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Integrating Diversity Analysis and Morphological Characterization for Strategic Trait Selection in Advanced Breeding Lines of Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: The depleting genetic diversity in cultivated chickpea highlights the need to identify and integrate novel variations into breeding programs. Morphological characterization remains a crucial tool for identifying elite lines with desirable traits, offering key insights for strategic crop improvement. The integration of morphological characterization with various methods of diversity assessment provides a robust framework for identifying genotypes with significant phenotypic diversity across key morphological traits.

Methods: The investigation was carried out during the *Rabi* seasons of 2022-2023 and 2023-2024 under three different sowing conditions, aiming to characterize thirty advanced breeding lines of *desi* chickpea according to DUS guidelines. Various analyses such as shannon weaver diversity index, principle component analysis, hierarchical clustering and phylogenetic assessment were integrated to identify diverse lines across all the traits studied.

Result: Significant phenotypic variability was observed for most of the traits. PCA revealed that first five components with Eigen values greater than one contributed to a maximum of 76.34 per cent of the variability with lines JG2016-9651, JG2022-2 and ICCV191609 being diverse for the multiple traits across several PCs. Hierarchical clustering analysis grouped genotypes into six clusters, with cluster III being the largest. Genotypes JG2020-55, ICCV181109, JG2022-12, ICCV191609 and JG2016-9651 showed the highest inter-cluster distances, indicating significant genetic divergence. Phylogenetic analysis validated these findings revealing six clusters and provided further insights into the genetic relationships and evolutionary divergence among the genotypes.

Key words: Chickpea, Clustering, DUS guidelines, PCA.

INTRODUCTION

Chickpea, a self-pollinating diploid pulse crop, belongs to the genus *Cicer*, family Fabaceae and subfamily Papilionoidae, with a genome size of 738 Mbp (Sajja *et al.,* 2017). Having high protein and micronutrients contents, chickpea offers a practical solution to combat malnutrition and have various potential health benefits (Jukanti *et al.,* 2012). India is the world's top producer in total production and the area under cultivation. In India, during 2022-23, chickpea was grown on approximately 14.8 million hectares globally, producing 18.09 million metric tons at an average yield of 1,222 kg/ha (Directorate of Pulses Development, 2024).

Recent advancements in crop improvement have reduced genetic diversity among cultivated chickpeas, underscoring the necessity to explore new sources of variation for integration into breeding programs. Morphological characterization plays a vital role in identifying elite lines with desirable attributes, providing valuable insights for targeted crop improvement. Using widely accepted descriptors for DUS testing are invaluable for varietal classification, estimating diversity and exploring phylogenetic relationships among diverse lines. Diversity assessment of traits is critical to identify genotypes with unique characteristics. The Shannon-Weaver diversity index (H) is a robust measure to assess the overall diversity of phenotypes (Dickman, 1968). Traits having greater value of Shannon-Weaver diversity index (H) signifies increased diversity, while a lower value indicates less diversity (Anand *et al.,* 2024). Principal component analysis was applied to identify the key traits contributing to the observed phenotypic variation, efficiently reducing the dimensionality of data while retaining critical information.

Cluster analysis complements PCA by evaluating the contributions of various traits to the overall diversity,

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measuring the extent of divergence and selecting genetically diverse lines for producing desirable recombinants (Vus *et al.,* 2020).

Phylogenetic analyses is important in evaluating genetic relationships and diversity among crop genotypes. By identifying discrete clusters of genetically distant genotypes, this analysis reinforces the results from clustering and PCA, ensuring accurate identification of diverse genotypes (Getahun *et al.,* 2021). This combined approach integrating morphological characterization, diversity assessment, PCA, clustering and phylogenetic analysis provides a comprehensive background for identifying genotypes that demonstrate phenotypic diversity across important morphological traits.

MATERIALS AND METHODS

The investigation was carried out at the Seed Breeding Farm, JNKVV, Jabalpur, Madhya Pradesh in *Rabi* 2022-23 and 2023-24, under three different sowings: normal sown, late sown and very late sown conditions. The experimental material consists thirty advanced breeding lines including four checks *viz.* JG14, JG24, JG18 and RVG204, sourced from AICRP on chickpea, JNKVV, Jabalpur and ICRISAT, Patancheru, Hyderabad. These lines were evaluated over three replications utilizing a randomized complete block design (RCBD) with a plot size of 4.8 m^2, comprising four rows, each 4.0 m in length with spacing at 30.0 cm × 10.0 cm. Standard agronomic and plant protection practices were implemented to ensure successful crop cultivation. Data pertaining to eighteen different morphological traits *i.e.*, stem anthocyanin colouration, plant growth habit, time of flowering, foliage colour, leaflet size (mm), leaf pattern, numbers of flower per peduncle, flower colour, flower stripes on standard, peduncle length (mm), plant height, pod size, seed colour, seed size, seed shape, seed testa texture, seed ribbing and seed type were recorded on 10 randomly selected plants, following the DUS (Distinctness, Uniformity and Stability) approved by the protection of plant varieties and farmers rights authority (PPV and FRA, 2007), from the commencement of the trial to harvest for the recording of all observations. All the traits were validated under three different sowing environments.

The Shannon-Weaver diversity index, denoted as (H), as described by Hutchenson (1970), is formulated in the following way:

$$H = \sum_{i=1}^{n} Pi \log e\ Pi$$

In this formula, Pi indicates the fraction of individuals belonging to the i^{th} category of an n class character, with n representing the total number of phenotypic categories for a particular trait. The H values were used to evaluate the diversity of individual characteristics. Morphological characterization-based hierarchical clustering and phylogeny tree were created using R Studio version 4.2.2. The NbClust package was used for clustering, igraph for phylogenetic trees and R Color Brewer for generating color palettes in the diagrams. PCA analysis was performed as propsed by Massy (1965) and Jolliffe (1986) using R studio 4.2.2 with the package Factoextra and FactomineR.

RESULTS AND DISCUSSION

Morphological characterization and trait significance analysis

Thirty advanced breeding lines were classified into distinct categories based on the variations in their morphological traits using DUS descriptors, which are presented in Table 1. Fig 1 visualizes the pictorial representation of all morphological traits.

Anthocyanin pigmentation was observed across all the stems of the plants, indicating a consistent expression of this trait among the studied lines. Anthocyanins work as scavengers of reactive oxygen species (ROS) and protect plants from oxidative impairment, improving their resilience under stressful environments.

Based on the flowering time, 60% of lines found early, while 40% were classified as medium. Flowering time is a critical trait influencing chickpea productivity. Early flowering genotypes fit well into short growing seasons, thereby enhancing land use efficiency. Medium flowering genotypes permit the plants to utilize the entire growing season for resource accumulation.

The growth habits of the chickpea plants exhibited diversity, 60% of the lines displayed a semi-erect growth habit, 26.67% exhibited an erect habit and 13.33% had a spreading habit. Plants with erect and semi-erect growth habits have a more upright structure that facilitates mechanical harvesting by reducing ground contact, minimizing pod losses during machine harvesting.

Foliage colour assessment revealed that a majority (86.67%) of the lines possessed medium green foliage, while light green and dark green foliage were each observed in 6.67% of the lines. Foliage colour itself may not directly impact yield, it can be an indicator of plant health and vigour and can aid in identification of genotypes. Leaflet size measurements indicated that 80% of the lines had medium sized leaflets, with small and large leaflets each existing in 10% of the lines. Variations in leaflet size among different genotypes can significantly affect photosynthetic efficiency and overall plant health. Larger leaflets may harness more sunlight, increasing biomass accumulation and higher yields. Notably, all lines displayed a consistent pinnate leaf pattern, with no variation in this trait.

Flower morphology was predominantly consistent across the lines. Among all the studied lines, JG 2022-1 exhibiting twin flowers per peduncle. The number of flowers per peduncle influences pod formation and, consequently, seed yield. All the studied genotypes exhibited pink flower colour, indicating uniformity in this trait in *desi* chickpea lines. Flower color is a significant morphological trait for

identification of genotypes. Each flower the lines under investigation displayed stripes on the standard petal, indicating 100% frequency of this trait. Peduncle length varied among the lines, 70% had medium-length peduncles, 23.33% had long peduncles and 6.67% had short peduncles. Optimal peduncle length may facilitate better pod development as it can influence nutrient allocation, contributing to improved yield potential.

Plant height assessments showed that 66.67% of the lines were of medium height, 26.67% were tall and 6.67% were short. Singh *et al.* (2019) observed that plant height is a critical trait influencing suitability for mechanical harvesting. Pod size evaluation revealed that 56.66% of the lines produced medium-sized pods, 26.66% had large pods and 16.67% had small pods. Larger pods may contribute to increased seed yield; however, factors such as number of seed per pod may influence the productivity.

Brown colour of seed coat was observed in 73.33% of the lines, while orange and dark brown seeds were present in 16.67% and 10% of the lines, respectively. Colour of seed coat determines the market quality and consumer acceptance, making it an important trait for chickpea breeding programs (Sastry *et al.,* 2014). Seed size analysis indicated that 56.66% of the lines had medium-sized seeds, 26.67% had small seeds, 13.33% had very small seeds and 3.33% had large seeds. Seed size is a significant trait influencing market value, with larger seeds often commanding higher prices due to consumer preferences. All seeds were angular in shape. The angular morphology influences various physical properties of the seed, including

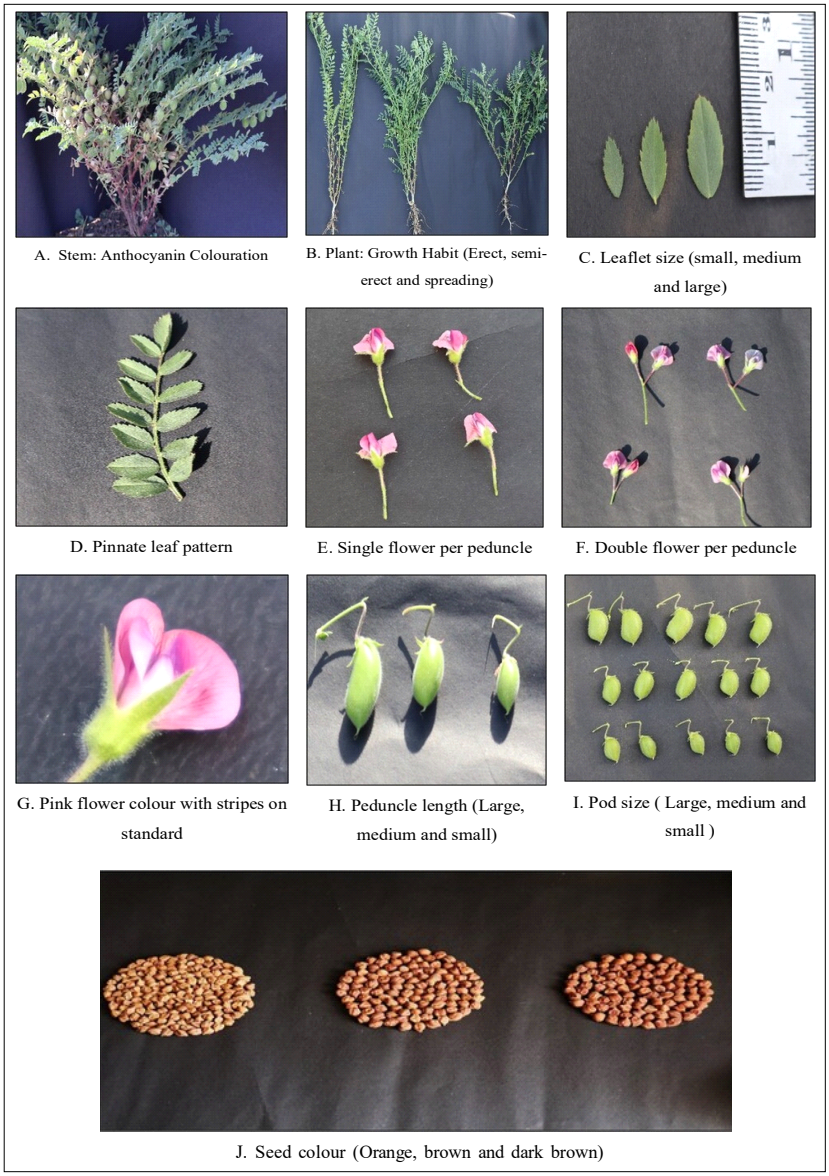


Fig 1: Depiction of morphological traits of the genotypes.

Table 1: Frequency analysis of morphological and seed traits in accordance with DUS guidelines and Shannon-Weaver diversity index (H).

Characters	Pattern	Score	Genotypic frequency	SWDI(H)	Genotypes
Stem: Anthocyanin colouration	Absent	1	0	0	-
	Present	9	30	-	JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8 , JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Erect (0-15 from vertical)	3	8	-0.92762	JG2022-2, JG2022-4, ICCV191609, ICCV181109, ICC181667, ICC191616, ICC191604, JG-24
Plant: Growth habit	Semi-erect (16-60 from vertical)	5	18	-	JG2022-1, JG2022-3, JG2022-5, JG2022-7, JG2022-8, JG2022-9, JG2021-1617, JG2016-14-16-11, ICCV15104, ICC191606, ICC181108-1, ICC181108-2, JG2016-9651, ICC191618, JG7413, RVG204, JG-18, JG-14, JG2020-55
	Spreading (61-80 from vertical)	7	4		JG2020-55, JG2022-26, JG2022-12 , ICCV181612
	Extra early	1	0	-0.67301	-
Time of flowering	Early	3	18		JG2022-1, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2016-14-16-11, JG2022-26, ICCV15104, ICC181108-1, ICC191618, JG7413, RVG204, JG-14, JG-24
	Medium	5	12		JG2022-2, JG2020-55, ICCV181109, ICC181667, ICC191606, JG2022-12 , ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICCV181612, JG-18
	Late	7	0		-
Plant: Foliage color	Light green	1	2	-0.48509	JG2016-9651, JG-14
	Medium green	2	26		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8 , JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, ICC191618, ICCV181612, JG7413, RVG204, JG-24
	Dark green	3	2		ICCV181109, JG-18
	Greenish purple	4	0		-
	Small (<10 mm)	3	3	-0.63903	JG2022-26, JG2022-12, JG2016-9651
Leaflet: Size	Medium (10-15 mm)	5	24		JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, ICC181667, ICCV15104, ICC191606, ICC181108-1, ICC191616, ICC191604, ICC181108-2, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14
	Large (>15 mm)	7	3		JG2022-1, JG2022-2, JG-24
	Simple	1	0		-
	Compound	2	0		-
Leaf: Pattern	Pinnate	3	30		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8 , JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104,

Table 1: Continue....

Table 1: Continue....

					ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Single	1	29	-0.14614	JG2022-1
Flower: No. per peduncle	Double	3	1		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	White	1	0	0	-
Flower: Colour	Pink	2	30		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Blue	3	0		-
	Absent	1	0	0	-
Flower: Strips on standard	Present	9	30		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Short (>5 mm)	3	2	-0.76978	JG2016-14-16-11, JG2022-26
Peduncle: Length	Medium (5-10 mm)	5	21		JG2016-14-16-11, JG2022-26, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, JG2021-1617, JG2020-55, ICCV181109, ICC181667, ICC191606, JG2022-12 , ICC181108-1, ICC191604, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204
	Long (>10 mm)	7	7		JG2022-1, ICCV191609, ICCV15104, ICC191616, ICC181108-2, JG-18, JG-24,
	Short (<45 cm)	3	2	-0.80331	JG2022-3, JG2022-26
Plant: Height	Medium (45-65 cm)		5	20	JG2022-1,JG2022-2, JG2022-5, JG2022-7, JG2022-8, JG2022-9, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-2, JG2016-9651, JG7413, RVG204, JG-18, JG-14
	Tall (>65 cm)	7	8		JG2022-4, ICCV191609, ICC181108-1, ICC191616, ICC191604, ICC191618, ICCV181612, JG-24
	Small (<15 mm)	3	5	-0.97295	JG2020-55, JG2022-12 , JG2016-9651, ICC191618, JG7413
Pod: Size	Medium (15-20 mm)		5	17	JG2022-3, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICC191606, ICC191616, ICC181108-2, ICCV181612, RVG204, JG-14,
	Large (> 20 mm)	7	8		JG2022-1, JG2022-2, JG2022-4, ICCV15104, ICC181108-1, ICC191604, JG-18, JG-24
	Orange	5	5	-0.75633	JG2020-55, JG2016-14-16-11, ICCV15104, JG2022-12, JG2016-9651,
Seed: Colour	Brown	6	22		JG2022-1, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, ICCV181109, JG2022-26, ICC181667, ICC191606, ICC181108-1, ICC191616, ICC181108-2, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24

Table 1: Continue....

Table 1: Continue....

	Dark brown	7	3		JG2022-2, JG2021-1617, ICC191604
Seed: Size	Very small	1	4	-1.05635	JG2020-55, JG2016-14-16-11, JG2022-12, JG2016-9651
	Small	3	8		JG2022-3, JG2022-7, ICCV181109, ICC181667, ICCV15104, ICC191616, JG7413, JG-14
	Medium	5	17		JG2022-1, JG2022-2, JG2022-4, JG2022-5, JG2022-8, JG2022-9,ICCV191609,JG2021-1617,JG2022-26, ICC191606, ICC181108-1, ICC191604, ICC181108-2, ICC191618, ICCV181612, RVG204,JG-24
	Large	7	1		JG-18
	Extra large	9	0		-
	Pea-shaped	1	0	0	-
	Owl's head	2	0		
Seed: Shape	Angular	3	30		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Rough	1	7	-0.54327	JG2022-8, ICCV191609, ICC181667, ICC191606, ICC191616, ICCV181612, JG7413
Seed: Testa texture	Smooth	2	23		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-9, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICCV15104, JG2022-12 , ICC181108-1, ICC191604, ICC181108-2, JG2016-9651, ICC191618, RVG204, JG-18, JG-14, JG-24
	Tuberculated	3	0		-
	Absent	1	0	0	-
Seed: Ribbing	Present	9	30		JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12 , ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
Seed: Type	*Desi*	1	30	0	JG2022-1, JG2022-2, JG2022-3, JG2022-4, JG2022-5, JG2022-7, JG2022-8, JG2022-9, ICCV191609, JG2021-1617, JG2020-55, JG2016-14-16-11, ICCV181109, JG2022-26, ICC181667, ICCV15104, ICC191606, JG2022-12, ICC181108-1, ICC191616, ICC191604, ICC181108-2, JG2016-9651, ICC191618, ICCV181612, JG7413, RVG204, JG-18, JG-14, JG-24
	Kabuli	3	0		-

bulk density and actual density (Sivakumar *et al.,* 2024) and primarily have utility for milling purposes.

The testa texture was smooth in 76.67% of the lines, with the remaining 23.33% exhibiting a rough texture. The rough testa safeguard the embryo from mechanical damage, pathogens (Smikal *et al.,* 2024). Seed ribbing was present in all the lines and all were classified as the *desi* type, characterized by small, coloured and angular seeds. These findings provide a detailed overview of the morphological characteristics of the chickpea lines studied, highlighting uniform and variable traits within the sample population. Behera *et al.* (2023); Asati *et al.* (2023); Kumawat *et al.* (2022) performed the similar characterization.

Diversity analysis by SWDI

The Shannon-Weaver diversity index, applied to 18 morphological traits (Fig 2; Table 1), ranging from 0 to 1.056. A higher index value indicates greater diversity, essential for broadening the genetic base in breeding programs. Notably, seed size demonstrated the highest diversity index (1.056), followed by pod size (0.973), plant growth habit (0.928), plant height (0.803), peduncle length (0.770), seed colour (0.756), flowering time (0.673), leaflet size (0.639), seed testa texture (0.543), plant foliage colour (0.485) and flower number per peduncle (0.146). Traits exhibiting a zero diversity index, such as stem anthocyanin colouration, leaf pattern, flower colour, flower stripes on the standard, seed shape, seed ribbing and seed type, indicate uniformity across the lines. Comparable analyses were performed by Awol (2018) and Asati *et al.* (2023) to estimate the phenotypic diversity in chickpea genotypes, highlighting the significance of these evaluations in crop improvement.

Principal component analysis

The PCA analysis indicated that the initial five components accounted for a total of 76.34 per cent of the variability across thirty lines, having threshold Eigen value greater than 1 (Fig 3 and 4). Genotype JG2020-55 obtained the maximum score in PC1, followed by JG2022-12, JG2016-9651 which indicates that these genotypes contributed significantly for the traits such seed colour, seed size and days to 50% flowering. PC2 was predominantly related to traits *viz*., plant growth habit and plant height with the maximum scores recorded for genotypes ICC191604, ICC191616 and JG24. The PC scores in PC3 were recorded the highest values for the characters such as leaflet size, flower number per peduncle, peduncle length and pod size through lines *viz.* JG2022-1, JG2022-26 and JG2022-2. In PC4, the genotypes JG 18, ICCV181109, ICCV191609 found diverse for colour of foliage. Genotypes JG7413, ICC191616, ICC181667, ICC191606 and ICCV181612 showed high

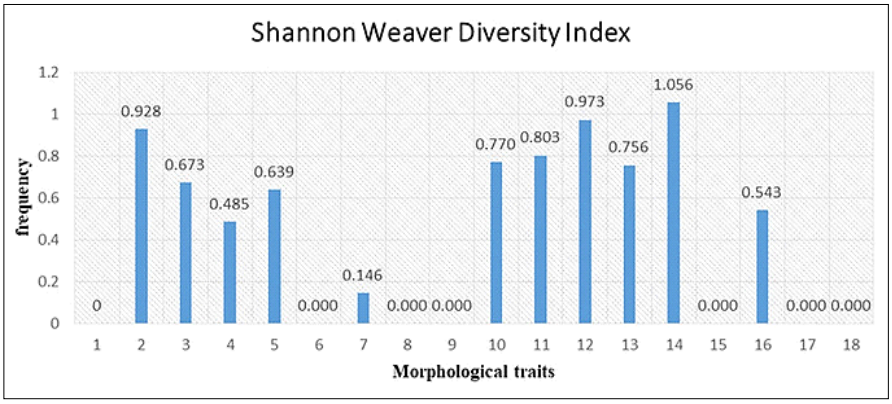


Fig 2: Shannon weaver diversity index for various morphological traits.

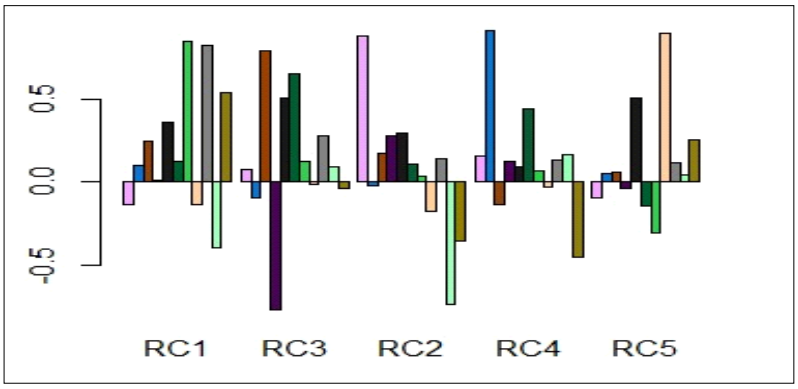


Fig 3: Rotated component matrix (RCM) demonstrates the contribution of various traits to the first five components (RC1-RC5).

values in PC5 being promising for traits such as pod size and testa texture.

Genotype JG2016-9651 was common in PC1, PC3 and PC4, while line JG2022-2 was shared by PC2, PC3 and PC5. Genotype ICCV191609 found mutual in PC1, PC4 and PC5. These lines showing Eigen values >1 in multiple PCs are diverse for the corresponding traits facilitated by these PCs. PCA biplot (Fig 5) also denotes that genotypes clustered near the center of the biplot reflect moderate contributions from multiple traits, while those positioned on the periphery (*e.g.* JG2022-1 and JG2016-9651) exhibit distinct profiles associated with specific traits. Dehbaoui *et al.* (2024) employed PCA to analyse diversity in morphological traits, emphasising its effectiveness in characterizing phenotypic variation.

Hierarchical clustering and phylogeny assessment

Hierarchical clustering analysis (Fig 6) based on morphological descriptors classified the studied lines into six distinct clusters, revealing substantial genetic variability within the studied population. Genotypes JG2020-55, ICCV181109, JG2022-12, ICCV191609 and JG2016-9651 exhibited the highest inter-cluster distances, indicating substantial morphological divergence. The phylogenetic tree (Fig 7) further validatez these results, visually depicting the six clusters. The clustering pattern, highlighted by different colors reveals the evolutionary relationships among genotypes. The light green cluster, forming the central groups, contain genotypes with high genetic similarity and minimal divergence, indicating shared ancestry or core germplasm. The red cluster (genotypes JG2020-55, JG2016-14-16-11, ICCV15104, JG2022-12 and JG2016-9651), dark blue cluster (genotypes ICC181108-1, JG2022-4, JG-24, ICC191604 and JG2022-2) and dark green cluster (genotypes JG2022-8 , JG7413, ICC191606, ICC181667, ICCV181612, ICC191616) comprises genetically distinct genotypes which may harbour unique

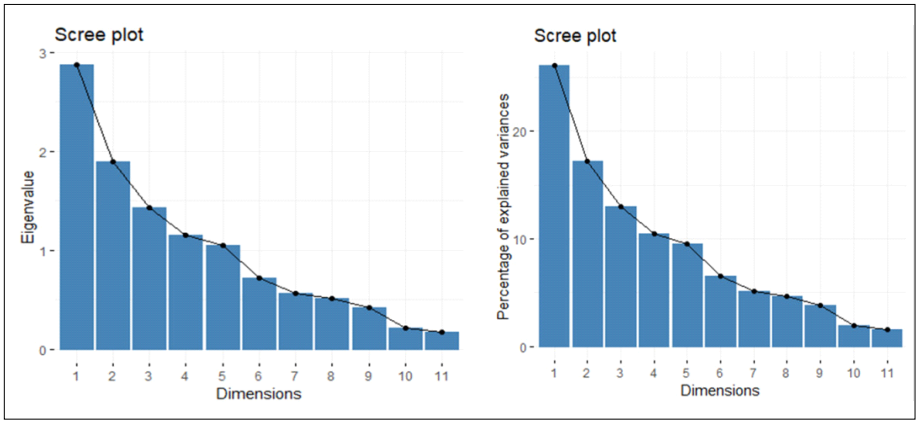


Fig 4: The scree plots illustrating the Eigen values and the percentage of explained variance for each principal component, depicting the first five components account for a higher degree of variation within the data set.

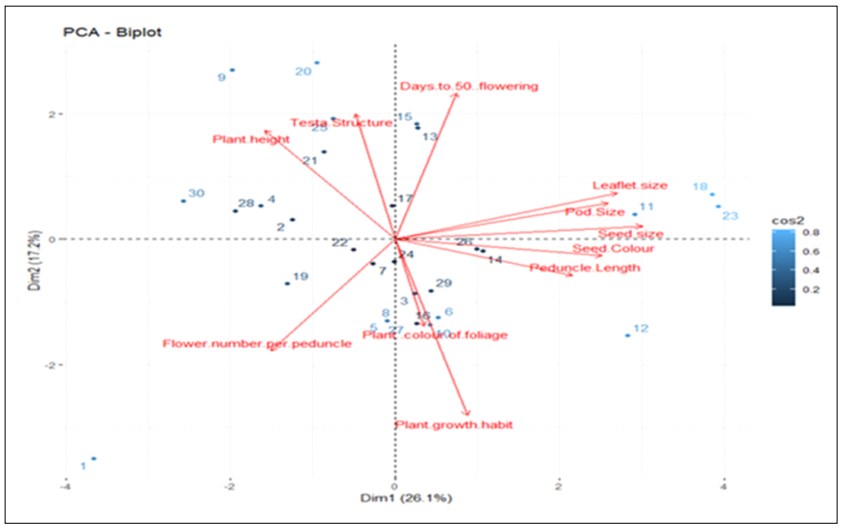
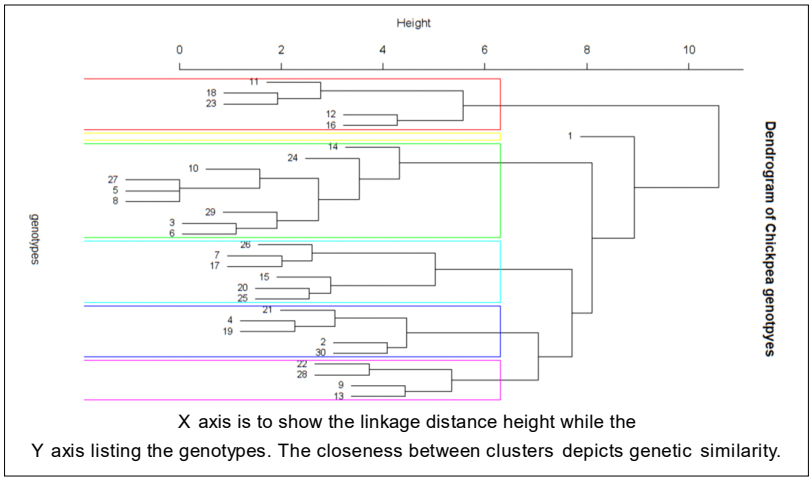
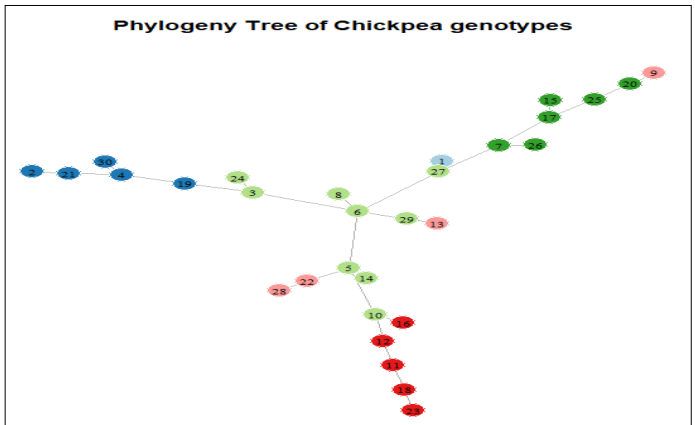


Fig 5: The PCA biplot showing the contribution of morphological traits (red vectors) to the first two principal components, Dim1 and Dim2, as well as the distribution of genotypes.



X axis is to show the linkage distance height while the Y axis listing the genotypes. The closeness between clusters depicts genetic similarity.

Fig 6: The dendrogram grouping the genotypes by their morphological traits.



Every node in the tree is a genotype and the branch lengths represent the genetic distances of these genotypes.

Fig 7: The phylogenetic tree illustrates the genetic relationships among genotypes.

or novel traits. The pink cluster, comprising genotypes ICC181108-2, JG-18, ICCV181109 and ICCV191609 reflect moderate differentiation, suggesting an intermediate level of genetic divergence. In contrast, the single genotype in the light blue cluster (JG2022-1) stands alone, representing a unique genetic lineage with considerable evolutionary divergence. Shorter branch lengths within clusters indicate a higher degree of genetic similarity. In contrast, longer branches between clusters suggest substantial divergence, which may imply that these genotypes evolved independently or harbour distinct genetic traits. These findings highlighted the diverse genetic architecture of the population, which is crucial for enhancing the genetic base in breeding programs. Jain *et al.* (2022) also utilized hierarchical clustering for the assessment of genetic diversity within chickpea genotypes. Similarly, Getahun *et al.* (2021) underscored the usefulness of phylogenetic clustering in distinguishing genetic lineages, emphasizing its importance in detecting unique genotypes for targeted improvement.

CONCLUSION

The present study indicated significant genetic diversity in traits seed size, pod size, plant growth habit, plant height, peduncle length, seed color, flowering time, leaflet size and testa texture. Lines ICCV191609 and JG2016-9651 have sown significant scores in several PCs, being promising for multiple traits. Similarly, line JG2020-55 found to be diverse as well as promising for traits seed colour, seed size and days to 50% flowering as showing the maximum score in PC1. These lines will produce the promising recombinants on hybridization, as having a wide cluster distances with other lines. Notably, line JG2022-1 stands out as a unique, diverse and most promising for traits like leaflet size, flower number per peduncle, peduncle length and pod size.

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Disclaimers

The opinions and conclusions presented in this article are strictly those of the authors and do not necessarily reflect the perspectives of their associated institutions. The authors are accountable for the precision and completeness of the information shared, yet they do not assume any liability for any losses, whether direct or indirect, that may arise from the use of this content.

Informed consent

All animal experimentation procedures were sanctioned by the Committee of Experimental Animal Care and were approved by the University of Animal Care Committee.

Conflict of interest

The authors state that there are no conflicts of interest concerning the publication of this article. The study's design, data collection, analysis, decision to publish and manuscript preparation were not influenced by any funding or sponsorship.

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