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Genotype × environment interactions and genetic variation reveal stable, high-yielding, and organosulfur-rich garlic cultivars adapted to tropical conditions

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Garlic is valued for its yield and sulfur-containing bioactive compounds, but the influence of genotype × environment (G × E) interactions on these traits remains poorly understood. This study evaluated seven garlic cultivars: Tawangmangu Baru (TB), Lumbu Kuning (LK), Sangga Sembalun (SS), Lumbu Putih (LP), Geol (G), Kusuma (K), and Lumbu Hijau (LH), across two agroclimatic regions in Mojokerto (cool) and Pasuruan (warm), Indonesia, during the 2022 and 2023 growing seasons. Key traits, including yield and organosulfur compounds (allicin, S-allyl cysteine [SAC], γ-glutamyl-S-allyl-L-cysteine [GSAC], alliin) were assessed. G × E interactions were analyzed using the Additive Main Effects and Multiplicative Interaction (AMMI) model. Single-nucleotide polymorphism (SNP) variation in the DELLA-like gene *Asa4G02090.1* was also examined. Results revealed significant G × E effects on yield and compound synthesis, with TB best performed in cooler environments, whereas LP exhibited stable production across the studied environments. Genetic variation in DELLA-like gene *Asa4G02090.1* was highest in high-yielding TB and LK (42.3%) and lowest in LP (26.9%), which corresponds with its phenotypic stability. This study emphasizes the significance of G × E interactions and genetic diversity in selecting high-quality cultivars, offering new insights for breeding programs to develop high-yielding, stable cultivars, particularly in tropical regions.

Keywords Allium sativum, AMMI model, *DELLA* gene, Genotype stability index, Organosulfur compounds

Garlic (*Allium sativum* L.) is one of the most important horticultural crops worldwide, valued for its culinary, medicinal, and economic contributions. It is widely used as both a spice and a vegetable, enhancing food flavor and providing numerous health benefits due to its rich content of bioactive compounds. Garlic is particularly known for its high levels of organosulfur compounds, such as allicin, S-allyl cysteine (SAC), γ-glutamyl-S-allyl-

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L-cysteine (GSAC), and alliin, which exhibit potent antioxidant, antimicrobial, cardioprotective, and anticancer activities[1,2]. Originally from Central Asia, garlic spread throughout Southeast Asia, Egypt, and the Mediterranean region, becoming the second-most widely consumed member of the *Allium* species after the onion.

In Asia, particularly Indonesia, garlic is a crucial cash crop that significantly impacts the nation's agricultural economy, especially during the off-season when demand often exceeds supply. Garlic cultivation in these regions, however, faces significant challenges due to varying environmental conditions, which are further intensified by the effects of climate change. Fluctuating temperatures, prolonged droughts, excessive rainfall, and temperature shifts directly impact plant growth, bulb yield, and the synthesis of bioactive compounds[3–5].

Genotype × environment (G × E) interactions are recognized as key determinants of phenotypic expression in many crops[6,7]. Such interactions significantly influence agronomic and quality traits, including bulb size, clove number, yield, and organosulfur content. Environmental variation, particularly in soil characteristics, temperature, and humidity, significantly affects garlic's adaptability, productivity, and quality[6–8]. For instance, suboptimal or low temperatures may restrict bulb development, whereas high temperatures accelerate maturation and increase evapotranspiration, resulting in smaller bulbs and reduced yields[9,10]. Consequently, environmental heterogeneity across agroclimatic zones complicates breeding programs aimed at achieving stable, high-yielding cultivars suited for tropical conditions[11,12].

Although garlic is vegetatively propagated and exhibits limited genetic recombination, it still shows substantial phenotypic variability in bulb morphology, yield, and organosulfur content[13,14]. Environmental factors, such as temperature and soil variation, have been reported to significantly influence garlic's productivity, adaptability, and organosulfur biosynthesis[8,15]. Notably, cooler temperatures tend to enhance the accumulation of sulfur-containing metabolites, such as allicin and SAC[16,17], whereas warmer environments promote rapid vegetative growth and larger bulb size, but reduce sulfur metabolism[18]. Despite these findings, the molecular mechanisms underlying these adaptive responses, particularly those linked to sulfur metabolic pathways, remain unknown.

Most previous studies have evaluated garlic phenotype plasticity, genotype stability, or biochemical composition separately[11,13,15,18]. However, to the best of our knowledge, no integrative analyses have been reported linking genetic variation with phenotypic and biochemical traits across different environments in garlic. In particular, the potential role of DELLA-like transcription factors in modulating garlic adaptability and organosulfur metabolism under various environmental pressures remains unexplored. Understanding how allelic diversity in DELLA-like genes interacts with environmental factors is therefore crucial for identifying molecular determinants of yield stability and bioactive compound synthesis in tropical garlic.

Recent studies have highlighted DELLA proteins as key repressors of gibberellin signaling and central regulators of plant growth, development, and stress adaptation[19,20]. As members of the GRAS (Gibberellic Acid Insensitive, Repressor of GAI, and Scarecrow) transcription factor family, DELLA proteins play central roles in the gibberellin (GA) signaling pathway[21,22]. This pathway controls various aspects of plant development, including cell elongation, flowering, and stress tolerance[21,23]. DELLA proteins also modulate meristem function, organ development, and environmental responses, highlighting their relevance in plant adaptability[19,23]. In garlic, the DELLA-like gene *Asa4G02090.1* has been identified through genome-wide analysis of GRAS transcription factors[22]. Functional analyses indicate that *Asa4G02090.1* responds to gibberellin (GA_3) treatment and correlates with bulb diameter and weight, implying a role in regulating bulb development. However, its potential involvement in sulfur metabolism and genotype-dependent adaptation remains unexplored.

As a tropical garlic-producing country, Indonesia encompasses diverse agroclimatic zones, from lowlands to high-altitude regions, providing an ideal setting for studying G × E interactions in garlic. These regions exhibit significant variations in temperature, humidity, and soil characteristics, providing an opportunity to evaluate how different environmental factors influence garlic yield and the production of bioactive compounds[11,18]. However, the genetic and phenotypic diversity of Indonesian garlic remains poorly characterized, limiting the development of breeding strategies to improve production stability and the biosynthesis of bioactive compounds.

Tropical regions, such as Indonesia, pose particular challenges for garlic production due to elevated temperatures and erratic precipitation. Garlic, which thrives in cooler climates (12–24 °C), matures more quickly and produces smaller bulbs in warmer environments, resulting in reduced yields. Extreme rainfall patterns associated with El Niño and La Niña events further intensify water stress and pest incidence[24]. Soil degradation due to fluctuating rainfall and temperature also affects nutrient dynamics and uptake[25]. Addressing these challenges requires adaptive cultivation strategies that are supported by an improved understanding of genetic, physiological, and biochemical responses across various environments.

Given Indonesia's diverse climate zones, ranging from the cooler highlands of Central Java to the tropical lowlands of East Java, selecting garlic varieties that suit these environments is essential for achieving stable yields. In this study, we examine the combined effects of G × E interactions and allelic diversity in the DELLA-like gene *Asa4G02090.1* on the phenotypic performance and biochemical profiles of Indonesian garlic cultivars grown across contrasting agroclimatic conditions. Phenotypic, biochemical, and molecular datasets were integrated to assess how environmental variability influences yield stability and organosulfur metabolism. The G × E interaction was further analyzed using the Additive Main Effects and Multiplicative Interaction (AMMI) model to identify cultivars with superior adaptability and consistent biochemical traits. Collectively, these analyses provide insights into the molecular, physiological, and environmental factors that shape garlic adaptability and productivity, thereby contributing to the development of climate-resilient cultivars suited to tropical agricultural systems.

Materials and methods

Plant material and experimental design

This study was conducted using seven garlic cultivars: Lumbu Putih (LP), Lumbu Hijau (LH), Lumbu Kuning (LK), Sangga Sembalun (SS), Tawangmangu Baru (TB), Geol (G), and Kusuma (K) taken from the National

Research and Innovation Agency (BRIN), Indonesia. These cultivars were selected based on their diverse geographical and altitudinal origins across Indonesia, ranging from lowland to highland growing conditions. They represent the most widely cultivated cultivars in their respective regions (Table 1). Planting materials were sourced from the Indonesian Agency for Agricultural Research and Development to ensure genetic authenticity and consistency across trials.

Field trials were conducted from April to October in two growing seasons of 2022 and 2023 across two contrasting agroclimatic locations in East Java, Indonesia: Mojokerto (550 m a.s.l.) and Pasuruan (631.3 m a.s.l.) (Fig. 1). These sites were chosen to represent distinct environmental conditions: Mojokerto, with cooler temperatures (21–23 °C) and high humidity (83–92%), and Pasuruan, with warmer temperatures (26–28 °C) and fluctuating moisture levels (67–81%). The field trials followed a randomized complete block design (RCBD) with four replications per cultivar at each location. Each experimental plot measured 1 m × 1.5 m and contained 80 plants, resulting in a total of 320 plants per cultivar at each study site. This design provided sufficient within-site replication to capture environmental and phenotypic variability while maintaining consistent plant density across locations. Overall, 20 individual plants per genotype (5 plants × 4 replications) were evaluated for phenotypic traits, and five bulbs per genotype were used for metabolite analysis.

Soil and environmental parameters

Before planting, soil samples were collected from each site to evaluate major soil parameters, including pH, organic carbon content, macronutrient levels (N, P, K), and sulfur availability. Soil pH was measured using a pH meter (1:1 soil-to-water ratio), while organic carbon was determined using the Walkley-Black method[26]. Sulfur availability was assessed using calcium chloride ($CaCl_2$) extraction and subsequent turbidimetric analysis[27]. Climate data (temperature, humidity, and rainfall) were continuously monitored at each site using installed weather stations.

Agronomic management

Standard agronomic practices were followed throughout the trials. Before planting, the soil was ploughed and leveled to improve aeration and root development. Fertilization was applied three days before planting with a pre-planting dose of SP36 (300 kg/ha) and NPK (500 kg/ha). Subsequent fertilization included NPK at 5, 30, and 45 days after planting (DAP), ZA and KCl at 60 and 75 DAP, and $MgSO_4$ at 70 and 80 DAP. Irrigation was provided bi-weekly, with adjustments based on local rainfall. Pest control was implemented using an integrated pest management (IPM) approach, which include manual weed removal and biopesticide applications.

Phenotypic data collection

Vegetative growth assessment

Key vegetative growth parameters, including plant height and leaf number, were recorded 90 days after planting (DAP). Plant height was measured from the soil surface to the tip of the longest leaf, while the number of leaves per plant was counted at the peak of vegetative growth.

Bulb traits and yield components

At 120 DAP, garlic bulbs were harvested and cured for 21 days in a well-ventilated, shaded area to facilitate uniform drying and improve post-harvest stability. Bulb size (diameter and length) was measured using a digital calliper, and the number of cloves per bulb was manually counted. Bulb weight was recorded following curing to assess individual bulb mass. The total yield per plot was determined by summing each plot's bulb weight and converting it to tons per hectare (tons/ha) to assess cultivar-specific productivity. The total yield was calculated in tons per hectare by the following formula:

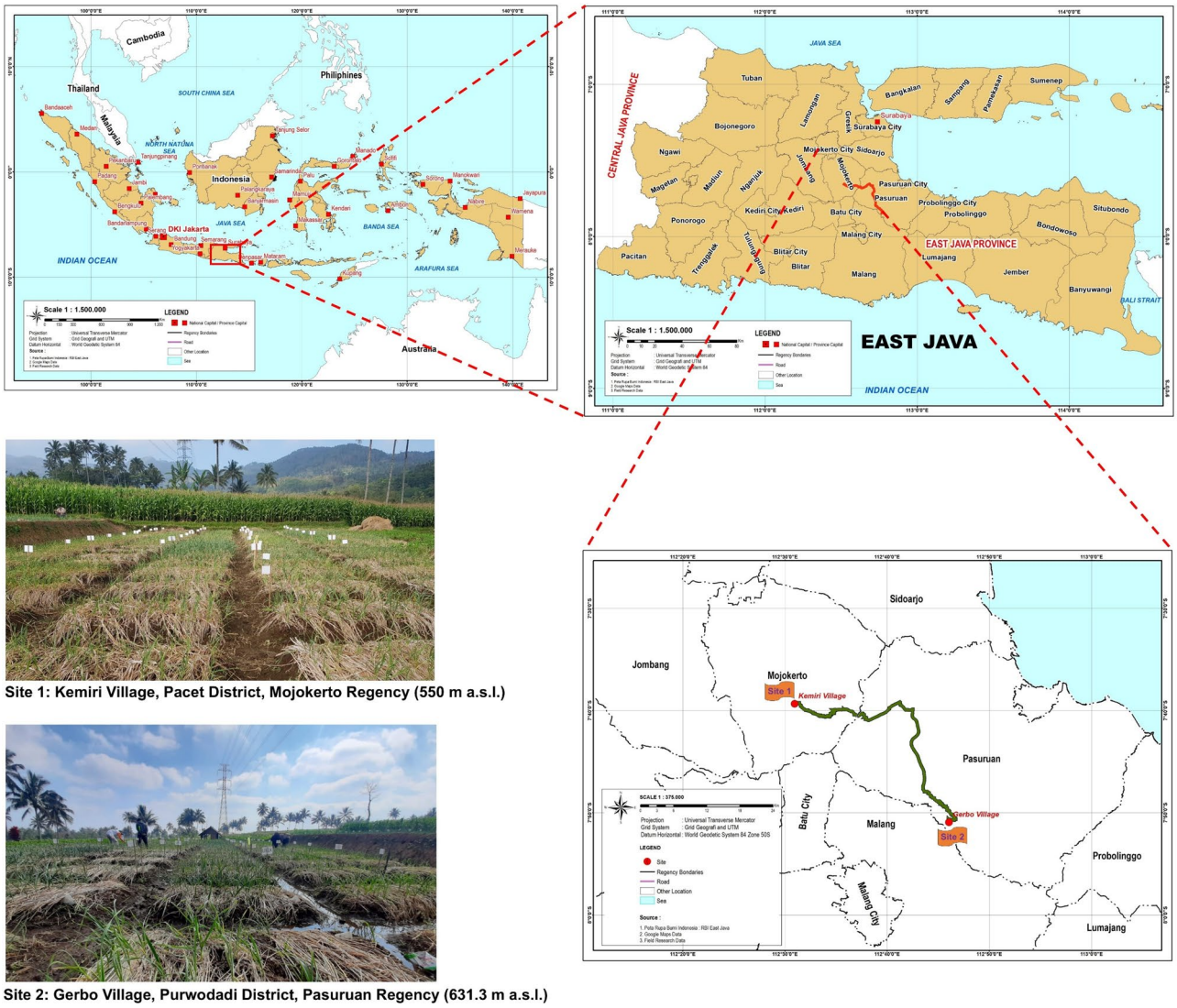
$$\text{Total yield } (tons/ha) = \frac{\text{Bulb yield/plot}}{\text{Area of plot } (\text{m}^2)} \times 10{,}000\ \text{m}^2$$

Additionally, the bulb ratio (%) was determined using the formula:

$$\text{Bulb ratio } (\%) = \frac{\text{bulb weight (g/plant)}}{\text{plant biomass (g/plant)}} \times 100$$

Cultivar	Origin	Growing altitude	Productivity capacity
Lumbu Putih (LP)	Yogyakarta, DIY	50–200 m a.s.l (low)	6–8 tons/ha
Lumbu Kuning (LK)	Batu, East Java	600–900 m a.s.l (medium)	6–8 tons/ha
Kusuma (K)	Malang, East Java	500–900 m a.s.l (medium)	8–10 tons/ha
Lumbu Hijau (LH)	Batu, East Java	900–1100 m a.s.l (high)	8–10 tons/ha
Sangga Sembalun (SS)	Lombok, West Nusa Tenggara	800–1200 m a.s.l (high)	8–9 tons/ha
Tawangmangu Baru (TB)	Tawangmangu, Central Java	1000–1400 m a.s.l (high)	8–12 tons/ha
Geol (G)	Temanggung, Central Java	1000–1500 m a.s.l (high)	6–9 tons/ha

Table 1. Geographical origins, growing altitudes, and productivity capacity of the seven garlic cultivars studied.



Site 1: Kemiri Village, Pacet District, Mojokerto Regency (550 m a.s.l.)

Site 2: Gerbo Village, Purwodadi District, Pasuruan Regency (631.3 m a.s.l.)

Fig. 1. Geographic distribution of garlic cultivation sites in East Java, Indonesia. Mojokerto (Site 1) and Pasuruan (Site 2) are highlighted, with an inset map showing the location within East Java. Representative field photographs are also included. Maps were created by the corresponding author (WH), using QGIS software (version 3.34.14; https://qgis.org/en/site/). All field photographs were taken by the authors.

Bioactive compound analysis

Sample preparation for targeted metabolomic profiling

Five bulbs per plot were randomly selected for analysis of bioactive compounds. Fresh garlic cloves (10–15 g) from each bulb were homogenized, and the homogenate was extracted using ultrasonic-assisted extraction in 30 mL of 0.01 M HCl at 40 °C for 25 min. The supernatant was collected after centrifugation (3500 rpm for 5 min). The remaining pellet was re-extracted with 15 mL of 0.01 M HCl, and the supernatants were combined. The final extract was filtered through a 0.22 µm membrane, diluted to 50 mL, and stored at 4 °C before analysis.

LC-MS/MS analysis

Bioactive compounds, including allicin, SAC, GSAC, and alliin, were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a Thermo MSQ Plus LC-MS system and equipped with an Accela pump (Thermo Fisher Scientific, US) for precise chromatographic separation. The separation of compounds was performed using a binary mobile phase consisting of A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid), with the following gradient: 0–1 min, 5% B; 1–4.5 min, increase to 85% B; 4.5–5.5 min, 85% B; 5.5–6.5 min, decrease to 5% B; and 7–8.5 min, 5% B. The flow rates were 100 µL/min for 0–1 min, 150 µL/min for 1–4.5 min, 300 µL/min for 4.5–5.5 min, and 300 µL/min for 5.5–8.5 min, with the system pressure maintained at 10 bars. A sample injection volume of 10 µL was used for each analysis, and detection was performed by electrospray ionisation (ESI) in positive-ion mode.

Quantification of bioactive compounds

The concentrations of organosulfur compounds were determined by comparing their chromatographic peak areas to a spectral library and internal standards. Quantification was performed based on relative peak areas obtained from LC–MS/MS chromatograms. The data were normalized by expressing each compound as a percentage of the total organosulfur signal intensity (% of total area), enabling consistent comparison of metabolite composition across genotypes and environments.

The relative organosulfur content was calculated using the following formula:

$$\text{Relative content } (\%) = \frac{\text{Peak area of individual organosulfur compound}}{\text{Total peak area of all detected organosulfur compounds}} \times 100$$

Genetic diversity analysis and dendrogram construction

DNA extraction and PCR amplification

Genomic DNA was extracted from the young leaves of 20-day-old plants using the Plant Genomic DNA Kit, following the manufacturer's instructions. For each genotype at each location, genomic DNA was extracted from five independent biological replicates. Equal amounts of DNA were then pooled prior to amplification and sequencing to represent the overall allelic composition of the genotype. The *Asa4G02090.1* gene, a putative DELLA-like gene involved in regulating plant growth and bioactive compound biosynthesis, was amplified using specifically designed primers[22]. Primers synthesized by Integrated DNA Technologies Pte. Ltd., Singapore, were used (Forward: GAACCATAAGCGTCAGAGAAC; Reverse: GAGCAAGCCATTAGAGTGTG). DNA amplification was performed using a Wee32® Thermal Cycler (HiMedia, India). The PCR reaction consists of a 25 µL PCR mixture, with 2 µL of template DNA (0.1–1.0 µg), 12.5 µL of MyTaq HS Red Mix (Bioline, London, UK), and 1 µL of each forward and reverse primer. The PCR program consisted of an initial denaturation step at 95 °C for 1 min, followed by 35 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The PCR products were analyzed via electrophoresis on a 2% agarose gel stained with GelRed® (Biotium, Fremont, CA, USA) (Supplementary Figure S1).

PCR product purification

The PCR products were purified using the DNA Clean & Concentrator-5 kit (Zymo Research, USA) according to the manufacturer's protocol to remove residual primers, nucleotides, and enzymes. The purified PCR products were quantified using a NanoDrop spectrophotometer.

Sanger sequencing

The purified PCR products were sequenced using the ABI 3500XL Sanger sequencer (Applied Biosystems, USA). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and the resulting sequences were analyzed to ensure the presence of high-quality reads.

Nucleotide variation analysis

The raw sequence data were processed and assembled using Geneious Prime software (Biomatters Ltd, New Zealand). The assembled sequences of the studied garlic cultivars were aligned using the ClustalW algorithm in Geneious[28]. The *Asa4G02090.1* marker gene was used to analyze nucleotide variation among the seven garlic cultivars. The degree of genetic variation, including single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels), was evaluated by comparing sequence differences across the cultivars.

Dendrogram construction

To assess the genetic relationships among the garlic cultivars, a dendrogram was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method[29]. A distance matrix was calculated based on the nucleotide variations identified through the sequence alignment. The distance matrix was used to construct the dendrogram, which visualizes the clustering of cultivars based on their genetic similarity. Bootstrap analysis was performed with 1,000 replicates to assess the reliability of the tree topology. The dendrogram was constructed using the MEGA11 software[30], which enabled visualization and evaluation of phylogenetic relationships among garlic cultivars.

Data analysis

Data pre-processing and assumption testing

All data from phenotypic, organosulfur, and genetic diversity assessments were subjected to statistical analysis to evaluate the significance of differences among garlic cultivars and their responses to environmental conditions. Before performing any analysis, datasets were tested for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. If assumptions of normality or homogeneity were violated, the data were log- or square-root-transformed to ensure statistical robustness.

Analysis of variance (ANOVA) and post-hoc comparisons

A two-way analysis of variance (ANOVA) was conducted to assess the effects of genotype, environment, and G × E interactions on plant growth traits, bulb morphology, yield performance, and organosulfur compound concentrations. Significant differences ($p < 0.05$) were further evaluated using Tukey's honest significant difference (HSD) post-hoc test, allowing for pairwise comparisons among cultivars and study locations.

Correlation between variables and genotypes

Correlation coefficients were computed using R version 4.4.2 in R Studio (version 2024.12.0 + 467) with the metan package[31]. Pearson correlation was classified as high (≥ ± 0.50 to ± 1), medium (≥ 0.30 to < ± 0.50), and low (< ± 0.30). Principal Component Analysis (PCA) was performed using R with the factoextra package[32].

Estimate selection differential, heritability, and selection gain

Estimation of variance, heritability, and selection gain for 12 traits across seven garlic genotypes was computed using the Matrix package in R Studio version 2024.12.0 + 467 (https://cran.r-project.org/web/packages/Matrix/index.html). The overall mean of genotypes (X0), individual genotype values (Xi), mean of selected genotypes (Xs), selection differential (SD), percentage selection differential (%SD), value of phenotypic variance(σ^2p), value of genotypic variance (σ^2g), heritability (h^2), selection gain (SG), and percentage of selection gain (%SG) were calculated using the following formulas:

1. Overall mean of genotypes

$$X0 = \sum \frac{Xi}{n}$$

This formula calculates the overall mean of the genotypes across all environments. It provides a baseline for comparing individual genotype performance.

2. Individual genotype values

$$Xi = \sum \frac{Xi}{e}$$

where *Xi* represents the mean value of a particular genotype across all environments. The formula calculates the average for each genotype, where *e* is the number of environments.

3. Mean of selected genotypes

$$Xs = \sum \frac{Xs}{ns}$$

4. Selection differential

$$SD = Xs - X0$$

The selection differential (SD) measures the difference between the mean value of selected genotypes (*Xs*) and the overall mean (X0), indicating the potential for selection gain.

5. Phenotypic variance

$$\sigma^2 p = \frac{MSg}{r}$$

Phenotypic variance (σ^2p) is calculated by dividing the mean square of genotype effects (MSg) by the number of replications (r). It quantifies the total variation in phenotypic traits.

6. Genotypic variance

$$\sigma^2 g = \frac{(MSg - MSe)}{r}$$

Genotypic variance (σ^2g) indicates the genetic variation among genotypes.

7. Heritability

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p}$$

Heritability (h^2) is the proportion of phenotypic variance attributed to genotypic variance. It indicates the potential for genetic improvement in the population. Heritability categories: low ($h^2 < 0.2$), medium ($0.2 \leq h^2 \leq 0.5$), and high ($h^2 > 0.5$). This classification helps in understanding the strength of genetic control over the trait, where higher heritability indicates a greater likelihood of genetic improvement through selection.

8. Selection gain and percentage of selection gain

$$SG = h^2 \times SD$$

$$\%SG = \frac{SG}{X0} \times 100$$

Selection gain (*SG*) quantifies genetic improvement by comparing the selection differential (SD) to the overall mean (X0) and is expressed as a percentage. Values of *SG* as a percentage of the mean from 0 to 10% are considered low, 10 to 20% as moderate, and 20% and above as high[33].

9. Mean total yield

$$MTY = \frac{TY_{E1} + TY_{E2} + TY_{E3}}{3}$$

The mean total yield (MTY) is the average yield from multiple environments: Mojokerto (E1), Pasuruan (E2), and the origin environments (E3). This formula helps evaluate a genotype's performance across various environmental conditions.

Analyzing multi-environment trials using the AMMI model

The AMMI model was used to analyze G × E interactions in R Studio using the metan package[31]. This model is commonly used for multi-environment trials (METs) and assists in understanding the interactions between genotypes and environmental factors critical for selecting high-yielding and stable genotypes[34]. The AMMI analysis was performed using total yield data to assess yield stability across environments. Missing values represented 0.8% of all observations. When individual genotype–environment cells were missing at random (e.g., when one of three replicates lacked a recorded value), the missing entries were imputed using the mean of the remaining two replicates within the same environment for that trait. The AMMI analysis was then conducted on the imputed complete dataset. In parallel, an additional AMMI analysis was performed without imputation to evaluate the robustness of the procedure. Both analyses demonstrated that genotype rankings and stability patterns remained consistent, indicating that the imputation method did not bias the analytical outcomes.

AMMI-based stability indexes

AMMI-based stability indexes were calculated in R Studio using the ammistability package with the AMMI_indexes(): function. The formulas for calculating each index are as follows:

1. AMMI stability index (ASI)

$$ASI = \sqrt{\left[\left[PC_1^2 \times \theta_1^2\right] + \left[PC_2^2 \times \theta_2^2\right]\right]}$$

where PC1 and PC2 are the first and second principal component scores of the genotype, and θ1 and θ2 are the proportions of variance explained by the first and second IPCAs[35].

2. AMMI stability value (ASV)

$$ASV = \sqrt{\frac{SS_{IPCA1}}{SS_{IPCA2}}} \times \sqrt{(IPCA1)^2 + (IPCA2)^2}$$

where SSIPCA1 and SSIPCA2 were the sums of squares of the first and second IPCAs, and IPCA1 and IPCA2 were the first and second interaction principal component scores[36].

3. Eigenvalues (EV)

$$EV = \sum_{n=1}^{N'} \frac{\gamma^2 in}{N'}$$

where $\gamma^2 in$ is the interaction principal component score for the n-th component, and N' is the number of significant IPCAs[37].

4. Modified AMMI stability index (MASI)

$$MASI = \sqrt{\sum {}^{N'}_{n=1} PC_n^2 \times \ \theta {}^{2}_{n}}$$

where PCn is the principal component score for the n-th IPCA, and θn is the proportion of variance explained by the n-th IPCA[38].

5. A sum of the interaction PC scores (SIPC)

$$SIPC = \sum {}^{N}_{n=1} |IPCA_{in}|$$

where *SIPC* is the summation of absolute *IPCA* scores across all components[39].

6. Weighted average of absolute scores (WAAS)

$$WAAS = \sum {}^{N}_{n=1} EV_n \times \ |IPCA_{in}|$$

where EVn is the proportion of variance explained by the n-th IPCA[40].

7. Ranking genotype (RTY)

$$RTY = Ranking\ of\ genotypes\ based\ on\ MTY$$

Ranking genotypes based on MTY (mean total yield) across environments. In RTY, the order is descending, and a lower rank means a greater yield.

8. Ranking WAAS (RWAAS)

$$RWAAS = Ranking\ genotypes\ based\ on\ WAAS$$

In RWAAS, the order is ascending order, and a lower rank means greater stability.

9. Genotype stability index (GSI)

The genotype stability index (GSI), also known as the yield stability index (YSI), and stability rankings were computed based on the AMMI analysis, allowing for the identification of genotypes with the highest adaptability and stability across environments. Genotypes were ranked by their performance in terms of yield and stability, with lower GSI values indicating more stable and higher-yielding genotypes. GSI was calculated using the following formula:

$$GSI = RTY + RWAAS$$

Results

Agroclimatic and soil characteristics at study sites

Field trials were conducted in East Java, Indonesia, at two contrasting tropical agroclimatic sites—Mojokerto and Pasuruan—during the 2022 and 2023 growing seasons from April to October. The average annual rainfall at Mojokerto (1,719 mm/year) classifies it as an intermediate region, whereas Pasuruan (2,155 mm/year) is classified as a rainy region[41]. Mojokerto has cooler temperatures (21–23 °C) and high humidity (83–92%), whereas Pasuruan experiences warmer temperatures (26–28 °C) and more variable moisture (67–81%). These contrasting agroclimatic conditions likely influenced the observed phenotypic variation in garlic cultivars. Detailed soil physicochemical properties for each site are provided in Supplementary Table S1.

Soil analyses revealed notable differences between the sites. Mojokerto's acidic clay loam (pH 4.1) has high nutrient retention but poor water drainage, whereas Pasuruan's loamy soil (pH 5.8) offers better drainage.

Mojokerto's soil has an organic content of 1.79%, with moderate nutrient levels. Potassium (0.26 Cmol+/kg) and magnesium (0.85 Cmol+/kg) are low. The high cation exchange capacity (CEC 27.86 Cmol+/kg) ensures adequate nutrient availability for plant growth. The soil in Pasuruan contains 2.12% organic carbon, with higher potassium (1.28 Cmol+/kg) and magnesium (2.23 Cmol+/kg) levels compared to Mojokerto, indicating a more nutrient-rich environment. However, its lower CEC (23.54 Cmol+/kg) may affect long-term nutrient retention. These factors may have impacted the garlic growth observed at each location.

Phenotypic and yield performance across environments

Significant phenotypic variation was observed among the seven garlic cultivars across the two environments (Mojokerto and Pasuruan), reflecting strong G × E interactions (Figs. 2 and 3). Differences in plant height, leaf number, bulb morphology, and yield performance were all statistically significant ($p < 0.05$).

Cultivar TB consistently exhibited the best vegetative vigor, producing the tallest plants and most leaves in both locations, with mean heights of 39.43 cm in Mojokerto and 47.72 cm in Pasuruan (Fig. 2a, b). These values were followed by SS and LK, which also demonstrated robust vegetative growth. In contrast, G showed relatively stable, but lower, vegetative performance across environments, suggesting a more conservative growth strategy. The higher leaf number in TB and K likely enhanced photosynthetic efficiency, contributing to their superior biomass accumulation (Fig. 2c). Previous studies have indicated that the number of leaves per plant is a key indicator of plant vigor and developmental stage[42,43].

Morphological and yield-related traits followed similar patterns (Fig. 3). TB produced the largest bulbs (3.91–4.65 cm in diameter and 3.07–3.29 cm length) and the highest plant dry weight (56.32 g/plant in Mojokerto), demonstrating efficient conversion of assimilates into bulb biomass (Fig. 3a–d). The number of cloves per bulb varied significantly among cultivars; K had the highest clove count across both locations, while LP exhibited a relatively higher clove number in Pasuruan (Fig. 3f). These differences confirm strong G × E effects on reproductive development.

Total yield varied markedly between cultivars and sites. TB achieved the highest yield, reaching 16.22 tons/ha in Mojokerto and 13.25 tons/ha in Pasuruan, representing a 91.27% yield advantage over Lumbu Hijau (LH) in Mojokerto (8.48 tons/ha) (Fig. 3g). The yield reduction of TB in Pasuruan underscores its sensitivity to warmer conditions, while LK maintained comparatively higher yields in Pasuruan, indicating better adaptation to heat and lower humidity. Overall, the yield patterns reveal that TB performs best under moderate temperature and humidity, whereas LK and SS combine both productivity and adaptability across environments.

Differences in biomass partitioning were also evident (Fig. 3e). The bulb ratio, representing the proportion of dry matter allocated to bulbs, was highest in K and LP, indicating a more substantial allocation of resources toward reproductive growth. In contrast, TB displayed a more balanced allocation between vegetative and reproductive organs, supporting both high vegetative vigor and substantial bulb formation (Fig. 3a, b). This balanced growth pattern likely contributes to TB's superior productivity under favorable conditions.

The integration of vegetative and reproductive traits demonstrates clear genotypic differentiation among garlic cultivars. TB consistently ranked as the highest-yielding cultivar with strong vegetative vigor but moderate environmental sensitivity, while LK and SS exhibited superior stability across contrasting agroclimatic regions. Conversely, LP, although yielding less, showed relatively stable performance, indicating suitability for areas with variable conditions. These results collectively emphasize that garlic productivity and adaptability are governed by complex G × E interactions, in which both genetic potential and environmental suitability play decisive roles in determining yield outcomes.

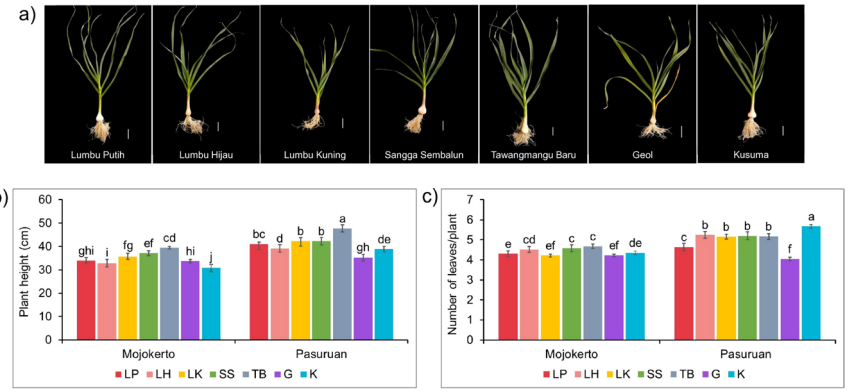


Fig. 2. Phenotypic variation and agronomic traits of garlic cultivars across two locations (Mojokerto and Pasuruan). (**a**) Visual representation of the seven garlic cultivars with distinct growth patterns. Scale bars represent 5 cm. (**b**) Plant height (cm) of the garlic cultivars. (**c**) Number of leaves per plant of the garlic cultivars. Different letters indicate significant differences among cultivars within each location based on Tukey's HSD test ($p < 0.05$). Data are presented as means ± SD, $n = 20$. Cultivar abbreviations: LP (Lumbu Putih), LH (Lumbu Hijau), LK (Lumbu Kuning), TB (Tawangmangu Baru), SS (Sangga Sembalun), G (Geol), and K (Kusuma).

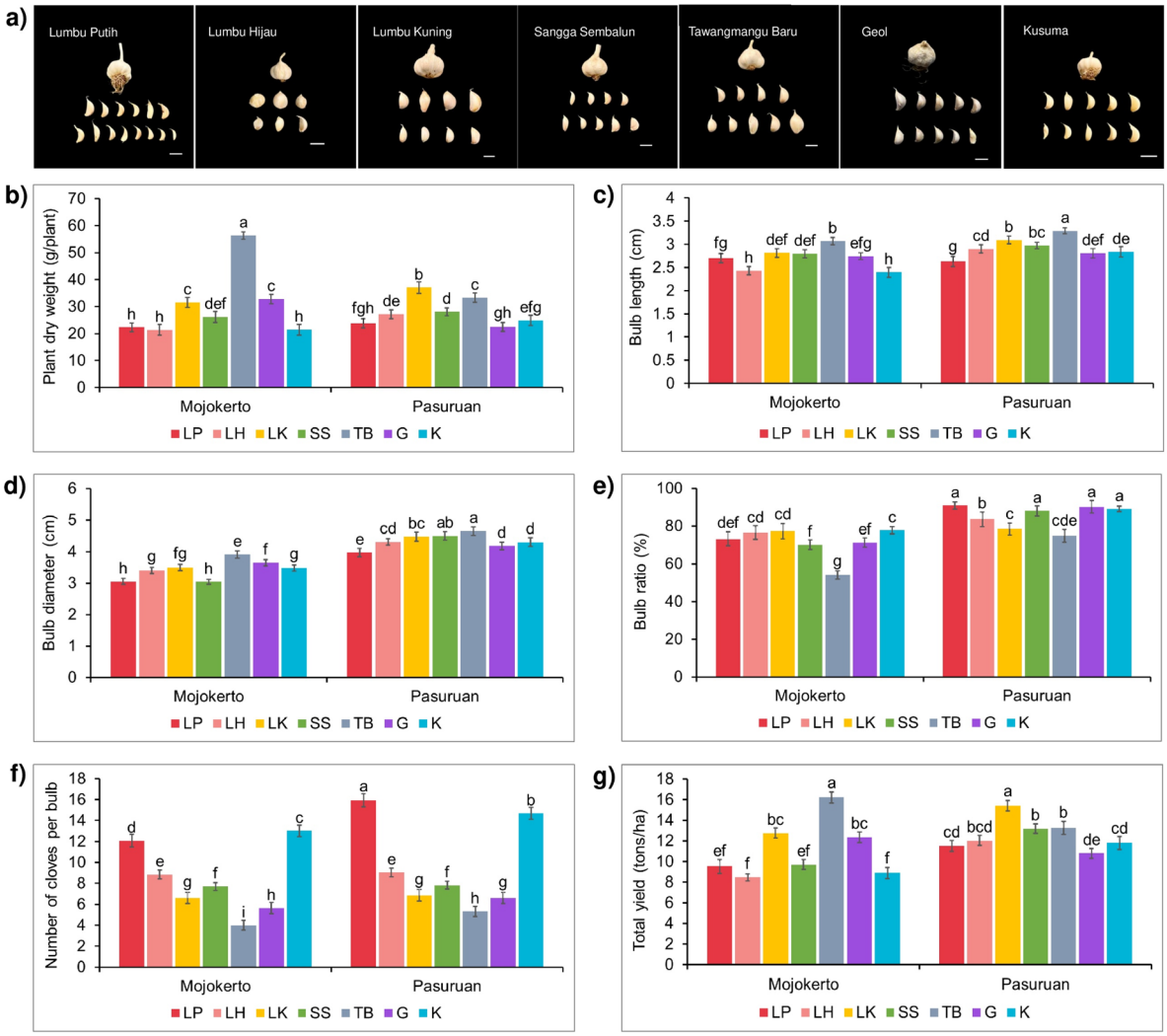


Fig. 3. Morphological and yield-related traits of seven garlic cultivars grown in Mojokerto and Pasuruan. (**a**) Representative bulbs of each cultivar illustrating size and clove structure (scale bar = 5 cm). (**b**) Plant dry weight (g/plant); (**c**) Bulb length (cm); (**d**) Bulb diameter (cm); (**e**) Bulb ratio (%); (**f**) Number of cloves per bulb; and (**g**) Total yield (tons/ha) across two agroclimatic environments. Bars represent means ± SD ($n = 20$). Different lowercase letters above bars indicate statistically significant differences among cultivars within the same environment based on Tukey's HSD test ($p < 0.05$). Cultivar abbreviations: LP (Lumbu Putih), LH (Lumbu Hijau), LK (Lumbu Kuning), TB (Tawangmangu Baru), SS (Sangga Sembalun), G (Geol), and K (Kusuma).

Bioactive compounds profiling

This study examined the interactions between genotype and environment on the bioactive compounds in garlic bulbs. Our findings demonstrated significant genotypic variation in the production of key organosulfur compounds (allicin, SAC, GSAC, and alliin) across two locations (Fig. 4). Notably, alliin synthesis fluctuated significantly among the seven cultivars ($p < 0.05$) (Fig. 4a). TB and SS cultivars had the highest alliin content in Mojokerto (98.07% and 96.06%, respectively), but both exhibited significant reduction in Pasuruan, with decreases of 45.36% and 47.53%, respectively. This dramatic reduction highlights the effect of agroclimatic factors on the stability of alliin synthesis.

In contrast, LP showed consistent SAC production across both locations, with LH (97.20%) and TB (99.53%) being the highest producers in Mojokerto and Pasuruan, respectively (Fig. 4b). Interestingly, while SAC production showed location-specific variation, LP demonstrated stability across both environments, making it a reliable cultivar for SAC production.

Similar variability was observed in GSAC and allicin synthesis. The highest GSAC producers were LK in Mojokerto (99.87%) and SS in Pasuruan (84.13%) (Fig. 4c). For allicin, the highest producers were LP (86.08%) in Mojokerto and TB (90.68%) in Pasuruan (Fig. 4d). Notably, TB demonstrated optimal performance in Pasuruan, with the highest production of both SAC and allicin, indicating a strong location-specific advantage for this cultivar.

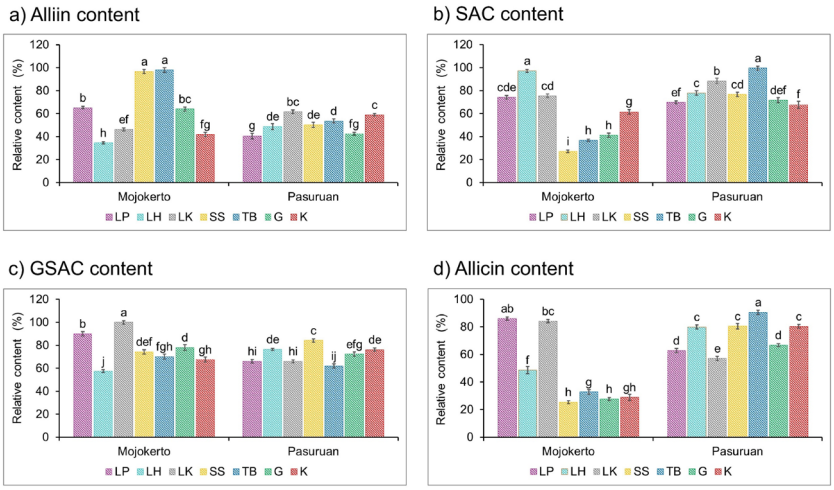


Fig. 4. Relative content (%) of major organosulfur compounds in seven garlic genotypes grown under two agroclimatic environments. Panels show relative concentrations of (**a**) Alliin, (**b**) S-allyl cysteine (SAC), (**c**) γ-glutamyl-S-allyl-L-cysteine (GSAC), and (**d**) Allicin in Mojokerto and Pasuruan. Data are presented as mean ± SD ($n = 5$). Different lowercase letters above bars denote statistically significant differences among cultivars within each environment, as determined by Tukey's HSD test ($p < 0.05$). These results illustrate genotype- and environment-dependent variations in sulfur metabolism, with TB showing the highest SAC and allicin production under warmer conditions.

These findings emphasize the role of G × E interactions in shaping garlic's medicinal properties. Agroclimatic factors significantly influence the synthesis of organosulfur compounds, with Mojokerto favouring alliin accumulation and Pasuruan favouring SAC and allicin. The superior SAC and allicin production observed in TB suggest its potential as a breeding candidate for enhancing garlic's medicinal quality.

Genetic diversity and polymorphism in the DELLA-Like gene Asa4G02090.1

Sequence analysis of the DELLA-like gene *Asa4G02090.1* revealed significant genetic polymorphisms across garlic cultivars, with LK, SS, and TB exhibiting the highest nucleotide variation (42.3%) and the highest number of SNPs (11; Table 2). This variation is correlated with superior performance in vegetative growth, yield, and the synthesis of bioactive compounds (Table 2). These trait variations are likely associated with DELLA protein function and gibberellic acid (GA) signaling, suggesting that this gene plays a critical role in cultivar adaptation and resilience under varying conditions.

The genetic relationships among seven garlic cultivars were assessed using the putative *Asa4G02090.1* gene and analyzed by UPGMA cluster analysis based on Jaccard's similarity coefficient (Fig. 5). The analysis categorized the cultivars into four genetic clusters:

- Cluster I: TB, LK, and SS, which indicate a shared genetic background. These cultivars are potentially promising in breeding programs that require genetic consistency, ensuring uniformity in traits such as yield and bioactive compound content.
- Cluster II: G and LH, which are genetically close and beneficial for targeted breeding to enhance specific traits.
- Cluster III consists solely of cultivar K, which has a unique genetic profile that can introduce genetic diversity into breeding programs, potentially leading to the development of new garlic varieties with distinctive traits, such as improved adaptability.
- Cluster IV comprises cultivar LP, which is genetically distinct and offers potential for introducing novel traits such as improved resilience and growth performance under various environmental conditions.

The dendrogram (Fig. 5) highlights significant genetic diversity within the cultivars. K and LP stand out as unique, with potential to develop new garlic varieties with enhanced agronomic traits. Our results demonstrate that the Asa4G02090.1 gene marker is a reliable tool for distinguishing genetic relationships and informing breeding programs.

Correlation and multivariate analysis

Correlation between traits and genotypes

Pearson correlation analysis revealed strong positive correlations between total yield and bulb traits (e.g., bulb diameter, plant height), and moderate correlations between bulb ratio and organosulfur content (Fig. 6a). Notably, plant dry weight was strongly correlated with both bioactive compound synthesis and total yield ($r = 0.84$, $p < 0.001$), highlighting the importance of biomass accumulation in driving yield and medicinal quality. PCA analysis (Fig. 6b) clearly distinguished genotypes based on their growth traits and bioactive compound synthesis, with TB showing the strongest correlation with high yield and organosulfur production.

Genotype	Nucleotide sequence																										SNP counts	Variation (%)
	5	52	53	54	55	73	85	86	87	91	99	100	110	115	123	124	132	136	137	147	148	149	150	151	165	192		
LP	C	A	T	C	**G**	G	G	-	C	C	C	**G**	T	C	T	**T**	-	C	G	-	A	**C**	T	C	G	G	7	26.9
LK	C	A	T	C	C	**C**	G	-	C	C	C	-	T	**G**	**C**	C	-	**T**	**C**	-	A	T	**C**	C	**T**	G	11	42.3
K	C	A	T	C	-	**C**	G	**G**	C	C	**G**	**C**	T	C	T	**T**	-	**T**	G	-	A	T	T	C	G	G	9	34.6
LH	C	A	T	C	C	G	G	-	C	C	C	-	**C**	**G**	**C**	C	-	C	G	-	A	**C**	T	C	G	G	8	30.8
SS	C	**G**	**A**	**T**	C	G	G	-	C	C	C	-	T	**G**	T	**T**	**C**	C	G	**G**	A	**C**	**C**	C	G	G	11	42.3
TB	**A**	A	T	C	C	G	G	**C**	**G**	**G**	**G**	**C**	**C**	C	T	C	**G**	C	-	-	A	T	T	C	G	**C**	11	42.3
G	C	A	T	C	C	G	**C**	**G**	C	C	C	-	T	C	T	C	**C**	C	G	**A**	**C**	T	**C**	**G**	G	G	8	30.8

Table 2. Nucleotide variations in the *Asa4G02090.1* gene sequence among seven Indonesian garlic genotypes. Sequence nucleotide variations (SNPs) are highlighted in bold, with the percentage of variation for each cultivar.

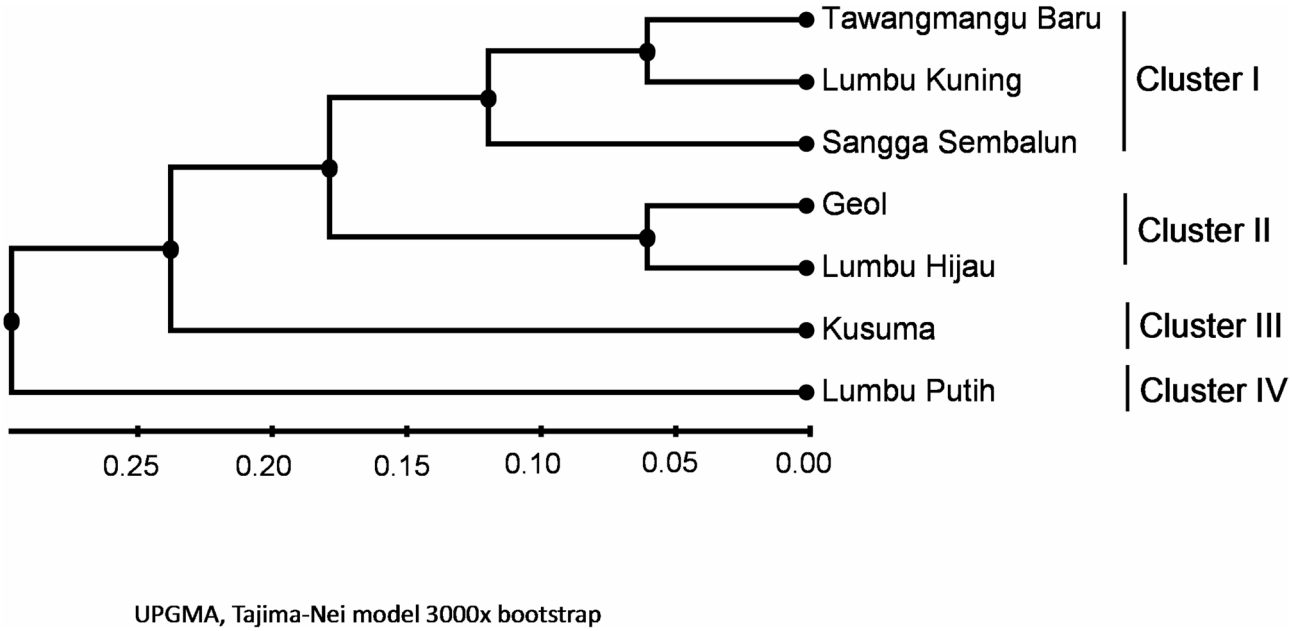


Fig. 5. UPGMA dendrogram cluster analysis showing the genetic relationship of seven garlic cultivars as determined by the putative DELLA-like gene *Asa4G02090.1*.

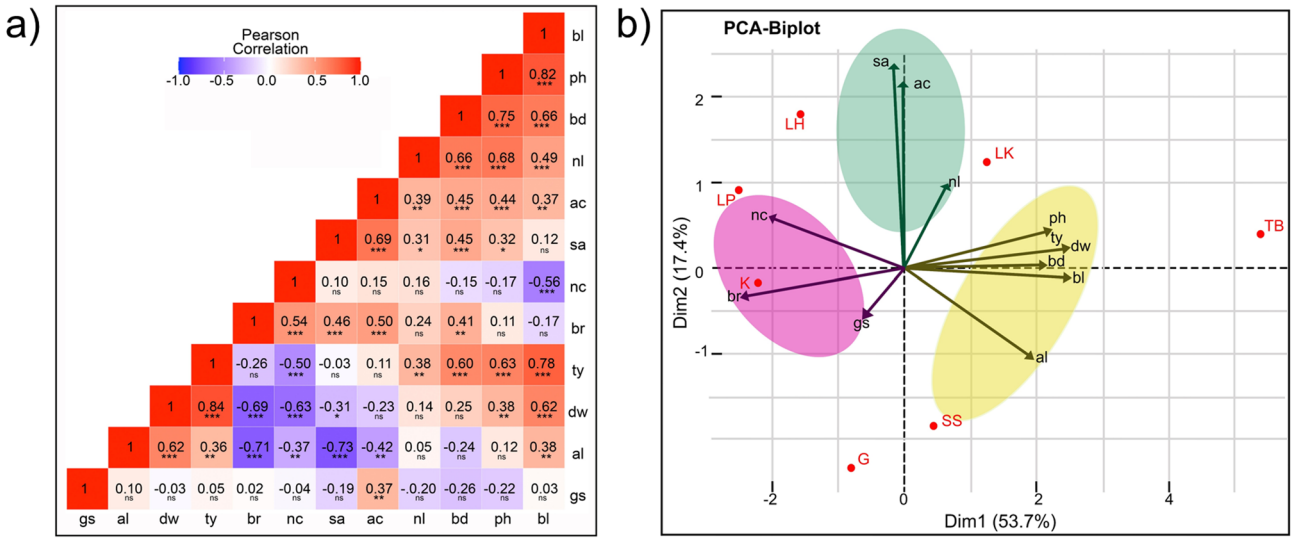


Fig. 6. Pearson correlation and principal component analysis (PCA) of morphological, yield, and bioactive compound traits in seven garlic genotypes. (**a**) Pearson's correlation matrix shows the relationships among morphological, yield, and bioactive compound traits across seven garlic genotypes grown in two environments. Correlation significance levels: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, ns = not significant. Correlation coefficients range from highly negative (blue) to highly positive (red). (**b**) PCA biplot showing trait associations and genotype clustering. Abbreviations: nl = number of leaves; ph = plant height (cm); dw = plant dry weight (g/plant); bd = bulb diameter (cm); bl = bulb length (cm); br = bulb ratio (%); ty = total yield (tons/ha); nc = number of cloves per bulb; al = alliin content; sa = SAC content; gs = GSAC content; ac = allicin content.

Heritability

All traits, except allicin content, exhibited high heritability ($h^2 > 0.7$), indicating that genetic rather than environmental factors strongly influence these traits. High selection differential (SD > 10%) indicates robust selection intensity, and high selection gain percentages (SG > 10%) imply significant phenotypic improvement, suggesting potential for rapid genetic advancement in breeding programs targeting these traits (Table 3).

Genotype × environment interactions

This study comprehensively evaluates G × E interactions to identify garlic cultivars with superior phenotypic plasticity, adaptability, and yield stability. The heatmap (Fig. 7a) of total yield across seven genotypes cultivated in two studied environments (E1 and E2) compared to the origin environment of each cultivar (E3) reveals distinct clustering patterns. The clustering of environments (E1 and E2 together, separate from E3) in the heatmap suggests that the yield responses in E1 and E2 are more similar to each other than those in E3 (Fig. 7a, d). Genotype clustering identified three distinct groups based on performance patterns:

- Cluster 1 (TB, LK, and SS) demonstrated the highest yield and interaction effects.
- Cluster 2 (LH and K) displayed moderate responses across environments.
- Cluster 1 (G and LP) showed stable but lower yield responses.

AMMI biplot analysis provides insights into the performance and stability of seven genotypes across three environments. The principal component scores (PC1 and PC2) reveal which genotypes exhibit stable performance and are influenced by environmental variations. PC1 explains 70% of the variation, suggesting that selection should focus on high-yielding genotypes, such as TB, which achieved a total yield of 16.22 tons per hectare (Fig. 7b). TB demonstrated superior and consistent performance across environments, making it the most productive genotype in the study. However, some environmental interaction remains (PC2 = 30%); therefore, genotypes with high or low PC2 values may require specific conditions to reach their full potential (Fig. 7c).

To assess genotype stability across environments, we analyzed the absolute values of PC2 (Fig. 7c). Genotypes with PC2 values closer to 0, such as LP (PC2 = -0.1322) and SS (PC2 = -0.1825), exhibited stable performance with minimal interaction effects, making them reliable for broad adaptation. In contrast, LK (PC2 = -1.543) exhibited the highest absolute PC2 value, indicating significant variation in yield depending on environmental conditions. TB (PC2 = 0.5968) also showed considerable interaction effects, suggesting that while it yields highly, its performance is less predictable across environments.

These findings suggest that TB has the potential to thrive in environments that favor its higher yield, such as medium-altitude regions. LP and SS, the most stable under diverse conditions, are more adapted for low-to-medium altitude regions.

Stability analysis using the AMMI model

AMMI indexes are useful for selecting genotypes based on their yield and stability. For instance, the breeder would want a specific genotype that either has a high yield at a particular location or balances high yield with stability. To monitor the relationship between yield and stability, we use RTY to rank genotypes based on the mean total yield. A higher yield (lower RTY) is typically desirable, but stability is critical, as indicated by a lower WAAS rank. TB has the highest mean total yield (MTY = 13.16, RTY = 1) but the lowest stability (RWAAS = 7), while LP has the highest stability (RWAAS = 1) but the lowest yield (RTY = 7) (Table 4). Furthermore, SS has a well-balanced genotype with a good yield (RTY = 3) and stability (RWAAS = 2), resulting in the lowest genotype stability index (GSI = 5), which offers an optimal trade-off between yield and stability.

Choosing the optimum genotype depends on the breeding target. TB is suitable for medium-altitude environments, providing stable yields in a specific area. In contrast, SS and LK may be suited for medium– to high–altitude regions, combining high yield and stability.

Traits	Xo	Xs	SD	SD_percent	h^2	SG	SG_percent
Plant height	37.92	43.74	5.83	15.36	0.98	5.69	14.99
Number of leaves	4.72	5.36	0.64	13.64	0.92	0.59	12.58
Plant dry weight	29.13	40.94	11.81	40.54	0.94	11.12	38.16
Bulb diameter	3.89	4.52	0.63	16.18	0.94	0.59	15.26
Bulb length	2.82	3.13	0.31	11.03	0.96	0.30	10.61
Bulb ratio	78.16	89.88	11.72	15.00	0.95	11.12	14.23
Number of cloves	8.86	14.06	5.20	58.64	1.00	5.17	58.37
Total yield	11.85	14.82	2.97	25.06	0.93	2.75	23.22
Alliin content	57.36	83.55	26.20	45.68	0.84	22.04	38.43
SAC content	68.93	92.78	23.86	34.61	0.83	19.92	28.90
GSAC content	74.33	89.66	15.34	20.64	0.71	10.93	14.71
Allicin content	60.81	86.29	25.48	41.90	0.57	14.60	24.01

Table 3. Selection differential, heritability, and selection gain for twelve traits of seven Garlic genotypes cultivated across two environments. Xo: Overall mean of genotypes; Xs: Mean of the selected genotypes; SD: Selection differential; SG: Selection gain or impact; h^2: Heritability. Heritability categories: high heritability ($h^2 > 0.5$), moderate heritability ($0.3 < h^2 < 0.5$), and low heritability ($h^2 < 0.3$). Low SG < 5, moderate SG 5–10, high SG > 10.

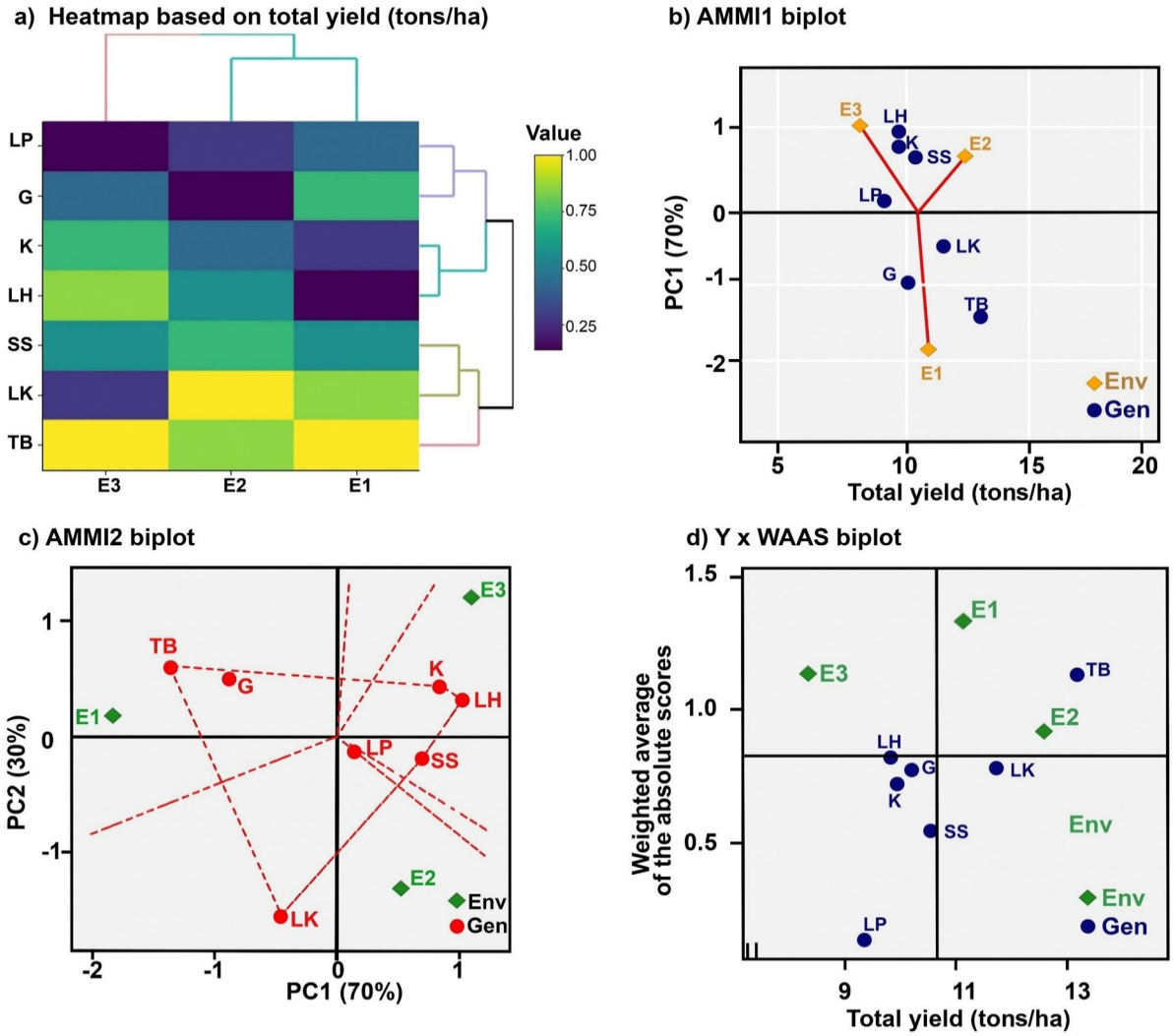


Fig. 7. Stability analysis of total yield (tons/ha) across two studied environments (Mojokerto and Pasuruan) and the origin environment of each genotype. This figure summarizes the G × E interaction patterns using multiple complementary models. (**a**) Heatmap illustrating the magnitude and direction of G × E interactions for total yield. The color gradient represents yield performance across environments, where darker shades indicate higher yields. (**b**) AMMI1 biplot of total yield (tons/ha) for seven garlic cultivars, showing the relationship between the main effects (mean yield) and the first principal component (PC1) of the G × E interaction. This plot identifies genotypes with high mean yield and evaluates their interaction stability. (**c**) Polygon view of AMMI2 biplot for total yield (tons/ha) constructed using PC1 and PC2 of the interaction effects. This view categorizes genotypes into "winning sectors," facilitating the visualization of which genotypes perform best in specific environments. (**d**) WAAS model-based biplot integrating yield performance and stability across environments. Genotypes located near the origin of the biplot are considered the most stable, while those positioned toward the high-yield region are both productive and stable. Environments: E1 = Mojokerto (medium altitude), E2 = Pasuruan (medium-moderate altitude), E3 = the original growing environment of each genotype. Genotypes: LP = Lumbu Putih; G = Geol; K = Kusuma; LH = Lumbu Hijau; SS = Sangga Sembalun; LK = Lumbu Kuning; TB = Tawangmangu Baru; PC = Principal Component; RTY = Rank of Total Yield; WAAS = Weighted Average of Absolute Scores; yield expressed in tons/ha.

Discussion

The present study comprehensively examined the G × E interactions of seven garlic cultivars grown under contrasting agroclimatic conditions in East Java, Indonesia, specifically in Mojokerto and Pasuruan. The findings reveal significant effects of both genetic factors and environmental conditions on garlic growth, yield, and bioactive compound production, demonstrating the complexity of G × E interactions in this crop. The study contributes to understanding how altitude, as a proxy for broader climatic factors, affects ga phenotypic expression and medicinal quality.

Mojokerto (550 m a.s.l.), with its cooler climate and high humidity, provided optimal conditions for vegetative growth and bulb development, particularly for the TB cultivar. In this environment, TB demonstrated superior

GEN	MTY	ASI	ASV	EV	MASI	SIPC	WAAS	RTY	RWAAS	GSI
G	10.22	0.63	2.12	0.11	0.63	1.39	0.77	4	4	8
K	9.90	0.60	2.01	0.10	0.60	1.27	0.72	5	3	8
LH	9.84	0.72	2.42	0.12	0.72	1.35	0.81	6	6	12
LK	11.73	0.56	1.88	0.38	0.56	2.00	0.78	2	5	7
LP	9.35	0.11	0.35	0.00	0.11	0.27	0.14	7	1	8
SS	10.55	0.49	1.63	0.05	0.49	0.88	0.54	3	2	5
TB	13.16	0.97	3.24	0.24	0.97	1.96	1.13	1	7	8

Table 4. Estimation on different genotypes (GEN), their yield (TY), and multiple stability indices. GEN: genotypes; MTY: mean total yield (tons/ha); RTY: the ranking of genotypes based on mean total yield; ASI (AMMI Stability Index): a measure of genotype stability from AMMI analysis; ASV (AMMI Stability Value): another AMMI-based index that considers the first two interaction principal components; EV (Eigenvalue) = the proportion of variance explained by the interaction components; MASI (Modified AMMI Stability Index) = a stability index derived from AMMI; SIPC (Sum of the Interaction Principal Components Scores): measures genotype interaction with environments; WAAS (Weighted Average of Absolute Scores): a stability index considering the entire genotype-environment interaction; RWAAS: the ranking of genotypes based on WAAS; GSI (Genotype Stability Index): a combined index that integrates yield ranking and stability ranking.

plant height, leaf number, bulb diameter, and yield (16.22 tons/ha). However, its yield declined in Pasuruan (13.25 tons/ha), located at a slightly higher altitude (631.3 m a.s.l.) with warmer temperatures and fluctuating humidity. These differences support the hypothesis that environmental factors interact with genetic potential to influence garlic growth. Notably, across all study sites, TB consistently showed the most significant plant height and leaf number ($p < 0.05$). This is consistent with previous findings showing that increased leaf number contributes to a larger leaf area, enhancing photosynthetic capacity and promoting crop growth[44]. Enhancing photosynthesis has long been recognized as a strategy to improve biomass and yield[45].

Interestingly, while TB's higher leaf number generally correlated with increased yield, this pattern did not hold for the G cultivar. Despite having fewer leaves, G maintained stable vegetative growth, suggesting that other factors, such as resource allocation, may play a more critical role in determining yield. These findings suggest that, unlike in TB, a higher leaf number in garlic does not consistently lead to greater bulb yield. This highlights the complexity of genotype-specific strategies for allocating photosynthates from leaves to bulbs and their impact on final yield. Overall, the results underscore the importance of altitude and climate in shaping garlic performance, with Mojokerto's cooler conditions proving more favourable for TB's productivity.

In contrast, the LK cultivar performed better in Pasuruan, achieving a higher yield of 11.73 tons per hectare, suggesting greater adaptability to warmer environments with lower humidity. This finding highlights the importance of selecting genotypes adapted to specific agroclimatic conditions in garlic breeding programs. Similar G × E interactions have been reported in other crops, such as rice, lentil, sugarcane, maize, and sweet potato, where simultaneous selection across diverse environments has proven effective[46–48]. Supporting this, a previous study reported that higher rainfall and temperatures enhanced growth and yield potential in maize[49]. Their findings align with the environmental conditions and yield patterns observed in the present study.

In addition to altitude, soil characteristics also played a critical role in shaping garlic's performance. The acidic clay loam in Mojokerto (pH 4.1) had a high cation exchange capacity (CEC), which promoted nutrient retention but posed challenges in water drainage, potentially affecting root development. In contrast, the nutrient-rich loam in Pasuruan (pH 5.8) supported better drainage, making it more conducive for root growth, especially for cultivars like LK, which thrived in this soil type. These differences underscore the importance of soil texture and nutrient availability in determining the overall productivity of garlic. Similar findings in other crops, such as sugarcane, have shown that soil characteristics significantly influence growth and yield[50,51].

The synthesis of bioactive compounds was also significantly influenced by G × E interactions, with cultivars exhibiting variable production of organosulfur compounds, including alliin, SSAC, GSAC, and allicin, across the two study sites. In Mojokerto, where the climate was cooler and more humid, TB and SS produced the highest alliin levels (98.07% and 96.06%, respectively). However, in Pasuruan, the production of alliin in these cultivars dropped significantly, with reductions of 45.36% and 47.53%, respectively, emphasizing the climate sensitivity of alliin synthesis. This suggests that the synthesis of alliin is highly sensitive to temperature and humidity, with cooler climates favoring its production. Interestingly, SAC production remained more stable across both locations, with LP showing consistent SAC levels. This suggests that specific cultivars, such as LP, exhibit resilience in synthesizing bioactive compounds regardless of environmental changes. This highlights the potential of LP as a reliable cultivar for medicinal garlic production, particularly in regions with climatic variability.

The present study also identified significant genetic polymorphisms in the DELLA-like gene *Asa4G02090.1*. Our findings from sequence analysis revealed specific single-nucleotide polymorphisms (SNPs), including C→T and A→G substitutions, in the regulatory regions of *Asa4G02090.1* in high-yielding cultivars TB and LK (42.3%). These polymorphisms may potentially influence DELLA protein function and gibberellin (GA) signaling (Table 2). Interestingly, these mutations were absent in the low-yielding and stable cultivar, such as LP,

suggesting that allelic variation in *Asa4G02090.1* is linked to yield potential, organosulfur compound synthesis stability, and environmental adaptability.

The genetic diversity observed among the cultivars further supports the significant role of genetic factors in determining garlic's performance. TB and SS demonstrated superior plant height, bulb size, and biomass accumulation due to their genetic potential for high yield and bioactive compound synthesis. This is consistent with findings in other crops, such as rice, where genetic diversity plays a crucial role in performance under varying environmental conditions[43]. The significantly high dry weight observed in TB, particularly in Mojokerto, supports the hypothesis that biomass accumulation is a key factor in determining yield in garlic. The increased biomass in TB also aligns with studies that indicate that greater biomass accumulation is positively correlated with higher yield in wheat genotypes[52,53]. However, unlike crops such as potatoes, where larger bulbs directly correlate with higher yield[54], garlic's yield potential appears more dependent on G × E interactions, which can cause yield variations across different environments. This was particularly evident in the yield reduction of TB in Pasuruan, highlighting that environmental factors beyond temperature can also influence garlic yield[55].

Furthermore, the AMMI model and multivariate analyses provided critical insights into the stability and adaptability of garlic cultivars. The AMMI biplot revealed distinct yield–stability patterns among seven garlic genotypes across different environments. PC1 (70%) and PC2 (30%) accounted for most of the G×E variation, distinguishing genotypes by adaptability. TB recorded the highest yield (16.22 tons/ha) and was associated with favorable environments, reflecting strong but specific adaptation. In contrast, LP (PC2 = −0.1322) and SS (PC2 = −0.1825) were close to zero, indicating stable and broadly adapted performance. AMMI-derived indices (ASI, ASV, MASI) further confirmed these patterns; SS and LK combined to yield high values, while the index values showed consistent responses across environments. TB exhibited high yield and significant interaction effects, making it suitable for high-input conditions. In contrast, LP's high stability and moderate yield favor marginal sites.

To quantify stability, we applied the GSI, which integrates both mean yield and the WAAS from the AMMI model to rank genotypes based on performance and stability simultaneously. According to GSI, SS demonstrated the best balance between yield and stability, making it a promising cultivar for wide-scale adaptation across different agroclimatic zones. The AMMI and GGE models are widely recognized as practical tools for detecting G × E interactions and evaluating the adaptability of garlic genotypes[56–58].

Based on our results, the contrasting performance among garlic genotypes reflects the trade-off between yield potential and stability. While more stable genotypes maintained moderate but consistent performance across sites, high-yield-potential genotypes tended to exhibit strong G × E interactions, indicating specific adaptation to suitable environments. Similar adaptation patterns have been reported in Ethiopian garlic, where high-yielding lines were more environmentally sensitive, whereas others showed better stability[56–58]. Under contrasting water regimes, phenotypic plasticity was found to regulate the balance between productivity and resilience[13]. Meanwhile, Turkish genotypes exhibited high diversity in morphological and biochemical attributes, validating the application of multivariate techniques in selecting genotypes[59]. Studies across Asia have also reported the importance of sulfur and mineral nutrition for yield and allicin composition[18,60,61].

Previous studies in other *Allium* crops have similarly reported that environmental variation—particularly soil pH, temperature, and rainfall—significantly influences both yield and sulfur metabolism[11,15,18]. Our findings extend these observations to tropical environments, emphasizing that genotype stability under fluctuating climatic conditions remains critical for sustainable garlic production.

Parallel with these findings, our multivariate analysis revealed strong correlations between vegetative growth and bioactive compound synthesis, particularly plant dry weight and alliin production. Such positive correlations between biomass and allicin yield indicate that biomass distribution is the most critical determinant of both yield and medicinal value[18,60]. Furthermore, environmental heterogeneity has a significant influence on sulfur metabolism and allicin accumulation[62–64], highlighting the role of G×E interactions in mediating biochemical responses. Overall, this result suggests that the association between yield potential, stability, and bioactive compound production observed in this study reflects a broader adaptive strategy in garlic across diverse agroecological environments.

This study provides valuable insights into G × E interactions and emphasizes the importance of selecting garlic cultivars based on their genetic traits and environmental suitability. The findings highlight the potential of genetic markers such *as Asa4G02090.1* in garlic breeding programs aimed at improving both yield and medicinal quality. Future research should focus on multi-trait and genomic selection strategies to enhance agronomic performance and bioactive compound production. Moreover, further investigation into the molecular mechanisms underlying G × E interactions, particularly those involving sulfur metabolism and DELLA-like gene polymorphisms, will provide deeper insights into optimizing garlic production for agronomic and medicinal purposes under changing climatic conditions. In addition, the observed association between DELLA polymorphism and organosulfur accumulation is correlative at this stage; functional validation (e.g., expression profiling, gene editing, or biochemical assays) will be required to establish causality.

Novelty statement

The novelty of this study lies in its comprehensive analysis of G × E interactions and genetic variation in garlic cultivars grown under contrasting agroclimatic conditions in East Java, Indonesia. By evaluating seven garlic cultivars cultivated at Mojokerto and Pasuruan, the study offers new insights into how environmental factors—including altitude, temperature, rainfall, and soil characteristics—influence vegetative growth, yield, and the production of essential bioactive compounds, such as alliin, SAC, GSAC, and allicin.

A novel aspect of this study is its integration of genetic analysis, particularly focusing on polymorphisms in the DELLA-like gene *Asa4G02090.1*, together with phenotypic data. This approach offers a more comprehensive understanding of how genetics and environmental factors influence garlic's growth potential, yield, and

medicinal properties. By identifying genetic markers linked to superior performance in both agronomic traits and bioactive compound synthesis, the study lays the groundwork for marker-assisted breeding programs. These programs have the potential to significantly enhance garlic's productivity and medicinal value through genetic selection adapted to specific agroclimatic regions.

Conclusion

This study highlights the significant G × E interactions that influence garlic growth, yield, and the biosynthesis of organosulfur compounds. TB is identified as a promising candidate for medium-altitude environments due to its high yield potential. In contrast, LP demonstrates stable SAC production across diverse conditions, making it a reliable cultivar for medicinal purposes. SS exhibits balanced performance, offering both high yield and stability, making it suitable for a variety of agroclimatic zones. Additionally, polymorphisms in the DELLA-like gene *Asa4G02090.1*, particularly in TB, LK, and SS, are associated with enhanced growth, the synthesis of bioactive compounds, and adaptability. The study also emphasizes the importance of multi-environment trials (METs) in identifying cultivars with high environmental adaptability, which is crucial for enhancing agricultural productivity and improving medicinal properties. Future research should employ transcriptomic and metabolomic approaches to elucidate the molecular mechanisms underlying the genetic diversity of garlic cultivars, especially in relation to the DELLA-like gene. This study presents an integrated framework that links genotype, environment, and the synthesis of bioactive compounds. It provides new insights for breeding programs aimed at developing adaptable, stable, resilient, and high-yielding garlic cultivars, particularly in tropical regions across varying altitudes.

Data availability

The data on agroclimatic, environmental, and soil parameters, as well as the agarose gel image of the *Asa4G02090.1* gene, are available in the Supplementary Information. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Baswarsiati: Conceptualization, Methodology, Validation, Investigation, Project Administration, Research Funding Acquisition; Wiwiek Harsonowati: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft of Manuscript, Writing – Review & Editing, Visualization; Dewi Pramanik: Validation, Formal Analysis, Data Curation, Writing – Original Draft of Manuscript, Writing – Review & Editing, Visualization; Zainal Arifin, Chendy Tafakresnanto, Amik Krismawati, Evy Latifah, Wahyu Handayati: Investigation, Data Curation, Project Administration; Farida Yulianti: Methodology, Validation, Investigation, Data Curation, Visualization, Project Administration; Aniswatul Khamidah: Investigation, Data Curation; Dunia A Al Farraj, Rashid Iqbal, Urszula Malaga-Tobola, Marek Gancarz: Writing – Review & Editing. All authors have reviewed and approved the final manuscript.

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Declarations

Permission

Permissions or licenses were obtained to collect Garlic Cultivars {Lumbu Putih (LP), Lumbu Hijau (LH), Lumbu Kuning (LK), Sangga Sembalun (SS), Tawangmangu Baru (TB), Geol (G), and Kusuma (K)} taken from the National Research and Innovation Agency (BRIN), Indonesia, before starting the research.

Statement on guidelines

All experimental studies and materials used in this research are in full compliance with relevant institutional, national, and international guidelines and legislation.

Competing interests

The authors declare no competing interests.

Additional information

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