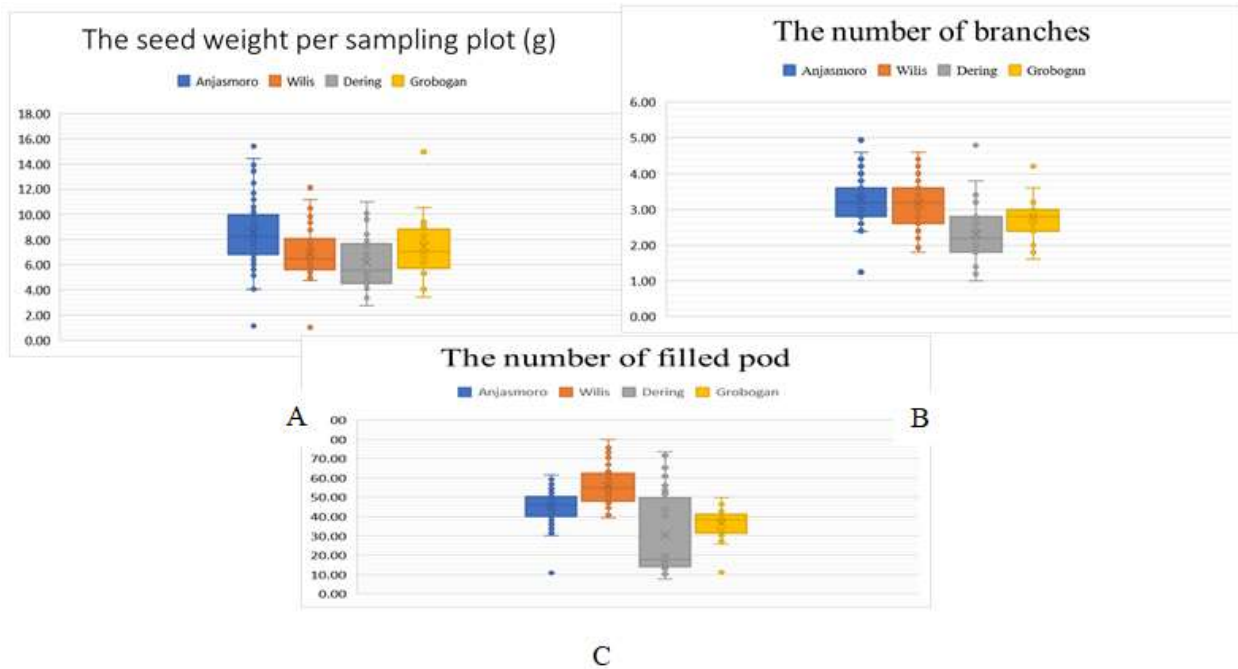


Figure 3. The boxplot of three main characters, i.e., the seed weight (A), the number of branches (B), the filled pods (C), among M4 soybean gamma ray-induced mutant lines.

cultivars Willis and Dering also demonstrated positive skewness properties. In contrast, the mutants group of cultivar Grobogan showed a centralized value, even though one positive outlier mutant exists. Based on Figure 3B, the mutants of the cultivar Dering showed the

highest diversity, whereas cultivars Anjasmoro and Grobogan mutant groups showed the lowest diversity values. The Willis mutant group had relatively greater diversity than Anjasmoro and Grobogan, although the diversity was not as widely found in the cultivar Dering mutant

Table 2. The selected genotype among M4 soybean gamma rays induced mutant lines.

No.	Genotypes	Wild type	Phenotype			Hybrid Vigor			LSD		
			SWSP	NFP	NB	SWSP	NFP	NB	SWSP	NFP	NB
1	A 179-1	Anjasmoro	53.70	54.40	3.60	20.05	16.57	2.23	tn	tn	tn
2	A 179-4	Anjasmoro	55.80	54.20	4.00	21.88	16.32	8.91	tn	tn	tn
3	A 180-6	Anjasmoro	59.40	53.80	4.20	25.01	15.81	12.25	tn	tn	tn
4	A 181-9	Anjasmoro	33.70	45.40	4.00	2.65	5.19	8.91	tn	tn	tn
5	A 181-11	Anjasmoro	44.00	61.60	4.20	11.61	25.68	12.25	tn	a	tn
6	A 182-13	Anjasmoro	72.20	43.20	3.20	36.15	2.40	-4.45	a	tn	tn
7	A 185-26	Anjasmoro	37.60	55.80	4.40	6.04	18.34	15.59	tn	tn	tn
8	A 186-27	Anjasmoro	64.10	40.00	2.40	29.10	-1.64	-17.82	a	tn	tn
9	A 188-32	Anjasmoro	58.50	51.00	4.40	24.23	12.27	15.59	tn	tn	tn
10	A 190-38	Anjasmoro	67.20	44.60	3.40	31.80	4.17	-1.11	a	tn	tn
11	A 190-39	Anjasmoro	77.20	58.60	3.60	40.50	21.89	2.23	a	tn	tn
12	A 191-40	Anjasmoro	47.20	46.80	3.80	14.39	6.96	5.57	tn	tn	tn
13	A 191-43	Anjasmoro	41.70	45.80	3.60	9.61	5.69	2.23	tn	tn	tn
14	A 195-50	Anjasmoro	54.60	56.00	3.60	20.83	18.60	2.23	tn	tn	tn
15	A 196-57	Anjasmoro	69.60	59.40	4.00	33.89	22.90	8.91	a	a	tn
16	A 197-58	Anjasmoro	56.00	60.00	3.60	22.05	23.66	2.23	tn	a	tn
17	A 202-73	Anjasmoro	41.70	53.60	3.60	9.61	15.56	2.23	tn	tn	tn
18	A 215-118	Anjasmoro	45.60	50.20	3.80	13.00	11.26	5.57	tn	tn	tn
19	A 215-119	Anjasmoro	49.70	46.60	3.60	16.57	6.70	2.23	tn	tn	tn
20	A 216-124	Anjasmoro	40.40	49.80	3.60	8.48	10.75	2.23	tn	tn	tn
21	A 216-125	Anjasmoro	54.50	54.80	4.00	20.75	17.08	8.91	tn	tn	tn
22	A 217-132	Anjasmoro	46.70	50.40	3.80	13.96	11.51	5.57	tn	tn	tn
23	A 218-142	Anjasmoro	49.80	48.60	3.60	16.66	9.24	2.23	tn	tn	tn
24	A 219-146	Anjasmoro	55.90	49.20	4.00	21.97	9.99	8.91	tn	tn	tn
25	A 219-150	Anjasmoro	38.70	53.40	3.80	7.00	15.31	5.57	tn	tn	tn
26	A 219-151	Anjasmoro	59.10	52.20	4.00	24.75	13.79	8.91	tn	tn	tn
27	A 220-154	Anjasmoro	43.40	46.00	4.00	11.09	5.95	8.91	tn	tn	tn
28	A 220-155	Anjasmoro	57.10	58.40	4.60	23.01	21.63	18.93	tn	tn	tn
29	A 220-156	Anjasmoro	62.40	56.80	4.40	27.62	19.61	15.59	tn	tn	tn
30	107	Wilis	40.50	53.80	3.60	8.32	3.86	16.18	tn	tn	b
31	109	Wilis	37.30	56.40	3.20	6.07	5.56	12.30	tn	tn	tn
32	111	Wilis	33.60	50.20	3.20	3.48	1.51	12.30	tn	tn	tn
33	112	Wilis	39.70	55.40	3.40	7.76	4.91	14.24	tn	tn	b
34	117	Wilis	31.20	48.80	2.80	1.80	0.59	8.41	tn	tn	tn
35	118	Wilis	39.20	63.40	3.40	7.41	10.15	14.24	tn	tn	b
36	121	Wilis	29.40	51.80	2.20	0.53	2.55	2.59	tn	tn	tn
37	122	Wilis	36.20	62.40	4.20	5.30	9.49	22.00	tn	tn	b
38	124	Wilis	33.20	60.80	3.20	3.20	8.44	12.30	tn	tn	tn
39	125	Wilis	32.20	56.20	2.60	2.50	5.43	6.47	tn	tn	tn
40	130	Wilis	33.10	55.00	2.60	3.13	4.65	6.47	tn	tn	tn
41	131	Wilis	43.80	67.00	4.20	10.63	12.50	22.00	tn	b	b
42	132	Wilis	35.30	71.20	3.00	4.67	15.25	10.36	tn	b	tn
43	133	Wilis	30.80	59.60	2.40	1.52	7.66	4.53	tn	b	tn
44	134	Wilis	52.40	73.20	3.60	16.67	16.56	16.18	tn	b	b
45	135	Wilis	60.60	80.00	4.20	22.42	21.01	22.00	tn	b	b
46	137	Wilis	55.80	70.60	3.60	19.05	14.86	16.18	tn	b	b
47	138	Wilis	46.70	68.20	4.20	12.67	13.29	22.00	tn	b	b
48	139	Wilis	49.40	75.60	3.80	14.56	18.13	18.12	tn	b	b
49	140	Wilis	44.10	55.60	2.80	10.84	5.04	8.41	tn	tn	tn
50	142	Wilis	49.10	63.40	4.60	14.35	10.15	25.89	tn	tn	b
51	143	Wilis	40.70	58.60	3.20	8.46	7.00	12.30	tn	tn	tn
52	144	Wilis	38.90	57.20	3.40	7.20	6.09	14.24	tn	tn	b
53	145	Wilis	38.00	56.80	4.40	6.57	5.83	23.95	tn	tn	b
54	146	Wilis	47.00	61.40	3.20	12.88	8.84	12.30	tn	tn	tn
55	148	Dering	42.00	65.40	3.40	5.27	2.70	4.68	tn	tn	tn
56	150	Dering	35.20	56.20	2.80	1.59	0.36	0.83	tn	tn	tn
57	152	Dering	50.50	73.60	3.40	9.87	4.79	4.68	tn	c	tn
58	154	Dering	55.00	71.60	3.80	12.31	4.28	7.24	tn	tn	tn
59	159	Dering	48.00	72.80	4.80	8.52	4.58	13.65	tn	tn	c
60	Anjasmoro (a)		30.20	43.27	3.47	0.00	0.00	0.00			
61	Wilis (b)		31.70	45.93	1.93	0.00	0.00	0.00			
62	Dering (c)		34.55	51.80	2.88	0.00	0.00	0.00			
63	Grobogan (d)		75.27	18.13	1.80	0.00	0.00	0.00			
The mean of selected genotype			46.70	56.12	3.55						
The mean of genotype population			38.52	44.21	3.04						
Differential Selection (S)			8.18	11.91	0.50						
Percentage of S			21.22	26.95	16.56						

Notes: NB = the number of branches; NFP = the number of filled pod; SWSP = the seed weight per sampling plot; ns = no significant; a,b,c,d = the means significantly different to wild type (a= Anjasmoro, b = Wilis, c = Dering, and d = Grobogan).

group. However, the comparison based on the mean values revealed that the Wilis mutant lines had the highest mean value among other mutant groups. Based on Figure 3C, the cultivar Wilis and Dering mutant groups had a wider diversity than the Anjasmoro and Grobogan mutant groups. Meanwhile, the cultivars Anjasmoro and Wilis mutant groups had a higher average number of branches than the Dering and Grobogan mutant groups. In addition, the Wilis mutant group showed no extreme outliers.

Selection based on three main agronomic characters showed only five lines of the soybean cultivar Anjasmoro mutant group had significantly higher seed weights than their parental genotype (Table 2). In contrast, no one mutant in the other group has significant improvement in terms of seed weight compared with their parents. Based on the positive heterosis values for the parents in three selection characters, 29 mutant lines in the cultivar Anjasmoro group, 25 mutant lines in the Wilis group, and five mutant lines in Dering group consistently have better heterotic responses than their parents (Table 2). In

total, the 59 mutant lines attained selection based on their performance. The selected mutant lines had a percentage of differential selection values for the traits of plant seed weight, number of seed contents, and number of branches, i.e., 21.22%, 26.95%, and 16.56%, respectively.

Validation of the selection made in the M4 generation in the M5

The results of PCA biplot analysis authenticated that the M4 generation achieved optimal diversity in PC2, while for the M5 generation the optimal diversity was adequately represented in PC1 (Table 3). Based on PC2 and PC1 in M4 and M5 generations, respectively, the selection indices were as follows:

- (a) M4 selection index = 0.933 SWSP + 0.337 NPF - 0.126 NB
- (b) M5 selection index = 0.571 SWSP + 0.605 NPF + 0.554 NB

Table 3. The principal component analysis of three selected characters in two generations of soybean gamma ray-induced mutant lines.

Characters	M ₄		M ₅
	PC1	PC2	PC1
The seed weight per sampling plot	-0.1477	0.9331	0.5712
The number of filled pods	0.6788	0.3368	0.6053
The number of branches	0.7193	-0.1262	0.5544
Cumulative proportion	0.5273	0.8824	0.8079
Eigenvalues	1.5818	1.0654	2.4237

Notes: M4 = the 4th Generation, M5 = the 5th Generation, PC = Principal component

Table 4 shows the results of both selection indices, grouped based on the scatter plot analysis as displayed in Figure 4. The scatter plot showed that both M4 and M5 selection indices on 29 Anjasmoro mutants have varying patterns, i.e., nine lines of the positive-positive pattern, seven lines of the positive-negative pattern, five lines of the negative-positive pattern, and eight lines of the negative-negative pattern. Based on the regression analysis of the displayed scatter plot, both indices have a significant regression with a fairly good b value of 0.667.

DISCUSSION

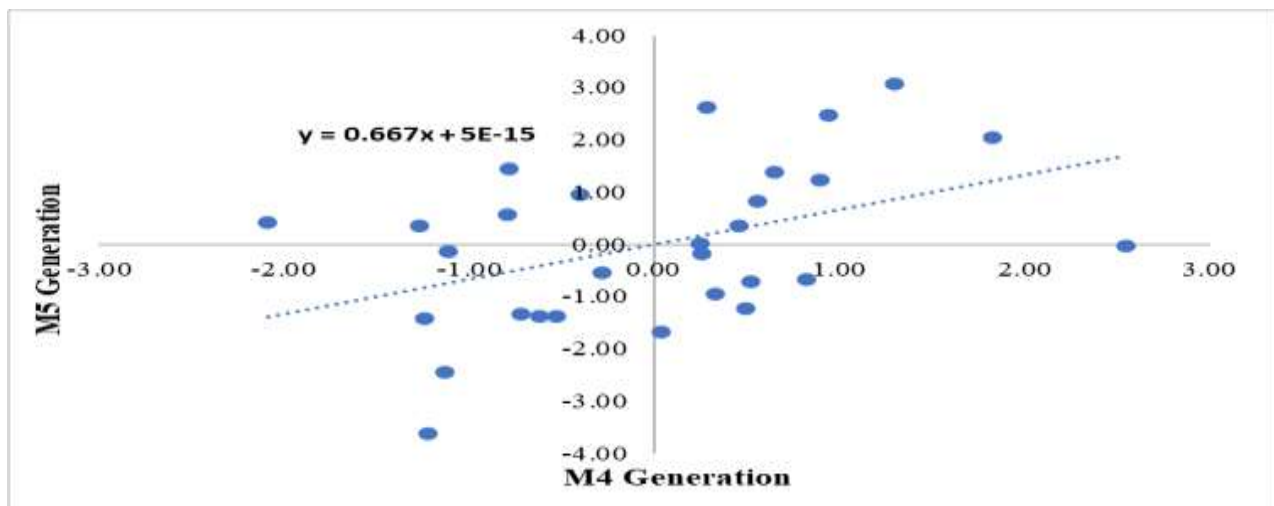
In plant breeding schemes, the augmented design served uniquely used for large

populations (You *et al.*, 2013; Ilker *et al.*, 2018). However, the design highly relates to the control treatment because the determination of error within the design and its effectiveness gets determined by the replications of each control cultivar used (Jambormias *et al.*, 2013; You *et al.*, 2016). In general, the use of control cultivars confirmed effectiveness, as proven by the significant effects of the varietal diversity on almost all observed traits with a low coefficient of diversity (below 20%). In addition, the impact of mutant lines and their interaction on several observed agronomical traits revealed diversity in both inter- and intra-lines. Based on the heritability value, all characters showed high heritability (>50%) (Syukur *et al.*, 2015). Heritability assessment was an important method to predict the degree of response of a

Table 4. Selection index value of M4 and M5 soybean mutant generation.

Genotypes	M4 Generation				M5 Generation			
	SWSP_M4	NFP_M4	NB_M4	SI_M4	SWSP_M5	NFP_M5	NB_M5	SI_M5
A 182-13	1.75	-1.55	-1.45	1.3	1.54	2.11	1.64	3.06
A 179-1	0.06	0.48	-0.51	0.29	1.27	1.08	2.25	2.63
A 220-156	0.86	0.91	1.35	0.94	1.08	1.43	1.79	2.48
A 196-57	1.52	1.39	0.42	1.83	1.57	1.51	0.42	2.05
A 202-73	-1.03	0.34	-0.51	-0.79	1.22	1.41	-0.19	1.45
A 186-27	1.01	-2.13	-3.31	0.65	0.39	1.09	0.88	1.37
A 190-38	1.3	-1.29	-0.98	0.9	1.11	1.18	-0.19	1.24
A 218-142	-0.29	-0.57	-0.51	-0.4	0.28	0.51	0.88	0.96
A 180-6	0.58	0.37	0.88	0.56	0.44	0.28	0.73	0.82
A 191-40	-0.53	-0.89	-0.05	-0.79	0.91	0.13	-0.04	0.58
A 181-9	-1.77	-1.15	0.42	-2.09	-0.05	0.34	0.42	0.41
A 191-43	-1.03	-1.08	-0.51	-1.26	0.11	-0.32	0.88	0.35
A 195-50	0.14	0.77	-0.51	0.46	0.02	-0.25	0.88	0.35
A 188-32	0.5	-0.13	1.35	0.25	-0.56	0.43	0.12	0
A 190-39	2.21	1.24	-0.51	2.55	0.36	-0.09	-0.34	-0.04
A 219-150	-1.31	0.3	-0.05	-1.11	1.38	-0.8	-0.8	-0.14
A 216-125	0.14	0.55	0.42	0.26	-0.22	-0.19	0.12	-0.17
A 181-11	-0.82	1.78	0.88	-0.28	-0.97	-0.09	0.12	-0.54
A 197-58	0.27	1.49	-0.51	0.82	-0.74	-0.39	-0.04	-0.68
A 220-155	0.37	1.2	1.82	0.52	-0.28	-0.87	-0.04	-0.71
A 179-4	0.25	0.44	0.42	0.33	-0.28	-0.7	-0.65	-0.95
A 219-151	0.56	0.08	0.42	0.49	-1.07	-0.57	-0.49	-1.23
A 215-118	-0.68	-0.28	-0.05	-0.72	-0.35	-0.6	-1.41	-1.34
A 215-119	-0.3	-0.93	-0.51	-0.53	-0.58	-0.3	-1.56	-1.38
A 217-132	-0.58	-0.24	-0.05	-0.61	-0.56	-1.17	-0.65	-1.38
A 185-26	-1.41	0.73	1.35	-1.24	-1.5	-0.62	-0.34	-1.42
A 219-146	0.26	-0.46	0.42	0.04	-1.09	-0.74	-1.1	-1.68
A 216-124	-1.15	-0.35	-0.51	-1.13	-0.98	-1.57	-1.71	-2.46
A 220-154	-0.88	-1.04	0.42	-1.22	-2.46	-2.24	-1.56	-3.62

SWSP = the seed weight per sampling plot; NB = the number of branches; NFP = the number of filled pod; SI = selection index; yellow background is the selected genotypes to yield test.

**Figure 4.** The scatter plot based on selection index (F value = 6.57 and P-value = 0.016).

population to both natural and artificial selections (Syukur *et al.*, 2015; You *et al.*, 2016; Gayosso-Barragán *et al.*, 2020). Based on the analysis of variance and heritability, the evaluation and selection of mutant lines in the M4 generation were considered effective.

The biplot analysis is used as the basis for mapping variance that helps visualize the complex data into a simple picture and as a dimensional basis based on PCA (Mendoza-Martinez *et al.*, 2021). The PCA successfully compressed the data with large variables into simpler ones. The compaction process could still maintain the diversity of the original data. The results of the said analysis also did not overlap, and its use was more effective than just based on correlation (Mattjik and Sumertajaya, 2011). The effectiveness of using this analysis has been reported by Hougue *et al.* (2019) in cassava, Fadhli *et al.* (2020) in maize, Donde *et al.* (2020) in rice, Laisina *et al.* (2021) in potatoes, Djarot *et al.* (2021) in sorghum, and Farid *et al.* (2021b) in cocoa. The biplot illustrated that irradiation positively affected the diversity in soybean mutants, with the greater effectiveness of the mutation found in cultivar Dering (Figure 1). However, this effectiveness also does not guarantee that the mutant lines had better heterosis than the wild and non-wild types. This condition also prevailed in the mutant lines obtained in the cultivars, i.e., Anjasmoro and Wilis. Therefore, the evaluation of mutants required a more in-depth assessment.

Assessment of mutants could be more effective if based on the combination of productivity and production attributes. Single character assessment based on productivity resulted in less consistent and stable selected lines (Fellahi *et al.*, 2018; Anshori *et al.*, 2021). Productivity characters displayed as highly polygenic, and the environment also strongly influenced the stability of potential lines, especially in the early generations (Fritsche-Neto and DoVale, 2012; Kassahun *et al.*, 2013; Faot *et al.*, 2019). Therefore, the use of several characters related to productivity ascertained the best solution in the mutant lines selection, with the said concept reported in the soybean (Kachhadia *et al.*, 2014; Teixeira *et al.*, 2017; Bizari *et al.*, 2017), chili (Hasan *et al.*, 2016), sunflower (Kose *et al.*, 2018), rice (Akbar *et al.*, 2019; Anshori *et al.*, 2021; Akbar *et al.*, 2021), and maize (Fadhli *et al.*, 2020).

Correlation analysis can determine secondary characteristics used to detect the association of the various traits in soybean (Faot *et al.*, 2019) and maize (Kinfé *et al.*,

2015; Padjung *et al.*, 2021). Based on correlation analysis, the traits, i.e., the number of branches and the number of filled pods, provided appropriate secondary characters. Plant height had a good correlation but was not included in the evaluation process because of the least diversity at the source in the lines and their interaction with parental cultivars. The number of branches and filled pods have the higher heritability and obtained seed weight, leading to the use of both traits to enhance the effectiveness of selection. According to Acquah (2012) and Syukur *et al.* (2015), the availability of wide genetic diversity with high heritability largely determines the effectiveness of selection activities. Therefore, the number of branches and filled pods were selected as secondary characters in the evaluation process with the seed weight. Previous reports using both secondary characters as selection criteria in soybeans existed in *Glycine max* (Yahaya and Ankrumah, 2015; Faot *et al.*, 2019).

The boxplot is a simple analysis to assess the distribution of obtained data, the diversity, means, and outliers on the dimensions of the XY graph (Praven *et al.*, 2017). This approach was previously used by Vad *et al.* (2016) in root growth, Ge *et al.* (2019) for the physiological properties of maize, and Zhang *et al.* (2020) for respiration flowering in soybean. Based on this analysis, the effectiveness of mutation not only focused on a wide variance but also on the role of the distribution pattern and the mean values of these mutants, which were also important in the assessment. This result analysis also sharpened the result of the biplot analysis. The biplot analysis showed that the diversity of the soybean cultivars Anjasmoro and Wilis mutants have relatively low distribution, whereas cultivar Dering mutants had a wide distribution. However, the Dering mutants have an upward skewness for all three characters, and most data were concentrated in the negative area. Therefore, cultivars Anjasmoro and Wilis mutant lines showed advantage for selection compared with the Dering mutants.

The role of the wild-type means was also crucial in the evaluation and selection process of mutant lines. Sayyad-Amin *et al.* (2017) and Espina *et al.* (2018) also reported this in soybean mutants and Hu *et al.* (2021) for rice mutants. It indicated that the mutant selection process was carried out independently and considered the mean values of the wild type in each mutant population. Based on this concept, the selected mutant

lines also reached 1/3 of the total evaluated mutant lines. In addition, the percentage of differential selection on characters, such as, seed weight and the number of filled pods, were categorized as good. Therefore, the selected mutant lines validate to have a greater genetic potential and recommended to be studied for further validation in future generations.

The selection made in the M4 mutant generation needs to be validated to test its effectiveness in the M5 generation. In the concerned study, the validation focused on the mutant lines of the cultivar Anjosmoro, which were considered better than the mutant lines of other soybean cultivars. Overall, validation can be by scatter plot and regression analysis on three selection characters, namely, the number of branches, the number of filled pods, and the seed weight. Previous reports on the use of scatter plots and regression analysis in the validation of mutation selection came from Fadhli *et al.* (2020), Wei and Molin (2020), Farid *et al.* (2021a), Mendoza-Martinez *et al.* (2021), and Okasa *et al.* (2021). The three mentioned characters were not analyzed independently but were combined to form a selection index through PCA. The selection index served as a good selection method for combining several selected characters, where the selection process proceeded through an index ranking of the genotypes in various crops (Rajamani *et al.*, 2016; Islam *et al.*, 2017). The said concept has also been reported by Akbar *et al.* (2021), Anshori *et al.* (2021), and Farid *et al.* (2021c) in rice, Fadhli *et al.* (2020) in maize, and Farid *et al.* (2021a) in wheat. Hence, the use of PCA based on both generations (M4 and M5) in validation comes highly recommended to enhance the effectiveness of selection. Considering the diversity of the main characters also caused the selection of an effective principal component (PC) as a selection index as reported also in earlier studies (Anshori *et al.*, 2021).

The formation of the selection index in the M4 generation resulted from PC2 with the highest eigenvalue (0.9331) of productivity. Meanwhile, in the M5 generation, PC1 was considered a good one because it accumulated a cumulative proportion of more than 0.8. Mattjik and Sumertajaya's (2011) findings showed that PC optimization occurred when the cumulative proportion of PC reached 0.8 or above. The PCA results in M4 generation showed a negative value for the number of branches because the said analysis took place on 29 selected genotypes. However, at the M5

index, the number of branches showed a positive eigenvalue, similar to the number of filled pods and seed weight. Therefore, the inclusion of the number of branches in the M4 generation remained in the selection index as a correction of the index value. Although, based on the PCA, the number of filled pods was considered successful and stable as a secondary character in soybean mutant selection.

Based on the scatter plot distribution and regression analysis, the selection of mutant lines at the M4 level based on the selection index was considered effective against the validation in the M5 generation. It was likely that the significance of the regression and the value of 'b' reached 0.667. The value of 'b' in the intergenerational regression can serve useful as a narrow sense heritability value in the parent-offspring analysis (Acquaah, 2012). Narrow sense heritability was an essential indicator in assessing the inheritance of traits between generations. Govintharaj *et al.* (2017) reported employing the parent-offspring estimation concept in soybean mutant line selection. In addition, the clustering error in the scatter plot analysis (positive-negative and negative-positive patterns) was rated lower than the correctness of the clustering between both generations. Therefore, based on the narrow sense heritability value and the clustering of the scatter plots, the relationship between the two indices was considered good, and the selection in the M4 generation was considered beneficial.

CONCLUSIONS

The selection based on multiple correlated characters proved highly effective at M4 soybean mutant lines. The number of filled pods as the secondary character showed effectiveness in the soybean mutant selection. The nine Anjosmoro mutant lines (A 182-13, A 179-1, A 220-156, A 196-57, A 186-27, A 190-38, A 180-6, A 195-50, A 188-32) attained selection based on significant genetic potential and require the further yield testing. In yield testing, the multiple characters could enhance the effectiveness of selection at the multi-locations.

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