

GENETIC VARIATION AND ASSOCIATION BETWEEN SEEDLING ROOT TRAITS AND YIELD IN CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

In the present study, 90 chickpea germplasm lines for yield and related traits, along with early plant growth root system architecture traits, under controlled conditions after 25, 30, and 35 days of sowing were evaluated. The analysis of variance revealed significant variation in traits such as shoot length, secondary root branches, days to 50 % flowering, days to maturity, seed index, plant height, and seed yield. Additionally, primary root length was positively correlated with shoot length and the number of root branches at all three seedling growth stages. The findings suggest that early shoot length and seedling root length, particularly more secondary root branches, are crucial traits that should be incorporated into chickpea breeding programs to develop new elite cultivars with improved yield potential, better resistance to biotic and abiotic stresses, and enhanced crop productivity.

Introduction

Chickpea (*Cicer arietinum* L.) is a major food legume crop grown in tropical to semi-arid regions of the world on approximately 12 million hectares of land (Siddique *et al.* 2012). However, chickpea production is challenged by various biotic and abiotic stresses, particularly terminal drought stress, which severely impairs plant growth and development and reduces productivity. The reproductive stages of the plant are particularly vulnerable to drought stress, leading to low productivity (Barnabas *et al.* 2008). To address these challenges, more water-use-efficient farming systems and drought-resistant chickpea cultivars are needed. Combining different drought resistance mechanisms, such as root traits, together with water-use efficiency, is a potential strategy for enhancing levels of drought resistance. Roots play a crucial role in numerous plant functions, including the uptake and mobilization of water and essential nutrients from the soil, mechanical support to prevent lodging, and nutrient storage. Plant species with deeper and more extensive root systems have adapted to grow in water and nutrient-limited soils, enabling them to absorb moisture from deeper soil layers during periods of stress and promote proper growth (EI-Hassouni *et al.* 2018). Root traits are the most important factors to protect plants under stress conditions and reduce yield losses. The identification of lines with better root architecture traits that can uptake soil moisture from the deeper layer would be beneficial for variety development for dry regions (Akman *et al.* 2017). Early seedling root traits serve as crucial indicators of plant drought tolerance and play a crucial role in enhancing chickpeas' drought

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resistance and yield potential (Gaur *et al.* 2016). In water-limited situations during the reproductive stage, a well-developed root system that penetrates deep soil can acquire residual soil moisture, positively affecting grain yield. Conversely, a shorter root system is correlated with reduced plant productivity and lower yields (Xie *et al.* 2017). Developing chickpea cultivars with improved root traits that can effectively access soil moisture from deeper layers could significantly enhance drought resistance and productivity in chickpea farming systems. The present study was aimed to assess early seedling root traits in chickpea germplasm and their correlation with seed yield and contributing traits under field conditions.

Materials and Methods

The study was carried out in Rabi 2018-19 at the experimental research farm of Banda University of Agriculture and Technology in Banda, Uttar Pradesh, India. The location has an altitude of 123 m above sea level, an annual rainfall of 850 mm, and is located at 25.4763° N and 80.3395° E. The region experiences dry and hot waves, particularly during the chickpea crop's flowering to maturity stages. The weather parameters, including mean maximum and minimum temperature (Max. and Min.), relative humidity (RH %), and rainfall distribution (RF), were recorded at the university's agro-metrological centre throughout the experiment (Fig. 1).

A panel of 90 chickpea diverse germplasm accessions included commercial cultivars, genetic stocks, advanced breeding lines and land races collected from local area; Punjab Agricultural University, Ludhiana; ICRISAT, Hyderabad and Indian Institute of Pulses Research, Kanpur. These accessions were utilised along with benchmark varieties to study seedling and agronomic traits.

During Rabi 2018-19, the germplasm set was screened for seedling root and shoot traits in germination trays. The trays had a size of 2.5" upper diameter, 1.8" lower diameter, and 3.8" depth, and were filled with a 70 % sand and 30 % vermi-compost mixture to support plant growth for up to 45 days. Each line was represented by ten visually healthy seeds that underwent surface sterilization using a 1.25 % sodium hypochlorite solution for 10-15 min. and rinsed with fresh tap water four times. Two seeds of each line were placed in five replicates with four checks. After 10 days, one seedling was uprooted, while the other was allowed to develop. At 25, 30, and 35 days after sowing, the seedling's root and shoot traits were measured by uprooting and washing them with tap water. The measurements, including root length, shoot length, secondary root branches, and root-to-shoot ratio, were taken on a flat surface using a measuring scale.

An experiment was conducted to evaluate accessions for yield and yield component traits under field conditions, using the same set of germplasm lines, in an incomplete augmented block design (Federer 1956). The accessions, along with four check varieties JG 14, JG 16, JAKI 9218, and Radhey, were planted in nine blocks, with each plot consisting of two rows of 2 m length and 30 cm row to row distance; the plant population was maintained through gap filling after 10 days of sowing, and recommended agronomical practices were adopted to ensure good plant population in each plot. Data on five key agro-morphological traits *viz.* days to 50 % flowering (DTF), days to maturity (DTM), plant height (PH), seed index (SI), and seed yield (SY) were recorded, with DTF and DTM being recorded on a whole plot basis and calculated when 50 % of plants in a plot appeared with flowers and physiologically matured, respectively. Plant height was measured on five randomly selected plants from the middle of each plot, and shoot biomass and seed yield were recorded at the maturity stage after drying of biomass and seed yield of every plot. Harvest index was calculated by dividing biomass with seed yield, and seed index was calculated by weighing 100 counted seeds from each genotype with an automatic seed counter machine (VT54, Indosaw) from each plot and weighed by electronic weighing balance.

The recorded observations were statistically analyzed following the guidelines of Johnson *et al.* (1955) and utilizing repeated checks to estimate the adjusted mean of each entry. The analysis of variance (ANOVA) was performed using the Statistical Package for Augmented Design (Rathore *et al.* 2004). Correlation was conducted to investigate the impact of seedling traits on productivity and identify the best-performing genotypes, using RStudio version 1.2.5033 and PAST 3 statistical software.

Results and Discussion

Analysis of variance showed significant differences among the tested genotypes for secondary root branches and field traits (Table 1). The accessions exhibited significant variation ($p < 0.001$) for secondary root branches (at 25, 30, and 35 DAS), days to 50 % flowering, days to maturity, seed index, and harvest index. The contrast analysis revealed significant differences in these traits among the test genotypes. These results were consistent with those reported by Ganjeali and Kafi (2007) for root length, total root length, and number of lateral branches among twenty chickpea genotypes. However, Yaqoob *et al.* (2012) found non-significant differences among two chickpea genotypes for seedling traits. The contrast analysis between the test and control also indicated a significant difference ($p < 0.001$) for seed index and seed yield. No significant variation was found for root length and root-to-shoot ratio at three sampling periods (25, 30 and 35 DAS) among genotypes, but significant differences were observed among blocks and test *vs.* block at 25 and 30 DAS. Similar findings were reported by Muriuki *et al.* (2020) in chickpea using ten genotypes.

Table 1. Analysis of variance for seventeen quantitative traits of test genotypes of chickpea.

Traits	Time ^a (DAS)	Mean Sum of square					
		Block (Adj.)	Treatments (Adj.)	Error	Among control	Among test genotypes	Test-vs control
Df		8	89	28	3	85	1
Seedling traits							
Primary root length	25	21.02	13.21	10.38	95.03***	8.99	122.99**
Primary root length	30	22.67	16.09	13.6	46.48*	13.67	112.58**
Primary root length	35	11.56	14.64	13.97	39.2	13.93	4.59
Shoot length	25	3.48	1.78	2.14	1.45	1.8	2.48
Shoot length	30	6.98**	2.57	1.63	1.19	2.63	0.78
Shoot length	35	16.70***	6.11*	3.39	6.44	6.11*	5.08
No. of root branches	25	13.00*	19.74***	4.35	14.35*	20.06***	7.6
No. of root branches	30	11.86	25.98***	5.67	72.26***	24.61***	5.1
No. of root branches	35	19.56*	28.96***	7.23	9.22	29.55***	34.76*
Root-to-shoot ration	25	0.18	0.12	0.21	1.15**	0.07	1.20*
Root-to-shoot ration	30	0.16	0.12	0.11	0.33*	0.1	1.11**
Root-to-shoot ration	35	0.07	0.09	0.07	0.17	0.08	0.1
Field traits							
Days to 50% flowering	FT	49.12**	101.00***	11.76	437.62***	89.82***	86.42*
Plant height	FT	95.27*	79.05**	30.82	314.48***	70.39*	143.08*
Days to maturity	FT	62.87***	24.71***	6.52	14.9167	25.32***	0.03
Seed index	FT	1.4	42.44***	1.02	14.22***	39.13***	422.40***
Seed yield	FT	5959.93	8936.34**	3237.75	17961.22**	8338.19**	35097.22**

^athe stage at seedling; FT = field traits; *significance at P = 0.05, **significance at P = 0.01, ***significance at P = 0.001

Descriptive statistics of measured parameters on seedling and field traits, including primary root length at 25 days, showed significant differences among genotypes with a mean value of 9.92 ± 3.39 , ranging from 2.56 (BG 5051) to 17.66 cm (GNG 1958), except for the check JAKI 9218 which measured 13.59 cm (Table 2). Akhtar *et al.* (2010) also reported the root length of more than 27 cm in two genotypes among studied hundred germplasm lines. At 30 days, primary root length varied from 5.46 (GNG 1581) to 22.66 cm (ICVT 181103) but root length at 35 days ranged from 6.50 (GNG 2171) to 27.21 cm (GNG 2391) with an average of 13.61 ± 4.41 and 15.98 ± 4.07 , respectively at 30 and 35 days after sowing. Among check varieties JAKI 9218 showed highest root length at 30 days (21.71 cm) and 35 days (22.09 cm). Ganjeali and Kafi (2007) also reported root length differences which varied from 6.8 to 15.4 cm in chickpea. At 30

Table 2. Descriptive statistics of seedling root-shoot and field response traits of 90 germplasm accessions of chickpea.

Trait	Check means				90 germplasm accessions	
	JG 14	JG 16	JAKI 9218	Radhey	Min-Max	Mean \pm StdDev
Primary root length (25DAS)	12.19	12.39	13.59	10.49	2.56-17.66	9.92 \pm 3.39
Primary root length (30DAS)	12.41	13.81	21.71	15.41	5.46-22.66	13.61 \pm 4.41
Primary root length (35DAS)	13.59	14.09	22.09	17.19	6.50-27.21	15.98 \pm 4.07
Shoot length (25DAS)	10.49	12.39	11.29	10.99	6.61-14.39	9.61 \pm 1.50
Shoot length (30DAS)	11.98	14.98	13.98	12.38	7.05-17.72	11.46 \pm 2.12
Shoot length (35DAS)	16.18	19.28	17.18	16.28	6.40-23.65	14.52 \pm 3.41
Root branches (25DAS)	9.58	10.58	12.58	7.58	6.47-31.97	14.07 \pm 4.91
Root branches (30DAS)	11.94	29.94	23.94	14.94	9.00-37.50	20.32 \pm 5.51
Root branches (35DAS)	14.64	32.64	24.64	28.64	11.50-41.00	24.78 \pm 6.03
Root-to-shoot ratio (25DAS)	1.16	1.00	1.20	0.95	0.30-1.82	1.04 \pm 0.31
Root-to-shoot ratio (30DAS)	1.04	0.92	1.55	1.24	0.48-2.44	1.20 \pm 0.37
Root-to-shoot ratio (35DAS)	0.84	0.73	1.29	1.06	0.52-1.91	1.12 \pm 0.32
Days to 50% flowering	75.82	78.82	71.82	81.82	52.74-97.99	80.84 \pm 9.78
Days to maturity	132.88	129.88	116.88	131.88	109.63-134.38	123.76 \pm 5.09
Plant height	36.59	45.79	60.39	76.99	25.39-76.99	49.22 \pm 8.16
Seed index	16.62	13.65	15.67	20.52	11.11-40.17	19.42 \pm 6.42
Seed yield	1225.00	700.00	991.67	1458.33	408.33-4083.30	2245.81 \pm 7.79

and 35 days, the maximum shoot length was recorded in genotypes ICVT 181111 (17.72 cm) and ICVT 181110 (23.65 cm). The maximum number of root branches (32, 38 and 41) was observed in JG 218 (25, 30 and 35 DAS). Among check varieties, JAKI 9218 recorded the maximum number of root branches at 25 days and JG 16 at 30 and 35 days. Root-to-shoot ratio varied from 0.3 (BG 5051) to 1.82 (EC 556270) with a mean value of 1.04 ± 0.31 at 25 days whereas, root-to-shoot ratio at 30 days varied from 0.48 (GNG 5081) to 2.44 (JG 11) with an average of 1.2 ± 0.37 . Root-to-shoot ratio varied from 0.212 to 0.770 in 10 genotypes of chickpea reported by Muriuki *et al.* (2020). In attaining early flowering, the variation among the genotypes varied from 52 days (ICVT 181112 and ICVT 181113) to 98 days (PG 184) with a mean value of 80.84 ± 9.78 whereas

the genotype ICVT 181114 matured in 109 days and GNG 2081 matured in 134 days with mean value 123.76 ± 5.09 . ICVT 181110 had the maximum plant height (76.99 cm) while ICVT 181108 had the minimum (25.39 cm). The highest seed index was recorded in genotype ICVT 181312 (40.17 g) and lowest in genotype EC 556270 (11.11 g) with a mean value of 19.42 ± 6.42 . Seed yield varied from 408.33 (GNG 312) to 4083.30 kg/ha (ICVT 181103).

Table 3. Pearson Correlation coefficient among seedling traits at each 25, 30 and 35 days after sowing.

25 DAS	Primary root length	Shoot length	Root branches	Root-to-shoot ratio
Primary root length.	1	0.478***	0.314**	0.883***
Shoot length		1	0.210*	0.027
Root branches			1	0.257*
Root-to-shootratio				1
30DAS				
Primary root length.	1	0.408***	0.292**	0.769***
Shoot length		1	0.341***	-0.234*
Root branches			1	0.035
Root-to-shootratio				1
35 DAS				
Primary root length.	1	0.369***	0.206*	0.653***
Shoot length		1	0.404***	-0.430***
Root branches			1	-0.106
Root-to-shootratio				1

*significant at $p = 0.05$, **significant at $p = 0.01$, ***significant at $p = 0.001$; DAS, days after sowing

The correlation coefficient between seedling root traits showed that at 25 DAS, a significant positive correlation was found between primary root length and root-to-shoot ratio (0.883***), primary root length and shoot length (0.478***), and primary root length and number of root branches (0.314**) (Table 3). In addition, the number of root branches was positively correlated with shoot length (0.210*) and root-to-shoot ratio (0.257*). Similarly, at 30 DAS, primary root length showed a positive significant correlation with root-to-shoot ratio (0.769***), shoot length (0.408***), and number of root branches (0.292**). The study by Lalitha *et al.* (2015) also reported a positive correlation among root traits in chickpea. Furthermore, root length was positively correlated with the total number of branches and total branch length (Chen *et al.* 2017). Akhtar *et al.* (2010) found that root length had a negative non-significant correlation with shoot emergence and seedling vigor, while shoot length was significantly correlated with root length.

Seed yield is a complex trait that is influenced by several secondary traits that can positively or negatively impact on seed yield. The observed significant differences in seedling and field traits among the genotypes were used to calculate the correlation between seedling root traits during the three observation periods (Table 4). In wheat, root length was observed to have a significant contribution to the increase in seed yield (Atta *et al.* 2013). The seed index was positively correlated with plant height (0.392***) and shoot length at 30 DAS (0.211*). On the other hand, days to maturity showed a negative correlation with shoot length at 30 DAS (-0.212*) and a

positive correlation with days to 50% flowering (0.776***). Ali *et al.* (2010) reported a positive correlation between root length and seedling biomass, root-to-shoot ratio, leaf area, leaf width, and leaf length.

Table 4. Pearson correlation coefficient among seedling and field traits.

Characters	SL.30. DAS	SRB.25. DAS	SRB.30. DAS	SRB.35. DAS	DTF	DTM	PH	SI	SY
SL.30.DAS	1	0.156	0.341***	0.410***	-0.310*	-0.212*	0.184	0.211*	0.245*
SRB.25.DAS		1	0.518***	0.495***	-0.019	0.050	0.008	0.095	0.214*
SRB.30.DAS			1	0.811***	-0.238*	-0.140	0.017	0.051	0.068
SRB.35.DAS				1	-0.217*	-0.131	0.125	0.183	0.132
DTF					1	0.776***	0.014	-0.051	-0.065
DTM						1	0.069	-0.097	-0.132
PH							1	0.392***	0.214*
SI								1	0.322**
SY									1

*significant at $p = 0.05$, **significant at $p = 0.01$, ***significant at $p = 0.001$, SL.30.DAS = shoot length at 30 days after sowing, RB.25.DAS = root branches at 25 days after sowing, RB.30.DAS = root branches at 30 days after sowing, RB.35.DAS = root branches at 35 days after sowing, DTF = days to 50% flowering, DTM = days to maturity, PH = plant height, SI = seed index, SY = seed yield

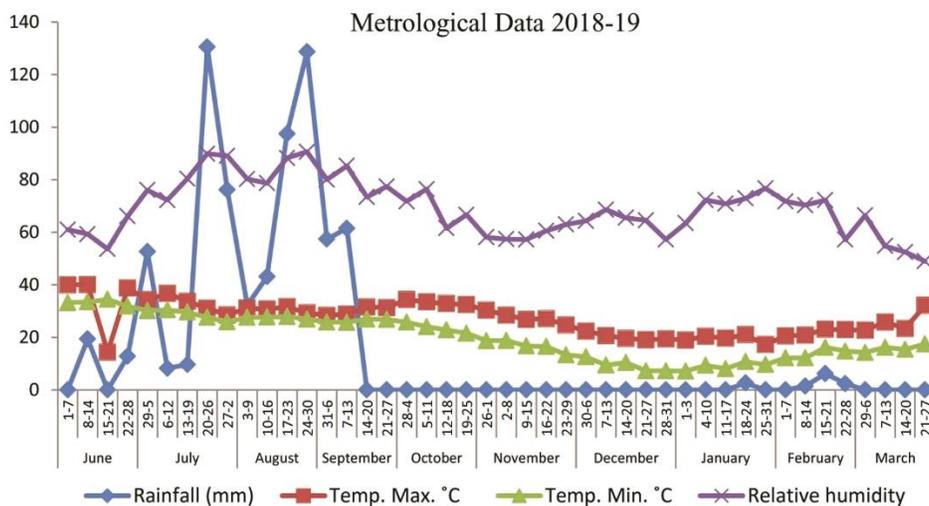


Fig. 1. Weekly temperature (Max. and Min.), air humidity and rainfall during study period *rabi* 2018-19.

In terms of seed yield, there was a positive and significant correlation with shoot length and number of root branches, while root length was significantly correlated with the number of root branches among root traits. Although there was a significant correlation among seedling root traits, the correlation of root length and root-to-shoot ratio with seed yield was found to be non-significant. Among the genotypes, ICVT 181103, RLBG-2, KGD 11-1 and ICVT 181102 were identified for their better secondary roots and higher yielding ability. Selecting genotypes based on root traits that showed positive correlation may directly improve their yield performance.

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