BIODIVERSITAS
Volume 25, Number 8, August 2024
Pages: 3476-3485

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250819

Evaluation of performance, diversity, and trait relationships of butterfly pea (*Clitoria ternatea*) genotypes M4 generations

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Manuscript received: 4 June 2024. Revision accepted: 11 August 2024.

Abstract. *Zulchi T, Husni A, Utami DW, Reflinur, Kosmiatin M, Maulana H, Suganda T, Concibido V, Karuniawan A. 2024. Evaluation of performance, diversity, and trait relationships of butterfly pea (*Clitoria ternatea*) genotypes M4 generations. Biodiversitas 25: 3476-3485.* Butterfly pea, a versatile forage plant with perennial growth and adaptability, is the focus of a crop improvement program aimed at enhancing production performance and nutritional contents through mutation polyploid induction. This study aimed to identify butterfly pea genotypes with higher agronomic values, genetic diversity, trait relationships, and traits-specific excellence. The mutants (M4) comprised 95 genotypes, and local varieties were used as controls. The experiments were conducted in an augmented design with local cultivars used as checks in Bogor, Indonesia, from March to August 2021. The results revealed that butterfly pea genotypes improved in stem diameter, fresh and dry weight biomass, and number of seeds per pod compared to local cultivars by 8.6%, 9.07%, 7.82%, and 5.37%, respectively. The nutritional content of protein, carbohydrates, and lipids was also enhanced. Additionally, early flowering times were detected for the butterfly pea genotypes. Further, principal component analysis indicated that the genetic diversity of butterfly pea mutants is broad, underscoring the richness of the research data. The four axes of the analysis had eigenvalues ranging from 2.573 to 1.129, with a cumulative value of 67.56% and a degree of variation contributed by dry-weight biomass. Genetically, butterfly pea genotypes can be categorized into four groups on genetic distance, with several variables displaying positive correlations. Butterfly pea genotypes such as BP78, BP70, BP58, and BP46 have been earmarked as genotypes possessing high biomass and good nutritional qualities. The butterfly pea genotypes in the current study have the potential to be selected for improvement or utilized as a parent in a hybridization program.

Keywords: Augmented, butterfly pea, dry weight, flowering, mutants

Abbreviations: BP: Butterfly Pea; PCA: Principal Component Analysis; GEI: Genetic and Environment Interaction; MASL: Meters Above Sea Level

INTRODUCTION

Butterfly pea (*Clitoria ternatea* L.) is a member of the legume family and an underutilized crop. Its perennial growth, nutritional benefits, and adaptability to diverse environments have prompted recommendations for its use as fodder or forage (Schultze-Kraft et al. 2018). Butterfly Pea (BP) field-grown plants yield fodder production that ranges from 4-6 t/ha dry matter or 8-12 t/ha/year fresh matter as a year-round source of forage adaptable to various climates and the biomass yield is higher than the herbaceous plant *Stylosanthe*s sp., *Lablab* sp., *Centrosema* sp. (Nulik et al. 2017). These plants are diploid 2n=2x=16 and largely self-fertile, but some accessions are partially outcrossing and have blue and white petals. As earlier indicated, the crops contain high nutrition, with protein content in the plant and seed ranging between 13-23% and 38-43%, respectively (Heuzé et al. 2016). These plants are highly palatable, digestible by livestock, and can be combined with grass and used as feed in hay or fodder forms (Capstaff and Miller 2018). Thus, this plant exhibits immense potential for livestock feed due to its biomass production abilities and nutritional qualities.

The ever-increasing livestock populations necessitate substantial forage resources. In 2022, the total livestock population comprised 65.3 million heads of cattle, buffaloes, horses, goats, sheep, and pigs (Ditjen PKH 2023). The demand for animal feed, particularly fresh forage, constitutes 10-12% of livestock weight (Nulik et al. 2013). Forage legumes like butterfly peas yield abundant biomass, fodder, and optimal nutrition and comprise essential feed nutrients such as carbohydrates, proteins, vitamins, fiber, and minerals (Shamnad 2019). Increased fodder resources are required due to the growing numbers of animals. Productive butterfly pea plants are important because they contribute significantly to the supply of plant biomass and nutrients, which can be essential for population livestock. Biomass production and nutritional qualities are necessary to produce superior forage crops, which can be accomplished by induction of genetic variation. Genetic diversity is the foundation for the selection and utilization of butterfly peas. Although the morphological characteristics of butterfly

pea petals are relatively diverse, there is little information regarding genetic diversity for its use as forage for livestock (Karuniawan et al. 2021). Polyploid induction technology has increased genetic diversity with cell size, chromosome duplication, greater plant morphology, and nutritional content. Polyploidy induction is useful in forming important traits and meeting market demand (Ghanbari et al. 2019). Polyploidy induction provides insight into genetic diversity, which indicates variation in individual genotypes (Acquaah 2012). Artificial mutagen induction has been used to enrich genetic diversity (Oladosu et al. 2016) and increase the metabolic efficiency of plants by doubling chromosomes (Hwang et al. 2015; Innes et al. 2021). Enhancement of genetic diversity through polyploid induction can provide an understanding of the potential of an individual plant.

The selection stage of genetic material requires character identification. Studies on butterfly pea germplasm diversity involve the morphological characterization of plants (Karuniawan et al. 2021; Nurhasanah et al. 2023). Such studies are combined with principal component analysis (PCA) and cluster analysis (Girgel 2021), which simplifies the assessment of each character's contribution to trait variation (Manivannan et al. 2016; Sattler et al. 2016). Clustering was used to create a PCA-based biplot to group the variables and to clearly visualize the relationship among variables (Baraki et al. 2023). Furthermore, genotype diversity can be evaluated through the Genotype-by-Yield Traits (GYT) biplot approach, where the traits combined with the yield crop can show the trait profile of the genotype (Yan and Frégeau-Reid 2018). Character variation in genotypes can inform parent selection for crossing or genotype selection.

Agronomic characterization of *C. ternatea* genotypes is essential in determining their genetic diversity and utility. The objectives of this study were to: (i) Identify BP genotypes that had higher values than check varieties for all traits; (ii) BP genetic diversity is identified based on morphological and nutritional traits; (iii) Identify relationships between traits tested; and (iv) Select a BP that has excelled in specific traits.

MATERIALS AND METHODS

Experimental methods and plant materials

The research was conducted at the Cikeumeuh experimental station in the Indonesian Center for Agricultural Biotechnology and Genetic Resources Development (ICABIOGRAD/BB Biogen), Bogor, Indonesia, from March to August 2021. This station lies within Bogor City, situated between 106°43'30 "East" and 106°51'00 "East," and 30'30 "LS" and 6°41'00 "S" with an elevation of 250 Meters Above Sea Level (MASL). This location's climate is classified in the tropical B1 category, per Oldeman's classification (Wijaya 2021). Climatic and soil conditions at the experimental site are listed in illustrated in Table 1. The research and conservation plant for M1 to M3 generation is located in the Cimanggu greenhouse BB Biogen, Bogor. Therefore, 95 M4 generation butterfly pea genotypes were developed from local East Nusa Tenggara (ENT), Indonesia cultivars with blue and white petals. The genotype butterfly pea M4 generation material mostly came from oryzalin induction treatment and some from colchicine induction. Some seeds of the ENT cultivar were induced with 12 µM oryzalin for 1-6 days in vitro (O1, O2, O3, O4, O5, and O6), while other seeds were induced with 0.5 Mm colchicine (C1, C2, C3, C4, C5, and C6). The meticulous process and continuous germination of butterfly pea mutants in each generation is crucial. The second and third-generation seeds experienced decreased seed vigor and were resistant to plant disease. Mutant plants are maintained, and third-generation seeds are mass-selected for plants with high biomass. The M3 generation material remains the butterfly pea genotype resulting from oryzalin induction (O1-O6), while colchicine induction takes 1 and 2 days (C1-C2).

Furthermore, 95 butterfly pea genotypes and three well-known control varieties were evaluated. These are blue and white petals ENT and 1 local Bogor. The experimental field was divided into six blocks. Each block contained replicated control varieties and a subset of the 95 new genotypes. Randomly assign the new genotypes to positions within each block. The three control varieties are systematically placed within each block. A total plant of fourth generations with bulk-selection had evaluated biomass production yield in the Bogor experimental field, Indonesia. Agronomic morphological characteristics such as plant height, stem diameter, flowering, pod and seed weight, and fresh and dry weight matter were evaluated for the M4 generation of butterfly peas.

When planting, urea and phosphate fertilizers were applied at rates of 100 kg/ha and 200 kg/ha, respectively, along with manure fertilizer. Bamboo provided a stand for the butterfly pea genotype 4 Weeks After Planting (WAP). The variables observed were various plant traits, including Plant Height (PH), Stem Diameter (SD), Early Flowering (EF), 50% Flowering (50%F), Pod Weight (PW), Pod Number (PN), Seed Number per Pod (SP), Fresh Weight (FW), Dry Weight (DW) biomass, branch number per plant (SVP), and 100 seed weight (Y100), conducted statistical analysis on the collected data (Muchugi et al. 2023). Then, the flowering time variable was calculated as the beginning of the flower, and 50% flowering time was calculated as 50% flowering of the total plant population. Carbohydrate, protein, and lipid content were analyzed from plant dry matter using rigorous methods; carbohydrate analysis uses the difference method, protein content uses the Kjeldahl method, and lipid content uses the Soxhlet method.

Table 1. Climatic conditions and soils in Bogor City, Indonesia in 2021

Parameter	Range
Climatic [a)]	
Temperature (°C)	25 - 32
Air humidity (%)	62 - 74
Rainfall (mm)	46.5 - 488.31
Soils [b)]	
Type soil	Inceptisol
Cation exchange	21.30
pH	4.9 - 5.7
C/N Ratio	12

Note: [a)]Bogor Annual Weather Averages - West Java, Indonesia (worldweatheronline.com), [b)]Laboratory of Soil Research Institute, Agriculture Department

Data analysis

In research design, an augmented design employs genotypes as treatments on check varieties. Therefore, to analyze the data, a correction factor was computed for the observation data within the groups. A variance (ANOVA) analysis of check varieties was performed with data processed using XL Stat version 19 and set an alpha value of 0.05. This analysis corrected for genotypes, treating three check varieties as having fixed effects. In each block, three checks and 16 genotypes were randomly distributed, such that genotypes were compared against varieties of checks using a design with six blocks. For each genotype, 5 plants were planted, and data was collected from three plants. The difference between the means of checks within each block and the entire trial was used to adjust for block disparities (Petersen 1994). This design's analysis needs an adjusted factor on the block observation data.

Data analysis results from calculating the adjusted factor for the observation data in each block. The adjusted factor data from each block was a useful comparison between the adjusted yield of a new selection and a check mean. Finding hypothetical variables that can explain as much of the variance in multidimensional data as possible is done through the Principal Component Analysis (PCA) process. Additionally, the level of similarity between genotypes was assessed using biplot analysis with data processed using XLStat version 19.

RESULTS AND DISCUSSION

Performance morphology, yield, and nutritive

The induction of polyploidy mutagens in butterfly pea genotypes led to notable changes in morphology, genetics, and plant nutrition. Various performance butterfly pea genotype traits experienced changes due to oryzalin (O) or colchicine (C) induction, with several plant traits superior to controls, as shown in Figure 1. Analysis of the results of an experiment designed to determine the growth performance and biomass yield of butterfly peas from the influence of various sources of oryzalin and colchicine induction compared to checks variety. Butterfly pea plant height was dwarfed unless immersion in oryzalin for 6 days. The stem diameter was large, induced by oryzalin for 3-4 days and colchicine for 1 day. The pod number was higher induced by oryzalin for 3 days and colchicine for 1 day. Pod weight and 100 seed weight were increased induced by oryzalin for 4-5 days and colchicine for 1-2 days. The increase in fresh weight of butterfly peas was caused by oryzalin induction for 3-4 days or colchicine for 1 day, while dry weight increased with oryzalin induction for 2-5 days.

The production yield was conducted to evaluate 95 butterfly pea genotypes. In the M4 generation, significant changes in various traits were observed, as illustrated in Figure 1. Notably, all genotypes exhibited a reduction in plant height compared to the check varieties. Similarly, pod number, pod weight, branch number, and 100 seed weight of all genotypes were decreased to compare control varieties, while the flowering time was elevated by 3 to 4 days. Polyploids grow slower, with less branching, or pod, than diploids since induction polyploids have less auxin than their diploid counterparts. Polyploids typically take flowers later, changing photoperiods and photosynthesis (Acquaah 2012). A loss-of-function mutation could be deleterious if the modified gene product no longer performs the same role as the wild-type gene product (Dwivedi et al. 2023). A selection of chemical mutation agents and their optimum concentration may be required for less deleterious mutations (Rauf et al. 2021).

Table 3 shows stem diameter as genotypes BP78, BP79 (O4), and BP69 (O5) exhibited significant improvement with induction oryzalin induced for 4 or 5 days. Fresh weight and dry weight of biomass as genotype (BP6 (O1), BP23 (O3), BP78 (O4), BP85 (O5) revealed higher values with induction oryzalin induced for 1 to 5 days. The number of seeds per pod as genotype (BP16 (O2) showed a considerable increase with induction oryzalin induced for 2 days. Based on Table 3, plant height traits (BP92, BP93 (O5); the number of pods (BP30 (O1), BP44 (O3), BP66 (O5), BP49 (C2); and pod weight (BP81 (O4), BP44 (O3) also increased, and flowering time appeared several days earlier except BP45 (O3) which had late flowering. The genotype BP84 (O5) had a high S100 due to immersion in oryzalin for 5 days. Butterfly pea plants are characterized as self-pollinated climbers, exhibiting year-round flowering, homozygous genetics, and diploid 2n=16. Polyploid induction technology has been deployed to increase genetic diversity, creating morphological and genetic variations in butterfly peas. A greater interest in creating synthetic polyploids for use in agricultural breeding operations has resulted from the improved traits of polyploid individuals. Oryzalin induction provides superior viability, growth, and yields compared to colchicine. These substances, oryzalin, have a higher affinity for plant tubulins (Sattler et al. 2016).

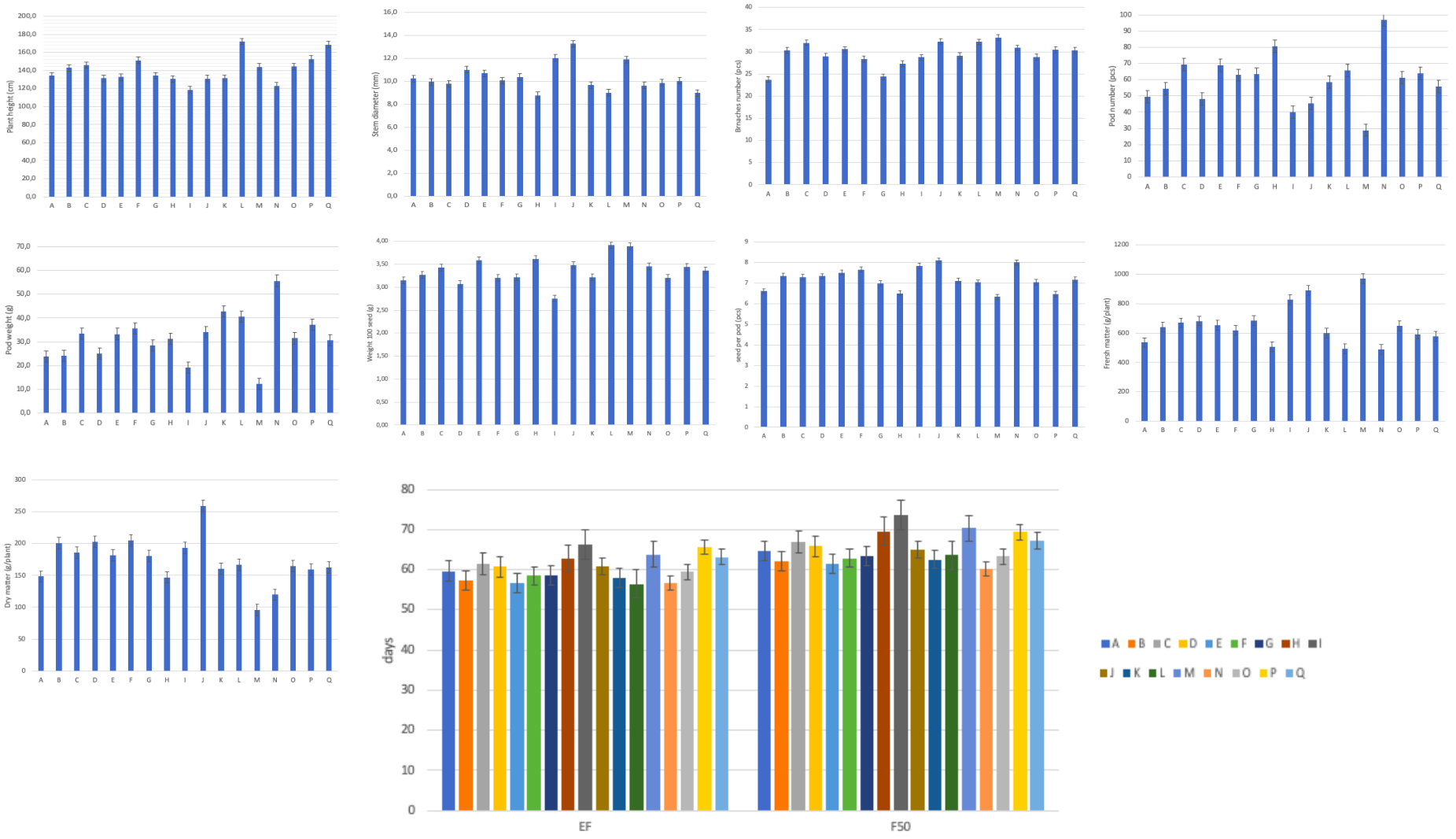

Figure 1. Performance morphology of butterfly pea genotype (A-N) and Checks (O, P, Q) in mean data. Note: PH: Plant height; SD: Stem diameter; EF: Early flowering; F50: 50% flowering; PW: Pod weight; PN: Pod number; FM: Fresh weight biomass; DM: Dry weight biomass; SP: Number seed per pod; SVP: Number branch/plant; S100: 100 seed weight. A: Immersion Oryzalin 1 day-b, B: Immersion Oryzalin 2 days-b; C: Immersion Oryzalin 3 days-b; D: Immersion Oryzalin 4 days-b; E: Immersion Oryzalin 5 days-b; F: Immersion Oryzalin 6 days-b; G: Immersion Oryzalin 1 day-w; H: Immersion Oryzalin 2 days-w, I: Immersion Oryzalin 3 days-w; J: Immersion Oryzalin 4 days-w; K: Immersion Oryzalin 5 days-w; L: Immersion Oryzalin 6 days-w; M: Immersion colchicine 1day-b; N: Immersion colchicine 1 day-w; O: Check varietal 1; P: Check varietal 2; Q: Check varietal 3

Table 2. Analyse of Variance (ANOVA) result of agro-morphology butterfly pea checks

Source of variance	Degree of freedom	Mean square										
		Plant height	Stem diameter	Early flowering	50% flowering	Pod weight	Pod number	Fresh weight biomass	Dry weight biomass	Number of seeds per pod	Branch number	Weight of 100 seed
Block	5	651.3 ns	11.657 ns	55.29 ns	61.52 ns	57.00 ns	329.64 ns	25,539 ns	1667.23 ns	21.756 ns	17.62 ns	0.28 ns
Check	2	2623.7 **	0.7946 ns	66.72 ns	66.72 ns	39.40 ns	27.26 ns	10,502 ns	66.01 ns	0.5293 ns	12.25 ns	0.37 ns
Error	10	365	1.852	46.32	39.12	32.51	178.36	13,068	522.92	1.2	12.63	0.19

Table 3. Agromorphology performance of butterfly pea genotypes and check varieties

Genotype	Induction	Plant height	Stem diameter	Early flowering	50% flowering	Pod weight	Pod number	Fresh weight biomass	Dry weight biomass	Number of seeds per pod	Branch number	Weight of 100 seed
		PH (cm)	SD (mm)	EF (days)	F50 (days)	PW (g)	PN (pcs)	FW (g)	DW (g)	SP (pcs)	SVP (pcs)	Y100 (g)
BP1	C1	144	11.9	63.8	70.3	12.3	29	972.7 abc	95.5	6.3	33	3.90
BP6	O1	144	11.3	52.8	57.6	13.0	34	729.7	224 abc	7.8	34	2.60
BP12	O2	167	10.3	52.1	53.3	22.9	46	545.4	210 c	7.7	29	2.90
BP13	O2	135	10.2	56.8	64.3	10.9	18	818.2	236 abc	7.4	26	2.90
BP14	O2	133	10.5	52.8	57.6	11.0	32	715.2	231 abc	6.8	32	2.00
BP16	O2	142	10.5	59.8	64.3	31.9	66	611.8	164	10 abc	34	3.29
BP23	O3	129	9.9	62.8	66.6	32.3	75	933.5 abc	243 abc	5.7	36	3.60
BP27	O1	140	12	66.8	69.6	16.5	38	936.0 abc	163	7	28	2.70
BP29	O1	136	12.3	52.8	60.6	26.6	57	843.9	237 abc	7.3	23	3.20
BP30	O1	121	10	62.8	66.6	40.7	94 abc	591.8	204	5.3	22	3.48
BP38	O2	154	13.6abc	56.8	64.3	25.6	54	805.9	319 abc	6.9	24	3.80
BP39	O2	134	9.7	49.8	57.6	19.4	46	684.2	289 abc	4.8	32	3.10
BP40	O2	144	9	58.8	64.3	15.1	16	752.9	234 abc	6.6	28	3.10
BP41	O3	117	10.2	57.8	64.3	30.15	81.52	748.51	157	10.3abc	35	3.36
BP42	O3	167	9.2	63.8	67.9	15.3	38	593.8	268 abc	8.4	30	3.30
BP44	O3	148	10.5	59.8	64.3	58.0 abc	132 abc	530.2	135	11 abc	32	3.22
BP45	O3	175	10.6	75.8 a	87.6 abc	40.0	78	797.3	190	7.5	28	4.08
BP46	O3	112	10.5	64.8	68.6	14.1	30	1040 abc	226 abc	9.3	27	2.80
BP48	O3	125	13.6 abc	67.8	78.6 a	24.3	49	618.48	160.88	6.3	31	2.76
BP49	C2	123	9.65	56.8	60.3	55.6 abc	97 abc	490.2	120	8.0	31	3.46
BP50	O4	124	11.2	56.8	60.6	17.8	45	910.6 abc	238 abc	5.2	33	2.60
BP51	O4	113	11.6	63.8	67.3	10.3	20	918.2 abc	222 abc	6.2	27	3.30

BP52	O4	118	12.1	56.8	64.3	10	24	832.4 c	237 abc	6.6	32	2.30
BP53	O4	190 ab	8.95	70.8	74.9	22.2	28	538.1	158	6.6	27	2.79
BP55	O4	152.6	10.4	63.8	67.3	38.9	65.1	477	133	9.7 abc	27	2.56
BP57	O4	110	12.2	56.8	60.6	22.4	48	781.4	246 abc	6.7	36	2.60
BP58	O4	107	13.8 abc	59.8	67.3	17.7	35	939.9 abc	250 abc	6.6	24	3.00
BP59	O4	106	13.3 abc	59.8	64.3	8.86	18	722.4	209	4.7	26	3.28
BP60	O5	107	11.3	49.8	57.6	20.9	45	933.9 abc	240 abc	5.5	33	2.50
BP61	O5	132	7.69	59.8	66.6	45.4 ac	109 abc	756	192	5.8	29	4.01
BP62	O5	13.6	11.01	56.8	60.3	29.24	70.10	596.84	161.7	10.7abc	28	3.49
BP66	O5	122	8.19	59.8	64.6	35.9	95 abc	811	212	5.8	32	3.75
BP68	O5	115	14.6 abc	52.8	57.3	14.33	37	609.06	173.00	6.4	21	3.48
BP69	O5	127	15.1 abc	54.8	60.3	21.7	23	909.9 abc	254 abc	7.6	32	2.90
BP70	O6	114	13.2 abc	49.8	53.6	23.2	63	953.1 abc	251 abc	6.8	30	2.90
BP76	O4	181	11	62.8	66.6	38.2	78	628.5	271 abc	11 abc	33	3.70
BP77	O4	113	11.9	49.8	57.6	20.3	55	740.6	279 abc	6.5	33	3.30
BP78	O4	120	15.6 abc	59.8	64.3	23.5	17	1062 abc	316 abc	9.6	27	3.10
BP79	O4	121	14.6 abc	59.8	64.3	15.5	18	974.9 abc	262 abc	9.6	32	3.40
BP81	O4	151	9.69	62.8	66.6	62.9 abc	102 abc	635.2	200	5.2	38 ac	3.95
BP84	O5	167	5.95	62.8	67.9	22.5	47	368.8	101	8.7	30	5.22 abc
BP85	O5	134	11.1	58.8	64.3	47.8 ac	66	875 bc	229 abc	9.3	31	3.60
BP86	O5	101	11	62.8	66.6	53.0 abc	106 abc	519.3	153	7.5	29	4.05
BP90	O6	123	8.5	55.1	57.3	33.0	54	701.2	237 abc	7.2	29	2.80
BP91	O6	129	8.3	55.1	57.3	40.1	84	562.9	299 abc	10 abc	24	3.60
BP92	O6	213 ab	8.6	55.1	60.3	34.5	29	431.4	282 abc	8.2	28	2.90
BP93	O6	197 ab	6.73	70.8	74.9	36.5	45	451.1	143	5.7	27	3.32
BP95	O6	192 ab	9.82	62.1	68.3	62.0 abc	94 abc	607.1	208	11 abc	31	3.37
LSI 5%		43.2	3.08	15.4	14.1	12.9	30	258.4	51.7	2.5	8	0.98
A+LSI		187	12.9	74.9	77.5	44.3	91	907	216	9.6	37	4.2
B+LSI		185	12.9	81.6	84.1	48.9	92	866.2	215	9.6	40	4.2
C+LSI		223	12.3	78.1	80.6	44.6	88	823.4	210	9.1	37	4.6

Note: PH: Plant height; SD: Stem diameter; EF: Early flowering; F50: 50% Flowering; PW: Pod weight; PN: Pod number; FM: Fresh weight biomass; DM: Dry weight biomass; SP: Number seed per pod; SVP: Number branches/plant; Y100: 100 seed weight. a: Exceed check A; b: Exceed check B, c: Exceed check C; nt: Notation

Conversely, the mean values of stem diameter, fresh and dry weight biomass, and the number of seeds per pod in the butterfly pea genotype demonstrated remarkable increases, as illustrated in Figure 1. This is the same thing that happens to the *Medicago* sp. plant; this increase in stem diameter could be attributed to mutations that affect cell division and elongation genes, leading to thicker stems. This enhancement of fresh and dry weight matter could be due to mutations that improve water uptake, retention, or photosynthetic efficiency, leading to larger plant sizes and greater biomass accumulation (Innes et al. 2021). The mutants have more structural biomass, potentially due to alterations in overall metabolic processes, resulting in more robust and resilient plant structures (Sattler et al. 2016). The application of mutagens can either promote or inhibit growth, depending on the plant material and type (Rauf et al. 2021). The main factors for chromosome doubling include the appropriate solution concentration, immersion time, pH, and adventitious organ involvement (Acquaah 2012). Combining immersion times with colchicine or oryzalin mutagens is an attempt to induce morphological diversity.

The ANOVA results for examining butterfly pea variety variables showed these insignificant effects except for plant height, and the environment variance on genotype traits was homogenous, as shown in Table 2. It is generally stated that genetic factors influence the growth and yield of all genotypes. Plant growth and development are a product of genetic and environmental interactions (Acquaah 2012). Polyploidy mutagenesis has significantly contributed to expanding both growth and genetic diversity (Oladosu et al. 2016; Rauf et al. 2021), resulting in notable changes in plant morphology (Diouf et al. 2021; Zulchi et al. 2022).

Genotype plants promoted by polyploidy allowed polyploidy individuals to exhibit often robust growth patterns, phenotypic variability, and heterosis (Sattler et al. 2016), leading to the emergence of new alleles as sources of character diversity within plant populations (Arumingtyas et al. 2023). However, it's important to consider the drawbacks of colchicine, a toxic compound that poses significant harm to humans and should not be employed in high concentrations. This is a serious matter that requires utmost caution. The compound oryzalin has been proposed as an alternative mutagen for polyploidization (Hwang et al. 2015). Higher genetic diversity is sought as a condition for selecting superior varieties

The average yields of the check varieties and yields adjusted for block differences resulting from genotype selection are given in Table 3. The significant, adjusted yields of fresh and dry weight for 29 new varieties were higher than check varieties. Using informative statistical methods to analyze crop yield stability is expected to identify high yielding. Any genotype yielding greater than that of check varieties (A, B, or C) of x+LSI would be declared to be significant (Petersen 1994). The butterfly pea genotypes' yield biomass was significantly higher than the checks varieties at the 0,05-probability level.

Table 3 indicates butterfly pea genotypes' growth and biomass production using the Least Significant Increase (LSI) test of the 0,05-probability level. Based on the LSI test, BP92, BP93, BP95, and BP53 genotypes of BP had significantly different PH compared to two local varieties as check varieties and must not be selected if the genotype is to look for short ones. Butterfly pea genotypes of BP70, BP59, BP68, BP69, and BP78-79 in SD traits; and BP45 genotype in EF and 50%F exceeding all check varieties. Then, BP44, BP49, BP81, BP86, and BP95 genotypes in PW trait; BP44, BP49, BP30, BP61, BP66, BP81, BP86, and BP95 genotypes in PN trait; BP16 and BP84 genotypes in the SP trait and BP81 genotype in the SVP trait are greater than all check varieties. Then, the Y100 trait had a BP84 genotype exceeding all check varieties.

Based on Table 3 shows the biomass production of genotypes using the Least Significant Increase (LSI) test. On the LSI 5% + checks varietal, the FW and DW traits were 12 and 26 genotypes greater than all check varieties. Furthermore, based on the inter-relation of FW and DW traits, 10 genotypes of butterfly peas were greater than the yield of the highest checks, and they were significantly higher. Crop yield is influenced by various environmental factors such as photoperiod, temperature, altitude, moisture, soil fertility, and plant genotype (Acquaah 2012). Polyploidization usually results in larger leaves and larger cells, so biomass yield can be increased (Innes et al. 2021). Their wide range of genetic diversity results in numerous alleles, which suggests the potential for increased productivity.

In addition to biomass production, the nutritional qualities of BF genotypes were excellent. Table 4 shows the range of protein, carbohydrate, and lipid nutritional contents in butterfly pea genotypes. The induction of polyploid mutagens has led to notable nutritional enhancements in butterfly pea genotypes. When the mean nutritional contents of genotypes are compared to the check varieties, there are remarkable increases in protein content by 29.6%, carbohydrates by 11.3%, and lipid content by 11.6%. Currently, animal feed plant breeding is oriented towards biomass production and nutrition. Genotype breeding has resulted in better commercial varieties and enhanced traits, including crop quality and nutritional attributes (Oladosu et al. 2016). Since polyploidization increases the number of chromosomes and the chromosomal homology, there is an improvement in phenotypic characteristics and nutrients (Manzoor et al. 2019). Its high nutritional value holds promise for enriching ruminant diets (Abreu et al. 2014) This plant can be used as a supplement to animal feed due to its good biomass production and nutrition.

Genetic diversity based on PCA

Mutational breeding, a significant evolutionary force, contributes to genetic diversity by inducing random changes in the DNA structure. This process can be effectively analyzed through principal component analysis (PCA). PCA can describe variations in variables (plant characters), reveal variations in traits, and explain possible variances in data (Acquaah 2012). When applied to butterfly pea genotypes, PCA revealed the presence of four significant principal components, each with eigenvalues exceeding one. The collective contribution of these four components

to the cumulative diversity is substantial, accounting for 67.566%, as depicted in Table 5, underscoring the impact of these findings.

Principal component one (PC1) contributes to the variation in pod number, while PC2 appears to contribute to diversity in dry-weight biomass. Further, PC4 is a diversity attribute for branch numbers, respectively, that supports diversification, as outlined in Table 6. In this table, in the Principal Component Analysis (PCA) of the genotypes of butterfly peas, variables identify the loadings of various factors on the principal components (PC1 to PC4). Broad genetic diversity is typically defined by a substantial dissimilarity coefficient or an Euclidean distance greater than 1 (Karuniawan et al. 2021). Particular note: PC1 primarily represents plant height (PH), with a loading of 0.269, and also shows influence from other traits. PC2 is heavily influenced by dry-weight biomass, with loadings of 0.506. PC3 is a narrow variety associated with early and 50% flowering. PC4 is linked to the branch number.

PC-1, with an eigenvalue of 2.57, contributed 23.38% of the total diversity of butterfly pea genotypes. PC-2, with an eigenvalue of 1.93, contributed 17.56% of the total diversity. PC-1 and PC-2, with a cumulative value of 40.94%, explained most of the accessions diversity. Cumulative PC-1 and PC-2 contribute highly to information on genetic diversity, relationships between traits, and genotypes that excel at certain traits in the biplot diagram.

The biplot in Figure 2 represents a genetic diversity value of 40.95%, with PC1 accounting for 23.39% and PC2 accounting for 17.56% of the variance, indicating a moderate influence on the resulting variance. The PC1-PC2 biplot shows that variables with short vectors from the center point, such as PH (plant height), Y100 (yield per 100 seeds), and SVP (seed volume per pod), exhibit narrow genetic diversity. Conversely, variables with long vectors, such as FM (flower morphology), DM (days to maturity), PN (pod number), and EF (early flowering), exhibit high genetic diversity. In this figure, the genetic diversity of variables can be identified by the length of their vectors. The butterfly pea genotypes are influenced by multigene interactions, primarily affecting quantitative plant traits. Genetic diversity has a minimal impact on these characteristics, potentially due to the emergence of desirable traits that suppress others (Tziouvalekas et al. 2022). The ability to distinguish between the assessed genotypes using these features was highly useful for identifying their genetic differences.

Relationship characteristics and selected genotypes

Distinct patterns in the relationships among various characteristics of butterfly peas are identified. Acute angles (less than 90º) indicated positive correlations, while right angles (equal to 90º) were thought to indicate that variables were not associated. Then, obtuse angles (>90º) reveal negative correlations. This determines the correlation magnitude between two sets of yield-related variables (da Cruz et al. 2020). Notably, the relatively small angles, or under 90°, between traits such as SD, FM, and DM indicate positive correlations. However, flowering time (EF and 50%F), PN, PW, and SP are positioned in low association with FM and DM. Conversely, there are inverse relationships between FM and DM related to flower production, seed production, and pod weight, as shown in Figure 2. This analysis provides valuable insights into the interplay of these key characteristics of the butterfly pea.

Table 4. Nutritional composition profile of butterfly pea genotype

Nutrients	Range	Genotypes	Range checks
Protein (%)	17,00	BP95, BP85, BP69, BP39, BP29	14.10
Carbohydrate (%)	67,69	BP92, BP70, BP57, BP50, BP29	64,77
Lipid (%)	2.40	BP95, BP92, BP69, BP46, BP39	2.23

Note: *Laboratory of Husbandry Research Institute, Agriculture Department

Table 5. Genetic variance revealed by Principal components

	PC1	PC2	PC3	PC4
Eigenvalue	2.573	1.931	1.799	1.129
Variability (%)	23.387	17.559	16.357	10.263
Cumulative %	23.387	40.946	57.302	67.566

Table 6. Principal component analysis of morphological traits in butterfly pea genotype

Variables	PC1	PC2	PC3	PC4	PC5
PH	0.269	-0.252	0.099	0.475	0.118
SD	-0.354	0.214	-0.038	0.132	-0.311
EF	-0.318	-0.401	0.457	-0.093	0.020
F50	-0.295	-0.377	0.493	-0.127	0.091
PW	0.381	0.103	0.361	-0.239	-0.391
PN	0.416	0.240	0.308	-0.329	-0.146
FM	-0.363	0.429	0.224	-0.043	0.113
DM	-0.287	0.506	0.188	0.036	0.000
SP	0.115	0.025	0.343	0.476	-0.411
SVP	0.126	0.239	0.250	0.541	0.352
Y100	0.229	0.146	0.215	-0.210	0.633

Note: PH: Plant height; SD: Stem diameter; EF: Early flowering; F50: 50% Flowering; PW: Pod weight; PN: Pod number; FM: Fresh weight biomass; DM: Dry weight biomass; SP: Number seed per pod; SVP: Number branch/plant; Y100: 100 seed weight

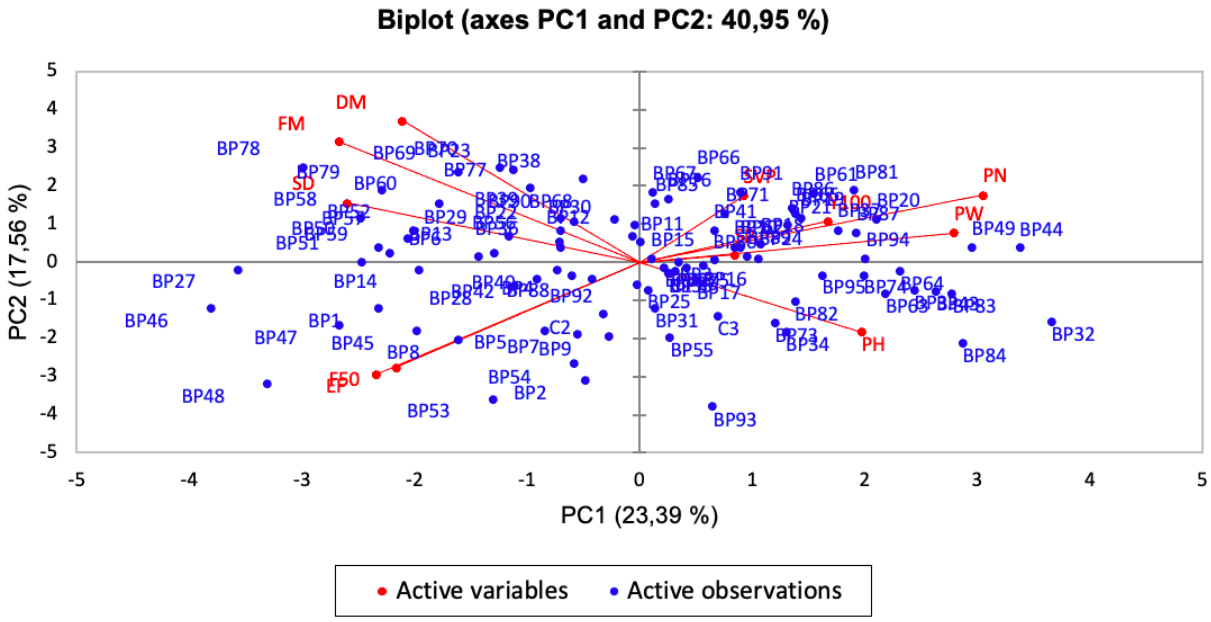

Figure 2. Principal Component (PC) biplot PCA 1 and PCA 2 on variable butterfly pea genotype. PH: Plant height; SD: Stem diameter; EF: Early flowering; F50: 50% Flowering; PW: Pod weight; PN: Pod number; FM: Fresh weight biomass; DM: Dry weight biomass; SP: Number of seeds per pod; SVP: Number branches/plant; Y100: 100 seed weight

Genotypes exhibited diverse characteristics in the yield component and yield. These genotypes will reveal nearly identical traits and display distinct potential features. Additionally, in Figure 2, the biplot graphic shows which genotypes excel in specific traits. For example, butterfly pea genotypes BP45, BP46/BP47, and BP48 are identified as having a trait of late flowering. In contrast, genotypes BP19, BP20, and BP21 are characterized by early flowering. Moreover, BP88 exhibits spreading growth patterns rather than climbing tendencies and a large stem diameter. Whereas BP1 possesses a notable few pods number, BP44 and BP95 had a large number of pods. It's important to note that BP1 is tetraploid, with thick leaves and dark blue petals; however, it produces a limited number of seeds. Further, BP1, BP23, BP46, BP50, BP51, BP58, BP60, BP69, BP70, BP78, and BP79 demonstrate promising genotypes related to high-production biomass potential relatives. These findings provide valuable insights into the genetic diversity and potential characteristics of butterfly pea genotypes induced by polyploidy mutagens, as illustrated in Figure 2.

Therefore, it is necessary to select sustainable forage production for the butterfly pea genotype as a forage crop. This means that the resilience of the forage plants is seen from the amount of production per year that can be achieved and the quantity and quality of the results. As an underutilized genetic and plant resource, butterfly pea plants require crop improvement through plant breeding programs by continuously evaluating available genotypes (Filio et al. 2023). Therefore, cultivating *C. ternatea* may be suggested to fill the gap between the demand and supply of forage legumes for livestock production (Shamnad 2019). Selecting sustainable production entails resilience regarding frequency, quantity, and yield quality to harness butterfly pea's potential as a forage crop.

Based on this study, we have discovered that cumulative diversity, which accounted for 67% of the variation in principal component analysis, was strongly influenced by biomass dry weight. This cluster analysis of butterfly pea genotypes has effectively categorized them into four distinct clusters. These clusters have identified potential genotypes that serve as valuable parental and genetic material for selection purposes, inspiring further research and development. Finally, we have identified butterfly pea genotypes such as BP78, BP70, BP58, and BP46 with high biomass and nutrient yields, offering a promising avenue for improvement as parents in hybridization programs.

ACKNOWLEDGEMENTS

The research leading to these results received funding from the Indonesian Agency for Agricultural Research and Development, c.q., Director of the Indonesian Center for Agricultural Biotechnology and Genetic Resources (ICABIOGRAD) for funding this research through the Indonesian State Revenue and Expenditure Budget (APBN) for the year 2021. Its experimental station is conducted to facilitate experiment research. The APC is paid by Universitas Padjadjaran, Bandung, Indonesia.

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