Adv. Hort. Sci., 2025 39(2): 137-148

DOI: 10.36253/ahsc-17277
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

Agro-morphological characterization of Indian garlic (*Allium sativum* L.) germplasm under mid hill of Northwest Himalaya

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Citation:
DEV R., HEDAU N.K., SANTHIYA S., PAL R.S., PASCHAPUR A., KANT L., 2025 - *Agro-morphological characterization of Indian garlic (Allium sativum L.) germplasm under mid hill of Northwest Himalaya.* - Adv. Hort. Sci., 39(2): 137-148.

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 6 February 2025
Accepted for publication 29 May 2025

Key words: Antioxidants, cluster analysis, garlic, principal component analysis, trait association.

Abstract: This study evaluated the genetic diversity of Indian long-day garlic genotypes based on agro-morphological and biochemical traits. A significant variation was observed across 23 traits, indicating high genetic diversity. Key traits such as bulb weight and 20-clove weight, leaf thickness, and clove showed substantial variability and antioxidant properties highlighted the potential for developing high-quality garlic varieties. Hierarchical cluster analysis grouped 94 genotypes into four clusters based on key traits, emphasizing their utility for breeding programs. Genotypes VGS-55 and VGS-43 excelled in growth traits, while VGS-51 and VGS-49 demonstrated superior biochemical content. Principal component analysis (PCA) revealed that the first two components accounted for 40.73% of the total variability, with yield-promoting traits dominating PC1 and biochemical traits influencing PC3. Trait association studies indicated strong positive correlations between bulb yield and traits like bulb weight (r=0.97 *) and equatorial diameter (r=0.73 ***), whereas no significant association was observed between bulb yield and biochemical traits. These findings underscore the immense genetic potential within Indian garlic germplasm for breeding programs targeting higher yields and improved biochemical traits, catering to the increasing demand for both bulbs and fresh garlic leaves in India.**

1. Introduction

Genus *Allium* includes about 918 species distributed all over the world (The Plant list, 2013), of which only 30 to 36 have been distributed in the Indian plains and Himalayan region (Santapau and Henry, 1973; Karthikeyan *et al.,* 1989). Among this garlic (*Allium sativum* L.) is one of the oldest and strongest flavoured *Allium* species. Owing to its typical

AHS - Firenze University Press
ISSN 1592-1573 (on line) - 0394-6169 (print)

flavor, garlic is mostly used as a spice worldwide in the form of fresh or stored bulbs (Block, 2010). In many regions, people also enjoy fresh garlic leaves in salad (Koch and Lawson, 1996). Additionally, there has been a recent increase in the consumption of dehydrated garlic (Ogar *et al.,* 2021). As a result of its versatility and popularity, garlic has become the second most important species in the *Amaryllidaceae* family, following onions, for both culinary and medicinal uses (Kamenetsky and Rabinowitch, 2001). Garlic is also an excellent source of vitamins and minerals (selenium), flavonoids, antioxidants, lectins, several enzymes, and amino acids (Pizzorno and Murray, 2005). It contains about 33 sulphur compounds (Albrecht *et al.*, 2017), of which allicin (diallyl-dithiosulfinate) is an important constituent. It is formed when non-proteinogenic acid alliin (S-allyl cysteine sulfoxide) is exposed to enzyme alliinase upon tissue damage (Fesseha and Goa, 2019). Allicin is responsible for garlic's characteristic pungent odour and medicinal value. However, composition and concentration of these compounds mainly depend on cultivar types, place of origin, and growing environments (Baghalian *et al.,* 2005; Khar *et al.,* 2011). Garlic consumption helps improve health by enhancing immunity (Percival, 2016), reducing cholesterol and triglycerides (Yeh and Liu, 2001), lowering blood pressure levels (Ried *et al.,* 2013), curing skin allergies (Lee and Park, 2003), and reducing cancer risk (Sengupta *et al.,* 2004). The numerous health benefits associated with this versatile plant highlight its significance in supporting human well-being.

Despite its health benefits, there exists a notable demand-supply gap in garlic production, particularly in India. This gap arises from the limited production in tropical regions, where cultivation faces constraints such as suboptimal climatic conditions and lower yields compared to temperate countries. Garlic requires a cold period (vernalization) for proper bulb development. The mild winters in much of India are less effective for vernalization compared to the prolonged and colder winters in temperate countries. Additionally, garlic cultivation in India is largely concentrated in specific regions, with the Indian Himalayan region contributing minimally to the overall supply. This geographical limitation contrasts sharply with high-yielding temperate countries, where garlic production is more prevalent and efficient (Lawande *et al.,* 2009). India is second only to China in terms of area and production of garlic, but the national average productivity is only 5 t/ha (FAOSTAT, 2017) and ranks 74th in the world (FAOSTAT, 2010).

Garlic plants are sensitive to photoperiod and temperature and thrive in temperate conditions. Fertile flowers and true seed formation in garlic are observed only in its primary diversity centre (Hong *et al.,* 2000). Garlic flowers are hermaphrodites and entomophilous and are pollinated primarily by bees, butterflies and moths. Flowering is controlled by several genetic factors such as photoperiod and temperature (Kamenetsky *et al.,* 2004). However, in a country like India, which is close to the place of origin, garlic clones often form bulbils (aerial bulbs) instead of flowers (Kamenetsky and Rabinowitch, 2001). Therefore, it is essentially regenerated by cloves or bulbils in the region. Such asexual propagation methods are generally favourable for maintaining the true-to-type identity and uniformity of a variety or accession because there is no segregation of alleles. Despite its sexually sterile nature, garlic has differences in various characteristics such as: morphological (maturity, plant growth) characteristics (Panthee *et al.,* 2006; Wang *et al.,* 2014) and biochemical characteristics (Bhusal *et al.,* 2019; Chadha *et al.,* 2019; Barboza *et al.,* 2020; Benke *et al.,* 2021), reproductive (maturity, bolting behaviour) (Kamenetsky and Rabinowitch, 2001), and bulb characteristics (bulb shape, bulb size, bulb colour, storage life) (Bradley *et al.,* 1996; Wang *et al.,* 2014). This diversity is thought to be due to sexual reproduction in the wild plant (Maab and Klaas, 1995), phenotypic plasticity (Bradley *et al.,* 1996), and extensive somatic mutations (Ata, 2005), mainly due to their apomictic nature.

However, this diversity presents challenges, particularly when considering the adaptation of garlic varieties to different climatic conditions. Garlic from temperate climates does not grow well in tropical and subtropical areas, making it difficult to compare different varieties of garlic in similar climate conditions. Although numerous studies have been conducted on genetic variation in garlic germplasm at various levels, there remains a significant gap in research focused on evaluation, selection and development of long-day garlic varieties or strains specifically suitable for Indian conditions. Bridging this gap requires a strategic effort to expand garlic production into underutilized regions (i.e. Himalayan

states), thereby ensuring wider availability of this health-enhancing crop. This highlights the need for targeted research to address the unique climate challenges faced by garlic farmers in India. To delve deeper into these aspects, this study aims to assess the genetic diversity and population structure of 94 garlic germplasms in the mid-hill regions of Uttarakhand, with special emphasis on morphological and biochemical traits. This information serves as a starting point for improving breeding strategies and developing high-yielding, regionally adapted long day garlic varieties. Ultimately, this research aims to address the challenges of low productivity and promote garlic crop improvement in Indian temperate conditions.

2. Materials and Methods

To identify suitable garlic material for the long day climatic conditions in mid-Himalayan region, an experiment was conducted using a total of 94 garlic accessions collected from the Indian Himalayan region and distributed under the All-India Network Research Project (AINRP- Onion and Garlic) and was maintained at Institute Genebank. The current study was conducted over two consecutive years, during the Rabi seasons of 2020-21 and 2021-22, at the experimental farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, located in Hawalbagh, Almora, Uttarakhand (latitude 29°35' N, longitude 79°39' E, and an elevation of 1250 meters above sea level). The region experiences a warm, temperate climate characterized by substantial but unpredictable rainfall, averaging 1132.5 mm annually. Summer lasts from late June to September, with an average yearly temperature of 23.5°C. The annual maximum temperature ranges between 20.0°C and 38.1°C, while the minimum temperature varies from 6.6°C to 25.2°C (Dev *et al.*, 2015). As per the Köppen Climate Classification, the area lies in the northern hemisphere and is classified under the "Cfa" subtype, indicating a humid subtropical climate.

Ninty-four garlic genotypes were cultivated in finely prepared soil with a planting depth of 20–30 cm. Prior to sowing, the experimental field was enriched with 20 t/ha of well-decomposed farmyard manure (FYM), which was thoroughly mixed into the soil. Consistent field management practices, including nutrient application, irrigation, and intercultural operations, were maintained throughout the cropping period. Nitrogen, Phosphorus, Potash, and Sulfur (NPKS) at 110:50:50:50 kg/ha were used as fertilizers. The first half of the N dose and the total doses of P and K were given at the time of transplantation, with the remaining N dose given 30 and 45 days later. Each genotype was planted in two rows, with row spacing of 15 cm and plant spacing of 10 cm, over a row length of 2 m. Cloves were directly sown in shallow line at a depth of 3-4 cm during the winter seasons of October 2020 and 2021. Watering was provided as required to prevent leaf wilting. The crop was harvested when the leaves reached senescence or necks fell off.

All morphological traits were recorded on five random plants in each replication. Data were recorded from five random plants in each replication for each 94 genotype to assess morphological, biochemical differences. These germplasms consist of varieties, landraces, improved materials, and cultivars (SM Table 1S). The observations were recorded on sixteen morphological and seven biochemical parameters. Morphological traits including number of leaves, fourth leaf width (mm), fourth leaf length (cm), pseudostem length (cm), pseudostem diameter (mm), plant height (cm), and neck thickness (mm) were recorded at 80 days of planting after completion of vegetative growth when the crop was in the field. While, other bulb traits viz. number of cloves per bulb, average weight of bulb (g), bulb polar diameter (mm), bulb equatorial diameter (mm), and weight of 50 cloves (g) were recorded after harvest at neck fall.

Clove samples were randomly taken from the harvested crop to quantify the above biochemical and antioxidant traits. Total soluble solids (TSS) were determined immediately after manual juice extraction from the macerated sample using a cotton cloth using a handheld digital refractometer model PAL-3 (ATAGO, Japan) and expressed in °Brix. The qualitative characteristics of the genotypes were recorded according to the descriptors of the Plant Varieties and Farmers' Rights Authority, Government of India. The biochemical properties, namely total soluble solids (TSS), 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), total antioxidant activity (TAA), ferric reducing antioxidant power (FRAP), and Total polyphenols (TPP) were estimated in the quality laboratory of the Institute.

Chemicals and reagents

All of the solvents employed in the research were of analytical purity, whereas the water was HPLC pure. The solvents and reagents were procured from Merck-Sigma (Bangalore, India) and used without further purification. The extraction and analysis were carried out using UV-vis spectroscopy. Different biochemical observations were made on the harvested fruits. The experiments were done in triplicate, and the data are shown as mean values ± standard deviations.

Preparation of extracts

Methanolic extracts of garlic were used to measure antioxidant metabolites and activities. Fresh samples (2 g) were ground in a pestle and mortar in 20 ml of 80% methanol. The methanolic extracts of the samples were placed in an orbital shaker overnight (16 hours) to completely extract antioxidant metabolites. The methanolic extract was centrifugated at 5000 rpm for 10 minutes, and the supernatant was kept at 4°C for further analysis. Methanolic pure (80%) extract was used for the study. All assays were performed out in triplicate, and results are expressed as mean ± standard deviations.

Total polyphenolic content and antioxidant activities

Total polyphenolic content. The Folin-Ciocalteau reagent was used to calculate total phenolic compounds (Singleton and Rossi, 1965). The final solution was vigorously stirred in a vortex mixer after adding 0.9 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium carbonate solution to the freshly obtained extract. The absorbance was measured at 725 nm after keeping the reaction at 300°C for 40 min. The standard curve for gallic acid was prepared by taking a different concentration of gallic acid (10-100 µg).

Determination of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH•). With some alterations, the DPPH test was carried out by detecting the reduction in absorbance of a methanolic DPPH solution at 515 nm in the presence of the extract (Brand-Williams *et al.,* 2005). The stock solution was made by dissolving 24 mg of DPPH in 100 mL methanol which was then stored at -20°C until needed, while, working solution was made by mixing 10 mL stock solution with 45 mL methanol to achieve an absorbance of 1.17±0.02 units at 515 nm. Garlic extracts (150 µL) were allowed to react for 24 hours in the dark with 2850 µL of DPPH working solution, and the absorbance was measured at 515 nm. The radical scavenging activity of DPPH• was calculated as a percentage of DPPH• discolouration using the equation:

$$\text{Radical scavenging (percent)} = (\text{A control} - \text{A sample}) / \text{A control} \times 100$$

Where, A sample is the absorbance of the solution recorded while adding extract/reference at a specific level. A control is the absorbance of the DPPH solution without adding extract.

Determination of scavenging effect on $ABTS^{\bullet+}$ radicals

The ABTS test was carried out by detecting the reduction in methanolic ABTS solution absorbance at 745 nm in the presence of the extract (Arnao *et al.,* 2001). The workable solution wa made by combining two stock solutions in equal amounts and allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted to attain an absorbance of 0.9 0.02 units at 745 nm by combining 1 mL ABTS solution with 3 mL methanol. Garlic extracts (200 litres) were allowed to react for 30 minutes in the dark with 2000 liters of newly made ABTS solution, and absorbance was measured at 745 nm.

The percentage inhibition was calculated using the equation:

$$\text{Radical scavenging (percent)} = (\text{A control} - \text{A sample}) / \text{A control} \times 100$$

Where, A sample is the absorbance of the solution recorded when adding extract/reference at a specific level, and A control is the absorbance of the ABTS solution without extract.

Determination of total antioxidant activity

The total antioxidant activity of the methanolic extracts of both samples was determined using a phosphomolybdenum technique (Prieto *et al.,* 1999), which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the creation of green phosphate / Mo (V) compounds. A sample extract of 0.3 mL was mixed with 2.7 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was sealed and incubated for 90 minutes in a boiling water bath at 95°C. The absorbance was measured at 695 nm after the samples had cooled to room temperature. Total antioxidant activity was measured in trolox

equivalents (mM/g of extract).

Determination of ferric-reducing antioxidant power (FRAP)

The FRAP test was performed with few changes according to Benzie and Strain. 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution were produced as stock solutions. 25mL acetate buffer, 2.5mL TPTZ solution, and 2.5mL $FeCl_3.6H_2O$ solution were mixed and warmed to 37^0C before use to make a fresh working FRAP solution. Methanolic extracts of samples (150 L) were allowed to react with 2850 L of FRAP solution in the dark for 30 minutes, and the coloured product (ferrous tripyridyltriazine complex) was measured at 593 nm. The FRAP value was calculated by drawing a standard curve formed by adding ferrous sulfate heptahydrate (20-200 mM) to the FRAP reagent, with the results represented in mM equivalent to $FeSO_4.7H_2O$.

Statistical analysis

The experiment employed a randomized complete block design with two replications, using a mean value over two years for each trait in the diversity analyses. Using multivariate methods, such as principal component analysis, agro-morphological diversity was analysed (Jolliffe, 1986). R software was used to do multivariate cluster analysis based on PCA charts and Ward's technique (Ward, 1963). Biplot-PCA, Variables-PCA, and Individuals-PCA graphics of PCA visualisations were created using the R packages "devtools,""ggplot2," and "factoextra." Trait correlations were computed using the "metan" package in R, while Principal component scores and descriptive analysis were obtained via PAST04 software (Hammer *et al.,* 2001).

3. Results

Genetic diversity based on agro-morphological and biochemical traits

The results showed a huge variation among the characters of interest of Indian long-day garlic in terms of the twenty-three morphological and biochemical characters as given in supplementary materials Table 1S and Table 2S. Descriptive data analysis revealed that most traits, except for a few variables, exhibit high genetic diversity. The most variable traits were stem thickness, leaf thickness, bulb weight, bulb diameter, clove width, 20-clove weight and DPPH (% inhibition) with CV values greater than 35 percent. Among the 20 agronomic traits, ABTS (percent inhibition) (CV=9.33%) achieved the lowest CV value. Yield characteristics differed significantly between garlic genotypes, with bulb weight ranging from 6.50 to 35.13 g (CV= 34.60%) and 20 clove weight ranging from 9.0 to 68.0 g (CV= 27.08%). The number of cloves per bulb varied between 5.80 and 39.60. The polar diameter ranged from 2.17 to 4.10 cm (CV= 11.54%), while the equatorial diameter varied from 2.27 to 5.07 cm (CV= 15.73%).The average clove length was 26.90 mm, ranging from 16.33 to 41.50 mm, while clove width varied from 2.83 to 39.33 mm, with an average of 12.77 mm. Genotypes VGS-43 and VGS-103 were noted for their clove length and width. Leaf thickness ranged from 0.70 mm (VGS-98) to 1.85 mm (VGS-55), length from 13.00 cm (VGS-51) to 45.13 cm (VGS-96), and width from 1.15 cm (VGS-91) to 3.75 cm (VGS-43), with coefficients of variation of 14.08%, 17.49%, and 23.66%, respectively. Plant height varied from 14.80 cm (VGS-82) to 44.80 cm (VGS-55), and the number of leaves ranged from 4.20 (VGS-49) to 9.0 (VGS-95), with coefficients of variation of 19.03% and 17.49%, respectively. The increasing demand for fresh garlic leaves in India highlights the potential of these diverse genotypes for producing leafy garlic varieties. Additionally, there was considerable variation in quality metrics like TSS and total carbohydrates, with TSS being crucial for assessing bulb quality in the tested accessions.

Among 94 genotypes, 43 genotypes had TSS values in-between 16.16 and 43.90°Brix, with VGS-67 having the highest at 43.90°Brix, followed by VGS-19 at 43.60°Brix, and VGS-5-2 with the lowest at 16.16°Brix. Garlic cloves primarily consist of carbohydrates, making up 33.06% of the total carbohydrate content (USDA National Nutrient Database).The study found that carbohydrate content in the genotypes ranged from 14.39% (VGS-8) to 28.81% (VGS-11B), with an average of 21.42%. Important antioxidant properties suggested potential for developing healthier varieties. Total polyphenols varied between 0.30 and 1.25 mg GAE/g DW, with a standard deviation of 0.15 and a coefficient of variation of 29.90%. The DPPH inhibition ranged from 10.08% to 49.23%, with a standard deviation of 10.08 and a coefficient of variation of 49.23%.The free radical inhibition percentage against ABTS in garlic

accessions ranged from 31.65% to 83.42%, averaging 62.14%, indicating low genotypic variation. The total antioxidant activity varied between 12.71 and 66.43 mM Trolox equivalent/g DW, with a standard deviation of 6.43 and a coefficient of variation of 24.97%. The FRAP values ranged from 95.04 to 269.32 mM FeSO4 equivalent/g DW, with a standard deviation of 32.77 and a coefficient of variation of 21.26%. VGS-51 had the highest FRAP values among the genotypes tested (Table 1).

Hierarchical cluster analysis

Cluster analysis using Ward's minimum variance (Ward, 1963) identified four main groups among 94 genotypes, explaining 29.78%, 51.06%, 17.02%, and 2.12% of total germplasm, respectively (Fig. 1). Cluster 1, consisting of 28 genotypes, showed associations with higher clove numbers (e.g., VGS-32, VGS-33), PST (VGS-33), PD (Bhima Omkar, VGS-06, VGS-103), BW (VGS-89), and TY (VGS-89). All released varieties, except Swarna-9, are in this cluster. The second cluster, the largest with 48 genotypes, is characterized by maximum plant height (VGS-55), LT (VGS-55), and higher TSS (VGS-35, VGS-36, VGS-37, VGS-13 and VGS-14), TAA (VGS-11-B), and (VGS-11-B). This cluster is advantageous for selecting genotypes with enhanced growth and bulb quality. The third cluster included thirteen genotypes with

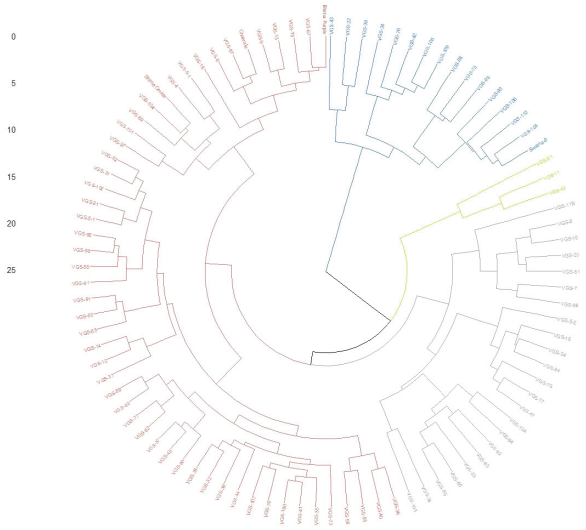

Fig. 1 - Dendrogram of cluster analysis of garlic germplasm based on morphological and biochemical traits (Ward Method).

Table 1 - Summary statistics morphological and biochemical characteristics of garlic accessions

Characteristics	Abbreviation	Min	Max	Mean	SE	Variance	SD	CV (%)
Plant height (cm)	PH	14.80	44.80	31.39	0.62	35.70	5.97	19.03
Leaf length (cm)	LL	13.00	45.13	32.01	0.58	31.35	5.60	17.49
Leaf width (cm)	LW	1.15	3.75	1.93	0.05	0.21	0.46	23.66
No. of leaves/plant	LN	4.20	9.00	5.65	0.09	0.74	0.86	15.27
Pseudostem thickness (mm)	PST	6.20	18.58	10.76	0.32	9.86	3.14	29.19
Leaf thickness (mm)	LT	0.70	1.85	1.33	0.02	0.03	0.19	14.08
Polar diameter (cm)	PD	2.17	4.10	3.25	0.04	0.14	0.37	11.54
Equatorial diameter (cm)	ED	2.27	5.07	3.62	0.06	0.32	0.57	15.73
20 clove weight (gm)	CW-20	9.00	68.00	25.41	1.18	131.02	11.45	45.04
Clove length (mm)	CL	16.33	41.50	26.90	0.40	14.94	3.87	14.37
Clove width (mm)	CW	2.83	39.53	12.77	0.49	22.85	4.78	37.44
No of clove layer/ bulb	CNL	1.00	3.00	2.38	0.04	0.19	0.43	18.23
No of clove/ bulb	CN	5.80	39.60	17.92	0.65	39.35	6.27	35.01
Bulb weight (gm)	BW	6.50	35.13	21.35	0.60	33.43	5.78	27.08
Total Yield (q /ha)	TY	32.50	175.67	105.67	3.09	899.52	29.99	28.38
Plant weight (g)	PW	8.00	52.67	23.99	0.87	71.52	8.46	35.25
TSS (°Brix)	TSS	16.16	43.90	38.27	0.46	19.77	4.45	11.62
Total Polyphenols (mg GAE/g)	TPP	0.30	1.25	0.51	0.02	0.02	0.15	29.90
DPPH (% Inhibition)	DPPH	10.08	49.23	27.49	1.01	96.64	9.83	35.76
ABTS (% Inhibition)	ABTS	31.65	83.42	62.14	0.60	33.62	5.80	9.33
TAA (mM Trolox equivalent/g DW)	TAA	12.71	66.43	25.74	0.66	41.32	6.43	24.97
FRAP Vale (mM $FeSo_4$ equivalent/g	FRAP	95.04	269.32	154.13	3.38	1074.06	32.77	21.26
Total carbohydrate (%)	TCA	14.39	28.81	21.42	0.35	11.76	3.43	16.01

distinctive leaf traits such as leaf length (VGS-96), leaf width (VGS-43), leaf number (VGS-95), and several yield-related traits such as bulb weight (VGS-96, VGS-105, Swarna-9) and total yield (VGS-39). In contrast, the fourth cluster contained only two genotypes, VGS-51 and VGS-49, which were characterized by their high biochemical content, including TPP, DPPH, ABTS, and FRAP.

Trait association study

The total bulb yield showed significant positive correlations with BW (0.97***), ED (0.73***), CW (0.24*), LN (0.39***), LL (0.29**), LW (0.24*), PST (0.48***), PW (0.66***), 20-CW (0.61***), and PD (0.25*). However, no association was found between bulb weight and various biochemical traits, including TAA, ABTS, total phenolics, and DPPH activities, suggesting that bulb weight has no influence on bulb quality traits. Furthermore, bulb weight, TSS, and bulb diameter had no relationship with plant height (Fig. 2). Conversely, TSS was negatively correlated with 20-clove weight (-33**), clove ABTS (-0.29**), and DPPH activity (-0.27**), while there was a non-significant negative correlation with clove phenol content (TPP), TAA, and FRAP values. Significant positive correlations were observed among DPPH (0.66**), TAA (0.39***), ABTS (0.55***), and FRAP (0.60***), indicating a complex gene action mechanism involving garlic quality parameters.

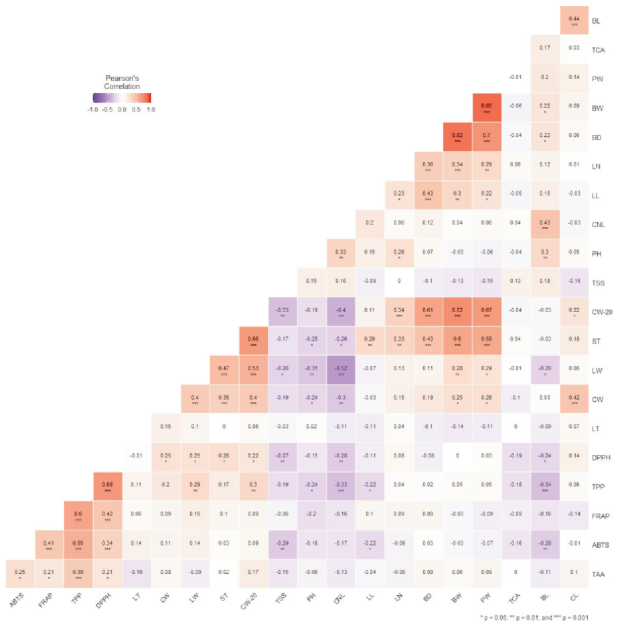

Fig. 2 - Pearson correlation matrix for morphological and biochemical traits in Indian long day garlic germplasm.

Principal component and Biplot analysis

Prior to performing Principal Component Analysis (PCA), Pearson correlation analysis was conducted to identify and remove highly correlated variables (BW and ED), as such variables can disproportionately influence the PCA results. The first principal component (PC1) accounts for 22.77% of the total variation, with the highest eigenvalue of 4.78. It includes features such as CW-20, PST, PW, TPP, DPPH and LW, which have positive contributions, while CN, CNL and TSS have negative effects (Table 2). The second principal component (PC2) has an eigenvalue of 3.22 and explains 15.31% of the variation. Positive contributions come from traits such as PD, TY, and PW, whereas most of the biochemical traits *i.e.,* TPP, DPPH, ABTS, TAA, and FRAP have negative contributions. The third principal component (PC3) explained 8.38% of the total variation, mainly due to biochemical features such as FRAP, DPPH (% inhibition), TPP, ABTS, TAA and a morphological trait *i.e.,* PH. The fourth principal component (PC4) accounted for 7.16% of the variation and was primarily influenced by clove length (CL), clove width (CW) and plant diameter (PD). Leaf thickness (LT) had the highest loadings in the fifth principal component (PC5), while total carbohydrates (TCA) and total soluble solids (TSS) dominated in the sixth principal component (PC6), highlighting their importance. Yield- promoting traits were mainly captured in PC1 and PC2, while PC3 focused on biochemical traits and highlighted their role in phenotypic variation. TPP, DPPH, ABTS, LW, and FRAP made the main positive contributions based on the squared cosine (Cos2), while CW-20, ST, BW, TY, and PW made notable negative contributions (Fig. 3). Genotypes VGS-10A, VGS-84, VGS-82, VGS-79, VGS-54, and VGS-70 had the highest square cosine values, while VGS-96, VGS-109, VGS-105, VGS-108, VGS-76, VGS-42, VGS-49, VGS-51, and Swarna-9 were the least contributions (Fig. 4). A biplot of PC1 and PC2 revealed distinct trait groups, including CNSS, TY, and various biochemical traits (TPP, DPPH, ABTS, TAA, FRAP). Genotypes VGS-96, VGS-43, VGS-34, VGS-51, VGS-49, VGS-44, VGS-12, VGS-14, VGS-97, VGS-104, and VGS-89 were in gaps, highlighting their uniqueness (Fig. 5).

Table 2 - Principal component loadings for morphological and biochemical traits of garlic

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
PH	-0.20	0.18	0.34	0.05	0.24	-0.16	-0.32	-0.14	-0.36	0.01
LL	-0.01	0.26	0.18	-0.28	-0.15	-0.23	0.49	0.29	-0.26	0.24
LW	0.32	0.03	-0.27	-0.12	0.20	0.02	0.01	-0.09	-0.06	-0.22
LN	0.09	0.27	0.25	-0.18	0.33	0.12	0.00	-0.06	-0.31	-0.06
PST	0.30	0.26	-0.05	-0.14	0.00	0.09	0.17	0.02	0.04	-0.02
LT	0.04	-0.06	-0.08	0.12	0.63	-0.12	-0.06	0.44	0.14	0.41
PD	-0.14	0.32	0.12	0.41	-0.04	0.12	0.22	0.03	0.17	-0.10
CW-20	0.36	0.25	-0.04	-0.04	-0.01	0.03	-0.20	0.01	0.03	0.11
CL	0.09	0.15	-0.01	0.68	0.00	-0.03	0.15	-0.01	-0.16	-0.03
CW	0.26	0.10	-0.11	0.33	0.16	-0.04	0.19	-0.14	0.01	0.18
CNL	-0.27	0.16	0.29	0.06	-0.11	-0.10	0.10	0.19	0.37	-0.20
CN	-0.33	0.07	0.25	-0.01	0.23	0.09	-0.09	-0.07	0.01	-0.06
TY	0.16	0.41	0.17	-0.11	0.04	0.00	-0.26	-0.06	0.16	-0.04
PW	0.21	0.37	0.04	-0.06	-0.19	0.02	-0.17	-0.01	0.37	0.04
TSS	-0.22	0.02	-0.01	-0.09	0.18	0.48	0.17	-0.48	0.27	0.37
TPP	0.29	-0.25	0.33	0.05	0.07	0.15	-0.03	-0.09	0.10	-0.02
DPPH	0.26	-0.17	0.28	0.09	0.05	0.00	0.16	-0.25	-0.18	-0.33
ABTS	0.18	-0.26	0.28	0.07	0.08	-0.07	-0.23	0.27	0.31	-0.16
TAA	0.13	-0.11	0.28	0.16	-0.44	0.22	-0.28	0.09	-0.24	0.52
FRAP	0.18	-0.21	0.37	-0.14	0.08	0.20	0.42	0.13	0.11	0.05
TCA	-0.07	0.09	-0.15	0.03	0.02	0.71	-0.08	0.48	-0.22	-0.27
Eigen value	4.78	3.22	1.76	1.50	1.32	1.12	0.98	0.90	0.81	0.75
Variance	22.77	15.31	8.38	7.16	6.26	5.35	4.67	4.29	3.85	3.57
Cumulative variance	22.77	38.08	46.47	53.63	59.89	65.24	69.91	74.20	78.05	81.63

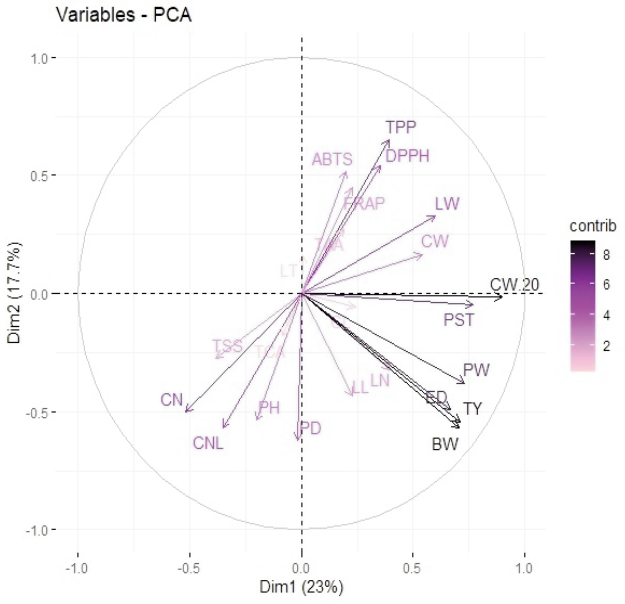

Fig. 3 - Contribution of different variables towards principal component analysis based on cos2 value.

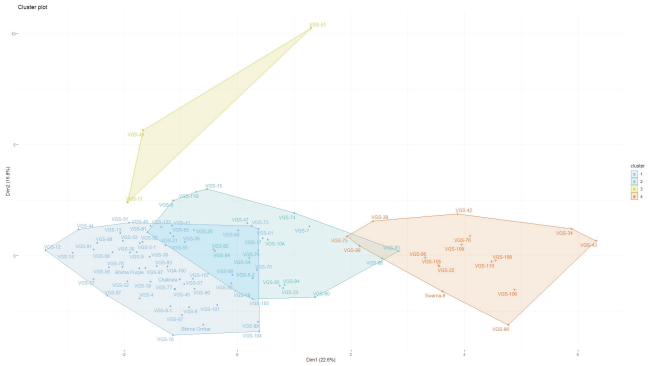

Fig. 4 - Contribution of different garlic accessions toward principal component analysis based on the cos2 value.

4. Discussion and Conclusions

The present study highlighted the significant genetic diversity among Indian long-day garlic

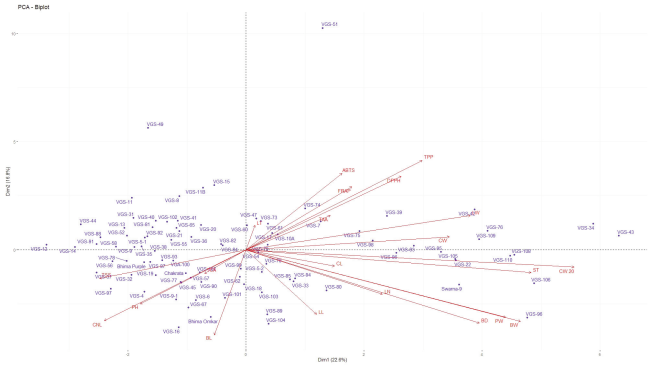

Fig. 5 - Segregation of 94 accessions of garlic according to morphological and biochemical traits determined by PC score and loadings.

genotypes based on agro morphological and biochemical traits. The wide range of mean and coefficient variations observed between traits highlights the potential for improving existing genotypes through clonal selection and paves the way for the development of new, tailored varieties suitable for high-altitude regions. Previously, Benke *et al.* (2020) reported similar genetic differences in garlic genotypes. Our results confirm the large variability found in morphological traits such as leaf width, stem thickness, bulb weight, 20-clove weight, clove width, and biochemical traits such as total phenolic content, DPPH inhibition, total antioxidant activity, and FRAP, and highlight their potential for selection and improvement. This is consistent with the findings of Bhusal *et al.*(2019); Narayan *et al.* (2019), who also found significant variability in yield-determining and biochemical traits in garlic genotypes and demonstrated the usefulness of such traits in the clonal selection highlighted programs. The variability of yield characteristics and biochemical properties offers opportunities for targeted selection. For example, features such as bulb equatorial diameter (CV = 56.54%) and clove length (CV = 34.82%), as reported by Ayed *et al.* (2019) indicate scope for clonal selection to develop new garlic varieties.

Furthermore, studies by Jabbes *et al.* (2012) and Baghalian *et al.* (2005) identified significant differences in plant height, number of cloves per bulb and bulb dimensions, consistent with the results of the current study. This variability forms the basis for improving both the agronomic and phytochemical properties of garlic. Present study also identified genotypes such as VGS-11B (high TAA and TCA content) and VGS-51 (high total phenolic content, DPPH, ABTS and FRAP activities) as candidates for developing healthier garlic varieties. Compared to existing commercial varieties, these genotypes showed significantly improved antioxidant and phenolic profiles, suggesting that they have the potential to meet growing consumer demand for nutrient-dense and health-promoting garlic products. These results echo Bhusal *et al.* (2019), who identified superior genotypes for antioxidant activity and suggested their use for health-oriented breeding programs. The analysis of the TSS (Total Soluble Solids) values in this study indicates a range of 16.16 to 43.90 °Brix, which relates these results to existing research and shows that they are slightly lower than those, observed in certain Indian genotypes from the western region (Bhusal *et al.,* 2019) and slightly higher than the values recorded for Egyptian garlic genotypes (Moustafa *et al.,* 2011).This variation highlights the potential for breeding programs aimed at growing garlic varieties with specific TSS characteristics. Such tailored varieties, particularly those with medium TSS and low pungency, can be particularly beneficial for improving post-harvest storage and processing, allowing growers to optimize quality and shelf life. Genotypes with high antioxidant activity and different TSS values are suitable for leaf purposes because TSS is not a crucial trait for selecting leaf genotypes. Our study found that VGS-55 (leaf thickness), VGS-43 (leaf width), VGS-95 (number of leaves) and VGS-96 (leaf length) are superior in leaf characteristics. Furthermore, Bhusal *et al.* (2019) identified PGS-105 as a genotype with higher antioxidant activity supporting improved health benefits in leaf production.

Despite the clonal propagation and inherent sterility of garlic, significant genetic variation has been observed, likely due to mutations, soma-clonal variations, or genetic transformation variations arising from sexual reproduction in the wild plant (Novak, 1990; Bradley *et al.,* 1996; Wang *et al.* 2014). These sources of variation are not unique to garlic and have also been reported in other clonally propagated crops such as potatoes (Zaag, 1987) and bananas (Ortiz and Vuylsteke, 1996). However, the extent and impact of such variations may vary depending on the genetic architecture and propagation method of each crop. The use of PCA and cluster analysis revealed four major groups

within the 94 genotypes, reflecting the diversity of morphological and biochemical traits. These clusters were dominated by the group of genotypes that were better in some traits. Fruit-related traits, for instance, were the most effective in differentiating across mango cultivars (Samsampour *et al.,* 2020). However, overlapping features were also recorded. Similarly, Benke *et al.* (2020) reported overlap in both qualitative and quantitative characteristics.

These findings align with Egea *et al.* (2017) and Wang *et al*. (2014), Oyetunde *et al*. (2021) on biochemical, color traits, and phenotypic diversity. Traits such as TPP, DPPH, and ABTS significantly influenced clustering patterns, emphasizing their role in defining genetic diversity, as seen in Chinese (Hassan *et al.,* 2015), Polish (Bozin *et al.,* 2008), and Serbian (Kim *et al.,* 2013) garlic genotypes.

Other studies also showed clustering based on various traits. For example, Bhusal *et al.* (2019) identified two clusters among 26 Indian garlic genotypes based on antioxidant and quality traits, Wang *et al.* (2014) clustered 212 garlic accessions into six groups based on morphological traits while Barboza *et al*. (2020) classified Argentinean genotypes into four groups using organo-sulfur and SSR markers. These studies highlight the value of clustering techniques in understanding genetic diversity and informing breeding programs. Furthermore, clustering patterns were unrelated to the geographic origins of the accessions, as noted by Benke *et al.* (2020). The clustering patterns and PCA results emphasize the importance of traits like TSS, antioxidant activity, and phenolic content in defining genetic diversity.

The high variability and significant correlations observed among yield and biochemical traits highlight the potential for using diverse genotypes in breeding programs. For example, strong correlations between bulb weight and clove dimensions could prioritize selection for these traits to enhance overall yield. Meanwhile, weak or non-significant correlations between yield and biochemical traits suggest that independent selection strategies might be necessary to balance productivity with quality improvements. This understanding enables breeders to design more focused and efficient strategies tailored to specific breeding goals. The total bulb yield showed strong positive correlations with traits like bulb weight, bulb diameter, and clove width, suggesting their importance in yield improvement. Conversely, the lack of association between bulb weight and biochemical traits indicates the need for independent selection strategies for yield and quality traits. Supporting this perspective, Jabbes *et al.* (2012), Imani and Shamili (2017) and Benke *et al.* (2021) found a strong positive correlation between marketable yield and yield-contributing traits in different garlic accessions. Panthee *et al.* (2006) reported comparable results in various Nepalese garlic accessions.

This study identifies the first two principal components (PCs) that encompass essential yield and biochemical traits, including bulb length, number of cloves, leaf count, plant weight, total soluble solids (TSS), clove length, total antioxidant activity (TAA), and total carotenoid content (TCA). Therefore, these PCs can aid in selecting genotypes with favorable horticultural and yield characteristics (Useche-Carrillo *et al.,* 2021). This collective evidence indicates that garlic's production potential is closely linked to its vegetative development, making these traits valuable for direct selection in garlic cultivation.

The study showed significant genetic diversity among 94 Indian garlic genotypes based on both agro-morphological and biochemical traits. Large differences were observed in traits such as bulb weight, clove size, leaf characteristics and biochemical parameters such as total polyphenols, antioxidant activity and total soluble solids (TSS). Notably, genotypes VGS-43 and VGS-103 had superior clove properties, while VGS-51 had the highest antioxidant properties. The cluster and principal component analyses identified distinct groups based on yield-promoting traits and biochemical content, providing a basis for selecting genotypes with specific desired traits. Yield-related traits such as bulb weight and 20-clove weight were positively correlated with several morphological traits but showed no association with biochemical traits such as total antioxidants, suggesting that these quality traits are independent of yield. The study highlights the potential of these different genotypes for breeding programs aimed at increasing both the yield and quality of garlic production, particularly improving antioxidant content and bulb quality. Future efforts should focus on integrating molecular markers into phenotypic assessments, which can help accelerate the development of high-yielding, nutrient-dense garlic varieties suitable for regional cultivation.

Acknowledgements

The authors thank the Indian Council of Agricultural Research (ICAR), New Delhi, and AICRP-Onion and Garlic for their support. Also, the authors thank Mr Manoj Bhatt, Mr Sher Singh Jeena, Mr Dinesh Nayal, and Mr Ajay Kumar for their contribution and unreserved involvement in all activities and data collection.

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