



UNVEILING GENETIC THREADS: PHENOTYPIC EXPLORATION OF CHICKPEA (*CICER ARIETINUM* L.) GERMPLASMS FOR HERITABILITY AND TRAIT ASSOCIATIONS

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Abstract The second most important self-pollinating legume crop in the world is the chickpea. Chickpea is an inexpensive source of protein that grows well in various climates. The current study aimed to evaluate the genetic advance and heritability of different characters at the seedling stage by analyzing their phenotypic attributes. During the 2022–2023 growing season, a randomized complete block design experiment was set up in the University of the Punjab's field area. The experiment included five different germplasms. At the seedling stage, data from seven morphological parameters were collected. ANOVA and correlation were performed using Statistix software. Seedling length, root length, and root-shoot ratio have shown low genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV), indicating a high environmental effect on these traits. Root length and leaf width had shown a strong correlation with seedling length. Path coefficient analysis directly affected root length and leaf width towards seedling length. Low genetic advance and high heritability were observed in root length, seedling length and root-shoot ratio, meaning selection should be delayed for one or more generations for such traits. Leaf width and leaf area have shown high heritability and genetic advance, suggesting that selecting such characters may lead to superior variety development based on phenotype.

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Introduction

Chickpea (*Cicer arietinum* L.) ranks first in South Asia and third globally among grain legumes (Qurban and Muhammad, 2011). Chickpea accounts for 12% of the world's pulse production. Since chickpea is high in protein, vitamins, minerals, and fiber, it is used as both human food and animal feed (Tsfamichael et al., 2015). Pakistan and India are global chickpea producers (Mohammed et al., 2019). Australia, Iran, Ethiopia and Turkey are the largest producers (Ali et al., 2010a; Ali et al., 2011; Ali and Malik, 2021; Damte and Ojiewo, 2016). Chickpea provides significant protein to those who cannot afford animal protein (Hama, 2019). Chickpea, the most important nutritional source in many nations, constitutes 22% protein and 63% carbohydrates (Shafiq et al., 2021). One of the types of desi constitutes up to 80–85% of chickpea production (Yousef et al., 2020). Animals are fed dried chickpea stalks that are abundant in protein (Li et al., 2015). Being rich in protein, fiber, vitamins, and minerals, chickpea is a staple food in

many cultures and helps support good health by contributing to a balanced diet (Kaur and Prasad, 2021). Additionally, Chickpeas have also drawn attention for their ability to reduce malnutrition and resolve problems about global food security (Bessada et al., 2019). Chickpea can withstand drought and grow well in various climates, making it a vital part of resilient agricultural systems. These characteristics highlight that chickpea fosters environmental sustainability and human nourishment (Ali et al., 2015; Ali et al., 2013; Ali et al., 2016; Asati et al., 2022).

In Pakistan, the major producer cities of chickpea include Mianwali, Bhakkar and Mankera. The demand for pulses is rising in tandem with the world's population rapid expansion. To meet the growing demand for pulses, new cultivars with higher production must be introduced to deal with protein deficiency (Joshi et al., 2018). Breeders are striving to increase the potential for heredity. Heritability

provides a more compelling explanation for variations observed due to the genetic makeup among plants and their interactions with the environment (Ahmad et al., 2012; Ahmad et al., 2021; Ali et al., 2013; Ali et al., 2014; Ali et al., 2010b; Asif et al., 2020; Saleem et al., 2002). Furthermore, genetic advance assumes a feasible improvement for new induction by selection. The current experiment was set up to assess heritability and genetic variability in diverse chickpea germplasm for distinct phenotypic features.

Materials and Methods

A field experiment was performed at the University of the Punjab Lahore, Pakistan, farm area from 2022 to 23. The experiment was set up in a randomized complete block design (RCBD) with five genotypes and three replications. Based on availability, five chickpea varieties, including Balkasar-2000, Aug-424, Noor-91, Paidar-91 and Punjab-2008 were utilized in the experiment taken from Ayub Agricultural Research Institute, Faisalabad. Fertilization and irrigation were applied as per crop demand. Data of various morphological traits was recorded viz. seedling length (cm), root length (cm), leaf length (cm), leaf width (cm), leaf area (cm²), seedling biomass and root-shoot ratio at early stage. Data of various parameters was analyzed through Statistix 8.1 software. The data of phenotypic characters was calculated using Fisher's analysis of variance at 5% probability for comparing treatment means (Steel et al. 1997). The correlation and pathway analysis were performed to check how various traits influence one another (Kashiani and Saleh, 2010). Phenotypic variances (σ^2_p) and genotypic variance (σ^2_g) were taken from a combined ANOVA table (Comstock and Robinson, 1952). The genetic analyses of phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV) and genotypic coefficient of variation (GCV) were determined using mean values (Pant and Singh, 2001). Broad sense heritability (H^2) was evaluated, as described by (Hill and Mackay, 2004). The calculation of genetic advance was done by the method described by (Parida et al., 2018)

Result and Discussion

Table 1. Combined ANOVA for seven phenotypic traits of five chickpea varieties

TRAITS	MS	GM	CV%	GV	GCV%	PV	PCV%	EV	ECV%	h ² bs%	GA
Seedling length	2.44011*	16.649	0.66	0.8094	5.4036	0.8214	5.4436	0.0120	0.6588	98.54	9.521
Root length	2.2754*	8.6467	8.73	0.5683	8.7187	1.1387	12.3413	0.5704	8.7345	49.91	10.933
Root Shoot ratio	0.00533*	0.4153	2.37	0.0017	10.0538	0.0018	10.3381	0.0001	2.4079	94.58	17.355
Seedling biomass	0.03471*	0.9773	1.06	0.0115	10.9888	0.0116	11.0411	0.0001	1.0732	99.06	19.413
Leaf length	0.005*	0.5767	1.23	0.0017	7.0436	0.0017	7.1495	0.0001	1.2261	97.06	12.317
Leaf width	0.01032*	0.3727	2.59	0.0034	15.6682	0.0035	15.8736	0.0001	2.5454	97.43	27.451
Leaf area	0.15022*	1.0507	18.63	0.0373	18.3821	0.0756	26.1710	0.0383	18.6285	49.33	22.918

Combined ANOVA for different parameters was performed (Table 1). The parameters, including root length, seedling length, root-shoot ratio, leaf area, leaf width and seedling biomass have shown significant results towards genotypes, meaning genotypes have greater genetic variation for these parameters. The parameter, shoot length, has shown the highest genotypic variance (0.8094) and the highest phenotypic variance was shown by root length (1.1387). The leaf length (0.0017) showed the lowest genotypic and phenotypic variance. The values of GCV and PCV ranged from 18.3821 (leaf Area) to 5.4036 (shoot length) and 26.1710 (leaf area) to 5.4436 (shoot length). PCV and GCV are divided into three categories: >20% as high, 10-20% as moderate, and <10% as low (Balqees et al., 2020; Deshmukh et al., 1986; Ghafoor et al., 2020; Iqbal et al., 2021; Iqra et al., 2020a; Iqra et al., 2020b). GCV value indicates the true genetic potential of genotypes. The current experiment showed higher values of PCV than that of GCV, proving the presence of environmental variation. Broad sense heritability and the genetic advance were calculated for all seven parameters. Estimation of heritability is categorized into three levels: high (30-60%), moderate (10-30%) and low 5-10%) (Dabholkar, 1992; Waseem et al., 2014). All the traits have shown high heritability. Heritability estimation for shoot length, root-shoot ratio, seedling biomass, leaf length and leaf width was higher than 90%. High heritability and genetic advance have already been reported in numerous studies (Mazhar et al., 2020; Naseem et al., 2020; Naveed et al., 2012; Waldia, 1991). Genetic advance has been classified into 0-10% as low, 10-20% as moderate, and >20% as high. Results indicated that shoot length had a low genetic advance percentage, root length and leaf length had a moderate genetic advance and leaf width and leaf area had a high genetic advance. The same results have already been reported in many findings (Kumar et al., 2011). The high value of heritability and genetic advance indicates that genetic gain would be high for selection in these traits if used for further breeding, and selection in these traits would be better because of additive gene action (Johnson et al., 1955).

*= Significant at 5% probability level, MS = mean square, GM = 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^2_{bs} = Broad sense heritability, GA = genetic advance

Plant breeders implement the important concept of correlation to determine if two morphological or physiological features influence one another positively or negatively. Correlation analysis can be utilized to check the significance level of two traits. Genotypic and phenotypic correlation for each trait was performed (Table 2). Leaf area and seedling biomass have strong and highly significant correlation ($p < 0.01$) with leaf width and seedling length, respectively. Leaf width and root length have a strong

and significant correlation towards seedling length, indicating an increase in seedling length will increase leaf width and root length, respectively. A strong and negative correlation was found in Root length and seedling length towards root-shoot ratio, indicating that an increase in root shoot ratio will decrease parameters like root length and seedling length. Seedling length has been positively and significantly correlated with leaf width and root length (Ali et al., 2010b).

Table 2: Pearson correlation to check positive or negative effects on parameters

Traits	LA	LL	LW	RL	SB	SL
LL	0.6228*					
LW	0.6569**	0.1740				
RL	-0.0329	-0.4929	0.2656			
SB	0.1625	-0.0359	0.3968	0.3057		
SL	0.2487	0.0333	0.5216*	0.5441*	0.7214**	
RSR	-0.3240	-0.1281	-0.3310	-0.6530**	-0.2846	-0.6591**

Where * is p value less than 0.05, ** is p value less than 0.01, LL is leaf length, LW is leaf width, RL is root length, SB is seedling biomass, SL is seedling length, RSR ratio is root shoot ratio.

Pathway analysis for all seven traits was performed (table 3). Results showed high positive direct effects of leaf length and leaf width for seedling length. Root shoot ratio and leaf area have a highly negative direct

effect on seedling length. Leaf area positively affects seedling length through leaf width and leaf length. Root length has a strong negative indirect effect on seedling length through leaf length.

Table 3. Path coefficient analysis for various traits on seedling length in chickpea

Traits	root length	S-R ratio	seedling biomass	leaf length	leaf width	leaf area
root length	0.095	5.367	1.268	-5.206	1.220	0.023
R-S ratio	-0.062	-8.219	-1.181	-1.353	-1.521	0.230
seedling biomass	0.029	-0.027	4.149	-0.379	1.823	-0.115
leaf length	-0.047	1.053	-0.149	10.562	0.799	-0.442
leaf width	0.025	2.721	1.646	1.838	4.594	-0.466
leaf area	-0.003	2.663	0.674	6.578	3.018	-0.709

Conclusion

Seedling length, root length and root-shoot ratio have low GCV and PCV values, indicating a high environmental effect. Seedling biomass, root length, and leaf width strongly correlate with seedling length. RL, SB and LW have direct effects on SL. RL, SL and R-S ratio had shown high heritability with low genetic advance, which indicates the presence of non-additive gene action and selection for these traits may not be rewarding, leaf width and leaf area had shown high heritability and high genetic advance, which shows the presence of additive gene action. Selection for such traits leads towards superior chickpea genotype through phenotypic 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

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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.



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