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Implications of Qualitative Traits Diversity for Future Chickpea Improvement and Conservation

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ABSTRACT

Morphological characterization and diversity estimation are essential in plant breeding. In this endeavor, the researcher performed a visual assessment of 18 phenotypic DUS listed traits of 32 Kabuli chickpea genotypes in *rabi* 2021-2022. Nine traits were monomorphic, four traits were dimorphic, while five traits were polymorphic. Shannon's diversity indices were estimated using Microsoft excel. The index ranged from 0.00 to 1.06, with a mean value of 0.36. Seed size (g) obtained the highest value of the 32 genotpyes, few were unique as they could be distinguished based on a single trait, while the majority were very closely related. Cluster analysis revealed that the 32 genotypes were grouped into two major clusters—similar genotypes concerning different traits clustered in the same clusters and vice versa.

Keywords Chickpea, DUS, Morphological characterization, Diversity, Qualitative traits.

INTRODUCTION

One of the first grain legumes humans domesticated in the old world was the chickpea (*Cicer arietinum* L.), also known as the Gram, Bengal gram, Egyptian pea, Chana, and Garbanzo bean. The family Leguminosae, currently referred to as Fabaceae (Bentham and Hooker 1970) and includes the subfamily Papilionaceae, consists of the genus Cicer. The crop is thought to have originated in Western Asia, from where it was spread to India and other regions. Pulses are far more vital in our country than in Asia or the rest of the globe since they contribute much more nutrients to the diet (Ali 2002). *Kabuli* (white-seeded) and *desi* (brown-seeded) are the two primary cultivars of chickpeas, representing two distinct gene pools. The traits that are used to identify a variety must, in practice, exhibit (DUS) variances. The certification officers, seed producers, and seed growers place great importance on chickpea characterization and varietal identification.

Due to the continuous use of some cultivars, the genetic basis among cultivated chickpea accessions is small, limiting the genetic improvement of chickpeas through breeding efforts (Bharadwaj *et al*. 2011a). Varietal purity, comprising morphological and genetic

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characteristics, determines seed quality. A variety or cultivar is a collection of grown plants identified by any characteristic (morphological, physiological, cytological, chemical, or other) that preserves its distinctive characteristics after sexual or asexual reproduction. A variety must exhibit distinct, uniform, and stable (DUS) differences in the features used to identify it as a particular variety. To carry out this aim, seed certification programs have been established to guarantee the identification and purity of cultivars in the marketplace. Historically, morphology has been the main factor in identity verification (Singh 2001). Field workers, certification officers, seed producers, and seed growers place a lot of importance on the characterization, varietal identification, and genetic purity assessment of chickpea cultivars to control the seed's quality. Seed analyzers lack access to standardized methods for evaluating cultivar purity, nevertheless. No cultivar can be recognized or disregarded solely by looking at its seeds or morphological characteristics. Therefore, it is crucial to understand stable visual diagnostic aspects of seed, seedling, and plant morphology to maintain the purity of cultivars (Lalitha 2007).

MATERIALS AND METHODS

During the *rabi* 2021–2022, thirty-two Kabuli chickpea lines were evaluated at the Breeder Seed Production Unit at the College of Agriculture, Jabalpur. The AICRP on Chickpea in Jabalpur and ICRISAT in Hyderabad donated the genotypes. In plots with four rows measuring 4.0 meters in length, genotypes were planted in three replications using a Randomized Complete Block Design (RCBD), with an inter- and intra-row spacing of 45.0 cm and 10.0 cm, respectively. The suggested package for agricultural and plant protection practices was followed. According to the DUS guidelines for chickpeas, data for each of the 18 descriptor qualities were collected on ten randomly chosen plants for each character in each replication. The calculated phenotypic frequencies were then utilized to generate Shannon's Diversity Index (H) following Negassa (1985) to evaluate the present diversity level.

$$H= -\Sigma [pi \times \log pi]$$

Pi is the percentage of entries corresponding to the ^{ith} class.

With the help of the Statgraphics Centurion XIX program, a cluster analysis was carried out according to Macqueen (1967) and Forgy's "k-means clustering" approach to categorize the genotypes (1965). The Corrplot package was used to assess Pearson correlation coefficients (r) in R studio version 2022.07.2-576.

RESULTS AND DISCUSSION

Eighteen attributes were scored visually for the distinctiveness test (Table 1). Nine attributes were discovered to be monomorphic in all 32 genotypes, including the absence of stem anthocyanin pigmentation, pinnate leaf pattern, single flower per peduncle, white flower color, stripes on standard, beige seed color, smooth testa texture, and ribbing on the seed surface. Four attributes were dimorphic: Plant height (15 medium genotypes and 17 tall genotypes), pod size (11 medium genotypes and 21 large genotypes), owl's head (28 genotypes) and the remaining genotypes were angular-seed shape. The polymorphism of the other five characteristics was evident. The genotypes were divided into three groups based on the height of the stem. Twenty-three genotypes showed medium initiation (8–15 nodes), two genotypes showed low initiation (8 nodes), and the remaining seven genotypes showed high initiation (>15 nodes) of the first flower. A distinguishing trait in varietal characterization is plant growth habit. Out of 32 genotypes, three were found to have an erect kind of growth habit, 20 genotypes have a semi-erect type, and the other nine have a spreading type. The degree of foliage color showed notable variance, with one genotype having light green foliage, 15genotypes having medium green, and the remaining 16 genotypes having dark green. Genotypes were divided into three categories based on leaflet size: Small, medium, and large. Eleven genotypes had medium leaflet sizes (10–15 mm), 19 genotypes had large leaflets (15 mm), and two genotypes had small leaflets (10 mm). Seed size is substantially genetically determined. After seed size (determined by 100 seed wt), genotypes were divided into three groups. Seven genotypes had large seed sizes (45-55 g), 11 genotypes had small (35 g), and 14 genotypes had medium seed sizes (35-45

Table 1. Frequency distribution of morphological traits of chickpea genotypes.

Characters	Score	Genotype frequency	Percentage contribution (%)	Shanon weiver diversity index
Stem anthocyanin coloration				
Absent	1	32	100	00
Present	9	-		
Stem height at initiation of first flower				
Low (<8 nodes)	3	2	6.25	0.74
Medium (8-15 nodes)	5	23	71.88	
High (>15 nodes)	7	7	21.87	
Plant: Growth habit				
Semi erect (20-40° from vertical)	5	3	9.375	0.87
Semi spreading (40-60° from vertical)	6	20	62.5	
Spreading (60-80° from vertical)	7	9	28.125	
Plant: Height				
Short (<45 cm)	3	-	-	0.69
Medium (45-65 cm)	5	15	46.875	
Tall (>65 cm)	7	17	53.125	
Plant: Color of foliage				
Light green	1	1	3.125	0.81
Medium green	2	15	46.875	
Dark green	3	16	50	
Greenish purple	4	-	-	
Leaflet size (mm)				
Small (<10mm)	3	2	6.25	0.84
Medium (10-15mm)	5	11	34.375	
Large (>15mm)	7	19	59.375	
Leaf pattern				
Simple	1	-	-	00
Compound	2	-	-	
Pinnate	3	32	100	
Flower: Number per peduncle				
Single	1	32	100	00
Twin	3	-	-	
Flower: Color				
White	1	32	100	00
Pink	2	-	-	
Blue	3	-	-	
Flower: Stripes on standard				

Table 1. Continued.

Characters	Score	Genotype frequency	Percentage contribution (%)	Shanon weiver diversity index
Absent	1	-	-	00
Present	9	32	100	
Peduncle Length (mm)				
Short (<5mm)	3	-	-	0.52
Medium (5-10mm)	5	7	21.875	
Long (>10mm)	7	25	78.125	
Pod: Size (length)				
Small (< 15 mm)	3	-	-	0.64
Medium (15-20 mm)	5	11	34.375	
Large (>20 mm)	7	21	65.625	
Seed color				
Beige (Kabuli)	1	32	100	00
Creamy beige	2	-		
Seed shape				
Pea shaped	1	-		0.37
Owl's head	2	28	87.5	
Angular	3	4	12.5	
Seed testa texture				
Rough	1	-	-	00
Smooth	2	32	100	
Tuberculated	3	-	-	
Seed ribbing				
Absent	1	-	-	00
Present	9	32	100	
Seed size (g)				
Small (<35 g)	3	11	34.375	1.06
Medium (35-45 g)	5	14	43.75	
Large (45-55 g)	7	07	21.875	
Very large (>55 g)	9	-	-	
Seed type				
Desi	1	-	-	00
Kabuli	3	32	100	

g). Individual scoring of each genotype is mentioned in Table 2.

We can readily distinguish between various chickpea genotypes by looking at these characteristics. Using these characters as a key to identification. A similar characterization pattern was adopted by Upadhyaya *et al*. (2003), Shrivastava *et al*. (2012), Bayahi and Rezgui (2015), Archak *et al*. (2016), Awol *et al*. (2018), Gediya *et al*. (2018), Adem and Tesso (2019), Gnyandev *et al*. (2019), Solanki *et al*. (2019), Janghel *et al.* (2020), Aktar-Uz-Zaman *et*

Table 2. Morphological characterization of Kabuli chickpea genotypes with scoring based on DUS descriptors.

Entry name	AC	SH	GH	PF	LS	LP	FPP	FC	SF	PL	PH	PS	SC	SSh	STT	SR	SS	ST
ICCV 211301	1	7	5	1	5	3	1	1	9	7	5	7	1	2	2	9	7	3
ICCV 211302	1	5	7	3	5	3	1	1	9	7	7	7	1	2	2	9	7	3
ICCV 211303	1	5	5	3	5	3	1	1	9	7	7	5	1	2	2	9	3	3
ICCV 211304	1	5	7	2	5	3	1	1	9	7	7	5	1	2	2	9	3	3
ICCV 211305	1	5	5	2	7	3	1	1	9	7	7	7	1	2	2	9	5	3
ICCV 211306	1	5	5	2	5	3	1	1	9	7	7	5	1	2	2	9	3	3
ICCV 211307	1	5	5	2	7	3	1	1	9	5	7	5	1	2	2	9	3	3
ICCV 211308	1	5	7	3	7	3	1	1	9	7	5	7	1	3	2	9	5	3
ICCV 211309	1	3	7	2	7	3	1	1	9	5	7	5	1	2	2	9	3	3
ICCV 211310	1	5	5	2	7	3	1	1	9	7	7	5	1	3	2	9	3	3
ICCV 211311	1	7	5	3	7	3	1	1	9	5	5	7	1	2	2	9	7	3
ICCV 211312	1	5	5	3	7	3	1	1	9	7	5	7	1	2	2	9	5	3
ICCV 211313	1	5	5	3	7	3	1	1	9	7	7	7	1	3	2	9	5	3
ICCV 211314	1	5	5	3	7	3	1	1	9	7	5	7	1	2	2	9	5	3
ICCV 211315	1	5	5	2	5	3	1	1	9	7	5	7	1	2	2	9	7	3
ICCV 211316	1	3	5	3	5	3	1	1	9	7	7	5	1	2	2	9	3	3
ICCV 211317	1	5	5	3	7	3	1	1	9	5	5	7	1	2	2	9	7	3
ICCV 211318	1	5	7	2	7	3	1	1	9	7	5	7	1	2	2	9	5	3
NBeG 119	1	5	7	2	7	3	1	1	9	7	5	7	1	2	2	9	5	3
JGK 5	1	5	7	2	7	3	1	1	9	7	7	7	1	2	2	9	5	3
FLIP 10-277C	1	7	5	2	3	3	1	1	9	7	7	5	1	2	2	9	3	3
FLIP 11-156C	1	5	3	2	5	3	1	1	9	5	7	7	1	2	2	9	5	3
FLIP 08-254C	1	5	5	2	5	3	1	1	9	7	7	7	1	2	2	9	7	3
FLIP 10-165C	1	7	5	3	7	3	1	1	9	5	7	5	1	2	2	9	3	3
FLIP 07-310C-81	1	7	5	3	7	3	1	1	9	7	7	7	1	2	2	9	5	3
RVSVT-K-105	1	5	7	3	3	3	1	1	9	5	5	7	1	3	2	9	5	3
ICCV 171312	1	5	3	2	7	3	1	1	9	7	5	7	1	2	2	9	5	3
FLIP-12-354C	1	5	3	3	7	3	1	1	9	7	5	7	1	2	2	9	5	3
FLIP-12-334C	1	7	5	3	5	3	1	1	9	7	5	5	1	2	2	9	3	3
FLIP-12-128C	1	5	5	2	5	3	1	1	9	7	5	7	1	2	2	9	5	3
ICCV181313	1	7	7	3	7	3	1	1	9	7	7	5	1	2	2	9	3	3
JGK-1 (Check)	1	5	5	3	7	3	1	1	9	7	5	7	1	2	2	9	7	3

Where,
AC = Stem anthocyanin coloration, SH = Stem height at initiation of first flower, GH =Plant: Growth habit, PF = Plant: Color of Foliage, LS = Leaflet: Size (length), LP = Leaf: Pattern, FPP = Flower: Number per peduncle, FC = Flower:Color, SF = Flower: Stripes on standard, PL = Peduncle: Length, PH = Plant: Height, PS = Pod: Size (length), SC= Seed:Color, SS = Seed size. STT = Seed: Testa texture, SR = Seed: Ribbing, SSh = Seed: Shape, ST = Seed: Type.

al. (2020), Kumawat *et al*. (2020), Chaudhary *et al*. (2021) and Thakur *et al*. (2021) taking distinguished morphological traits.The published literature also supports these claims in the case of chickpea.

Shannon's diversity indices

Any breeding program should be designed with diversity in mind. For 18 morphological characters,

Table 3. Distribution of genotypes into 6 clusters as per K-means clustering.

Clusters	No of genotypes	Genotypes
1	9	ICCV 211301, ICCV 211302, ICCV 211311, ICCV 211315, ICCV 211317, FLIP 11-156C, FLIP 08-254C, 30, JGK1
2	1	RVSVT-K-105
3	6	ICCV 211303, ICCV 211304, ICCV 211306, ICCV 211316, FLIP 10-277C, FLIP-12-334C
4	5	ICCV 211307, ICCV 211309, ICCV 211310, FLIP 10-165C, ICCV181313
5	11	ICCV 211305, ICCV 211308, ICCV 211312, ICCV 211313, ICCV 211314, ICCV 211318, NBeG 119, JGK5,FLIP 07-310C-81 ,ICCV 171312, FLIP-12-354C

the calculated Shannon's diversity indices (Table 3) ranged from 0.00 to 1.06, with a mean value of 0.36. The highest diversity index was found for seed size (g), which was 1.06. The genotypes showed no variations for the nine monomorphic features, which is why they all obtained the lowest diversity index value of 0.00. As a result, the disclosed diversity index values can reveal high diversity in the morphological features under study. Therefore, improvements in these attributes may be explained by the effective use of diverse genotypes.

Cluster analysis for distinctiveness

Using cluster analysis, the variation was split into distinct subgroups. According to the cluster analysis, the 32 genotypes were divided into two main groups (Fig. 1). There are two sub-clusters made up of 15 genotypes in the first cluster (Fig. 1). There were 17 genotypes in the second cluster, which was further separated into two sub-clusters. Similar genotypes for related phenotypes were grouped together, and vice versa.

K-means clustering

K-means clustering divides the set of n items into k clusters, each of which contains the one with the closest mean. K-means is a clustering technique based on centroid data. An input parameter is the cluster count, indicated by the letter "K." Each data point is assigned to the cluster center that is nearest to it during collection. By using this strategy, k unique clusters can be formed at most. The K-means clustering method was used to divide all 32 genotypes, including three check entries, into five different groups, as shown in Table 3. Cluster V was the richest with 11 genotypes, followed by cluster I with 9 genotypes. Our results are similar to those of Muhammad *et al*. (2016),Wanga *et al*. (2017), Kaur *et al*. (2018), Sharma *et al*. (2018), and Mohan *et al*. (2019).

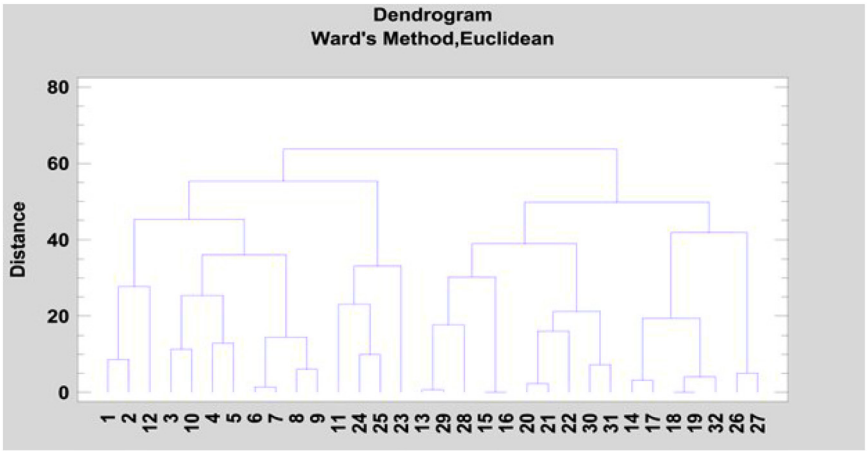

Fig. 1. Dendrogram representing distinctiveness of chickpea genotypes.

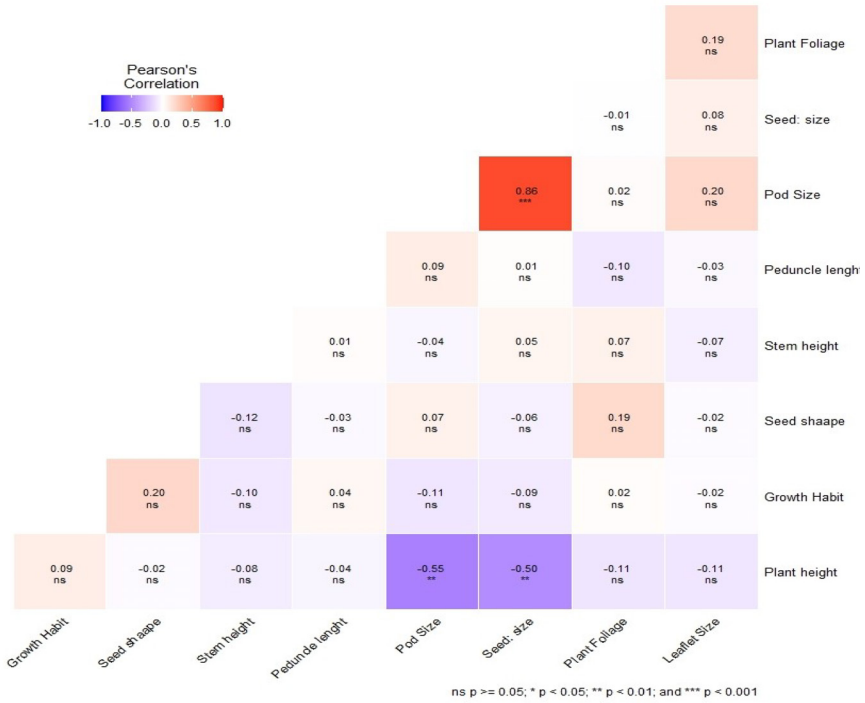

Fig. 2. Represents pearson's correlation coefficients of the 18 DUS traits as a heat map.

Correlations among the different traits studied

Significant correlations were found after the attributes under consideration were assessed for Pearson's correlation coefficients. Combinations of attributes, such as pod size and seed size, showed noticeably strong positive associations (p = <0.05) (Fig. 2). Significantly strong negative associations were inferred for trait combinations such seed size and pod size with plant height (p = < 0.05). One of the key yield-determining factors in crop improvement, particularly chickpea, is seed size (measured through 100 SW) (Saxena *et al*. 2021). Capturing evolutionary divergence and figuring out the genetics of 100SW in chickpea have been major goals of several traditional breeding techniques (Upadhyaya *et al*. 2006, Biçer and Tuba 2008, Kivrak *et al*. 2020).

CONCLUSION

Showing polymorphism, high diversity, and strongly correlated traits, such as seed size, pod size, and plant height, should all be taken into account in order to identify a genotype. Findings indicates that most characters had sufficient and desirable variation, indicating sufficient room for selection. It is possible to use DNA fingerprinting to identify the key differences between molecular and biochemical markers. Breeders will find it easier to identify the variable sources to employ in breeding programs and to apply for protection under the Protection of Plant varieties and farmers rights act with the aid of the current study.

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