PHENOTYPIC CHARACTERIZATION OF CHICKPEA GERMPLASMS FOR HERITABILITY AND ASSOCIATION AMONG TRAITS

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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 used 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.*

Introduction

Chickpeas, scientifically known as *Cicer arietinum*, hold significant importance in global agriculture, nutrition, and culinary traditions. Chickpea is a self-pollinated annual legume crop from the Fabaceae family (Varshney et al., 2013). Chickpea is most extensively cultivated as it ranks 3rd in pulses worldwide (Tesfamichael et al., 2015). Chickpea accounts for 12% of total pulses productivity all over the globe. Pakistan and India are global chickpea producers (Mohammed et al, 2019). Other major chickpea producers are Iran, Australia, Ethiopia, and Turkey (Ojiewo, 2016).

Chickpea is a major and cheap source of protein for people who can't afford animal protein (Hama, 2019). Chickpea contains 63% carbohydrates and 22% protein, considered the most essential nutritional source in various countries (Shafique et al., 2016). Beyond their nutritional value, chickpeas play an essential role in sustainable agriculture because of

their ability to fix nitrogen in the soil, enhance soil fertility, and promote healthier crop rotations (Gul et al., 2011). Furthermore, chickpea contain fibers and minerals, including zinc, potassium, magnesium, and phosphorus, which are also most important for health (Kousar et al., 2019). Dried stalks of chickpea, rich in protein, are utilized as animal feed (Deb and Khaleque, 2009).

Chickpeas are a staple food in many cultures. They are rich in protein, fiber, vitamins, and minerals, contributing to a balanced diet and promoting health (Kaur and Parasad, 2021). The legume's versatility makes it a key ingredient in various dishes, from the popular Middle Eastern hummus to Indian chana masala (Ali et al, 2023). Additionally, chickpeas have garnered attention for their potential in addressing global food security challenges and combating malnutrition (Bessada et al, 2019). As a drought-resistant crop, chickpeas thrive in diverse climates,

making them essential to resilient agricultural systems. These qualities underscore chickpeas' importance in promoting human nutrition and environmental sustainability (Asati et al., 2022). In Pakistan, the era of Thal is the major chickpea producer, including tehsil Choubara, Mankera, Bhakkar, and Mianwali. As the world's population increases rapidly, the demand for pulses also increases. To overcome this increasing demand of pulses, it is essential to introduce new cultivars with enhanced productivity to overcome protein deficiency (Ali et al., 2018). The breeders are in a struggle to enhance heredity potential. Variations noted due to the different genetic makeup of plants and their interaction with the environment can be better explained through heritability (Saleem et al., 2002). Genetic gain also predicts acceptable improvement for new induction by selections. Therefore, the current experiment was designed to evaluate the genetic variability and heritability in various chickpea germplasm for different phenotypic characters.

Materials and methods

A pot experiment was executed at the Department of Plant Breeding and Genetics, University of the Punjab greenhouse, from 2022 to 23. The study was laid out in a complete randomized block design that was further replicated three times. Based on availability, five chickpea varieties, including C-612, C-727, C-44, Punjab-91, and Punjab-2000, were collected for our experiment from Ayub Agricultural Research Institute, Faisalabad. Irrigation and fertilization were applied as per crop demand. Data on various morphological parameters was collected viz. seedling length (cm), root length (cm), seedling and root ratio, leaf length (cm), leaf width, and leaf area (cm^2) at the early stage. Data of various parameters was analyzed through Statistix 8.1 software. Morphological traits data was evaluated using Fisher's analysis of variance and Tukey test check at 1% probability for comparing treatment means (Steel et al. 1997). The correlation and pathway analyses were evaluated to check how various parameters influence each other (Kashiani and Saleh, 2010). Phenotypic variances ($\sigma^2 p$) and genotypic variance ($\sigma^2 g$) were taken from a combined ANOVA table (Comstock and Robinson, 1952). The genetic analyses of the phenotypic coefficient of variation (PCV), Environmental coefficient of variation (ECV), and genotypic coefficient of variance (GCV) were determined using mean values, as described by Singh and Choudhary (1985) as:

$$ \text{GCV} \quad = \quad \frac{\sqrt{\delta 2g}}{\bar{x}} x 100 $$

$$ \text{PCV} \quad = \quad \frac{\sqrt{\delta 2p}}{\bar{x}} x 100 $$

$$ \text{ECV} \quad = \quad \frac{\sqrt{\delta 2e}}{\bar{x}} x 100 $$

$\bar{x}$ mentions the grand mean of a character. Estimation of broad scene heritability (H^2) was calculated, as described by Falconer and Mackay (1996), as follows:

$$ H^2 = \frac{\delta^2 g}{\delta^2 p} \text{ x } 100 $$

The genetic advance was calculated according to the method described by Allard (1960), Singh and Choudhary (1985):

$$ \text{GA} \quad = \quad K \cdot \sqrt{\delta^2 p} \text{ x } H^2_{bs} $$

Where value of *K* is 2.64 at 1% selection intensity.

Results and Discussions

Analysis of variance depicted that the parameters, including root length, root-shoot ratio, seedling length, leaf length, leaf width, and leaf area, have shown significant results towards genotypes, which means that genotypes have greater genetic variation for these parameters. As genetic variability is secured, the breeders have to isolate the heritable portion for non-heritable to improve specific traits containing high heritability (Workie, 2018; Ali et al., 2014b; Naeem et al., 2022). A combined analysis of variance of seedling length, root length, root-shoot ratio, seedling biomass, leaf length, leaf width, and leaf area were performed (Table 1). The parameter root-shoot ratio has shown the highest genotypic variance and phenotypic variance, 0.8343 and 2.7210, respectively, while leaf length and leaf width have the lowest genotypic variance. The values of the genotypic coefficient of variance and phenotypic coefficient of variance ranged from 60.4913 (root-shoot ratio) to 0.6373 (leaf width) and 109.24 (root shoot ratio) to 0.6854 (leaf width). PCV and GCV are classified into three categories:>20 as high, 10-20% as moderate, and 0-10% as low (Deshmukh et al. 1986). The high genotypic coefficient of variance and phenotypic coefficient of variation were observed in the root length, seedling length, and root-shoot ratio, while the low PCV and GCV were observed in leaf width, leaf area, seedling biomass, and leaf length. The same results have already been reported in numerous findings (Kumar et al, 2011). GCV value indicates the true genetic potential of genotypes. The current experiment showed higher values of PCV than that of GCV, proving the existence of environmental variation.

Broad sense heritability and genetic advance were estimated for all seven parameters. Estimation of heritability is categorized into three levels: high (30-

60%), medium (10-30%), and low (5-10%) (Dabholkar, 1992; Ali et al., 2011; Ali et al., 2014a). All the traits have shown a high heritability. Waldia et al. (1991) have already reported high heritability and genetic advance for the parameters including seedling biomass, root length, and seedling length. Heritability estimation for seedling length, root length, seedling biomass, leaf length, leaf width, and leaf area was higher than 85%. Genetic advance has been classified into 0-10% as low, 10-20% as moderate, and >20 as high. Results indicated that leaf length and leaf area

had low genetic advance percentage, seedling biomass had moderate GA percentage, while RS ratio, seedling length, and root length had high GA value. The high value of genetic advance and heritability indicates that genetic gain would be high for selection in these traits if utilized for further breeding. The traits with high genetic advance and heritability could result in better selection variation in these traits because of additive gene action (Johnson et al., 1955).

Table 1. Combined analysis of variance for seven phenotypic traits of five chickpea genotypes

TRAITS	MS	GM	CV	GV	GCV%	PV	PCV%	EV	ECV%	H2bs%	GA%
Seedling length	0.4009	1.0400	16.729	0.1236	33.7982	0.1437	36.4540	0.0303	16.7291	85.9601	55.621
Root length	0.3894	1.0200	9.4240	0.1267	34.9016	0.1329	35.7397	0.0092	9.4240	95.3647	60.498
Root shoot ratio	5.3330	1.5100	111.408	0.8343	60.4913	2.7210	109.2414	2.8300	111.4080	30.6627	59.456
Seedling biomass	0.0369	2.3400	0.9838	0.0121	4.7034	0.0125	4.7715	0.0005	0.9838	97.1658	8.229
Leaf length	0.0039	3.5600	0.4700	0.0012	0.9771	0.0014	1.0498	0.0003	0.4700	86.6348	1.614
Leaf width	0.0029	4.6900	0.3090	0.0009	0.6373	0.0010	0.6854	0.0002	0.3090	86.4516	1.052
Leaf area	0.0388	3.9900	0.7477	0.0126	2.8163	0.0132	2.8817	0.0009	0.7477	95.5119	4.885

Where MS = mean square, GM = grand mean, CV = covariance, GV = genotypic variance, PV = phenotypic variance, EV = environmental variance, GCV = genotypic coefficient of variance, PCV is the phenotypic coefficient of variance, ECV = environmental coefficient of variance, H²bs = heritability, GA = genetic advance

Correlation is an important term plant breeders use to check whether two morphological or physiological parameters have positive or negative effects on each other. 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 length had a strong and highly significant correlation with leaf width (p<0.01). The parameters like root length, root shoot ratio, and leaf length have shown a strong correlation toward seedling length, indicating that an increase in seedling length will increase root length, root-shoot ratio, and

leaf length. A negative and significant correlation was found in seedling biomass and leaf width toward root shoot ratio, indicating that an increase in root-shoot ratio will decrease parameters like seedling biomass and leaf width. Toker and Cagirgan (2004) have found that grain yield had a positive and significant correlation with biological yield. Ali et al., (2010ab) reported that seedling length positively and significantly correlated with seedling biomass, leaf width, leaf area, and root shoot ratio at the genotypic level.

Table 2: Pearson correlation to check positive or negative effects parameters of each other

Traits	SL	RL	SR ratio	SB	LL	LW
SL	0.0697					
RL	0.2127	-0.8164**				
RS ratio	0.4301	0.4435	-0.1453			
SB	0.0025	0.7779**	-0.6001*	0.0335		
LL	0.3865	0.4212	-0.2879	0.1122	0.5798*	
LW	0.1195	0.6610**	-0.5445*	0.0897	0.7980**	0.8945**

*Where * p value < 0.05, ** p value < 0.01, SL = seedling length, RL = root length, RS ratio = root shoot ratio, SB = seedling biomass, LL = leaf length, LW = leaf width*

Path analysis shows how different traits/parameters are correlated with the yield. Correlation and regression analysis were performed in path analysis to check the direct and indirect effects of different traits (Ali et al., 2013; Ali et al., 2016; Ali et al., 2010; Naveed et al., 2012; Waseem et al., 2014). Pathway

analysis for all seven parameters was performed (Figure 1). Results showed a high direct effect of RS ratio and leaf width for seedling length. Traits like root length and leaf area affect seedling length through leaf width. A negative indirect effect was found in root length, seedling biomass, and leaf length

with leaf length. The other positive indirect effects are seedling biomass through root length, leaf length through leaf width, and leaf area through R-S ratio.
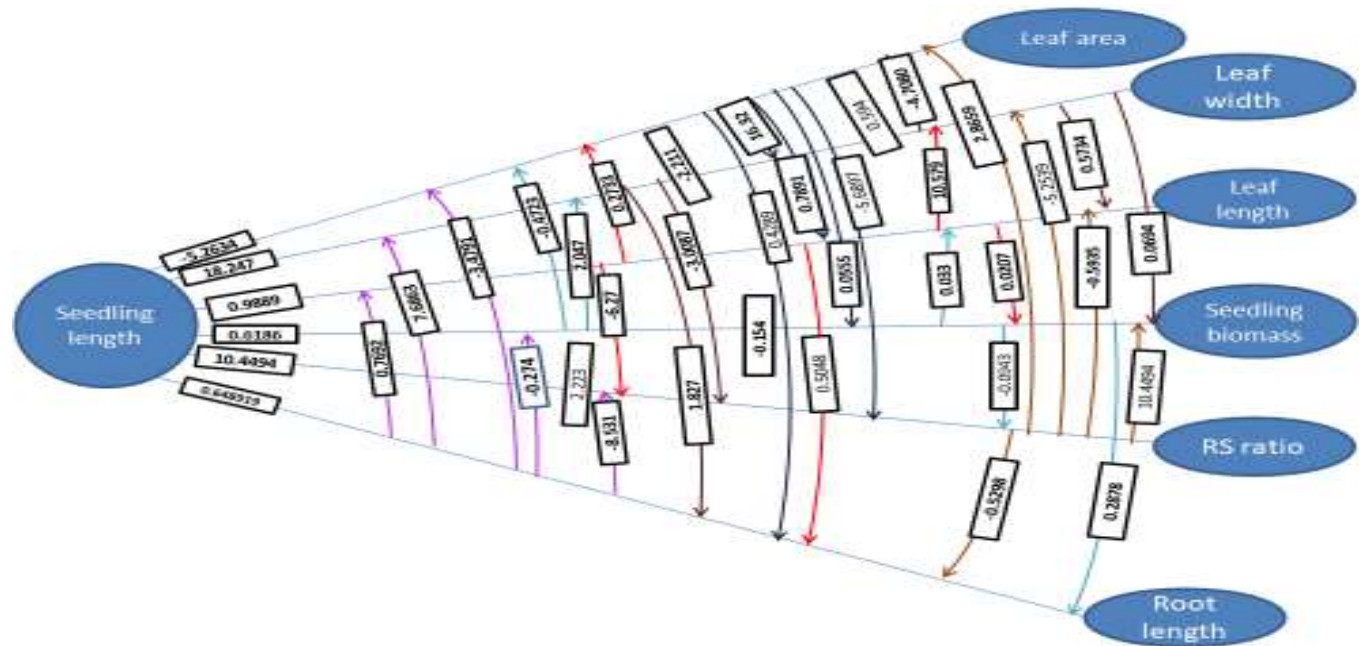

Figure 1: Path coefficient analysis showing direct and indirect effects of various traits on seedling length in chickpea

Conclusions

Current findings show high variation and genetic variability in all parameters that can lead to the direct selection of yield-attributing characters. Seedling length, root length, and soot root ratio have high GCV and PCV values. Root length, root-shoot ratio, and leaf length had a strong correlation with seedling length. R-S ratio and leaf width have a high direct effect on seedling length. Seedling length, root length, and root-shoot ratio had shown high heritability and genetic advance. The traits with high heritability, and genetic advance may lead to superior chickpea variety via phenotypic selection.

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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.