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GENETIC VARIABILITY AND INTERRELATION OF PHENOTYPIC CHARACTERS IN *CICER ARIETINUM L.* AT EARLY STAGE

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Abstract *Cicer arietinum L., commonly known as chickpea, is the 2nd essential legume throughout the globe and is vital for global food security and sustainable practices due to its high nutritional value, adaptability to various climates, and nitrogen fixation. A pot experiment was conducted at the Experimental area of University of the Punjab Lahore to assess the genetic-variability and association of various morphological traits in different chickpea genotypes at the seedling stage during October 2023. A Completely Randomized Block design (RCBD) was applied along with three replications. Following traits were evaluated; shoot-length(SL), root-length(RL), seedling-biomass(SB), R-S ratio, leaf-length(LL), leaf-width(LW), and leaf-area(LA). Standard statistical analysis calculates genetic variability, heritability(Hbs2), and genetic advance(GA). Out of the seven characters studied, leaf area, seedling biomass, and root length exhibited a high value of genotypic-coefficients of variation(GCV), heritability, and high genetic-advance, indicating that these parameters depicted potential traits for effective selection to developing desirable chickpea varieties or genotypes. The path analysis revealed that leaf length, root-to-shoot ratio, and seedling biomass had the greatest positive direct effect on seedling length and thus may be considered useful traits for improvement against drought stress tolerance in chickpea.*

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Keywords: *Chickpea; Genetic variability; selection; heritability; correlation*

Introduction

Chickpea, a legume a member of the Fabaceae family, is the world's second widely cultivated pulse crop & 1st in South Asia in terms of cultivated area and productivity (Hama, 2019; Purdy et al., 2023). It is a nutrition-rich pulse crop containing 64% total carbohydrates, 23% proteins,5% fat, 6 % crude fiber, 3% ash, and a high level of vital minerals and vitamins(Kaur & Bhardwaj, 2019). Chickpea is the world's leading pulse crop in acreage and production. Globally, chickpea cultivation covers 15million-hectares, producing 15 million tons with an average of 10578 kg/ha productivity. In Pakistan, 0.8 million hectares are cultivated, resulting in 0.2 MT & average yield of 2650 kg per hectare, according to the Food and Agriculture Organization (FAO, 2021). India is the world's top producer and consumer of chickpeas, while Pakistan Ranked 7th in production("Chickpeas: Production volume by country worldwide 2021," 2023). It is produced under rain-fed and irrigated production systems in different regions of the World. Chickpeas are classified into two groups based on seed size, shape, and color. The first relatively few seeds are known as desi(brown), while the larger seeds are known as Kabuli (White)(Naveed et al., 2012; Qurban & Muhammad, 2011; Shah et al., 2007).

Despite having the world's 2nd largest cultivation area for chickpea, Pakistan's total output is inadequate, inconsistent, and far insufficient to meet local needs (Rasool et al., 2023). This yield gap can be narrowed by manipulating diverse genotypes and incorporating them into a breeding program. Thus, assessing genetic diversity using proper methodologies such as genetic coefficient of variation, heritability estimates, and genetic progress is necessary for increasing agricultural output (Ahmad et al., 2012; Ali et al., 2011; Ali et al., 2008; Waseem et al., 2014). The present study was carried out to explore genetic variability for various seedling parameters and their associations in chickpea germplasm to provide the necessary data that could be helpful in a program to improve grain yield in chickpea.

Materials & Methods

The pot experiment was carefully designed and executed at the experimental area of the Depattment of Plant Breeding and Genetics, Faculty of

Agricultural Sciences, University of the Punjab, Lahore (31°29'42.5"N 74°17'46.7"E) during rabi 2023 using a Completely Randomized Block Design(RCBD) to ensure stringent control over potential causes of variability. Our plant material comprised fifteen carefully selected genotypes of Chickpea, namely (Paidar91, Thal 2020, Bittal 2021, Bittal 2022, Noor 2013, Noor 2019, Noor 2022, Tamman, Balkassar2000, Wanhar 2000, Bhakkar 2011, Thal 2006, Punjab 2008, C44, CM2008) chosen for their diverse genetic backgrounds from different Research institute. Each experimental unit comprised standardized pots filled with 12kg of sand. Pots were randomly distributed to establish independence between nearby pots while keeping consistency in pot size, sand content, and environmental variables within the experimental area. 10 Seeds of each genotype were sowed at a consistent depth at different intervals in sand-filled pots. 100 percent germination percentage was noted for all genotypes. Throughout the experiment growth conditions were kept across all pots, including consistent watering, nutrient supply, and environmental factors. Data collection during the seedling stage covered key traits such as Seedling-length (SL), Root-Length (RL), Root to shoot ratio(R-S ratio), Leaf Length(LL), Seedling-Biomass(SB), Leaf-width(LW), Leaf-Area(LA). The collected data were then subjected to a thorough statistical analysis, applying analysis-of-variance to measure the significance of genotype and replication effects. All Statistical analyses, such as ANOVA, Heritability, Genetic Advance, were applied and Calculated using Statistix 8.1 to assess genotype variations. The results were interpreted to conclude genetic variability and trait associations among the genotypes. Analysis of variance(A.N.O.V.A) utilizing C.R.D was calculated for each of the analyzed variables via statistics. As per Comstock and Robinson (1952), GV and PV were derived. According to Singh and Choudhury (1985), mean results for genetic analysis were utilized to calculate GCV, PCV and ECV.

H^2 was computed as described by(Allard, 1961; Falconer, 1996) as also mentioned by(Bello et al., 2012)

$$\mathbf{h^2_{bs}\ (\%) = (\sigma^2 g / \sigma^2 p)\ x100}$$

GA is estimated as given by Allard (1960); Singh and Choudhary (1985) as mentioned by(Parida et al., 2018).

$$\mathbf{GA} = K\ \mathcal{X}\ \sqrt{\ }\ \sigma^2 p\ x\ h^2 bs$$

Where the value of K. is 2.06; similarly, GA as a percentage of the mean (GAM) was determined as,

$$\mathbf{GAM\ (\%) = \frac{GA}{\bar{x}}\ x100}$$

Results and Discussions

Analysis of Variance

In the ANOVA, the mean sum of squares (Table 1) resulting from the genotypes was discovered to be significant at both the 5% and 1% significance levels for all the characters examined. The notable variations observed imply that the genotypes evaluated include a large level of genetic variability.

Genetic parameters

Genetic parameters for all seven parameters are presented in Table 1.

It is evident from all the observations that for all of the characteristics under discussion, the phenotypic coefficient of variance (PCV) is higher than the corresponding genotypic coefficient of variance (GCV). Among the seven traits, the greatest GCV and PCV were found in Leaf Area (19.16, 19.64), which was followed by the Seedling Biomass(13.89, 13.98) and Root length(13.68, 14.05), while less GCV and PCV were noted for seeding length(5.11,5.16) respectively. Furthermore, No highest GCV and PCV values were noted in any parameter, but moderate GCV were noted for leaf area (19.16), Seedling biomass(13.89), Root length(13.68), root to shoot ratio(12.78) and Leaf length(10.69) while low GCV value for Seedling shoot length(5.11) and Leaf Width(9.94). Likewise, moderate PCV was noted for leaf area(19.64) followed by Root length(14.05), seedling biomass(13.98), root to shoot ratio(13.23), leaf length(11.67), and leaf width(10.87), and Low PCV for seedling shoot length(5.16) as suggested by Sivasubramanian and Madhava (1973). Less significant variations in GCV and PCV values were seen for all attributes, indicating increased variability and minimal environmental effects on the trait expressions.

Heritability and Genetic advance

Heritability and genetic advance was computed for the seven quantitative traits under study. The heritability values were classified according to (Johnson et al., 1955) as low, moderate, and high. The estimates for heritability ranged from 83.58% to 98.64% (Table 1). All of the study traits, including seedling-biomass (98.64%), seedling-length (98.08%) leaf-area (95.11%), root-length (94.87%), RS ratio (93.43%), leaf-length (83.87%), and leaf-width (83.58%), had high heritability. High heritability was observed in all assessed traits, indicating heritable variation in all the studied traits. GAM ranged 10.42 % to 38.49% in seedling length and leaf area, respectively. Falconer and Mackay (1996) classified GAM as; low (0-10%), medium (10 - 20%), and high (above 20%). Leaf Area exhibited the highest value of genetic advance (38.49%), followed by Seedling biomass(28.42%), Root length (27.45), RS ratio (25.45), and leaf length(20.17), while moderate GAM was found for Leaf width(18.72) and Seedling length(10.42). High heritability traits, when coupled with genetic advance, may yield greater genetic gain through selection because the variation in these traits arises from additive gene action(Vimal & Vishwakarma, 1998), and selection pressure could be profitably applied to these traits to improve yield outcome.

Correlation Coefficient Analysis

Correlation analysis is crucial in plant breeding and genetics, measuring the strength and direction of associations between traits. It aids in selecting plants with favorable traits, guiding breeding programs towards improved yields and disease resistance (Ali, Ali, Awan, et al., 2014; Asif et al., 2020; Naeem et al., 2022; Shafique et al., 2020). In the present study, the Seedling length is positively and strongly correlated with Seedling biomass (0.705), Leaf length(0.666), leaf Area (0.630), Root length(0.629), and Leaf width(0.499), which means that an increase in seedling length will also increase seedling biomass, leaf length, leaf area, root length, and leaf width as well. A similar association of Seedling length with seedling biomass, leaf width, leaf area, and root shoot ratio at the genetic level was also reported (Abbas et al., 2021; Ali et al., 2010). A negative and weak association was found between Root length and RS ratio, indicating that the RS ratio tends to decrease with an increase in root length, but the relationship is not robust. The low correlation indicates that the relationship between the variables is inconsistent, and other factors may influence their behavior independently. Also, root length hada positive and strong correlation with Leaf Length (0.658), Leaf Area(0.619), Seedling biomass(0.543), and leaf Width(0.511). Positive and weak correlation between RS ratio and Seedling biomass, indicating that with an increase in one trait, the other will also increase but there will be a weak association between them. RS ratio had a negative and very weak correlation with leaf length(-0.005), Leaf Area(-0.036), and Leaf width (-0.101), which indicates that when one trait changes, the other generally tends to follow, but this relationship is so weak that predicting one variable based on another may not be meaningful (Ali et al., 2013; Ali et al., 2016; Ali, Ali, Ahsan, et al., 2014).

Path Analysis

Path analysis is a detective tool that helps determine how various variables influence agricultural output. Correlation and regression studies examine the relationships between characteristics and yield. These analyses help us determine each trait's direct and indirect effects on the final yield. Results have shown that a high direct effect was found for seedling biomass(2.125), RS ratio(3.311), and leaf length(5.009). Leaf area showed a high direct negative effect on Seedling length. The trait-like seedling biomass indirectly affects seedling length through leaf length. Traits like leaf width(-4.702), leaf length(-4.744) & root length(-3.154) have an indirect negative effect on seedling length through leaf area. Similarly, leaf area(4.661), leaf width(3.614), and root length(3.297) showed an indirect positive effect on seedling length through leaf length.

Table1: Variance components, genotypic, environment, and phenotypic coefficients of variation, and heritability estimates for seven Seedling parameters of 15 chickpea genotypes

Traits	M.S	G.M	CV	GV	GCV	PV	PCV	EV	ECV	$h^2bs\%$	GA
Seedling Length	2.27	16.96	0.88	0.75	5.11	0.77	5.16	0.02	0.88	98.08	10.42
Root length	5.20	9.50	3.90	1.69	13.68	1.78	14.05	0.14	3.90	94.87	27.45
RS ratio	0.01	0.40	4.12	0.00	12.78	0.00	13.23	0.00	4.15	93.43	25.45
Seedling biomass	0.06	1.01	1.99	0.02	13.89	0.02	13.98	0.00	2.00	98.64	28.42
Leaf length	0.01	0.56	5.74	0.00	10.69	0.00	11.67	0.00	5.74	83.87	20.17
Leaf width	0.00	0.39	5.41	0.00	9.94	0.00	10.87	0.00	5.40	83.58	18.72
Leaf Area	0.12	1.04	7.43	0.04	19.16	0.04	19.64	0.00	5.32	95.11	38.49

M.S = mean square, G.M = grand mean, CV = coefficient of variation, GV = genotypic variance, PV = phenotypic variance, EV = environmental variance, GCV = genotypic coefficient of variance, PCV = phenotypic coefficient of variance, ECV=environmental coefficient of variance, H^2bs = heritability(broad sense, GA = genetic advance

Table 2: Pearson Correlation

Traits	SL	RL	RTSR	SB	LL	LW
RL	0.629**					
RTSR	0.196	-0.263				
SB	0.705**	0.543**	0.230			
LL	0.666**	0.658**	-0.005	0.520**		
LW	0.499**	0.511**	-0.101	0.454**	0.722**	
LA	0.630**	0.619**	-0.036	0.529**	0.931**	0.922**

* = > p value < 0.05, **=> is p value < 0.01, SL is seedling length, RL is root length, RTSR is root-to-shoot ratio, SB is seedling biomass, LL is leaf length and LW is leaf width LA is Leaf Area

Table 3. Path table showing direct(Bold and Highlighted) and indirect effects of various traits on seedling length in chickpea

Traits	Root length	RS- ratio	Seedling biomass	leaf length	leaf width	leaf area
Root length	**0.196**	-0.871	1.154	3.297	0.952	-3.154
RS- ratio	-0.051	**3.311**	0.488	-0.023	-0.188	0.183
Seedling biomass	0.106	0.045	**2.125**	2.604	0.845	-2.696
leaf length	0.129	-0.015	1.105	**5.009**	1.344	-4.744
leaf width	0.100	-0.335	0.964	3.614	**1.863**	-4.702

leaf area	0.121	-0.119	1.124	4.661	1.718	**-5.098**

Conclusion

The study reveals significant variation and genetic diversity in chickpea traits, highlighting the importance of direct selection for yield-contributing characteristics. Leaf area, root length, and seedling biomass show high genotypic and phenotypic coefficients of variation. Root-shoot ratio, leaf length, and seedling biomass are influential factors that directly affect seedling length. These traits demonstrate substantial heritability and genetic advance, offering the potential for developing superior chickpea varieties through effective selection.

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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Conflict of Interest

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.