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GENETIC VARIABILITY AND SELECTION IN GAMMA-IRRADIATED GARLIC (*ALLIUM SATIVUM* L.) MUTANTS FOR LOWLAND ADAPTATION

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SUMMARY

Garlic breeding suffers from limited genetic variability and poor adaptability to tropical lowland environments due to high temperatures and humidity. This study aimed to compare the genetic variability and selection efficiency of the M2 garlic mutants in two contrasting areas (highland and lowland) in Central Java, Indonesia. The experiment has an augmented research design. The results showed the analysis of genetic parameters confirms that the highland environment optimizes the expression of the genetic potential of the M2 garlic mutants. Conversely, with the environmental stress, the garlic mutants still exhibited sufficient variability for selection based on bulb weight. Obtaining a total of 29 mutants came from the highlands, and 13 were from the lowlands. The PCA biplot successfully validated the $G \times E$ interaction by explaining 55.41% of the total variance. Mutants H28, H29, H15, and H23 express potential as putative highland-adapted mutants. Meanwhile, mutant L9 displays adaptability to lowland areas. As a plant propagated vegetatively, garlic allows for the genetic fixation of induced superior traits in subsequent generations. Although there is a single-season constraint, screening these early generations using biplot analysis provides an efficient framework for initial selection.

Keywords: Garlic (*A. sativum* L.), gamma irradiation, M2 mutants, genetic variability, heritability and genetic gain, lowland adaptation, augmented design, yield traits

Key findings: The M2-generation garlic (*A. sativum* L.) mutants exhibited reduced heritability for vegetative traits due to high temperature and humidity stress in lowland areas. Nevertheless, the 13 mutants maintained the high bulb weight despite environmental stress, demonstrating that direct selection in the target environment was highly effective for isolating mutants resistant to high temperature and humidity.

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INTRODUCTION

The genetic improvement of garlic (*Allium sativum* L.) faces severe challenges from inherent reproductive constraints and limited adaptability to target environments. Garlic rarely produces flowers and viable seeds. It results in a narrow genetic base that limits conventional breeding approaches applied for varietal improvement (Wu *et al.*, 2016). Conversely, garlic is a vegetatively propagated crop through cloves derived from the bulbs harvested in previous years. It results in lower genetic variations and enhances risks of pathogen accumulation and yield decline throughout the successive planting cycles (Parreño *et al.*, 2023).

In addition to biological constraints, garlic cultivation considerably receives influences from agroclimatic conditions. Being a crop of subtropical regions, garlic generally requires relatively cool temperatures (approximately 22 °C–25 °C) and moderate humidity (60%–70%) for optimal growth and bulb development. Cultivation outside its typical ecological range, particularly in lowland environments with the higher temperature and humidity (24 °C–33 °C) than highland, often leads to suppressed vegetative growth, poor bulb formation, and reduced yield (Rafiuddin *et al.*, 2023). Consequently, the development of garlic cultivars adapted to lowland conditions is a crucial prerequisite for expanding garlic production in suboptimal environments.

Mutation breeding using gamma irradiation has gained wide application to overcome genetic constraints in vegetatively propagated crops by generating novel heritable variations. Gamma irradiation has successfully improved the barley (Ansari *et al.*, 2023) and various ornamental plants (Vishwanath *et al.*, 2024) through enhanced heat tolerance and adaptive traits. Several previous studies in garlic revealed that gamma irradiation can effectively improve genetic variability through morphological and yield-related traits (Mostafa *et al.*, 2015; Benke *et al.*, 2019; El-Sayed *et al.*, 2021). However, the mutation breeding success primarily depends on both the induction of variability and effective selection in early generations. Individual mutants with

morphological variations provide a practical basis for estimating genetic parameters, such as genotypic and phenotypic coefficients of variation, heritability, and genetic gain, which collectively determine the effectiveness of selection (Shiwani *et al.*, 2025).

Although reports on gamma-irradiated garlic have come out, studies on the genetic variability and selection efficiency of M2 mutants under lowland tropical conditions remain few. Furthermore, comparative evaluations across contrasting altitudes are insufficient to understand how divergent environmental pressures affect trait expression. Based on the previous studies described, this study provides insight into quantifying genetic variability and selection efficiency of M2 gamma-irradiated garlic mutants under two contrasting regions (highland and lowland of tropical environments), with emphasis on lowland adaptation. Hence, this study aimed to evaluate the genetic variability and selection potential of M2 gamma-irradiated garlic mutants grown under highland and lowland environments and identify the putative mutants exhibiting superior growth and bulb formation under lowland conditions.

MATERIALS AND METHODS

Experimental site

A gamma irradiation treatment of garlic commenced at the Nuclear Energy Research Organization (ORTN), Jakarta, Indonesia. Field evaluations performed took place during 2024–2025 at the two contrasting agroecological locations in Central Java, Indonesia. The highland site has a location at a latitude of 7°09'50.1"S (longitude 109°08'50.3"E) in Tuwel Village, Tegal Regency. Meanwhile, the lowland site sits at a latitude of 7°21'08.2"S (longitude 109°16'14.1"E) in Gandatapa Village, Banyumas Regency, Indonesia. The first location represents a relatively optimal environment for garlic growth, characterized by an altitude of 929.7 m above sea level (masl), a rainfall of 412.21 mm, a temperature of 27 °C, humidity of 78.2%, and light

intensity reaching approximately 100,000 lux at midday. Meanwhile, the second location was a lowland area, serving as a less-than-optimal target environment for evaluating lowland adaptation in higher temperature and humidity conditions. It has an elevation of 320 masl, a rainfall of 945.9 mm, a temperature of 31.3 °C, a humidity of 82.18%, and a light intensity of 135,000 lux.

Materials and the experimental design

Induction of mutations in four garlic genotypes used three doses of gamma radiation. The M1 harvest from the highland served as experimental material for M2, undergoing planting at two locations: the highland and the lowland. M2 mutants planted in varying quantities depended on the survival rate of the previous generation. This study used an augmented randomized complete block design (ARCBd). This design was a choice because it allows evaluation of numerous genotypes with limited clove availability; in this design, mutant genotypes had no replication, whereas check genotypes entailed replications in each block to assess environmental effects. The experiment consisted of 21 blocks; each block tended to have relatively homogeneous environmental conditions.

Assessed traits and measuring procedure

Measurements took place on all M2 mutants individually using the following methods: plant height (cm)—measured from the base to the tip of the tallest leaf; the number of leaves—calculated as the total count on the plant; pseudostem length (cm)—measured using a caliper; and root length (cm)—measurement continued after harvest by measuring the root from the base of the stem to the tip of the longest root. Measuring the horizontal and vertical tuber diameters (cm) used a Vernier caliper. Total biomass and bulb weight (g) determination utilized a digital scale, with the number of cloves determined by separating the bulb into individual cloves.

Statistical analysis

Analysis of variance (ANOVA) ensued separately for each location. Mean comparisons between mutants and check genotypes, as conducted by the study, used the least significant difference (LSD) test at a 0.05 significance level. Using genetic parameter estimates ran in the augmented RCBD package in RStudio (Aravind *et al.*, 2018). The parameters calculated included genotypic variance (GV), phenotypic variance (PV), environmental variance (EV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (h²), genetic advance (GA), and genetic advance as a percentage of the mean (GAM). A PCA biplot analysis sought to examine the G × E interaction.

Genetic parameters' calculation employed the following formulas:

$$LSD = t_{\alpha, df} \sqrt{\frac{2 \times MSE}{r}}$$

Selection criteria:

Genetic variance

$$GV = PV - EV$$

Genotypic (GCV) and phenotypic coefficient of variance (PCV) (Burton, 1952)

$$GCV = \frac{\sqrt{GV}}{\bar{x}} \times 100 \%$$

$$PCV = \frac{\sqrt{PV}}{\bar{x}} \times 100\%$$

Broad sense heritability (Johnson *et al.*, 1955)

$$h^2 = \frac{GV}{PV} \times 100\%$$

Genetic advance (Johnson *et al.*, 1955)

$$GA = (k \times \sqrt{PV} \times h^2)$$

Genetic advance as percentage of the mean (GAM)

$$\text{GAM (\%)} = \frac{GA}{\bar{x}} \times 100$$

Where $\bar{x}$ is the trait mean, r is the number of replications of the check genotypes, and k is the standardized selection differential at 5% selection intensity ($k = 2.063$). The magnitude of GCV and PCV attained classification as low ($< 10\%$), moderate ($10\%–20\%$), and high ($> 20\%$). Categories for heritability were as low ($< 30\%$), moderate ($30\%–60\%$), and high ($> 60\%$). Genetic advance mean (GAM) values classification comprised low ($< 10\%$), moderate ($10\%–20\%$), and high ($\geq 20\%$).

Selection criteria

The selection criteria for identifying promising M2 mutant individuals relied entirely on bulb weight, the primary economic trait. A mutant individual becomes a choice if its bulb weight exceeds the average weight of the best-performing check genotype in that environment, plus the least significant difference (LSD) at the 5% significance level. The 'best control' is according to the highest bulb weight among the four checks at each experimental site.

RESULTS

Analysis of variance and mutants' survival

Analysis of variance, performed separately at the two locations, used an augmented design (Table 1). At the high altitude, significant differences appeared between the garlic mutants and check genotypes for all morphological traits. In the lowland, mutants and check genotypes showed significant variations for most traits (plant height, the number of leaves, vertical diameter of bulb, horizontal diameter of bulb, total biomass, bulb weight, and the number of cloves), except pseudostem diameter and root length. On average, at the harvest, the number of surviving garlic M2 mutants was strikingly

lower at the lowlands (216 individuals) than at the highlands (526 individuals).

Mean performance of M2 mutants

The garlic mutant exhibited consistently higher growth and yield in the highlands than in the lowlands (Table 2). In the highland versus the lowland, average plant height was 32.58 versus 30.68 cm, the number of leaves was 5.77 versus 4.13, and the pseudostem diameter was 4.68 versus 3.48 cm. Significant decreases in lowland areas were also evident in root length (from 11.4 to 6.32 cm), vertical diameter of bulb (from 2.06 to 1.55 cm), and horizontal diameter of bulb (from 1.64 to 1.33 cm). The sharpest declines were notable in total biomass (75.77%, from 13.62 g to 3.30 g) and bulb weight (62.16%, from 5.18 g to 1.96 g).

Genetic parameters

Genetic parameter estimates, such as GCV, PCV, and heritability, underwent independent analysis for each location to assess the local genetic variability. These two estimates represent the phenotypic variance of mutants in highland and lowland habitats, respectively (Tables 3). Genetic parameters' (GCV, PCV, and heritability) analysis by location sought to assess local variability (Table 3). Consistently, phenotypic variance in the highlands was higher than in the lowlands for all traits. In the highlands, all GCV and PCV values exceeded 20% (high variability), with the highest values observed for the number of cloves (GCV = 67.53%; PCV = 80%).

Inversely, in the lowlands, PCV values remained high, but GCV values for the number of leaves, pseudostem diameter, and root length were moderate. Broad-sense heritability (h^2_{BS}) and genetic advancement (GAM) in the highlands were high for most traits, except for leaf number and pseudostem diameter, acquiring a classification as moderate. In lowland areas, heritability decreased to low levels for pseudostem diameter (14.91%) and root length (5%) and remained moderate for the number of leaves (35.62%). Although heritability decreased in lowland areas, the

Table 1. Mean squares of garlic mutants in high and low altitude generations.

Source	d.f.	Mean squares								
		Plant height (cm)	Number of leaves	Pseudostem (cm)	Root length (cm)	Diameter vertical of bulb (cm)	Diameter horizontal of bulb (cm)	Biomass total (g)	Bulb weight (g)	Number of cloves
Highland										
Blocks	20	248.25**	6.61**	3.24**	1012.05**	8.51**	6.31**	1579.79**	21.17**	82.29**
Treatments	529	109.06**	3.69**	3.62**	28.12**	0.63**	0.39**	43.17**	8.89**	25**
Treatments: Check	3	15.74ns	0.67ns	10.66**	45.46**	1.02**	0.4*	54.79*	18**	434.63**
Treatments: Mutants and mutants vs. check	526	109.59**	3.71**	3.58**	28.02**	0.63**	0.39**	43.11**	8.84**	22.66**
Residuals	60	33.35	1.94	1.49	11.85	0.21	0.12	16.78	2.27	7.32
Lowland										
Blocks	20	77.73**	1.08ns	2.39*	39.24**	0.66**	0.44**	53**	3.42**	11.1*
Treatments	219	74.91**	1.31**	1.5ns	13.16ns	0.24**	0.15**	8.92**	2.35**	12.6**
Treatments: Check	3	741.17**	17.95**	4.68*	25.79ns	1.28**	1.24**	43.05**	17.36**	61.89**
Treatments: Mutants and mutants vs. check	216	65.66**	1.08*	1.46ns	12.99ns	0.22**	0.14**	8.45**	2.14**	11.92**
Residuals	60	26.52	0.7	1.26	13.17	0.07	0.06	4.99	0.67	1.35

*, ** = Significant at $P \leq 0.05$ and $P \leq 0.01$, ns = $P > 0.05$ **Table 2.** Mean values, standard deviation, and maximum values of quantitative traits of the second-generation population of gamma-irradiated garlic on highland and lowland.

Traits	Highland		Lowland	
	Mean $\pm$ SD	Max. value	Mean $\pm$ SD	Max. value
Plant height (cm)	32.58 $\pm$ 11.48	64.71	30.68 $\pm$ 8.65	58.76
Number of leaves	5.77 $\pm$ 2.36	16.53	4.13 $\pm$ 1.15	7.06
Pseudostem (cm)	4.68 $\pm$ 2.07	12	3.48 $\pm$ 1.37	7.70
Root length (cm)	11.4 $\pm$ 5.95	33.41	6.32 $\pm$ 4.04	20.47
Diameter vertical of bulb (cm)	2.06 $\pm$ 0.97	4.82	1.55 $\pm$ 0.53	3.03
Diameter horizontal of bulb (cm)	1.64 $\pm$ 0.79	3.5	1.33 $\pm$ 0.4	2.27
Biomass total (g)	13.62 $\pm$ 7.44	44.44	3.3 $\pm$ 3.14	21.29
Bulb weight (g)	5.18 $\pm$ 3.18	21.02	1.96 $\pm$ 1.55	11.92
Number of cloves	6.28 $\pm$ 4.97	25.28	4.16 $\pm$ 3.51	16.97

Table 3. Estimation of genetic parameters for various agronomic traits in garlic mutant populations.

Location	Traits	Mean	PV	GV	EV	GCV	PCV	ECV	hBS	GA	GAM
Highland	PH	32.58	114.82	81.47	33.35	27.71	32.89	17.73	70.96	15.69	48.15
	NL	5.77	3.6	1.66	1.94	22.3	32.85	24.12	46.09	1.80	31.23
	PD	4.68	3.62	2.13	1.49	31.16	40.63	26.07	58.82	2.31	49.30
	RL	11.4	60.04	48.19	11.85	60.89	67.97	30.2	80.26	12.83	112.54
	DVB	2.06	0.94	0.73	0.21	41.27	46.88	22.23	77.51	1.55	74.95
	DHB	1.64	0.61	0.49	0.12	42.94	47.93	21.31	80.24	1.30	79.34
	TB	13.62	95.22	78.44	16.78	65	71.62	30.07	82.38	16.58	121.72
	BW	5.18	9.67	7.4	2.27	52.49	59.99	29.04	76.57	4.91	94.76
	NC	6.28	25.31	17.99	7.32	67.53	80.09	43.07	71.08	7.38	117.45
Lowland	PH	30.68	68.8	42.28	26.52	21.2	27.04	16.78	61.46	10.52	34.28
	NL	4.13	1.09	0.39	0.7	15.1	25.3	20.3	35.62	0.77	18.59
	PD	3.48	1.48	0.22	1.26	13.51	34.97	32.26	14.91	0.37	10.76
	RL	6.32	13.86	0.69	13.17	13.17	58.87	57.38	5.00	0.38	6.08
	DVB	1.55	0.27	0.2	0.07	29.12	33.64	16.83	74.95	0.81	52.01
	DHB	1.33	0.17	0.1	0.06	23.86	30.56	19.1	60.95	0.51	38.43
	TB	3.3	12.24	7.25	4.99	81.5	105.87	67.57	59.27	4.28	129.44
	BW	1.96	2.4	1.73	0.67	67.1	79.06	41.81	72.04	2.30	117.49
	NC	4.16	12.45	11.1	1.35	80.15	84.88	27.94	89.16	6.49	156.13

Note: PV: Phenotypic Variance; GV: Genotypic Variance; EV: Environmental Variance; GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; ECV: Environmental Coefficient of Variation; hBS: Heritability in Broad Sense; GA: Genetic Advance; and GAM: Genetic Advance as Percentage of Mean.

GAM values—which remained moderate to high for yield-related traits—ensure the potential for sustained genetic improvement in that environment.

Selection of putative mutants

M2 garlic mutants selected totaled 29 in the highland, while in the lowland, 13 mutants with bulb weight advantages exceeded those of the best comparison genotype (Table 4). The identification of 13 superior individuals in lowland areas in this study demonstrates that M2-generation mutants can reach direct selection in lowland locations that limit morphological performance. Although early-generation selection from a single economic trait (bulb weight) typically limits claims of definitive genetic stability, garlic's vegetative propagation nature ensures that induced mutations and superior phenotypes identified at the M2 stage are potentially genetically fixed without further segregation. The provision of a strong statistical validation of the cross-environmental adaptability and trait stability of these provisionally selected candidates, their subsequent evaluation used a biplot analysis.

Genotype-by-Environment (G×E) evaluation via biplot analysis

As shown in Figure 1, the PCA biplot exhibits distinct environmental differences along PC1, separating the genotypes into two mutant groups: the highland location in the negative half of PC1 (black circles) and the lowland location in the positive half of PC1 (blue triangles). To simplify the visual representation in the Principal Component Analysis (PCA) biplot (Figure 1), the mutant genotypes evaluated in the highlands (Table 4) were coded H1 through H29, while the mutant genotypes in the lowlands were coded L1 through L13 according to their order in the rows of Table 4. This diversity represents 55.41% of the total phenotypic variance. Principal component 1 (PC1) and PC2 contributed 29.6% and 25.81%, respectively. A formation of a narrow acute angle arose from several vectors representing the yield components, including bulb weight (BW), vertical bulb diameter (DVB), and horizontal bulb diameter (DHB). This shows a substantial positive association between these dimensions.

Table 4. Mutants selected in the highlands and lowlands based on bulb weight and other morphological traits.

No.	Mutants Highland	PH (cm)	NL	PD (cm)	RL (cm)	DVB (cm)	DHB (cm)	TB (g)	BW (g)	NC
1	G6B13G1,5a	39.6	7	5	5	3.41	2.2	13	10.7	12
2	G10B16G2,2.5	32	6	5.5	4	2.65	2.27	22.5	10.71	11
3	G2B5G3,7.3	23	4	2.6	12	1.17	1.1	34.3	10.73	14
4	G2B1G1,12b	35	4	4.4	23.8	1.2	1.4	31.6	10.8	10
5	G2B3G1,10b	29	6	4.6	24.6	1.25	1.5	30.1	10.8	9
6	G10B16G3,15e	29	5	2.6	18	3.2	2.5	17	10.97	8
7	G2B3G1,10a	48.2	4	7.8	9.4	1.4	1	11.5	11.1	14
8	G6B12G1,8b	33.3	6	5.4	11	3.4	2.4	15.6	11.1	22
9	G6B13G1,5b	43.5	6	6	6	3.13	2.54	12.9	11.1	12
10	G6B9G1,13c	36.6	5	4.3	10	3.47	2.56	18.5	11.25	18
11	G6B8G2,6d	39.2	8	2.3	16	3.92	3.41	21.2	11.28	23
12	G6B8G2,6e	41	9	4.1	13	3.52	2.52	14.4	11.35	13
13	G2B1G1,1	40.6	4	4.3	21.6	2.3	2.2	30.3	11.6	14
14	G10B2G3,3a	35	6	4.7	12	3.79	2.75	17.6	11.67	7
15	G6B8G2,6c	13.7	6	2	15	3.67	3.22	19.6	11.88	20
16	G6B12G1,15b	37	6	4.3	8	3.05	2.5	13.4	11.98	13
17	G6B9G1,13b	28.9	9	2.8	8	3.75	3	28.4	12.35	19
18	G6B12G1,8a	44.5	7	5.9	9	3.22	2.17	18.1	12.4	6
19	G6B8G1,5b	32.2	8	4.2	23.7	1.38	1.2	30	12.82	8
20	G10B6G3,11a	45.6	7	7.3	17	4.22	3	22.2	12.97	15
21	G6B8G2,6b	24.7	4	9	9	3.97	2.85	18.4	13	22
22	G6B8G2,6a	53.9	13	7.4	10	3.76	2.39	15.8	13.08	21
23	G6B12G1,15a	28	6	3.6	12	3.57	2.4	19.3	13.19	22
24	G2B2G1,11.2a	12	3	2.5	25.5	1.5	1.6	24.8	13.3	15
25	G2B1G1,12a	48	6	7	31.1	1.7	1.8	35.7	14	24
26	G6B8G1,5a	40.5	7	6	27	1.68	1.6	35.8	15.32	13
27	G10B15G3,15	42.5	6	6.2	28.8	2.34	1.1	37.6	15.38	6
28	G6B9G1,4	50.6	6	7.4	11	2.84	1.9	24.2	16.45	20
29	G6B9G1,13a	36.5	5	4.6	10	2.51	1.9	24.9	19.45	20
LSD _{0.05}		13.22	3.19	2.8	7.88	1.05	0.8	9.38	3.45	6.19
LSD+xLPS		40.55	8.54	6.38	16.49	2.99	2.62	26.04	8.54	19.84
LSD+ xSS		41.78	8.94	8.01	17.6	3.26	2.59	27.82	8.46	10.29
LSD+ xLH		42.44	8.64	6.81	18.93	3.51	2.8	27.72	9.74	11.35
LSD+xCWD		42.34	8.6	6.72	19.84	3.12	2.46	24.41	7.48	10.71
S.No.	Mutants Lowland	PH (cm)	NL	PD (cm)	RL (cm)	DVB (cm)	DHB (cm)	TB (g)	BW (g)	NC
1	G2B3G1,12a	50	6	5	10	2.7	2.1	11.4	8.4	12
2	G2B3G1,12b	30	6	3	8	2.4	2	10.6	5.4	9
3	G2B3G1,4a	36	5	3.6	8	2.2	2	7.4	5.1	8
4	G2B3G1,4b	51	6	5.1	7	2.5	2	9.4	5.5	10
5	G2B3G1,4c	40	5	4	6	2.3	2.1	6.5	5	8
6	G6B2G1,13a	31	4	5.7	2	2.6	1.8	6.4	6	10
7	G6B2G1,13b	30	3	4.5	8	2.3	2.3	6	5.8	17
8	G6B3G1,4	45	6	7	4	2.4	2	6.5	6.2	13
9	G6B5G1,14	32	4	3.2	12	1.7	1.7	13.7	11.6	16
10	G6B7G1,2a	30	5	3.7	2	2.3	2	5.3	5.1	11
11	G6B7G1,2b	27	4	3.2	2	2.5	1.6	5.3	4.9	13
12	G6B7G1,3	40	6	4.8	11	2.4	2.2	6.8	5.5	12
13	G10B7G1,4.7	19	3	3	10	2.5	2.1	7	4.8	8
LSD _{0.05}		11.8	1.9	2.6	8.3	0.6	0.6	5.1	1.9	2.7
LSD+xLPS		50.3	6.5	6.8	14.7	2.3	2.1	11.8	4.8	8.1
LSD+xSS		48.1	7.3	7.2	15.2	2.1	1.9	8.9	3.4	5.3
LSD+xLH		42.8	6	6.2	13.6	2.1	1.8	9	3.5	4.8
LSD+xCWD		37	5.1	6.4	16.3	1.7	1.5	8.8	2.7	4.3

Note: PH: plant height; NL: number of leaves; PD: pseudostem diameter; RL: root length; DVB: diameter vertical of bulb; DHB: diameter horizontal of bulb; TB: total biomass; BW: bulb weight; NC: number of cloves. G2, G6, and G10 represent the gamma irradiation doses. A "B" followed by a number represents the block number. G1, G2, G3, and G4 denote the origin of mutant genotypes: G1 (LPS), G2 (SS), G3 (LH), and G4 (CWD).

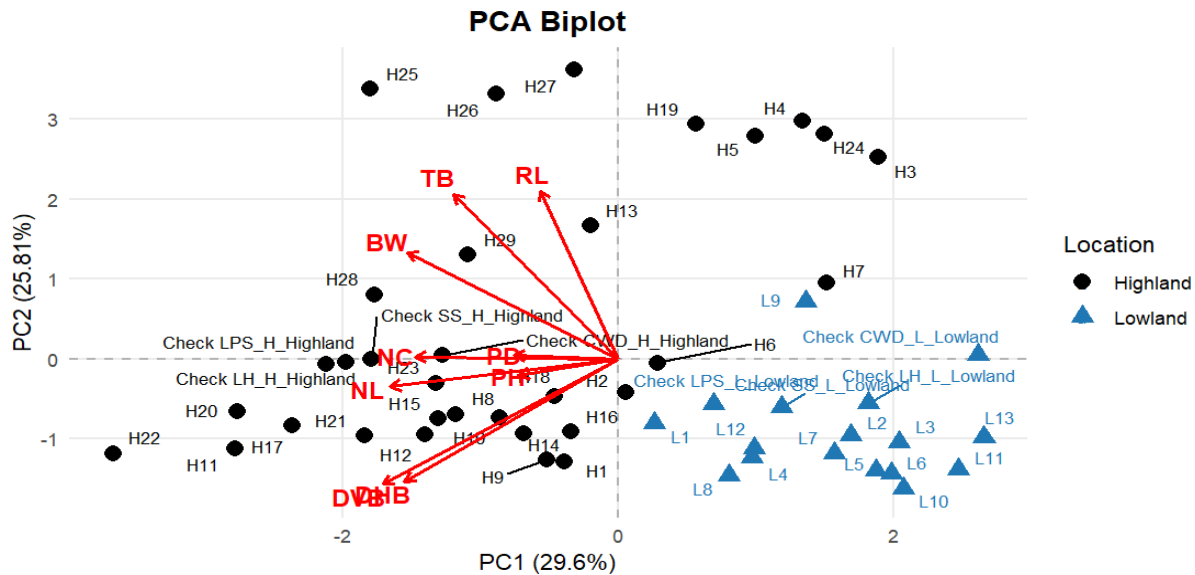


Figure 1. PCA biplot evaluating genotype-by-environment (G x E) interactions of garlic M2 mutants based on agro-morphological vectors under highland (●) and lowland (▲) conditions.

The vectors for the nine morphological variables identified in this study all point to the highland quadrant. The mutant genotypes H28, H29, H15, and H23 all cluster in the same vectors, indicating a substantial phenotypic reduction caused by environmental stress in the lowlands. However, mutant L9 stands out by moving away from the extremely stressed lowland cluster and into the upper-right quadrant near H7. This demonstrates that directed screening successfully isolated select, hardy individuals capable of maintaining better bulb characteristics despite high-temperature and high-humidity stress in the lowlands.

DISCUSSION

The phenotypic expression and selection efficiency of garlic mutants have a strict control of macro-environmental changes, primarily altitude-related differences in temperature and humidity (Ahmad *et al.*, 2023). The lowland site, characterized by the highest temperatures (exceeding 30 °C) and humidity, forced substantial physiological stress on garlic plants' growth and development. These results emphasized the importance of evaluating garlic mutants under the target lowland environment.

direction, showing improved performance under ideal conditions. In contrast, the clustering of the M2 population in the lowland quadrant is precisely opposite to the yield trait. Under lowland conditions, reduced survival and suppressed trait expression were particularly evident for pseudostem diameter and root length, suggesting these traits were highly sensitive to heat and humidity stress. Previous studies have reported similar responses with the high-temperature stress, where elevated humidity exacerbates stress effects and negatively affect the crop performance (Oh *et al.*, 2015; Eom and Hyun, 2023). Conversely, high temperatures can improve some aspects of bulb formation under certain light conditions (Atif *et al.*, 2020). This study found that keeping garlic at temperatures above 30 °C exceeded the optimal range for its growth.

Evaluation of genetic parameters is vital for determining whether observed phenotypic variation obtains influences from heritable components or environmental factors. Higher genotypic and phenotypic variance values in the highlands indicate optimal expression of genetic potential under favorable conditions. Conversely, moderate GCV values and lower heritabilities for some traits in the lowlands reflect strong environmental

modulation of phenotypic expression (Table 3). These findings are consistent with the patterns reported by Diouf *et al.* (2020), which show that environmental stress triggers pronounced phenotypic modifications and alters the expression of genetic parameters in response to adverse conditions. These differences help identify genotypes that are more suitable to the environment. Despite reduced broad-sense heritability in lowland conditions, moderate-to-high genetic gain values for key yield traits, particularly bulb weight, revealed that effective selection remains possible. In this study, bulb weight showed high PCV and GCV values at both locations, making it a strong trait for selecting garlic. This result is similar to what Fufa *et al.* (2025) found about using bulb weight as a reliable selection marker in vegetative crops.

As a vegetative plant, garlic does not inherit traits resulting from gene interactions in subsequent generations. Consequently, in clonally propagated crops, yield-related traits with higher genetic gain are often priorities in early-generation selection because of the integration of multiple physiological processes associated with adaptation and productivity (K *et al.*, 2024). The successful identification of 13 M2 mutants exceeding the best check genotype under lowland conditions enunciated that early-generation selection under target environments was feasible and informative, even under severe environmental constraints. These results supported the principle that effective selection for stress adaptation should take place directly under the target stress environment. This approach is especially crucial for mutation-derived populations where segregation and phenotypic expression were robustly environment dependent (Kraemer *et al.*, 2016).

Figure 1 shows the PCA biplot, which represents genotype-environment interactions. The considerable spatial divergence between the highland and lowland groups suggests that agroecological altitude is a primary stressor, influencing how mutations express themselves. Mutants H28, H29, H15, and H23, which are in a close relation to the yield-component vectors (BW, DVB, and DHB), show promise for development in highland locations.

Furthermore, mutation L9 was successful in its identification as capable of maintaining optimal growth and bulb formation despite being under lowland stress. The superior bulb weight and size traits demonstrated by L9 make it the most promising mutant candidate for lowland adaptive garlic breeding programs. Therefore, evaluating individual mutations using PCA biplots can reduce the selection bias inherent in mutation screening during early generations. In line with the findings of Rasheed *et al.* (2025), their findings highlight the potential for genetic variability in the development of high-yielding, adaptive, and marketable varieties using PCA biplot analysis.

CONCLUSIONS

The analysis of genetic parameters confirms that the highland environment optimizes the expression of the genetic potential of M2 garlic mutants. Conversely, although the environmental stress of tropical lowlands inhibits growth and yield, the garlic mutants still exhibit sufficient variability for selection based on bulb weight. The PCA biplot successfully validated this $G \times E$ interaction by explaining 55.41% of the total variance. Mutants H28, H29, H15, and H23 show potential as putative highland-adapted mutants. Meanwhile, mutant L9 can adapt to lowland areas. Garlic is a vegetatively propagated crop; these induced superior traits have the potential to be genetically fixed if passed on to the next generation. Thus, while restricted to a single-season observation, early-generation selection supplemented by biplot analysis serves as an efficient preliminary step toward identifying candidate lines for future multi-environment validation.

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