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MULTIVARIATE SELECTION STRATEGY FOR GENETIC DIVERSITY AND HIGH YIELD IN F6 MULTIPLE-CROSS BIRD'S EYE CHILI (CAPSICUM FRUTESCENS L.) LINES

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ABSTRACT

Developing bird's-eye chili lines using a multiple-cross concept generates high genetic diversity. Selection in advanced generations, such as F6, requires a systematic multivariate approach. This study aimed to identify superior F6 chili pepper (*Capsicum frutescens* L.) lines using genetic diversity analysis, correlation, path analysis, principal component analysis (PCA), and selection indices. The research was conducted at the Experimental Farm of the Faculty of Agriculture, Hasanuddin University, in Makassar City, South Sulawesi Province, Indonesia, from June to October 2025 using an Augmented Design with five blocks, comprising 59 F6 lines and four check varieties. Observations covered 13 agronomic traits. Adjusted means for the unreplicated F6 lines were derived from the Augmented Design analysis, correcting for block effects using the repeated check varieties. Analysis of variance showed highly significant differences (P<0.01) in most traits, with high broad-sense heritability (>50%) for yield (95.57%), fruit weight per plant (87.07%), total fruit quantity (73.68%), fruit stalk length (73.85%), and number of productive branches (71.84%). Path analysis indicated that total number of fruits per plant (TFQ) had the strongest direct effect on yield (0.928), followed by fruit weight per plant (0.363). PCA showed that two principal components explained 63.17% of the total variation. Based on a selection index integrating yield, fruit weight, and fruit number, six lines (G16, G9, G84, G57, G25, and G28) demonstrated superior performance. These lines are recommended as promising candidates for further multi-environment testing. The multivariate approach effectively screened F6 chili lines with high yield potential under the tested conditions. From a practical breeding standpoint, these six lines represent ready-to-use genetic resources for accelerating the development of high-yielding, multiple-cross bird's eye chili cultivars.

***Keywords*:** Augmented design, Bird's eye chili, Genetic variability, Selection index, Yield components.

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1. INTRODUCTION

Bird's eye chili is a horticultural commodity that plays a strategic role in the economy and daily life of Indonesian people (Siddik et al., 2022; Maulana et al., 2025). Bird's eye chili (*Capsicum frutescens* L.) possesses high economic value owing to its content of bioactive compounds such as capsaicinoids, phenolic compounds, and vitamin C, which confer documented health benefits (Olszewska et al., 2021). High consumer demand for both fresh consumption and processed forms positions this commodity as a strategic horticultural crop (Asir et al., 2022; Kirana et al., 2024; Mardiyati & Natsir, 2024). Although national production levels are generally high, fluctuations attributable to agronomic factors, seasonal variations, and pest and disease pressures remain prevalent (CSA, 2025). The perishable nature of the commodity, coupled with supply–demand imbalances in the market, further exacerbates price volatility (Lestari et al., 2024; Mardiyati & Natsir, 2024). Consequently, efforts to enhance productivity and stabilize bird's eye chili production remain a critical priority (Ahmed et al., 2025).

Plant breeding utilizing a multiple-cross scheme is recognized as an effective strategy for broadening the genetic diversity base within complex breeding programs. This method combines superior alleles from multiple parents with distinct genetic backgrounds into a single segregating population, thereby increasing the likelihood of transgressive segregation that yields individuals that surpass the performance of the best parents (Arrones et al., 2020). By involving more than two parents, the multiple-cross scheme facilitates extensive genetic recombination, which is critical for

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breaking linkage drag and accumulating genes governing quantitative traits, such as yield. Although multiple-cross techniques have been widely applied in chili breeding, empirical studies regarding the efficiency of multivariate selection in advanced generations (F6) derived from this scheme remain very limited. Most previous studies have tended to focus on early-generation evaluation (F2–F4) (Amas et al., 2023; Anshori et al., 2024; Musdir et al., 2024), where heterozygosity levels remain high and phenotypic stability is not yet assured, or they rely solely on simple univariate analyses that assess traits independently. Univariate approaches often overlook complex inter-trait correlations and genetic interactions that become evident only as the population approaches homozygosity; consequently, the hidden genetic potential in advanced generations remains suboptimally explored (Zafar et al., 2022; Zafar et al., 2025). As a result, many potential lines may be overlooked due to imprecise early selection (Meier et al., 2022). The present study employs a comprehensive multivariate approach integrating various statistical parameters within an F6 population. This strategy is expected to enhance the accuracy of identifying prospective lines with high genetic stability, thereby enabling more efficient selection to produce candidate varieties for further evaluation.

The population used in this study was developed through a multiple-cross scheme involving four parental lines with distinct characteristics: a hybrid cultivar (Dewata F1), an inbred line (Bara), an ornamental accession (Ungara), and *Capsicum chinense* (Katokkon), the latter noted for its exceptionally high pungency level (Amier, 2022). Generational advancement was conducted progressively, beginning with the F2–F3 populations (Amas et al., 2023; Anshori et al., 2024). Subsequent selection and advancement to the F4–F5 generations were carried out by (Musdir et al., 2024) and (Saharani, 2025) respectively, with the population now reaching the F6 generation. The F6 generation was specifically selected for this study because, at this stage, lines are expected to have attained a substantially higher level of homozygosity compared to earlier generations. This genetic stability renders the F6 population more suitable for preliminary phenotypic stability evaluation prior to multi-location testing.

In this study, the selection index was developed by integrating information from key yield components. The weighting of each trait was determined based on direct effects derived from path analysis and principal component analysis (PCA), further adjusted using determination coefficients (R^2) to prioritize traits that exert the most substantial direct influence on yield (Alsabah et al., 2019; Anshori et al., 2019). This approach enables selection efforts to remain focused on yield improvement while still accounting for the contributions of supportive agronomic traits. Therefore, this study aims to integrate genetic diversity, correlation, path, and principal component analyses to develop a multivariate selection index within an F6 population. This framework is designed to identify lines exhibiting superior yield components and select stable, high-performing bird's eye chili lines, thereby providing promising candidate varieties for advanced evaluation.

Globally, interest in multi-parent and multiple-cross breeding schemes has grown substantially over the past several years as breeders seek to combine complementary alleles from more than two founders within a single recombinant population (Arrones et al., 2020). Compared with simple bi-parental crosses, multi-parent populations broaden the allelic pool available for selection and increase the probability that superior recombinant genotypes will segregate beyond the performance of any single parent, a principle demonstrated across several field and horticultural crops advancing from early to intermediate inbred generations (Meier et al., 2022). In this multiple-cross bird's eye chili population specifically, genetic characterization at the F2 stage confirmed wide segregation for qualitative and quantitative traits (Amas et al., 2023) and the population was subsequently tracked through the F3 and F4 stages using a selection index and qualitative-trait mapping, respectively (Anshori et al., 2024; Musdir et al., 2024), before its production potential was evaluated at F5 (Saharani, 2025). Collectively, these studies established that useful genetic variability persists through the early and intermediate generations of this population, but none has evaluated it once it approaches homozygosity at F6, nor tested whether an integrated multivariate framework combining diversity, correlation, path, and principal component analyses into a single selection index can reliably identify superior lines once segregation has stabilized. This represents an important gap, because selection decisions made in early segregating generations are frequently revised as populations advance and genotype-by-environment noise diminishes; a line that appears promising at F2–F4 does not always retain its rank once homozygosity is reached (Meier et al., 2022). The present study addresses this gap directly: rather than repeating the largely univariate, single-generation screening approach used in the F2–F5 stages of this population, it applies an integrated multivariate selection framework at F6 for the first time, providing an empirical test of whether indices built from path-analysis and PCA loadings can consistently and efficiently flag superior lines at the stage immediately preceding candidate-variety development.

2. MATERIALS AND METHODS

2.1. Experimental Design

This research was conducted at the Experimental Farm of the Faculty of Agriculture, Hasanuddin University, Tamalanrea Subdistrict, Makassar City, South Sulawesi Province, Indonesia (5°7'40" S, 119°28'59" E) from June to October 2025. Fig. 1 shows a map of the experimental site. The experimental design used was the Augmented Design, which is effective for evaluating populations with limited seed availability, with the experimental environment arranged in randomized blocks. The experiment consisted of five blocks containing 59 F6 lines selected from the F5 population of multiple crosses, which were planted without replication; a total of 79 experimental units were derived from 59 unreplicated lines plus 4 check varieties replicated in each of the 5 blocks (59 + [4 × 5] = 79 plots). as well as four check varieties (Bara, Taruna, Maruti, and Dewata) as controls that were repeatedly planted in each block to control for environmental variability. Checks were distributed systematically within each block to control for environmental variability. The details of the genotypes used are listed in Table 1.

Table 1: Genotype lists and codes in this study

GENOTYPE	CODE	GENOTYPE	CODE	GENOTYPE	CODE
G1.2.4.3.3.4	G1	G4.11.1.11.2.12	G25	G7.7.10.8.4.6	G57
G1.9.2.3.3.3	G2	G4.11.1.13.3.8	G27	G7.12.3.3.1.7	G58
G1.9.2.3.3.4	G3	G4.11.1.13.4.2	G28	G7.12.3.3.1.8	G59
G1.9.5.10.3.1	G4	G4.11.1.13.4.4	G29	G8.3.9.10.4.7	G60
G1.9.5.10.3.2	G5	G4.11.1.4.5.7	G30	G8.3.9.14.4.1	G63
G1.9.5.5.3.2	G7	G5.3.3.8.2.3	G31	G10.2.4.6.3.1	G69
G1.12.7.4.4.4	G8	G5.5.8.3.1.3	G33	G10.2.4.6.3.5	G70
G1.12.9.7.4.2	G9	G5.12.1.12.3.8	G37	G10.5.4.11.5.5	G71
G2.1.10.2.4.5	G10	G5.12.4.4.5.2	G40	G10.5.4.11.5.6	G72
G2.1.10.2.4.6	G11	G5.12.4.4.5.6	G41	G10.5.4.11.5.7	G73
G2.6.10.1.5.1	G12	G6.11.2.1.2.5	G42	G10.5.8.4.3.5	G74
G3.12.7.4.5.5	G14	G6.11.2.2.5.2	G43	G10.5.8.5.1.3	G76
G4.3.8.10.7.7	G15	G6.11.2.3.1.1	G45	G10.5.8.6.3.3	G78
G4.3.8.11.3.2	G16	G7.3.4.2.2.7	G46	G10.9.6.1.7.4	G82
G4.3.8.11.3.7	G17	G7.3.4.3.3.6	G47	G10.9.6.1.7.8	G83
G4.3.8.12.5.8	G19	G7.3.4.5.1.4	G49	G10.9.6.11.1.4	G84
G4.3.8.3.1.2	G20	G7.3.4.5.1.6	G50	G10.9.6.11.1.7	G85
G4.3.8.3.1.7	G21	G7.3.4.6.5.2	G51	DEWATA	DW
G4.11.1.10.6.4	G22	G7.6.3.11.2.7	G54	TARUNA	TR
G4.11.1.10.6.7	G23	G7.7.10.3.5.1	G55	BARA	BR
G4.11.1.11.2.1	G24	G7.7.10.8.4.4	G56	MARUTI	MR

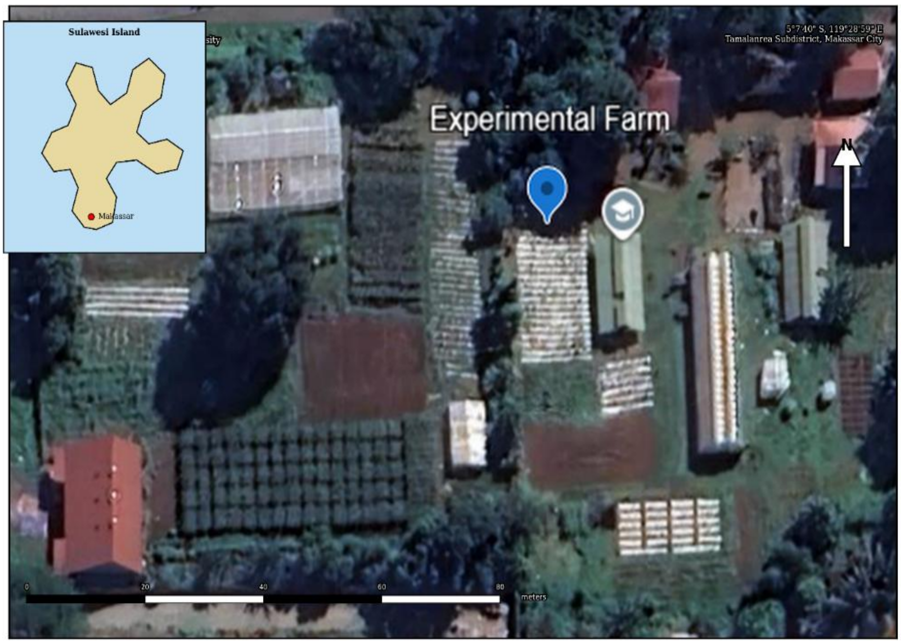

Fig. 1: Map of the experimental site at the Experimental Farm, Faculty of Agriculture, Hasanuddin University, Tamalanrea Subdistrict, Makassar City, South Sulawesi (5°7'40" S, 119°28'59" E). Source: Google Earth satellite imagery (2026), processed by the author. Available at: https://earth.google.com/web/@-5.127421,119.482943,536d,35y.

2.2. Procedural Design

F6 generation Bird's eye chili seeds were selected based on the criteria of being healthy, full, and pure, both physically and genetically, and then germinated for approximately one week. Subsequently, the seeds were sown in trays using a mixture of soil, compost, and burnt rice husks (1:1:1) that had been moistened. At 10 days after sowing (DAS), the seedlings were given NPK Mutiara fertilizer (5 ml/L). Seedlings were maintained in trays until 20 days after sowing (DAS), then transplanted into polybags. Final transplanting to raised beds was conducted at 21 days after transplanting (DAT) from the tray. The raised beds for F6 measured 13.4 m × 1 m with a planting distance of 60 cm

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× 60 cm. The beds are covered with perforated silver-black plastic mulch (10 cm in diameter) for planting, and each plant is provided with a support stake. Maintenance included watering twice a day (morning and evening), replanting sick or abnormal plants at 14 DAP (replaced with plants of the same genotype and age), regular fertilization (NPK Mutiara and KNO_3 10 g/L every week), pruning of shoots to focus growth on the main stem, manual weeding around the plants or using herbicide (Gramoxone 276 SL, 2 g/L) outside the beds, as well as pest and disease control using a mixture of abamectin and curacron when necessary. Harvesting is performed when the fruit reaches physiological maturity, which is when 90% of the fruit is red.

2.3. Observation Data

Observations were conducted on 13 agronomic characteristics, with field observations carried out in each experimental plot using measurement methods. Plant height (cm) was measured from the soil surface to the highest growing point at the stage just before harvest, while dichotomous height (cm) was determined from the soil surface to the main branching point. Plant canopy width (cm) was obtained by measuring the widest span of the canopy just before harvest. Stem length (mm) was measured using a caliper at the stem segment after the second harvest. The number of productive branches was recorded by counting branches bearing fruit. Days to flowering and days to harvest were recorded in days after transplanting (DAT), with each noted from transplanting until the appearance of the first flower and until the first harvest, respectively. Fruit characteristics were observed on five ripe fruits per plant, including fruit length (cm) measured from base to tip, fruit diameter (mm) at the midpoint using a caliper, as well as fruit stalk length (cm) from base to stalk tip. Fruit weight per plant represents the total weight of all harvested fruits per plant (g) and was accumulated over five harvests. The number of fruits per plant and yield per plant (g) were calculated as the accumulation from the first to the fifth harvest, with yield representing the total fruit weight per plant during that harvest period.

2.4. Data Analysis

The data obtained from all observed variables were analyzed using analysis of variance (ANOVA) according to the Augmented Design to test for significant differences between genotypes. To account for the unreplicated nature of the test lines, adjusted means for the F6 lines were calculated based on the performance of the replicated check varieties within each block. This adjustment controlled for spatial environmental variability and block effects, following standard augmented design procedures (You et al., 2016). The check varieties provided the necessary degrees of freedom to estimate the experimental error and reference variation used for significance testing. Mean Square values from the ANOVA were used to calculate the components of genetic variance, phenotypic variance, and broad-sense heritability values. Correlation analysis was used to identify relationships between traits, whereas path analysis was employed to separate the direct and indirect effects of variables on yield. Principal Component Analysis (PCA) was used to detect patterns of diversity among lines and to identify groups of traits that contributed most to the total phenotypic variation. Traits that consistently contributed positively to yield based on correlation, path analysis, and PCA were used as the foundation for developing a selection index. All analyses were conducted using Microsoft Excel, SAS 9.0 software for ANOVA, and STAR-R version 2.0.1 for correlation analysis and estimation of genetic parameters.

3. RESULTS

Based on Table 2, the analysis of variance revealed highly significant differences (**) at the 1% probability level across the treatment, line, and line × check interaction sources of variation for most of the evaluated agronomic traits in the chili genotypes. Estimates of genotypic (σ^2g) and phenotypic (σ^2p) variances varied across the traits. The ratio of σ^2g to σ^2p determines broad-sense heritability (H^2), where traits with σ^2g values closely approaching σ^2p indicate strong genetic control and high heritability. This pattern was particularly evident in yield (Y) (σ^2g = 11784.21; σ^2p = 12330.33; H^2 = 95.57%), total fruit quantity (TFQ) (σ^2g = 8259.11; σ^2p = 11209.86; H^2 = 73.68%), fruit weight per plant (FWP) (σ^2g = 0.15; σ^2p = 0.17; H^2 = 87.07%), fruit stalk length (SL) (σ^2g = 0.31; σ^2p = 0.42; H^2 = 73.85%), and the number of productive branches (NFL) (σ^2g = 26.39; σ^2p = 36.74; H^2 = 71.84%), all of which exhibited high heritability estimates (>50%). Additional traits demonstrating high heritability included dichotomous height (DH; 76.27%), days to flowering (FD; 58.79%), fruit diameter (FDM; 67.67%), and fruit length (FL; 61.74%). In contrast, stem diameter (SD; 41.34%), plant canopy width (PC; 48.94%), plant height (PH; 22.22%), and days to harvest (HD; 12.58%) exhibited moderate to low heritability, indicating that the phenotypic variation for these specific traits is predominantly governed by environmental factors. The predominance of high heritability estimates across most evaluated traits suggests that direct phenotypic selection would be highly effective for genetically improving these characteristics in bird's eye chili breeding programs.

The results of the correlation analysis in Table 3 show that most agronomic traits were positively and significantly correlated with yield, making them potential selection criteria. These traits include the number of fruits per plant,

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plant habit, stem diameter, plant height, number of productive branches, pedicel length, branch height, fruit length, fruit weight, and fruit diameter, all of which are significantly correlated with yield traits. The analysis indicates that these characteristics contribute directly to yield formation and should be considered when selecting superior lines. In contrast, flowering and harvest times were not significantly correlated with most major agronomic traits, including yield. This suggests that these two phenological characteristics have a limited impact on yield improvement in the observed population and are therefore less relevant as the basis for selection in advanced breeding stages, which are focused on increasing yield.

Table 2: Analysis of variance, genetic parameters, and heritability estimates for agronomic traits in chili genotypes

Character	MS Trt.	MS L	MS L X C	CV (%)	σ^2g	σ^2p	H^2 (%)	Heritability Category
PH	144.71	123.60	181.57	19.09	35.14	158.12	22.22	Moderate
DH	91.71**	72.60**	312.19**	18.98	57.25	75.07	76.27	High
SD	6.64*	6.37*	36.13**	17.95	3.74	9.06	41.34	Moderate
PC	178.84	168.43ns	376.42**	19.24	84.28	172.21	48.94	Moderate
NFL	35.10**	29.03**	14.25*	11.18	26.39	36.74	71.84	High
FD	254.49**	219.03*	372.56*	16.48	153.64	261.33	58.79	High
HD	255.07**	223.12**	474.07*	10.10	31.65	251.51	12.58	low
FWP	0.16**	0.16**	0.29**	12.62	0.15	0.17	87.07	High
FDM	1.88**	1.84**	12.13**	9.07	1.35	1.99	67.67	High
FL	2.08*	2.04*	0.003	21.39	1.32	2.14	61.74	High
SL	0.42**	0.38**	1.54**	10.76	0.31	0.42	73.85	High
TFQ	9530.87**	8909.73**	22917.30**	19.81	8259.11	11209.86	73.68	High
Y	12447.25**	11899.40**	13590.55**	7.81	11784.21	12330.33	95.57	High

Notes: ns: not significant, **: significant at α = 1%, *: significant at α = 5%, MS Trt: mean square treatment, L: Lines, C: Check, L x C: interaction of Lines and Check, MS L: mean square line; MS L x C: mean square Line x Check; CV: coefficient of variance, σ^2g: genotypic variance, σ^2p: phenotypic variance, H^2: heritability, $H^2 > 50\%$ (high heritability), $20\% \le H^2 \le 50\%$ (moderate heritability), $H^2 < 20\%$ (low heritability), PH: plant height, DH: dichotomous height, SD: stem diameter, PC: Plant Canopy, NFL: number of productive branches, FD: flowering day, HD: harvest days, FWP: fruit weight per plant, FDM: fruit diameter, FL: fruit length, SL: stem length, TFQ: total fruit quantity, Y: yield.

Table 3: Pearson's correlation coefficient between agronomic traits and traits related to yield in chili genotypes

	PH	DH	SD	PC	NPB	FD	HD	FWP	FDM	FL	SL	TFQ	Y
PH	1.00												
DH	0.28**	1.00											
SD	0.44**	-0.05ns	1.00										
PC	0.6**	0.09ns	0.49**	1.00									
NPB	0.11*	0.09ns	0.2**	0.18**	1.00								
FD	-0.03ns	-0.06ns	-0.11*	-0.29**	-0.18**	1.00							
HD	-0.01ns	-0.05ns	-0.11ns	-0.27**	-0.17**	0.97**	1.00						
FWP	-0.02ns	-0.01ns	0.03ns	0.03ns	0.05ns	-0.09ns	-0.1ns	1.00					
FDM	0.15*	0.07ns	0.07ns	0.14*	0.14*	0.05tn	0.07ns	0.24**	1.00				
FL	0.13*	0.04ns	0.16**	0.21**	0.15**	-0.28**	-0.29**	0.17**	0.04ns	1.00			
SL	0.33**	0.05ns	0.22**	0.34**	0.09ns	-0.33**	-0.33**	0.06ns	0.14*	0.25**	1.00		
TFQ	0.46**	0.11ns	0.44**	0.53**	0.33**	-0.18**	-0.18**	-0.21**	0.05ns	0.23**	0.27**	1.00	
Y	0.45**	0.11*	0.47**	0.55**	0.34**	-0.2**	-0.2**	0.16**	0.11*	0.26**	0.27**	0.88**	1.00

Notes: ns: not significant, **: significant at α = 1%, *: significant at α = 5%, PH: plant height, DH: dichotomous height, SD: stem diameter, PC: Plant Canopy, FD: flowering day, HD: harvest days, NFL: number of productive branches, FWP: fruit weight per plant, FDM: fruit diameter, FL: fruit length, SL: stem length, TFQ: total fruit quantity, Y: yield.

The path analysis results for chili plants indicated that the total fruit quantity per plant (TFQ) had the most dominant direct effect on yield, with a direct influence coefficient of 0.928 (Table 4). This suggests that TFQ is the main determinant of yield and should be the top priority when selecting superior genotypes for chili breeding. Additionally, fruit weight per plant (FWP) also contributes directly to yield, although its effect is smaller (0.363). The indirect influence on yield was mostly channeled through the number of fruits, where plant habit and plant height showed significant indirect effects (0.495 and 0.426, respectively) through this pathway. The determination coefficient value ($R^2 = 69.47\%$) indicates that the eight agronomic characters analyzed can explain approximately 70% of the variation in yield, making this path analysis model quite representative and suitable as a basis for designing selection strategies. Therefore, in chili breeding programs, the number of fruits per plant and the fruit weight per planting should be the main focus of selection, supported by vegetative traits such as plant habit and plant height, which play a crucial role in strengthening the indirect contribution to the yield. This approach is expected to improve the efficiency of selection and accelerate the development of superior varieties with high productivity.

The results of the principal component analysis (PCA) showed that the first two components (PC1 and PC2) explained 63.17% of the total variation in agronomic traits among the F6 chili lines (Table 5). This indicates that most of the genetic diversity can be efficiently represented using just two dimensions. In PC1, traits such as SD (0.4079), SL (0.4381), and FWP (0.4292) are the most significant contributors. This suggests that PC1 reflects plant structural

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vigor and fruit capacity, both of which play a role in supporting high production. The results for PC2 are influenced by TFQ (-0.4372) and Y (-0.4602), which have negative values, as well as by FD (0.2143) and HD (0.2699), which have positive values. This pattern indicates an inverse relationship between developmental timing and yield. Biologically, the negative loading values for TFQ and Y on PC2 indicate that these two traits are contrasted with the traits with positive loading values (FD, HD). In other words, lines with high PC2 scores tend to have later flowering and harvest times (positive values for FD/HD) but lower fruit number and yield (due to negative contributions to PC2). Conversely, lines with low (negative) PC2 scores tend to flower earlier and produce higher yields.

Table 4: Path analysis of various production traits and yield components of chili genotypes

Character	Direct	Indirect effect										Total
	Effect	PH	DH	SD	PC	NFL	FWP	FDM	FL	SL	TFQ	Effect
PH	-0.005		0.005	0.018	0.020	0.001	-0.008	-0.004	-0.003	-0.002	0.426	0.449
DH	0.019	-0.001		-0.002	0.003	0.001	-0.003	-0.002	-0.001	0.000	0.099	0.112
SD	0.041	-0.002	-0.001		0.017	0.001	0.011	-0.002	-0.004	-0.001	0.409	0.469
PC	0.034	-0.003	0.002	0.020		0.001	0.011	-0.004	-0.005	-0.002	0.495	0.550
NFL	0.007	-0.001	0.002	0.008	0.006		0.017	-0.004	-0.004	0.000	0.305	0.337
FWP	0.363	0.000	0.000	0.001	0.001	0.000		-0.007	-0.004	0.000	-0.192	0.162
FDM	-0.028	-0.001	0.001	0.003	0.005	0.001	0.085		-0.001	-0.001	0.048	0.113
FL	-0.026	-0.001	0.001	0.007	0.007	0.001	0.060	-0.001		-0.001	0.215	0.262
SL	-0.005	-0.002	0.001	0.009	0.012	0.001	0.021	-0.004	-0.006		0.247	0.273
TFQ	0.928	-0.002	0.002	0.018	0.018	0.002	-0.075	-0.001	-0.006	-0.001		0.882
R^2		69.470										

Notes: PH: plant height, DH: dichotomous height, SD: stem diameter, PC: Plant Canopy, NFL: number of productive branches, FWP: fruit weight per plant, FDM: fruit diameter, FL: fruit length, SL: stem length, TFQ: total fruit quantity, Y: yield.

Table 5: Principal component analysis (PCA) for chili agronomic traits

Variables	PC1	PC2	PC3	PC4
PH	-0.2592	-0.2561	0.3347	0.0819
DH	-0.1546	-0.2386	0.0024	0.6816
SD	0.4079	-0.1911	0.1697	0.0225
PC	-0.2564	-0.3502	0.1074	0.0401
NFL	0.2778	-0.3178	0.0151	0.134
FD	0.1178	0.2143	0.6861	-0.0916
HD	-0.3087	0.2699	0.4063	-0.118
FWP	0.4292	-0.1194	0.0583	0.1134
SDH	-0.2695	-0.0435	0.0742	0.4403
FL	-0.1522	-0.2534	-0.3476	-0.3474
SL	0.4381	-0.1234	0.14	0.0656
TFQ	-0.0928	-0.4372	0.2001	-0.292
Y	-0.0993	-0.4602	0.1491	-0.2632
Standard deviation	2.1491	1.8955	1.2149	0.9668
Proportion of Variance	0.3553	0.2764	0.1135	0.0719
Cumulative Proportion	0.3553	0.6317	0.7452	0.8171
EigenValues	4.6186	3.5931	1.4759	0.9347

Notes: PH: plant height, DH: dichotomous height, SD: stem diameter, PC: Plant Canopy, FD: flowering day, HD: harvest days, NFL: number of productive branches, FWP: fruit weight per plant, FDM: fruit diameter, FL: fruit length, SL: stem length, TFQ: total fruit quantity, Y: yield.

Analysis of the selection index on F6 chili lines and four comparison varieties showed considerable variation in agronomic performance based on the combination of three main traits: yield, fruit weight per plant, and number of fruits per plant. The selection index developed in this study was constructed through the following steps:

1. Data standardization: The actual values for each character (Yield, number of fruits, fruit weight per plant) are first standardized to eliminate differences in units.
2. Determination of character results: The results for each character are determined based on a combination of the PCA loading values and the direct effect coefficients from path analysis.
3. Index calculation: The PCA loading value for yield from PC2 (0.4602) is multiplied by the standardized yield value. Similarly, the weights for the number of fruits and fruit weight are calculated based on their contributions in PCA and path analysis.

The selection index developed in this study is as follows:

$$SI = (W_Y \times L_Y \times Y_{std}) + (W_{FWP} \times L_{FWP} \times FWP_{std}) + (W_{TFQ} \times L_{TFQ} \times TFQ_{std})$$

or

$$SI = (1 \times 0.4602 \times Y) + (0.363 \times 0.119 \times FWP) + (0.928 \times 0.437 \times TFQ)$$

Note:
W: the weight from path analysis direct effect

L: the loading from PCA
Std: indicates standardized values
FWP: Fruit weight
TFQ: total fruit quantity per plant
Y: Yield

The lines with the highest selection index (SI) values were G16 (SI = 3.62), G9 (SI = 2.30), G84 (SI = 1.54), G57 (SI = 1.12), G25 (SI = 0.99), and G28 (SI = 0.89) (Table 6). These high positive values reflect outstanding performance across all three traits, with yield, fruit weight, and fruit count consistently being above the population average. Conversely, several lines show negative selection index values, such as G40 (SI = –1.08), G58 (SI = –1.10), G71 (SI = –1.18), G55 (SI = –1.27) and G60 (SI = –1.50), indicating that the performance of these three main traits in those lines is below average. Reference varieties such as DEWATA (SI = 0.00) and TARUNA (SI = –0.02) also displayed relatively low index values compared to the superior lines. These findings confirm that G16, G9, G84, G57, G25, and G28 are the most promising candidates for further selection or the development of new varieties, as they simultaneously excel in the key yield components that are the focus of chili pepper breeding in this generation.

4. DISCUSSION

The observed genetic diversity in agronomic traits, particularly yield components in F6 chili lines, indicates that substantial variability remains available for effective selection, even though the population has reached an advanced generation (Hasan et al., 2020; Amier et al., 2025). In the F6 generation, the level of homozygosity is expected to be higher than earlier generations; however, phenotypic variation may still be influenced by environmental factors, and stability should be confirmed through further testing. High broad-sense heritability estimates for yield and its components indicate strong genetic control over phenotypic variation, potentially facilitating selection response. However, broad-sense heritability encompasses all genetic variance components (additive, dominance, and epistatic), not solely additive effects. Therefore, this interpretation must be understood within the context that phenotypic selection can be effective, although the relative proportion of additive vs. non-additive variance cannot be determined solely from broad-sense heritability. These findings align with previous studies stating that integrating diversity analysis with heritability estimates can effectively identify traits with high selection potential (Kaushik & Dhaliwal, 2018; Sidhdharth et al., 2025). Relatively low coefficients of variation for yield traits reflect adequate experimental precision and indicate that genetic factors dominate phenotypic variation. Conversely, the absence of significant diversity and low heritability values for plant growth type indicate that these traits are largely controlled by micro-environmental factors, making them less suitable as primary selection criteria (Sharma et al., 2025). These findings direct selection efforts toward high-heritability yield components rather than vegetative traits in chili line development.

The mean values of yield component traits, heritability estimates and correlation patterns obtained in this study are consistent with trends reported in published chili breeding studies. The broad-sense heritability for yield and number of fruits per plant observed in this study tends to be comparable to values reported by Chowdhury et al. (2023) and Anshori et al. (2024) in advanced generation chili populations, who also found high heritability (>75%) for yield-related traits. The strong positive correlation patterns between fruit number, fruit weight, and total yield are in agreement with the findings of Fadhila et al. (2022) and Rosero et al. (2023). Minor differences in the magnitude of correlation coefficients may be attributed to differences in genotypes, environmental conditions, or cultivation methods used. This comparison indicates that, although contextual variations exist, the fundamental principles of relationships among agronomic traits in chili remain consistent, thereby strengthening the validity of the applied selection approach.

The selection criteria in this study were identified through a multivariate approach that combined correlation analysis, path analysis, and principal component analysis (Zafar et al., 2023; Zafar et al., 2024). This approach is a standard strategy in modern plant breeding to ensure that selected traits are not only correlated with yield but also have a strong direct effect and reflect the main dimensions of genetic variation within the population. The finding that traits related to yield components show positive correlations with yield is in line with many previous reports stating that traits, such as the number of fruits and fruit weight, are generally the main determinants of yield in fruit-bearing plants (Fadhilah et al., 2022; Rosero et al., 2023). The dominance of the direct influence of the number of fruits per plant on yield, as revealed by path analysis, is consistent with the physiological principle that increasing the number of reproductive organs generally contributes more to the overall yield than the size of individual organs (Chowdhury et al., 2023). The role of vegetative characteristics, such as habitus or plant height, which primarily have an indirect influence, is also consistent with findings from various chili studies, where plant architecture supports yield through light efficiency and resource distribution (Sobir and Syukur, 2015; Hulagannavar et al., 2024). The

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Table 6: Actual values, standard values, and selection index scores of chili genotypes and four control varieties

Genotype	Actual Value			Standardization			Selection
	Y	FWP	TFQ	Y	FWP	TFQ	Index
G1	83.01	1.53	51	-0.68	0.04	-1.08	-0.75
G2	120.60	1.06	124	-0.13	-0.02	0.05	-0.04
G3	102.20	1.09	98	-0.40	-0.02	-0.34	-0.32
G7	92.88	1.19	80	-0.54	0.00	-0.63	-0.50
G8	80.99	0.82	105	-0.71	-0.05	-0.24	-0.43
G9	330.49	1.24	266	2.98	0.00	2.28	2.30
G10	181.93	1.27	154	0.78	0.00	0.53	0.58
G11	163.12	1.07	153	0.50	-0.02	0.51	0.44
G12	68.50	0.86	84	-0.90	-0.04	-0.57	-0.65
G14	112.85	1.49	77	-0.24	0.03	-0.68	-0.39
G15	131.43	0.91	149	0.03	-0.04	0.44	0.19
G16	419.85	1.07	378	4.31	-0.02	4.04	3.62
G17	103.11	1.51	75	-0.39	0.03	-0.71	-0.47
G19	156.05	0.85	186	0.40	-0.05	1.03	0.60
G20	168.53	1.32	128	0.58	0.01	0.11	0.31
G21	193.83	1.25	152	0.96	0.00	0.49	0.64
G22	157.81	1.14	136	0.42	-0.01	0.25	0.29
G23	129.04	1.00	123	0.00	-0.03	0.04	0.01
G24	140.97	1.22	119	0.17	0.00	-0.02	0.07
G25	197.69	1.26	202	1.01	0.00	1.29	0.99
G27	132.11	1.17	118	0.04	-0.01	-0.03	0.01
G28	187.26	1.09	198	0.86	-0.02	1.21	0.89
G29	129.89	1.10	110	0.01	-0.02	-0.15	-0.06
G30	38.97	0.68	61	-1.34	-0.07	-0.94	-1.00
G31	119.18	0.84	126	-0.15	-0.05	0.09	-0.03
G33	138.68	1.05	127	0.14	-0.02	0.10	0.10
G37	66.38	1.05	70	-0.93	-0.02	-0.79	-0.75
G40	39.74	1.00	46	-1.33	-0.03	-1.16	-1.08
G41	138.98	0.85	159	0.14	-0.05	0.61	0.31
G42	149.30	1.50	103	0.30	0.03	-0.28	0.03
G43	102.57	1.27	90	-0.40	0.01	-0.47	-0.37
G45	83.79	0.95	83	-0.67	-0.03	-0.59	-0.55
G46	172.04	1.02	184	0.63	-0.03	1.00	0.70
G47	132.84	1.04	129	0.05	-0.02	0.13	0.08
G49	226.15	1.66	124	1.44	0.05	0.06	0.69
G50	125.78	7.28	126	-0.05	0.72	0.09	0.04
G51	126.44	0.90	139	-0.04	-0.04	0.29	0.10
G54	69.60	0.87	79	-0.88	-0.04	-0.65	-0.67
G55	33.98	1.50	23	-1.41	0.03	-1.52	-1.27
G56	165.18	0.94	174	0.53	-0.03	0.85	0.59
G57	215.61	1.07	203	1.28	-0.02	1.30	1.12
G58	48.40	1.55	34	-1.20	0.04	-1.36	-1.10
G59	191.54	1.41	130	0.92	0.02	0.15	0.49
G63	95.30	1.05	88	-0.50	-0.02	-0.50	-0.44
G69	125.46	1.16	98	-0.06	-0.01	-0.36	-0.17
G70	125.11	1.17	117	-0.06	-0.01	-0.05	-0.05
G71	40.83	1.51	30	-1.31	0.03	-1.42	-1.18
G72	95.33	1.38	80	-0.50	0.02	-0.63	-0.49
G73	44.27	1.51	30	-1.26	0.03	-1.42	-1.16
G74	76.62	1.30	62	-0.78	0.01	-0.91	-0.73
G76	133.64	0.96	137	0.07	-0.03	0.26	0.13
G78	113.56	0.81	136	-0.23	-0.05	0.25	-0.01
G82	99.87	1.08	98	-0.44	-0.02	-0.35	-0.34
G83	127.33	1.07	117	-0.03	-0.02	-0.05	-0.03
G84	221.28	0.82	264	1.36	-0.05	2.26	1.54
G85	50.80	1.16	45	-1.16	-0.01	-1.17	-1.01
G5	57.35	1.17	54	-1.07	-0.01	-1.03	-0.91
G4	115.98	1.37	98	-0.20	0.02	-0.35	-0.23
G60	10.70	0.89	12	-1.76	-0.04	-1.70	-1.50
MARUTI	181.67	1.41	130	0.78	0.02	0.16	0.42
DEWATA	121.53	0.96	129	-0.11	-0.03	0.14	0.00
BARA	210.78	0.84	257	1.21	-0.05	2.15	1.43
TARUNA	125.37	1.05	121	-0.06	-0.02	0.01	-0.02

Y: yield. FWP: fruit weight per plant, TFQ: total fruit quantity.

PCA results, which show that major patterns of phenotypic variation can be summarized in two principal components one reflecting structural vigor and the other the relationship between development time and yield, strengthen the validity of this approach, as similar patterns have been reported in various Solanaceae species (Laila et al., 2023; Amal et al., 2025).

The dominant direct effect of fruit number per plant on total yield suggests a pattern consistent with source-sink dynamics reported in indeterminate-type chili plants. Yield depends on fruit number and fruit weight; however, path analysis helps distinguish the direct physiological contributions of each component to selection priorities. As strong sinks, each fruit creates competitive demand for photosynthates, thereby increasing fruit number and optimizing the participation of reproductive organs in assimilate allocation, rather than accumulation in vegetative biomass. Furthermore, since fruiting occurs cumulatively throughout the plant cycle, yield variation is more deterministically determined by the frequency of successful fruit set than by individual fruit weight, which has stricter physiological limits (Kumar & Srivastava, 2021; Syukur et al., 2023). In the context of an F6 population that has reached a high level of homozygosity, the consistency of fruit number expression reflects the efficiency of genetically controlled biomass partitioning, making it a more precise and responsive selection indicator than other yield components in chili breeding (Kuswantoro et al., 2018).

The combination of these three aspects ensures that the selected traits are not only statistically correlated with outcomes but also have a strong biological and genetic foundation, making them suitable as the basis for selection in breeding programs. This integrative approach aligns with modern breeding principles, which emphasize the need for dual validation of genetic, physiological, and statistical data to avoid biased selection resulting from spurious correlations or uncontrolled environmental influences (Alsabah et al., 2019; Anshori et al., 2019). This strengthens the selection priority for both traits while ensuring that the selection decisions made are efficient, accurate, and sustainable in efforts to obtain superior chili pepper lines in the genetically stable F6 generation.

Based on these findings, a selection index was developed that integrated the relative weight of each criterion based on the magnitude of its direct influence (from path analysis) and the primary component loadings (from PCA). The selection index formula is designed as follows:

$$SI = (1 \times 0.4602 \times Y) + (0.363 \times 0.119 \times FWP) + (0.928 \times 0.437 \times TFQ)$$

The application of the developed selection index can enable the objective and efficient identification of superior lines by taking into account the genetic and physiological contributions of each yield component. Lines such as G16, G9, G84, G57, G25 and G28, which ranked highest based on their index values, tend to exhibit outstanding

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performance in the three key traits: yield, number of fruits per plant, and fruit weight per plant. This indicates that these lines not only have high productivity but also demonstrate consistency in the expression of major yield components governed genetically (Ambrósio et al., 2024; Farid et al., 2024). The implications of this selection are considered strategic. At the F6 generation, which has reached a high level of homozygosity, selection of lines based on a multivariate index can increase the chances of obtaining candidate varieties that are responsive to selection and have agronomic stability, thus potentially advancing to further testing stages, such as preliminary yield trials (Sayekti et al., 2021).

This F6 population retains sufficient genetic variability despite undergoing advanced generation inbreeding, with coefficients of variation ranging from 7.81% to 21.39% across traits. The identified superior lines represent valuable genetic resources for breeding programs targeting chili yield improvement. Conservation and utilization of this genetic diversity contribute to chili germplasm preservation efforts, which have important implications for food security and adaptation to environmental changes. In the future, it is recommended that selected lines be further evaluated in multi-location and multi-season trials covering specific environments, such as lowland to midland areas and conditions vulnerable to biotic and abiotic stresses, to test adaptation stability. Traits that can be evaluated in subsequent generations include resistance to major diseases and fruit quality parameters not currently covered in the index. The results of these tests can contribute to a more comprehensive candidate variety development process (Rosero et al., 2023: Hulagannavar et al., 2024). The integration of fruit quality parameters (such as capsaicin content, color, and shelf life) into future selection indices will also enhance the competitiveness of the resulting varieties, especially in both the fresh and processed markets (Suraj et al., 2024; Vega-muñoz et al., 2025). Thus, the proposed selection strategy is not only relevant for the current F6 population but can also serve as an adaptive framework for future chili breeding programs.

5. CONCLUSION

This study demonstrates that the F6-generation bird's eye chili lines evaluated exhibited substantial phenotypic variation under the tested conditions, with promising yield potential. Through the integration of diversity analysis, correlation analysis, path analysis, principal component analysis (PCA), and selection index methodology, two key traits, total fruit quantity per plant (TFQ) and fruit weight per plant (FWP), were identified as primary determinants of productivity. Both traits exerted dominant direct effects on yield and were consistently detected across multiple analytical approaches, underscoring their effectiveness as selection criteria in breeding programs aimed at enhancing production. Based on selection index values, lines G16, G9, G84, G57, G25, and G28 exhibited the most favorable phenotypic performance among the evaluated population and are recommended as prospective candidates for advanced evaluation. These six lines represent promising candidates requiring further validation through multi-location testing and comprehensive agronomic assessment prior to variety release.

Declarations

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