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Marker-trait association study for root-related traits in chickpea (*Cicer arietinum* L.)

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Abstract: Root structure modification can improve important agronomic traits including yield, drought tolerance and nutrient deficiency resistance. The aim of the present study was to investigate the diversity of root traits and to find simple sequence repeat (SSR) markers linked to root traits in chickpea (*Cicer arietinum* L.). This research was performed using 39 diverse accessions of chickpea. The results showed that there is significant variation in root traits among chickpea genotypes. A total of 26 alleles were detected 26 polymorphic bands were produced by 10 SSR markers in the eight linkage groups (LG). The results indicated that there is substantial variability present in chickpea germplasm for root traits. By analyzing the population structure, four subpopulations were identified. PsAS2, AF016458, 16549 and 19075 SSR markers on LG1, LG3, LG2 and LG1 linkage group respectively were associated with root traits**.** The research findings provide valuable information for improving root traits for chickpea breeders.

Key words: linkage groups; drought tolerance; population structure; SSR markers; subpopulations; variation

Raziskava povezave genskih označevalcev in lastnosti korenin pri čičerki (*Cicer arietinum* L.)

Izvleček: Sprememba zgradbe korenin lahko izboljša pomembne agronomske lastnosti vključno s pridelkom, strpnost za sušo in odpornost na pomanjkanje hranil. Namen raziskave je bil preučiti raznolikost lastnosti korenin in najti enostavne označevalce ponavljajočih se zaporedij (SSR) povezanih z lastnostmi korenin pri čičerki (*Cicer arietinum* L.). Raziskava je bila opravljena na 39 različnih akcesijah čičerke. Rezultati so pokazali, da obstaja značilna spremenljivost v lastnostih korenin med genotipi čičerke. Celokupno je bilo ugotovljeno 26 alelov. 10 SSR označevalcev je dalo 26 polimorfnih prog v osmih povezanih skupinah (LG). Izsledki so pokazali, da obstaja v dednem materialu čičerke znatna variabilnost v lastnostih korenin. Z analizo zgradbe populacije so bile ugotovljene štiri podpopulacije. PsAS2, AF016458, 16549 in 19075 SSR označevalci v LG1, LG3, LG2 in LG1 povezanih skupinah so bili povezani z lastnostmi korenin. Ugotovitve raziskave prispevajo žlahtniteljem čičerke pomembne informacije za izboljšanje lastnosti korenin.

Ključne besede: povezane skupine**;** toleranca na sušo; zgradba populacije; SSR označevalci; podpopulacije; variabilnost

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L., 2n = 16) as a third major legume in the world widely used for food and fodder. Numerous biotic and abiotic stresses affect the production and yield of chickpea of which drought is one of the most important abiotic constraints. Drought causes heavy production losses, about 45–50 % in chickpea (Ahmad et al., 2005).

For drought management, genetic improvement over crop options for better adaptation to drought can be a sustainable and low-cost solution. But, it is very difficult to understand the maintenance of potential yield under drought stress, due to the different mechanisms used by plants to maintain growth under limited water resource, (Tuberosa & Salvi, 2006**).** The major challenges in identifying drought tolerance genotypes is drought interaction with the environment and its quantitative inheritance (Varshney et al., 2014).

Root structure modification can improve important agronomic traits including yield, drought tolerance and nutrient deficiency resistance (Tuberosa et al., 2002; Beebe et al., 2006; Ghanem et al., 2011). Despite, approximately small populations and inaccurate phenotyping cause it difficult to make large scale use of root genetic information in plant breeding (de Dorlodot et al., 2007). From now, correct phenotyping and characterization of root traits is necessary for translating novel physiological and genetic progresses into a conception of the role of root systems in increasing yield and productivity (especially in dry environments). The effect of diverse root features on drought tolerance were found to be high under final drought stress condition, mainly in environment where plant only confide in the stored soil water (Ludlow & Muchow, 1990; Kashiwagi et al., 2006; Passioura, 2006; Wasson et al., 2014). For example, Kirkegaard et al. (2007) indicated using root traits and soil moisture assessments in the field, that a 30 cm enhance in root depth increased the uptake of 10 mm more underground soil moisture and thus increased the yield by 0.5 t ha^{-1} grain yield. it also was demonstrated that Large root system effect on shoot biomass production and harvest index (HI) under terminal drought stress (Kashiwagi et al., 2006; Zaman-Allah et al., 2011). Although plant breeders are aware of the worth of the root system offering, but due to the low heritability of root traits, high variation in expression in different soils and soil moisture environments, and the difficulty of measuring these traits in the field has been less pay attention to these traits selection (Tuberosa et al., 2002; Malamy, 2005; Gaur et al., 2008).

Genetic diversity has been investigated using diverse types of *DNA markers,* including SSR in chickpea (Sefera et al., 2011; Keneni et al., 2012; Ghaffari et al., 2014; Hajibarat et al., 2015). *DNA* markers have been found for many agronomic traits (Thudi et al., 2014a).

Majority of the breeding attempts made in chickpea have been, and are being, focused on improving yield, resistance to diseases like *Ascochyta* blight and *Fusarium* wilt (Varshney et al., 2014a) and on tolerance to various abiotic stresses (such as drought (Varshney et. al., 2014; Jaganathan et al., 2015), cold (Mugabe et. al., 2019) and heat tolerance (Jha et al., 2018)). However even with the value of root traits and their critical roles in drought and heat adaptation in chickpea (Maphosa et. al., 2020), their genetic control has been less studied. Consequential associations between markers and quantitative traits led to the identification of locus significantly associated with drought tolerance. The root phenotyping problems has reduced the identity of root trait genomic locus in chickpea thus the aim of this research was to identify of the SSR markers associated with root-related traits in a various chickpea germplasm.

2 MATERIAL AND METHODS

Plant material contains 39 chickpea genotypes, including accessions from ICARDA (International Center for Agricultural Research in the Dry Areas) chickpea germplasm (Table 1). These entries were selected based on the results of previous drought tolerance trials in Kabuli type chickpea genotypes.

2.1 GENOTYPING

2.1.1 DNA extraction and SSR primers, PCR and agarose gel electrophoresis

Genomic DNA was extracted from young leaflets of chickpea genotypes plant leaves (4 plants of each genotypes) using a CTAB method according Doyle and Doyle (1987) with a slight modification. On the basis of their locations on the eight linkage groups (LGs) of the integrated genetic linkage map of chickpea (*Cicer arietinum* L.), altogether 10 SSR markers were select (Sefera et al., 2011) (Table 2). PCR was carried out in a 14 µl reaction mixture that contain 100 ng of DNA, 100 pmol of each primer (forward and reverse), 7µl of CinnaGen PCR master mix, 2 X (0.08 units μl^{-1} Taq DNA polymerase in reaction buffer, 3 mmol $MgCl_2$, and 1.6 mmol dNTPs). The amplifications were performed with a Thermal Cycler (Applied Bio Rad, Foster City, CA,

USA), with an initial denaturation at 94 ^{0}C for 240 sec that was followed by 10 cycles of: at 94 ^{0}C for 30 s, 45 s at annealing temperature (Ta) (Table 2), 120 s at 72 ^{0}C, and then was followed by 25 cycles of: 30 s at 94 ^{0}C, 45 s at Ta, 120 s at 72 ^{0}C and a final extension step at 72 ^{0}C for 420 s In 2.5 % agarose gel by 1X TBE running buffer, amplified fragments were resolved and quantity one software (Bio-Rad, CA 94547, USA) analyzed images.

Table 1: The list of genotypes used in the present study

NO.	Genotype Name	Pedigree
1	FLIP97-706C	X04TH62/X03TH-130XFLIP97-116
2	FLIP03-77C	X04TH65/X03TH-133XFLIP96-154
3	FLIP03-130C	X04TH65/X03TH-133XFLIP96-154
4	FLIP06-158C	X04TH65/X03TH-133XFLIP96-154
5	FLIP07-19C	X04TH66/X03TH-134XFLIP97-116
6	FLIP07-20C	X04TH66/X03TH-134XFLIP97-116
7	FLIP07-22C	X04TH66/X03TH-134XFLIP97-116
8	FLIP07-28C	X04TH67/X03TH-135XFLIP99-34
9	FLIP07-31C	X04TH67/X03TH-135XFLIP99-34
10	FLIP07-44C	X04TH76/X03TH-144XFLIP97-116
11	FLIP07-239C	X04TH77/X03TH-145XFLIP99-34
12	FLIP07-261C	X04TH79/X03TH-147XFLIP96-154
13	FLIP07-280C	X04TH110/X03TH-178XFLIP97-116
14	FLIP08-46C	X04TH110/X03TH-178XFLIP97-116
15	FLIP08-200C	X04TH114/X03TH-182XFLIP97-116
16	FLIP09-70C	X04TH115/X03TH-183XFLIP99-34
17	FLIP09-81C	X04TH117/X03TH-185XFLIP96-154
18	FLIP09-85C	X04TH123/FLIP97-205XFLIP97-116
19	FLIP09-90C	X04TH124/FLIP97-229XFLIP99-34
20	FLIP09-98C	X04TH126/FLIP98-229XFLIP96-154
21	FLIP09-148C	X04TH129/FLIP98-233XFLIP99-48
22	FLIP09-149C	X05TH7/X04TH-126XFLIP01-18
23	FLIP09-189C	X05TH106/FLIP97-131XFLIP00-14
24	FLIP09-191C	X05TH106/FLIP97-131XFLIP00-14
25	FLIP09-192C	X05TH106/FLIP97-131XFLIP00-14
26	FLIP09-194C	X05TH106/FLIP97-131XFLIP00-14
27	FLIP09-214C	X05TH131/FLIP97-118XFLIP00-17
28	FLIP09-216C	X05TH152/FLIP98-107XUC27
29	FLIP09-218C	X04TH31/X03TH-31XFLIP97-116
30	FLIP09-219C	X06TH100/FLIP02-47XFLIP98-230
31	ILC482	ILC482
32	FLIP 82-150C	X79TH101/ILC 523 X ILC 183
33	FLIP88-85C	X85 TH143/ILC 629 x FLIP 82-144C
34	FLIP93-93C	X89TH258/ (FLIP 85-122CXFLIP 82-150C)/FLIP 86-77C
35	FLIP07-180C	X04TH12/X03TH-12XFLIP99-48
36	FLIP09-88C	X04TH40/X03TH-40XFLIP99-34
37	FLIP09-115C	X04TH50/X03TH-50XFLIP99-34
38	FLIP09-337C	X04TH53/X03TH-53XFLIP97-116
39	FLIP09-386C	X04TH59/X03TH-59XFLIP99-48

Table 2: The list of genotypes used in the present study

NO.	Marker name	Primer sequences(5'-3')	Linkage Group	Annealing temperature (^{0}C)
1	19075	F:CACGAGTACAACATGGAGTGAAG R: CAAGCTCAACCTCCTCATACC	LG1	57.75
2	18363	F:CATGCATGGAGTTGGAAGAG R: GTCCCAAAATGCAGCCAATA	LG3	55.6
3	16549	F:CAATGAGATGCTGGCGATAA R: GTTCGGTGTTGTGGGTTTTT	LG2	55.7
4	C24	F:GCTACTGGAGGAGGCTTTCA R: GCCTTCTACACAACGGCTTC	LG4	58.2
5	PsAS2	F: CTAATCACACGTTTAGGACCGG R: CGAAATCCAAACCGAACCTAATCC	LG1	58.9
6	PSAB60	F:AATTAATGCCAATCCTAAGGTATT R: GGTTGCACTATTTTCGTTCTC	LG6	53.95
7	PD23	F:ATGGTTGTCCCAGGATAGATAAR: GAAAACATTGGAGAGTGGAGTA	LG 5	54.9
8	PSAD147	F: AGCCCAAGTTTCTTCTGAATCC R: GAAAACATTGGAGAGTGGAGTA	LG7	57.55
9	17605	F:CGCCCTTCATCATCATCTTC R: AAATTCGCAGAGCGTTTGTTAC	LG 8	57.35
10	AF016458	F:CGCCCTTCATCATCATCTTC R: CGAATCTTGGCCATGAGAGTTGC	LG 3	57

2.2 PHENOTYPING

2.2.1 Root sample extraction and processing

The experiment was conducted in Glasshouse at Ilam university. The average daily temperature was 25/16 ^{0}C (day/night), and the humidity was 70 %. Experiment was carried out in completely randomized design (CRD) with four replications. The seeds of each genotype were sown in split drain pipes (SDP) with 60 cm height and 10 cm diameter. The soil used in SDP was a mixture of sand and Jons Innes No-2 (1:1 ratio). Each SDP was put together with a single plant. The plants were harvested 30 days after germination. Plants were harvested on 35 day after germination based on taproot length increments for the growth period (Chen et al., 2017).

2.2.1 Root-related traits

Chickpea root samples were taken to record root traits. Using a water shower, the soil was separated from the roots and then the fresh mass of the roots was measured. Then, by floating the root samples in water in a tray, organic debris and weed roots were removed manually from chickpea roots. The fresh soil and roots were thereupon dried in an oven at 65 °C for 72 hours and the percentage of soil and root moisture was obtained. The root characteristics are showed in Table 3.

2.3 STATISTICAL ANALYSIS

Analysis of variance was performed with the SAS 9.2 software to evaluate the factor 'GENOTYPE". The genotype means were compared by a Duncan's multiple range post hoc test and used for the association analyses.

2.4 ASSOCIATION ANALYSES

The polymorphism information content (PIC) value was calculated using $PIC = 1\text{-}\Sigma\ (P_{ij})^2$ (Where Pij is the frequency of jth allele in ith primer and summation extends over 'n' patterns) (Nei , 1973) for each primer. PIC describe content of 'gene diversity'.

NTSYSpc 2.02e was used to compute Jaccard similarity coefficients to report genetic relationships among

the chickpea genotypes. Also using this software and based on genetic distances, cluster analysis was carried out using the unweighted pair-group (UPGMA) method and the dendrograms were drawn (Rohlf, 2000).

The marker–trait association between the SSR markers and each of root related traits tested using TASSEL 4.0. (Bradbury et al., 2007). General linear model (GLM) and mixed linear model (MLM) approaches used for association analysis. Covariates in GLM and MLM analyses were the corresponding *Q* values. Manhattan plots present association between a SSR marker and phenotypic trait that was significant at $p \leq 0.05$. STRUCTURE version 2.3.4 used for determine the population structure of the 39 accessions using the Bayesian clustering method (Pritchard et al., 2000). The STRUCTURE analysis separated the population based on ΔK method (Evanno et al., 2005).

Table 3: The root related trait measured in the present study

No.	Trait	Formula	Unit of measurement	References
1	Root length (RL)	Total RL of each sample was measured using a ruler.	cm	-
2	Root fresh mass (RFM)	The fresh weight of the roots was measured with a digital scale to the nearest thousandth	g	-
3	Root dry mass (RDM)	The roots were kept for oven drying at 70 ◦C for 72 h (to constant mass) then was estimated.	g	Ramamoorthy et al., 2017
4	Dry mass of plant shoots (SDM)	The shoots were kept for oven drying at 70 ◦C for 72 h (to constant mass) then SDW was estimated	g	Ramamoorthy et al., 2017
5	Root volume (RV)	$RV = B - C$	cm^3	-
6	Root area (RA)	$RA = 2 \times SQRT \langle RV \times 3.14 \times RL \rangle$	cm^2	Akhavan et al., 2012
7	Root fineness (RF)	$RF = \frac{RL}{RV}$	cm root /root fresh mass	Hajabbasi, 2001
8	Root diameter (Rd)	$Rd = SQRT \frac{\langle 4 \times RFW \rangle}{\langle RL \times 3.14 \rangle}$	cm	Schenk & Barber, 1979
9	root length (SRL) Specific	$SRL = \frac{RL}{RDW}$	cm root length cm^{-3} soil volume	Mahanta et al., 2014
10	Root water content (RWC)	$RWC = \frac{RFW - RDW}{RDW}$	g	Lovelli et al., 2012
11	Root length density (RLD)	$RLD = \frac{RL}{SV}$	cm RL cm^{-3} soil volume	Mahanta et al., 2014
12	Specific root volume (SRV)	$SRV = \frac{RDW}{SV}$	g RDW cm^{-3} soil volume)	Hasanabadi et al., 2010
13	Root tissue density (RTD)	$RTD = RDW \times RV$	g RDW× cm^3 soil volume	Paula & Pausas, 2011
14	Root volume density (RVD)	$RVD = \frac{RFW}{SV}$	cm m^{-3}	Hajabbasi, 2001
15	Root area density (RAD)	$RAD = RL \times RD \times 3.14$	$cm^2 cm^{-3}$	Akhavan et al., 2012
16	Root density (RD)	$RD = \frac{RDW}{RV}$	g cm^{-3}	Akhavan et al., 2012

B =water and root volume, C = water volume, SQRT = root square

3 RESULTS

3.1 ANALYSIS OF ROOT TRAITS DATA

Root morphological traits differed significantly among genotypes. All of 16 measured root related traits differed significantly among genotypes ($p \leq 0.001$) (Table 4). The average root length was 50.69 cm and ranged from 27 to 72 cm (Table 5). The variation (Coef. Var.) in RL among genotypes was 20.7 % (Table 5). Root volume (RV) and root fresh mass (RFM) varied significantly among genotypes (Table 5). RV ranged from 3.75 cm^3 (FLIP07-28C) to 22 cm^3 (FLIP07-31C), with an average root volume of 11.5 cm^3. The root fresh mass (RFM) averaged 10.93 g across all genotypes. RFM varied among genotypes and ranged from 2.69 g (FLIP07-28C) to 22.52 g (FLIP09-192C). Root dry mass (RDM) was 0.15 g (ILC482) to 3.93g (FLIP09-192C) (average 1.33 g). The average leaf dry mass (LDM) was 0.91g, ranging from 0.17 g (FLIP07-31C) to 2.28 g (FLIP09-192C), and root fineness (RF) ranged from 2.07 FLIP97-706C to 13 (FLIP88-85C) (mean 4.95 cm root / root fresh mass). The average specific root length (SRL) was 50.37 cm and ranged from 15 cm (FLIP97-706C) to 238.71 cm (FLIP 82-150C). Root water content (RWC) averaged 8.30 g across all genotypes. RWC ranged from 2.58 (FLIP07-31C) to 30.88 (FLIP07-20C). The average root tissue density (RTD) ranging from 0.61 (ILC482) to 86.53 (FLIP07-31C) (mean 16.47 g RDW × cm^3 soil volume). Root diameter (Rd) ranged from 0.038 cm (FLIP88-85C) to 0.27 cm (FLIP09-192C), with an average root volume of 0.12 cm. The average root area (RA) was 83.69 cm^2 and ranged from 36.83 cm^2 (FLIP07-28C) to 127.68 cm^2 (FLIP07-31C). The average root density(RD) was 0.52 g cm^{-3} and ranged from 0.29 g cm^{-3} (ILC482) to 0.71 g cm^{-3} (FLIP07-31C). Root length density (RLD) ranging from 0.05 (FLIP07-28C) to 0.13 (FLIP07-20C) (mean 0.094 cm RL cm^{-3} soil volume). The average specific root volume (SRV) was 0.0025 g RDM cm^{-3} soil volume and ranged from 0.0028 (ILC482) to 0.0073 g RDM cm^{-3} soil volume (FLIP09-192C). Root volume density (RVD) ranged from 0.0050 cm m^{-3} (FLIP07-28C) to 0.042 cm m^{-3} (FLIP09-192C), with an average RVD of 0.020 cm m^{-3}. Root area density (RAD) averaged across all genotypes 82.14 cm^2 cm^{-3}. RAD ranged from 30.20 cm^2 cm^{-3} (FLIP07-28C) to 129.19 cm^2 cm^{-3} (FLIP09-192C).

3.2 SSR MARKER SCREENING AND GENETIC DIVERSITY ASSESSMENT

Using the SSR marker system the genetic diversity of 39 chickpea genotypes analyzed. Detected alleles were 26. 2-3 bands with an average number of 2.6 alleles per locus observed. AF016458، 17605، PSAD147، 19075، 16549 and PD23 had 3 alleles.

All of the amplification products (100 %) showed polymorphism, denoted high variation among chickpea accessions at the DNA level. Size of fragments produced varied from 110 to 150 bp (Table 6). The highest PIC was for primer 16549 and PSAD147 (0.54) and the lowest PIC was for the primer C24 (0.38). Hence, primer 16549 and PSAD147 were effective and useful markers for determining the genetic differences among the chickpea genotypes (Table 6).

The cluster analysis showed that the 39 accessions were divided into five clusters (Fig. 1). The first cluster included FLIP97-706C and FLIP03-77C. The second cluster included only FLIP09-148C. The third cluster included FLIP09-85C, FLIP09-90C, FLIP09-98C, FLIP09-115C, FLIP09-337C and FLIP09-386C. The forth cluster included FLIP03-130C, FLIP09-214C, FLIP09-216C, FLIP09-218C, FLIP09-219C, ILC482, FLIP 82-150C, FLIP88-85C, FLIP93-93C, FLIP07-180C and FLIP09-88C. The fifth cluster included FLIP06-158C, FLIP07-20C, FLIP07-239C, FLIP07-280C, FLIP08-200C, FLIP09-149C, FLIP09-189C, FLIP09-191C and FLIP09-192C.

3.3 POPULATION STRUCTURE

The marker segregation data was used for the population clustering, the STRUCTURE analysis separated the population into four cluster (Fig. 2). The 39 chickpea genotypes were grouped in to four subpopulations, as viewed in STRUCTURE analysis (Fig. 2).

Genotypes 39, 38, 20, 19, 18 and 37, respectively, were named as FLIP09-386C, FLIP09-337C, FLIP09-98C, FLIP09-90C, FLIP09-85C and FLIP09-115C, respectively. Genotypes 31, 32, 33, 28, 30, 27, 29, 26 and 34 respectively with the letters ILC482, FLIP 82-150C, FLIP88-85C, FLIP09-216C, FLIP09-219C, FLIP09-214C, FLIP09-218C, FLIP09 -194C and FLIP93-93C belonged to the second subpopulation. Genotypes 13, 11, 12, 14, 16, 6, 17, 15, 8, 5, 7, 9 and 10 respectively with the names FLIP07-280C, FLIP07-239C, FLIP07-261C, FLIP08-46C, FLIP09-70C, FLIP07-20C, FLIP09-81C, FLIP08-200C, FLIP07-28C, FLIP07-19C, FLIP07-22C, FLIP07-31C and FLIP07-44C were in the third subpopulation and genotypes 23, 3, 24, 25, 22, 2 , 1, 4, 35, 36 and 21 respectively with the letters FLIP09-189C, FLIP03-130C, FLIP09-191C, FLIP09-192C, FLIP09-149C, FLIP03-77C, FLIP97-706C, FLIP06-158C, FLIP07-180C, FLIP09 -88C and FLIP09-148C were also included in the fourth subpopulation (Figure 2).

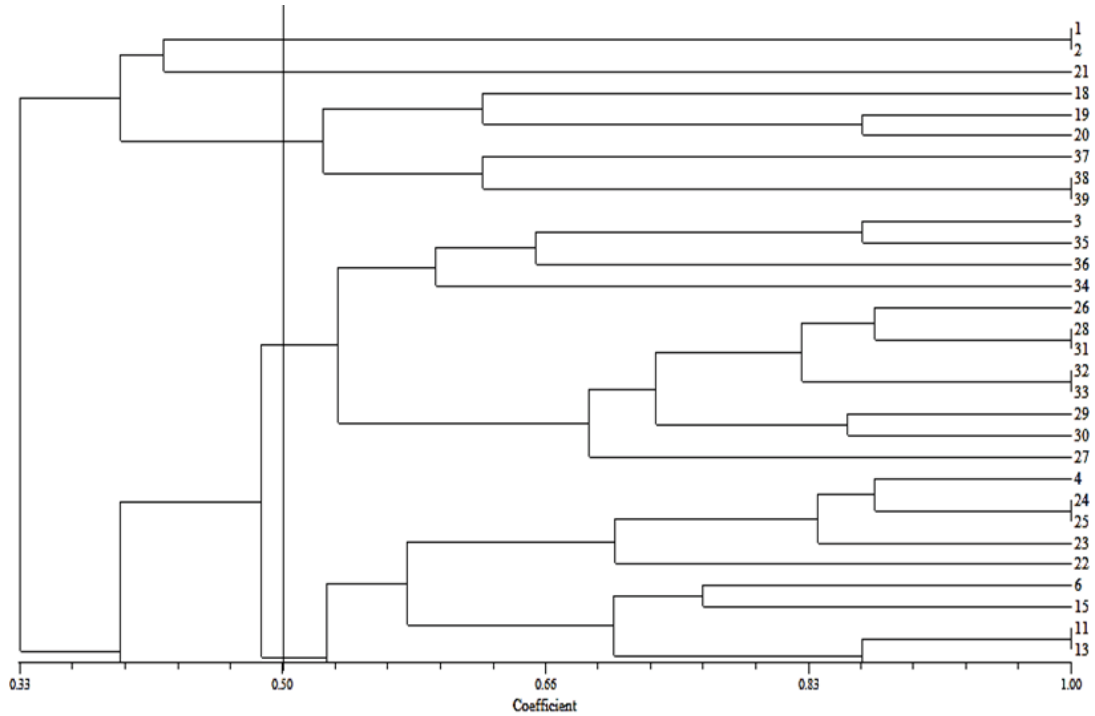

Figure 1: A dendrogram based on SSR markers of the 39 chickpea genotypes by UPGMA method

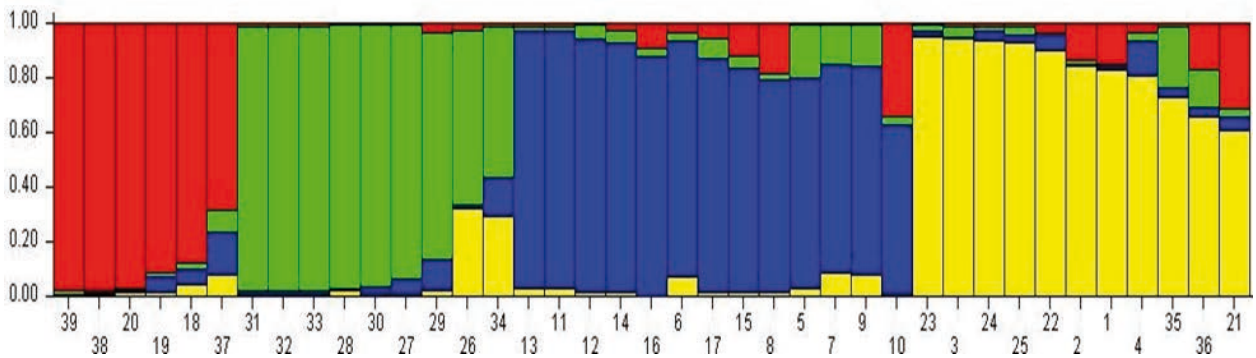

Figure 2: Genetic relatedness of 39 genotypes of chickpea with 10 SSR primer combinations as analyzed by the STRUCTURE program

3.4 ASSOCIATION ANALYSIS

The markers with minor allele frequency less than 5 %, remove so 21 marker loci retained for association analysis (Table 7). As in table 7 seen, AF016458 significantly associated with root fresh masst, root diameter, root volume density, root area, root length density, root area density, root length and root flavor. The 16549 marker was significantly associated to root fresh mass, root volume density, root area, root volume, root fineness and root area density. Significant associations were observed to the marker 19075 with root flavor. PsAS2 was significantly associated with root flavor, root volume density, root area, root volume, root fresh mass and root area density.

4 DISCUSSION

Several putative root traits contributing to drought

Table 4: Analysis of variance of root morphological traits in 39 chickpea genotypes

Source of variation	Degree of freedom	Mean square															
		RTD	RWC	SRL	RF	LDW	RDM	RFM	RV	RL	RAD	RVD	SRV	RLD	RD	RA	Rd
genotype	38	387.26**	30.67**	4018.71**	10.72**	0.69**	1.26**	48.61**	40.71**	399.72**	1308.67**	0.0001**	0.000 004**	0.001 **	0.027**	1193.69**	0.005**
Experimental error	113	16.65	2.97	73.50	0.84	0.03	0.04	2.97	2.90	7.95	54.59	0.00001**	0.0000 001	0.000 02	0.001	55.13	0.0007

RL: Root length, RFM: Root fresh mass, RDM: Root dry mass, DMS: Dry mass of plant shoots, RV: Root volume, RA: Root area, RF: Root fineness, Rd: Root diameter, SRL: specific root length RWC: Root water content, RLD: Root length density, SRV: Specific root volume, RTD: Root tissue density, RVD: Root volume density, RAD: Root area density, RD: Root density. **: significant at 0.01

Table 5:. Descriptive statistics of 16 measured root traits in 39 chickpea genotypes grown in a greenhouse condition

Variable	Mean	SE Mean	Coef. Var. (%)	Minimum	Maximum
RL (cm)	50.69	0.85	20.7	27	72
RV (cm^3)	11.30	0.29	32.1	3.75	22
RFM (g)	10.93	0.31	35.14	2.69	22.52
RDM (g)	1.33	0.051	47.85	0.15	3.93
LDW (g)	0.91	0.036	49.98	0.17	2.28
RF (cm root /root fresh mass	4.95	0.15	38.41	2.07	13
SRL (cm)	50.37	3.02	74.17	15	238.71
RWC (g)	8.30	0.31	46.22	2.58	30.88
RTD (g RDM× cm^3 soil volume)	16.47	0.95	71.70	0.61	86.53
Rd (cm)	0.12	0.0036	37.91	0.038	0.27
RA (cm^2)	83.69	1.52	22.41	36.83	127.68
RD (g $cm^{-3)}$	0.52	0.0074	17.56	0.29	0.71
RLD (cm RL cm^{-3} soil volume)	0.094	0.0016	20.75	0.05	0.13
SRV (g RDM cm^{-3} soil volume)	0.0025	0.000095	47.85	0.0028	0.0073
RVD (cm $m^{-3)}$	0.020	0.00058	35.14	0.0050	0.042
RAD (cm^2 $cm^{-3)}$	82.14	1.57	23.70	30.20	129.19

RL: Root length, RFM: Root fresh mass, RDM: Root dry mass, DMS: Dry massof plant shoots, RV: Root volume, RA: Root area, RF: Root fineness, Rd: Root diameter, SRL: Specific root length C: Root water content, RLD: Root length density, SRV: Specific root volume, RTD: Root tissue density, RVD: Root volume density, RAD: Root area density, RD: Root density

Table 6: The number and size range of bands produced by the SSR primers among the 39 chickpea genotypes

Marker name	Number of observed alleles	Polymorphism information content (PIC)
19075	3	0.57
18363	2	0.47
16549	3	0.66
C24	2	0.38
PsAS2	2	0.48
PSAB60	2	0.48
PD23	3	0.59
PSAD147	3	0.66
17605	3	0.65
AF016458	3	0.54
Mean	2.6	0.55

resistance in chickpea has been found (Benjamin and Nielsen, 2006; Fukai et al., 1995; Ali et al., 2005; Kashiwagi et al., 2008). Phenotypic selection for root traits is difficult because of the laborious, time-consuming and destructive methods involved in root studies (Gaur et al., 2008). An effort has been made in this study to identify the markers showed association with root traits in chickpea using a diverse set of genotypes. All of the measured root related traits differed significantly among genotypes ($p \leq 0.001$) (Table 4). The variation (Coef. Var) in all root-related traits (17.56-74.17 % (Table 5)) observed in the genotypes in the present study justifies its use for association analysis. Breseghello & Sorrells (2006) suggested use of diverse genotypes for the purpose of association mapping.

FLIP09-192C had the highest root fresh mass, root dry mass, the average leaf dry mass, root diameter, the average specific root volume, root volume density and root area density and FLIP07-31C had the highest root

Table 7: Marker-trait associations with MLM and GLM models

Traits	Marker name	No. of Associations	P. value		R^2(%)
			MLM	GLM	
Root fresh mass	PsAS2	2	0.047	0.039	44.9
Root fresh mass	AF016458	3	-	0.042	44.1
Root fresh mass	16549	3	0.021	0.038	45.2
Root diameter	AF016458	3	0.042	0.045	34.8
Root volume density	16549	3	0.025	0.037	45
Root volume density	AF016458	3	-	0.048	42.2
Root volume density	PsAS2	2	0.047	0.039	44.8
Root flavor	AF016458	3	0.039	0.025	37.2
Root flavor	16549	3	0.039	0.042	32.5
Root flavor	19075	2	0.034	0.046	31.7
Root flavor	PsAS2	2	-	0.044	32
Root area	AF016458	3	-	0.038	40.1
Root area	PsAS2	2	0.046	0.016	49
Root area	16549	3	0.027	0.014	49.9
Root length density	AF016458	3	0.038	0.029	31.7
Root volume	16549	3	0.050	0.017	51.6
Root volume	PsAS2	2	0.029	0.019	50.9
Root area density	16549	3	0.036	0.025	46.2
Root area density	AF016458	3	0.041	0.019	48.9
Root area density	PsAS2	2	-	0.026	45.9
Root length	AF016458	3	0.049	0.030	31.6

volume, the average leaf dry mass, root water content, the average root area. Generally, tolerant genotypes have high root growth vigor and deeper soil root proliferation under drought stress, allowing them to extract water from all soil depths and maintain yield and HI (Maphosa et al., 2020). The marker segregation data grouped FLIP07-31C and FLIP09-192C in the third and fourth subpopulation respectively.

In this research, a total of 26 alleles with a mean of 2.6 alleles per locus were found. Also, the mean PIC value was 0.55 (Table 6). So that according to indicated genetic diversity among cultivated chickpea genotypes was lesser than the wild chickpea genotypes (Ghaffari et al., 2014 and Hajibarat et al., 2015) and the wild chickpea species showed greater PIC value and number of allele count per locus (Upadhyaya et al. (2008) and Ghaffari et al. (2014)).

The 39 genotypes used for association analysis were split in to four distinct subpopulations at K = 4 (Fig. 2). Genotypes in a subpopulation often have similar pedigrees (Table 1). The presence of subpopulations within a population can be due to reasons such as the different geographical origin of the genotypes, natural or human selection, or genetic drift (Flint-Garcia et al., 2003; Buckler & Thornsberry, 2002).

In the present study, a total of 10 SSR markers have been used for genotyping the 39 chickpea The microsatellite markers showing association with root traits were detected using TASSEL software. A total of 21 marker-trait association have been found in this study at $p < 0.05$. The markers, PsAS2, AF016458, 16549 and 19075 on LG1, LG3, LG2, LG1 linkage group respectively was linked with root fresh mass root diameter, root volume density, root area, root length density, root area density, root length and root flavor.

Several QTLs controlling root traits have been reported (Kale et. al., 2015; Gaur et al., 2008; Varshney et al., 2014). Chandra et al. (2004) reported that a SSR marker, TAA 170, was associated with root mass and root length under drought stress in chickpea. Li et al. (2018) found that several SNPs from auxin-related genes were associated with yield and yield-related traits under drought condition. H6C-07 (on LG3) and H5G01 (on LG4) markers found that associated with QTLs for many drought-related traits (Hamwieh et al., 2013). Thudi et al. (2014b) discovered over 200 SSR, DArT, and SNP markers associated with drought-related traits. The most of highly expressed ESTs encoded proteins involved in cellular organization, protein metabolism, signal transduction, and transcription in the chickpea under drought stress (Jain & Chattopadhyay, 2010). The role of hypothetical abscissic acid and stress ripening (ASR) protein NP_001351739.1 in mediating drought responses as a transcription factor were recognized in chickpea (Sachdeva et al., 2020). A "QTL-hotspot" containing quantitative trait loci (QTL) for several root and drought tolerance traits was transferred through marker assisted backcrossing into JG 11, a leading variety of chickpea (*Cicer arietinum* L.) in India from the donor parent ICC 4958. some introgression lines were identified that may be released as improved variety with enhanced drought tolerance (Varshney et al., 2013).

5 CONCLUSIONS

In conclusion, this study demonstrated the existence of genetic diversity exists in the current chickpea germplasm for root traits. The present study has helped in identification of significant marker-trait associations on LG1, LG2 and LG3. This shows that these chromosomes are potential candidate ones for emphasizing future studies. The research findings provide valuable information for marker-assisted selection improving root traits after validation for chickpea breeders.

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Authors' Contributions: Zahra Shekari: Collection of experimental data. Zahra Tahmasebi: supervision of the study and writing of manuscript. Homayon Kanoni: review of the manuscript. Ali Asherf Mehrabi: molecular and statistical analysis.

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