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QUANTITATIVE TRAIT LOCI FOR YIELD AND YIELD-ASSOCIATED TRAITS IN CHICKPEA UNDER DROUGHT STRESS

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ABSTRACT

Crop yield is a complex phenomenon, controlled by several genes, each contributing to the overall phenotypic expression; which is affected by environment and genotype x environment interactions. Identifying and mapping quantitative trait loci (QTLs), make tracing these traits during breeding possible and easy. The objective of this study was to identify QTLs associated with chickpea (*Cicer arietinum* L.) grain yield and its associated traits, under drought stress. The experiment was conducted using 188 $F_{3:5}$ genotypes from ICCV 05107 x ICCV 94954 crosses. Genotypic data were from 49 polymorphic simple sequence repeat (SSR) markers; while phenotypic data were obtained from a field evaluation designed in a 19 x 10 alpha lattice. The study was replicated thrice on three sites, namely at Koibatek Agricultural Training Centre, Muserech; and at the Kenya Agricultural Research and Livestock Organisation, Marigat. Eight QTLs were mapped on a linkage map spanning a total length of 335.04 cM, with varying phenotypic variation expression (PVE%). These QTLs include, one each for days to maturity, 100-seed weight, and two each for above-ground biomass, harvest index, and grain yield. Five major QTLs having PVE ranging from 10.37 to 32.39%, were identified for days to maturity, 100-seed weight above-ground biomass, harvest index (HI), and grain yield. Four of the eight QTLs were mapped on linkage group 4 (LG4); days to maturity, 2 for above-ground biomass, and grain yield. The QTLs mapped are useful in genomic-assisted breeding for chickpea yield improvement. However, there is a need for marker saturation on LGs and specific genes identified for effective marker-assisted breeding.

Key Words: *Cicer arietinum*, genomic-assisted breeding, linkage map

RÉSUMÉ

Le rendement des cultures est un phénomène complexe, contrôlé par plusieurs gènes, chacun contribuant à l'expression phénotypique globale, qui est affectée par l'environnement et les interactions génotype x environnement. L'identification et la cartographie des locus de caractères quantitatifs (QTL) rendent possible et facile le traçage de ces caractères pendant la sélection. L'objectif de cette étude était d'identifier les QTL associés au rendement en grains du pois chiche (*Cicer arietinum* L.) et à ses caractères associés, sous stress hydrique. L'expérience a été menée en utilisant 188 génotypes

$F_{3:5}$ issus de croisements ICCV 05107 x ICCV 94954. Les données génotypiques provenaient de 49 marqueurs polymorphes de répétition de séquence simple (SSR) ; tandis que les données phénotypiques ont été obtenues à partir d'une évaluation sur le terrain conçue dans un réseau alpha 19 x 10. L'étude a été reproduite trois fois sur trois sites, à savoir au Centre de formation agricole de Koibatek, Muserech; et à l'Organisation de recherche agricole et d'élevage du Kenya, Marigat. Huit QTL ont été cartographiés sur une carte de liaison couvrant une longueur totale de 335,04 cM, avec une expression de variation phénotypique variable (PVE%). Ces QTL comprennent, un pour chacun des jours jusqu'à maturité, le poids de 100 graines et deux pour chacun des éléments suivants : biomasse aérienne, indice de récolte et rendement en grains. Cinq QTL majeurs ayant un PVE allant de 10,37 à 32,39 %, ont été identifiés pour les jours jusqu'à maturité, le poids de 100 graines de biomasse aérienne, l'indice de récolte (HI) et le rendement en grains. Quatre des huit QTL ont été cartographiés sur le groupe de liaison 4 (LG4) ; jours jusqu'à maturité, 2 pour la biomasse aérienne et le rendement en grains. Les QTL cartographiés sont utiles dans la sélection assistée par génomique pour l'amélioration du rendement du pois chiche. Cependant, il est nécessaire de disposer d'une saturation des marqueurs sur les LG et d'identifier des gènes spécifiques pour une sélection assistée par marqueurs efficace.

Mots Clés : *Cicer arietinum*, sélection assistée par génomique, carte de liaison

INTRODUCTION

Chickpea (*Cicer arietinum* L., $2n = 16$), is a self-pollinated crop, whose outcrossing rate is less than 1% (Singh *et al.,* 2008); and genome size is 740 Mbp (Gaur *et al.,* 2011). It is a preferred legume due to its multiple uses related for instance to nutrition, health and for N fixation (Merga and Haji, 2019; Zhang *et al.,* 2024), especially for resource-poor farmers. It is the most popular in arid and semiarid regions, under rain-fed conditions, on residual moisture (Korbu *et al.,* 2021; Singh *et al.,* 2021), and has great potential to moderate climate change and food security.

Chickpea production is generally low in Africa, accounting for only half a million hectares, against world production of 14.56 million hectares (Marga and Haji, 2019; Fikre *et al.,* 2020). Ethiopia leads in Africa in both lands under chickpea cultivation and level of production of 492.69 thousand metric tonnes production (Singh *et al.,* 2021; Statista, 2024).

Chickpea production in Kenya is scantly documented and is mainly promoted in low-altitude regions, under dry conditions; and in the highlands as a relay crop (Kimurto *et al.,* 2014). The average yield in Kenya is approximately 1.8 metric tonnes ha^{-1} during the long rainy seasons (Onyari *et al.,* 2010), and 0.55 t ha^{-1} during the short rainy seasons; under low altitude areas (Thagana *et al.,* 2009).

The major challenges to chickpea production are low and unstable yields, impacted largely by diseases and pests, drought and heat stress (Kosgei *et al.,* 2021; Manjunatha *et al.,* 2022; Jain *et al.,* 2023); all of which are expected to worsen with the rising wave of global warming. Kenya is not an exception to climate change and chickpea production is affected by these factors. Several chickpea varieties have, therefore, been introduced, evaluated and some released for drought tolerance, resistance to pod borers, as well as resistance to Ascochyta blight (Mulwa *et al.,* 2010; Kimurto *et al.,* 2013; Kimurto *et al.,* 2014). However, more high-yielding varieties for varied agro-ecological zones are needed, and this calls for the adoption of new and more robust breeding techniques.

Several breeding technologies have been employed to improve chickpea yields, mainly using conventional and molecular breeding techniques. The use of novel molecular breeding techniques such as marker-assisted selection, marker-assisted backcrossing, gene pyramiding, genetic engineering and gene editing (Asati *et al.,* 2022; Kosgei *et al.,* 2022; Singh *et al.,* 2023) has been studied and some

of the methods have been applied in traits that are difficult to breed, for instance, grain yield (Pratap *et al.,* 2017; Bharadwaj *et al*., 2021).

Grain yield traits are controlled by multigenes but with low heritability; and their expression is highly affected by environment and genotype x environment interactions (Kindie *et al*., 2021; Ligarreto-Moreno and Pimentel-Ladino, 2022), thus slowing the process of breeding for high yields. Indirect selection, using highly correlated traits for grain yield, has been one of the option to overcome this slow process. However, the use of technologies such as molecular markers that are closely linked to quantitative trait loci (QTLs) trait of interest, makes it possible to fast-track breeding with the help of marker-assisted approaches (Ribaut *et al.,* 2010; Salgotra and Stewart, 2020; Istanbuli *et al.,* 2024). The prerequisite to this success is the identification of these QTLs. Quantitative trait loci have been defined as regions within genomes that contain genes associated with a particular quantitative trait (Collard and Mackill, 2008).

Several markers have been used in chickpeas breeding, to generate genetic maps and detect QTLs linked to important quantitative traits, with the common ones being simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). SSR and SNP markers have been used in identifying reliable QTL, for example, drought tolerance (Varshney *et al.,* 2013; 2014; Kushwah *et al.,* 2022), harvest index (Rehman, 2009), yield per plant (Imtiaz, 2010; Gowda *et al.,* 2011; Yadava *et al.,* 2023), pods per plant (Gowda *et al.*, 2011), seed traits (Verma *et al.,* 2015), flowering time (Mallikarjuna, 2017), 100-seed weight, and plant height (Jingade and Ravikumar, 2019; Barmukh *et al.,* 2021). The identification of QTL linked to yield and its related traits is critical in deploying marker-assisted selection (MAS) in chickpea improvement. The objective of this study was to identify QTLs associated with chickpea grain yield and its associated traits under drought stress.

MATERIALS AND METHODS

Parent plant materials. The parental materials used in this study were two inbred lines developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India; and evaluated for adaptation in Kenya. These included ICCV 94954, also JG 130 (female); and ICCV 05107 (male parent). The ICCV 94954 parent was developed from a cross between ICCV 42 and BG 256, also known as Pusa 256; both being Desi type and high-yielding (Thudi *et al.,* 2014).

The pedigree, ICCV 42, has resistance to Fusarium wilt; while BG 256 has both Ascochyta blight and Fusarium wilt resistance (Pratap *et al.,* 2017). On the other hand, ICCV 05107 (Desi type) is a variety developed from a cross between ICC 4958 (Desi type, with a large rooting system), and ICCV 92311, a Kabuli type, early maturing, and with large seeds (Sharma *et al.,* 2012).

Development of the population. The two parents, ICCV 94954 and ICCV 05107, were crossed at ICRISAT in India, to generate 186 $F_{1,}$ and selfed to produce F_2. The F_2 population was advanced by single seed descent (SSD), to generate F_3 families. The purpose of using SSD was to advance faster the generations and ensure that a random sample from F_2 was retained. Seed multiplication of $F_{3:4}$ and the $F_{3:5}$ families was carried out and evaluated in Kenya. A total of 188 genotypes, which included the two parents, were evaluated.

Genotyping of parents and $F_{3:5}$ families. Screening for polymorphic markers between the two parents, ICCV 94954 and ICCV 05107, was done using 72 simple sequence repeat (SSR) markers. The F_3 families were genotyped with the polymorphic markers. The

DNA extraction protocol was carried out according to Chakraborti *et al.* (2006). Lambda DNA standard (MBI Fermentas, USA) was used to quantify, check the quality and normalise DNA to 5 ng il^{-1} on agarose gel (0.8%) (Upadhyaya *et al.,* 2008).

PCR amplification and genotyping protocol were done according to Varshney *et al.* (2009). Genotyping was performed at the Applied Genomics Laboratory at ICRISAT, Patancheru, Hyderabad, in India. Data scoring was done with GeneMapper software, version 4.0 (Applied Biosystems, 2005). Deviations from expected ratios were tested using the Chi-square test.

Site description and field layout. Field evaluation of $F_{3:5}$ families was carried out at three sites, namely Koibatek Agricultural Training Centre (KATC), Muserech, in Eldama Ravine and Kenya Agricultural and Livestock Research Organisation (KALRO), Perkerra, Marigat. The three sites are located in extensive Baringo County in the Rift Valley, in Kenya. KATC and Muserech lie in latitude 1° 35' S, longitude 36° 66' E, and altitude 1890 m in the upper midland four agroecological zone (UM4), with low agricultural potential.

The average annual rainfall is 767 mm; mean annual minimum and maximum temperatures are 10.9 and 28.8 °C, respectively. The soils are mainly Vitric Andosols, with moderate to high soil fertility, and well-drained deep loam to sandy loam soil (Jaetzold and Schimdt, 1983). However, Muserech is in the lower region of Eldama Ravine, towards the dry areas of Mogotio, which is the transition zone between the Eldama Ravine and Mogotio. The area receives unpredictable and thus unreliable rainfall.

Kenya Agricultural Livestock and Research Organisation, Perkerra, Marigat lies at 0.5° N and 36° E, in Lower Midland 5 agro-ecological zone (LM5), approximately 1067 m above sea level (asl). The area receives a bimodal mean annual rainfall of 650 mm, with the first rainy season between April and June; and the second season between November and early December. The area has a mean annual maximum and minimum temperature of 32.4 and 24.6 °C, respectively. The mean annual temperature is 25 °C, with the hottest season (37.7 °C) occurring between January and April. Soils are mainly volcanic Fluvisols of sandy/silty clay loam texture, slightly acidic to slightly alkaline, and highly fertile with adequate P, K, Ca, Mg, but low in N and carbon (Jaetzold and Schmidt, 1983).

Treatments and experimental design. Treatments applied included irrigated and non-irrigated (rain-fed) regimes in two sites KATC and KALRO-Perkerra; while Muserech was planted under rain-fed conditions. The experiment was laid out in a 19 x 10 alpha lattice design. Plot size was 90 cm x 360 cm and planting were done at a spacing of 40 cm x 10 cm, single row each with ten seeds per row; replicated thrice.

Plants were irrigated after planting, until about 7 days after planting. The rain-fed conditions were maintained without irrigation. However, one slot of irrigation of up to 70% field capacity was applied at 50% flowering. There was no further irrigation applied until maturity. Data on rainfall were provided by area weather stations in both KALRO Marigat and Koibatek ATC (data not presented).

The irrigated conditions were achieved by applying and maintaining water at or near 70% field capacity. This was monitored using the gravimetric method; whereby furrow irrigation was applied and soil sampling was done. The soil was collected and packed in an airtight container and weighed. The soil sample was collected two days after irrigation, then oven-dried at 105 °C for 24 hours, and reweighed. The soil water content as a percentage of dry mass or gravimetric content, Pw, was determined using Equation 1.

$$Pw = \frac{WSW - DSW}{DSW} \times 100 \quad \text{..... Equation 1}$$

Where:

WSW = wet sample weight (g), and DWS = dry sample weight (g).

A repeat of soil sampling was done every 15th day after irrigation, and whenever moisture dropped below 70%, the plants were irrigated to restore field capacity. The water utilised for irrigation was from KALRO Marigat Perkerra irrigation scheme; while at Koibatek ATC, irrigation water was piped from river Chemasusu thus quality was presumed to be uniform for relevant treatments.

Data collection. Data were collected on morphological traits important for yield expression; including days to maturity (DM), above-ground biomass, 100-seed weight, grain yield per plot, and harvest index (HI). Days to maturity were recorded from emergence to when 90% of the plants attained physiological maturity. The above-ground biomass was assessed from biomass harvested at 90% physiological maturity from 5 plants per plot, harvested randomly within the plot, and packaged in labeled sampling bags.

The samples were then oven-dried at 60 ºC for 48 hours, to a constant dry weight. They were kept in labeled air- and water-tight zip-lock bags. Similarly, grain yield field dry pods were harvested per plot, threshed, and oven-dried at 105 ºC for 3 hours, to a constant dry weight, and kept in labeled air and water-tight zip-lock bags. The grain samples were then weighed and converted into kg ha^{-1}.

The 100-seed weight per sample was obtained by weighing randomly selected 100 seeds, after drying the grain. Harvest index (HI) was determined by dividing grain dry weight by the total dry weight of above-ground biomass per plot.

The analysis of the phenotypic data was done using PROC GLM of SAS software version 9.2. Significant differences among genotypes and G x E were tested using the Least Significant Difference (LSD) at $P < 0.05$. The model used was:

$$Y_{ijkl} = \mu + t_i + r(l)_{jl} + b(r)_{jk} + l_l + (cxl)_{il} + e_{ijkl} \quad \text{..... Equation 2}$$

Where:

Y_{ijkl} = Observation of treatments; μ = Overall mean; t_i = effect of i^{th} genotypes; $r(l)_{jl}$ = jl^{th} effect of j^{th} replication within environment; $b(r)_{jk}$ = effect of jk^{th} block within replication; l_i = effect of l^{th} environment, $(cxl)_{il}$ = effect of il^{th} interaction between genotypes and environment; and e_{ijkl} = error term.

Linkage map construction. A linkage map was constructed based on genotypic data from the polymorphic markers, using WinQTL Cartographer version 2.5 (Wang *et al.,* 2012). A Microsoft Excel workbook file was prepared to contain general information, genotype, and anchor markers. The following parameters were used:

a) grouping was achieved using the logarithm of odds (LOD) of 2.5; where any two markers with LOD higher than the threshold were grouped;
b) ordering option nnTwoOpt whereby the nearest neighbor was used for tour construction, and two-opt was used for tour improvement; and
c) rippling option SARF (sum of adjacent recombination fractions) was used to fine-tune the ordered chromosomes.

The linkage map was generated using the 'QTL mapping input file' outputting options. Chi-square values and probability were generated by pairwise distance outputting options.

Quantitative trait loci (QTLs) detection. QTL was detected using IciMapping (Inclusive Composite Interval Mapping), version 3.2 (Wang *et al.,* 2011), based on the linkage map

generated, genotypic and phenotypic data. The phenotypic data used included, days to maturity, grain yield (kg ha^{-1}), above-ground biomass (kg ha^{-1}), HI, and 100-seed weight.

The IciMapping data/file were supplied with the following information, with the options chosen bracketed: (i) General information i.e indicator (mapping), mapping population type (selfing), mapping function (Kosambi), marker space (positions), marker space unit (centiMorgan) (cM), number of chromosomes (8), size of mapping population (188) and number of traits (6 traits listed were used). The other data included (ii) Chromosome information indicating the linkage group (LG) and the number of markers on each LG; (iii) A linkage map specifying the marker, linkage group, and distance; (iv) genotype data; and (v) phenotype data.

This information, prepared in one Excel workbook, was uploaded to ICIM software. The missing phenotypes were set to be deleted and ICIM additive and dominance mapping (ICIM-ADD) were used for QTL detection and estimation of additive and dominance effects. Scanning of the genome was done every 1cM with probability stepwise regression of 0.001 by 1000 permutations of data, which maintained the chromosome type 1 error of 0.05. Kosambi's functions were used to convert recombination percentage to centiMorgan (cM) map unit distances.

RESULTS

Phenotypic evaluation of $F_{3:5}$ genotypes

Irrigated conditions. Genotypes differed significantly ($P<0.05$) for measured traits under irrigated conditions (Table 1). Days to maturity (DM), and 100-seed weight (SDWT) differed significantly for genotype x environment interaction. However, there were no significant genotype x environment interactions ($P>0.05$) for above-ground biomass (BHYA), grain yield (SHYA), and harvest index (HI) traits.

Rain-fed conditions. There were significant differences ($P < 0.05$) among genotypes and genotype x environment interaction, across the three sites; namely: Koibatek ATC, Museresh and KALRO, Perkerra under rainfed conditions (Table 2). Genotypes differed significantly in days to maturity (DM), 100-seed weight (SDWT) and grain yield (SYHA); but not for above-ground biomass (BYHA). The genotype x environment interactions differed

TABLE 1. Mean squares and genotypes mean for yield and yield-associated traits under irrigated conditions under different sites in Kenya

Source	df	DM	BYHA	SYHA	SDWT	HI
Envt	1	20525***	5.5E+08***	1.4E+07***	4.70^{ns}	3.056***
Rep(Envt)	1	7.24^{ns}	3630473^{ns}	434728^{ns}	20.77^{ns}	0.001^{ns}
Block(Rep)	18	10.17^{ns}	1305032^{ns}	171013^{ns}	6.55^{ns}	0.005^{ns}
Genotypes	187	47.36***	2112402**	216341*	18.29***	0.014*
Envt *Gen.	187	36.05***	1579667^{ns}	152058^{ns}	18.51***	0.012^{ns}
Error	364	19.66	1490097	167002	8.97	0.011
Mean		86.513	2291.158	739.633	23.399	0.361
LSD		0.899	247.592	0.608	82.888	0.021

*, ** and ***: Significance level at $P < 0.05$, 0.01 and 0.001, respectively. DM = days to maturity, BYHA = above-ground biomass (kg ha^{-1}), SDWT = 100-seed weight, SYHA = yield in kg ha^{-1} and HI = harvest index

significantly for the measured traits; namely: days to maturity (DM), 100-seed weight (SDWT), above-ground biomass (BYHA) and grain yield (SYHA).

Identification of QTLs

General features of genetic linkage map. A total of 49 SSR markers were mapped into eight linkage groups (LG) that spanned a length of 335.04 cM of the chickpea genome, at an average marker density of 7.21 cM (Table 3). Linkage group three (LG 3) was the smallest (8.73 cM) and had few markers (3) with an average marker density of 2.9 cM. On the other hand, Linkage group two (LG 2) was the longest, spanning a length of 90.63 cM with a marker density of 15.1 cM. Linkage groups 4 and 5 (LG 4 and LG 5) spanned a length of 41.28 cM and 41.29 cM, respectively. These two had marker densities of 5.2 cM each. A total of 32 markers out of 45 were common with one or more of the maps produced by other researchers, represented by an asterisk, while 13 new markers were mapped (Fig. 1a, b).

TABLE 2. Mean squares and genotypes mean for yield and yield-associated traits and phenological traits under rainfed conditions in different sites in Kenya

Source	df	DM	BYHA	SYHA	SDWT	HI
Envt	2	25975***	1.8E+08***	2.5E+07***	220***	1.09***
Rep(Envt)	2	14.62ns	751771ns	159357ns	2.51ns	0.00ns
Block(Rep)	18	7.13ns	702963ns	94695ns	3.91ns	0.01ns
Genotypes	187	31.61***	747280ns	126537**	32.32***	0.01*
Envt *Gen	374	28.39***	900579**	146221**	9.71***	0.01***
Error	555	9.344	691449	113968	3.267	0.006
Mean		81.488	2195.693	803.124	23.590	0.373
LSD		0.618	168.102	68.247	0.365	0.016

*, ** and ***: Significance level at $P < 0.05$, 0.01 and 0.001, respectively; DM = days to maturity, BYHA = above-ground biomass (kg ha^{-1}); SDWT = 100-seed weight, SYHA = yield in kg ha^{-1} and HI = harvest index

TABLE 3. General features of the genetic map of chickpea developed from 49 SSR markers from 188 $F_{3:5}$ population for ICCV 94954 x ICCV 01507

Linkage group	Length (cM)	Number of mapped markers	Average marker density (cM)
LG1	35.99	3	11.9
LG2	90.63	6	15.1
LG3	8.73	3	2.9
LG4	42.25	8	5.2
LG5	41.29	8	5.2
LG6	51.82	10	5.2
LG7	36.27	7	5.2
LG8	28.06	4	7.0
Total	335.04	49	7.21

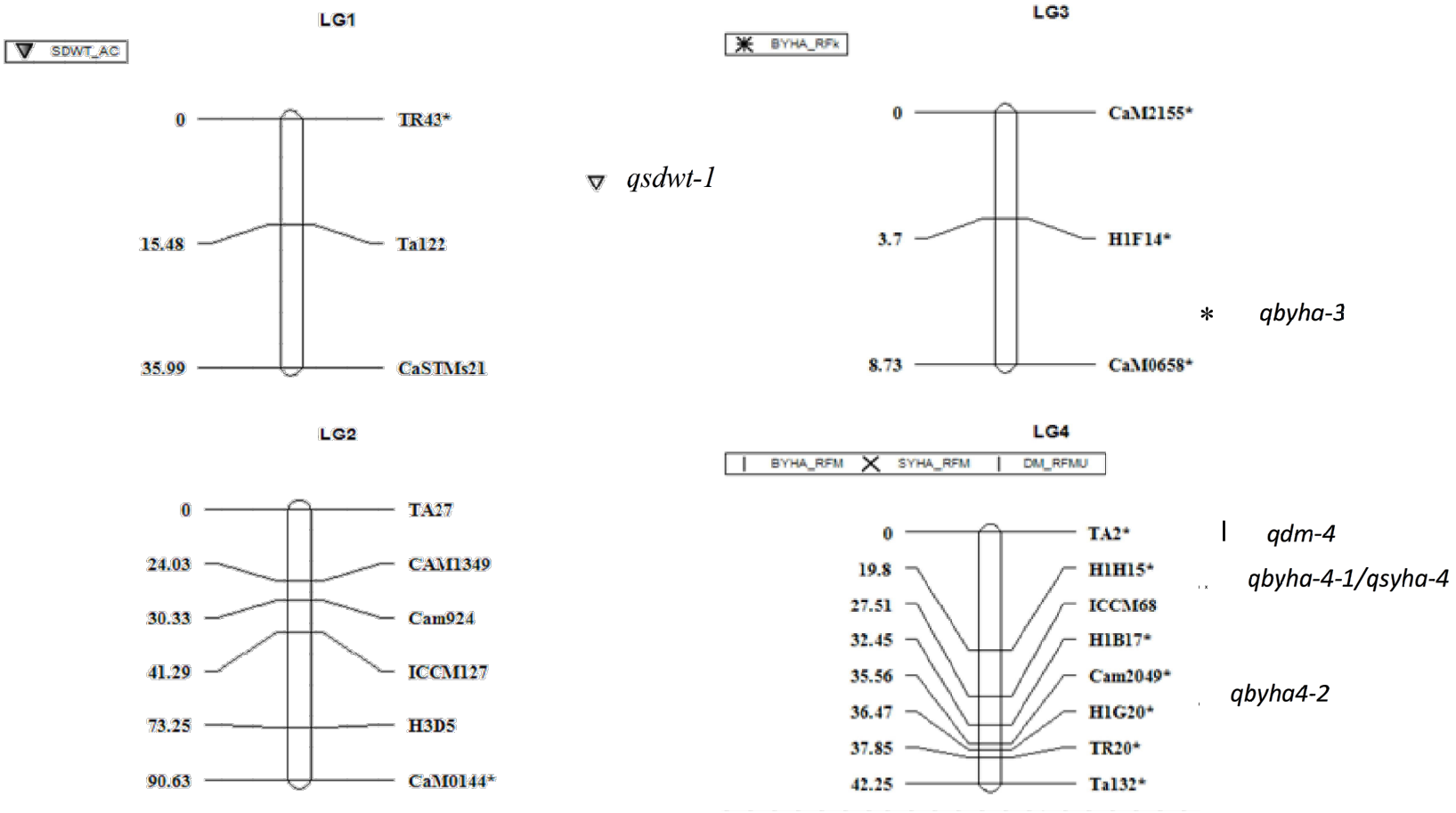

Figure 1a. Linkage map showing QTL on LG1: *qsdwt-1* (QTL for 100-seed weight) LG3: *qbyha-3,* LG4: *qbyha-4-1* and *qbyha4-2* (QTL for above ground biomass) and *qdm-4* (QTL for days to maturity).

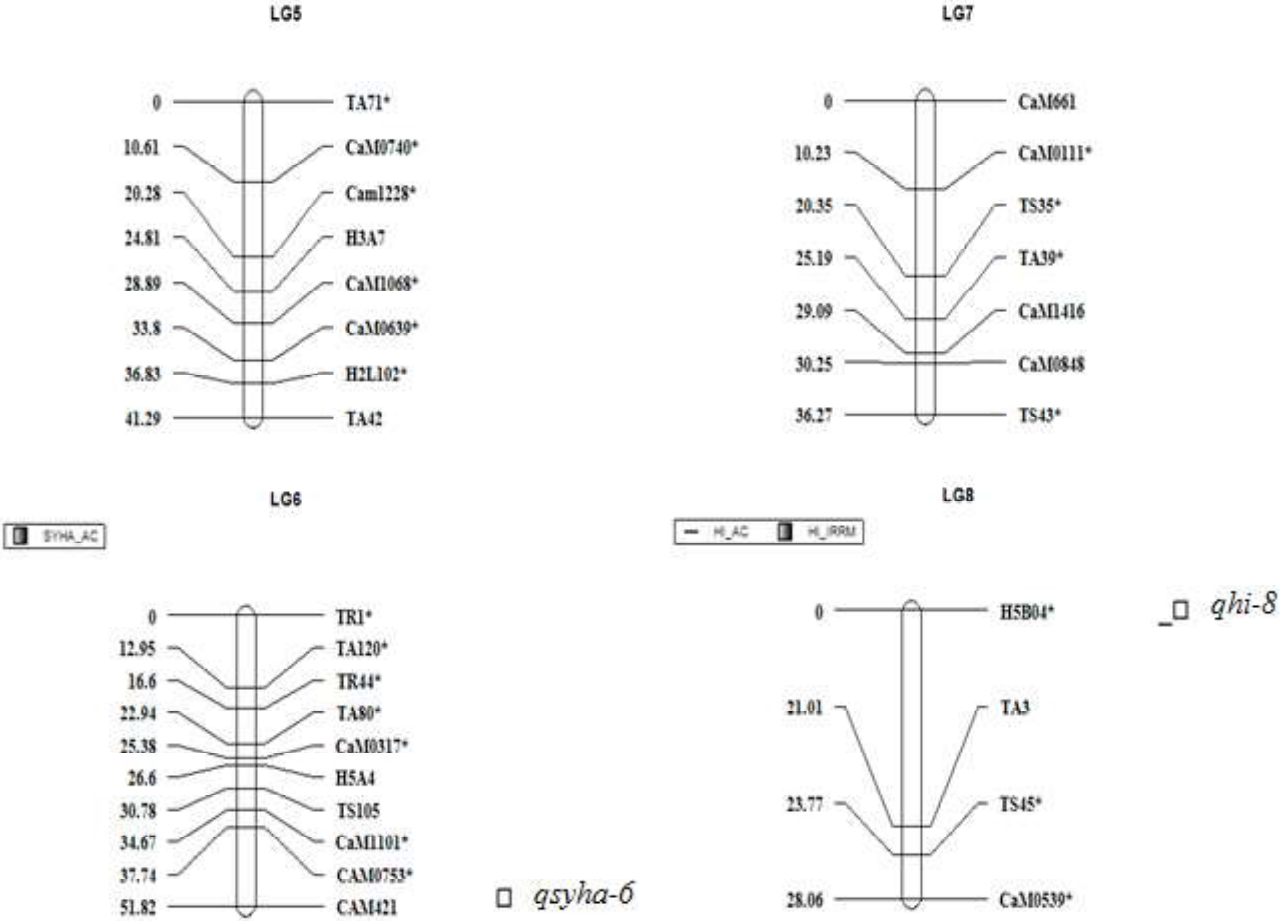

Figure 1b. Linkage map showing QTL on LG6: *qsyha-6* (QTL for 100-seed weight) and LG8: *qhi-8* (QTL for harvest index). *Markers mapped by Winter *et al.* (2000), Tar'an *et al.* (2007), Rehman (2009), Nayak *et al.* (2010) and Hiremath *et al.* (2012).

Mapping of QTLs. Eight QTLs were mapped under different environments (Table 4). Three QTLs were detected for above-ground biomass (BYHA) under rain-fed conditions at KATC and KALRO. One QTL, *qbyha-3,* was detected on LG 3 flanked by H1F14 - CaM0658 at an interval of 5.03 cM with a logarithm of odds (LOD) of 3.3 and 8.7% phenotypic variation expressed (PVE). Two other QTLs were detected on LG 4, *qbyha-4-1* and *qbyha-4-2*, between H1H15 - ICCM68 and H1G20 - TR20, respectively. QTL *qbyha-4-1* had 32.5% PVE while *qbyha-4-2*, had 13.5% PVE and were both considered major QTLs.

Two QTLs for grain yield (SYHA) were identified, one under rain-fed at KALRO and the other in both treatments (rain-fed + irrigation) under the three study locations (Table 4). Under rain-fed conditions, it was found on LG 4 (*qsyha-4*) between H1H15 - ICCM68 with LOD of 3.4 and PVE of 8.2%. This QTL was mapped on the same position as QTL (*qbyha4-1*) for above-ground biomass (BYHA) under the same environment. A second QTL was found on LG 6, *qsyha-6,* mapped between CAM0753 - CAM421 flanking markers, expressing 11.08% phenotypic variation with a LOD of 3.8.

TABLE 4. Quantitative trait loci mapped for grain yield and yield-associated traits; linkage group, position of mapped QTL, LOD, percentage variation expressed, and contributing parent allele

Trait	QTL	Environment	Linkage	Interval markers	Interval	LOD*	Additive^	PVE(%)
BYHA	*qbyha-3*	Rainfed	LG3	H1F14 -CaM0658	5.03	3.2936	152.0293	8.6668
	qbyha-4-1	Rainfed	LG4	H1H15 -ICCM68	7.71	12.7822	-51.9556	32.3926
	qbyha-4-2	Rainfed	LG4	H1G20 - TR20	1.38	5.8727	-37.4196	13.4941
SYHA	*qsyha-4*	Rainfed	LG4	H1H15 -ICCM68	7.71	3.3952	-13.0127	8.2405
	qsyha-6	Irrigated and rainfed	LG6	CAM0753 - CAM421	14.08	3.8	-36.179	11.08
DM	*qdm-4*	Rainfed	LG4	TA2 - H1H15	19.8	4.2902	-0.5971	13.3181
HI^{+}	*qhi-8*	Irrigated	LG8	H5B04 - TA3	21.01	4.2585	0.0006	9.906
	qhi-8	Irrigated and Rainfed	LG8	H5B04 - TA3	18.25	3.4	-0.002	10.37
SDWT	*qsdwt-1*	Irrigated and Rainfed	LG1	TR43 -Ta122	15.48	3.2	0.038	12.19

Key: * = LOD score >3.0, ^ = positive value denotes contribution from the female parent while a negative value is a contribution from donor parent, SDWT = 100-seed weight, SYHA = grain yield, HI = harvest index where HI^{+} = the HI QTL was treated as one

One QTL for HI was mapped under irrigated conditions at KALRO on LG 8 (*qhi-8*) flanked by H5B04 - TA3 with a PVE of 9.9% and a LOD of 4.3 (Table 4). This was the same QTL identified on the same linkage group across environments. One QTL for SDWT was mapped on LG 1 (*qsdwt-1*) on LG 1 between two flanking markers, TR43 and Ta122, with a distance of 15.48 cM in both rainfed and irrigated with a 12.19% PVE. One QTL for DM was mapped on LG 4 (*qdm-4*) under rainfed conditions at Muserech which represented 13.3% PVE. The marker traits were TA2 - H1H15 with an average distance of 19.8 cM and a LOD of 4.3.

Contributions to the expressions of traits mainly came from the parent, ICCV 05017 (donor), rather than ICCV 94954 (recurrent) (Table 4). The parent, ICCV 94954, contributed to high above-ground biomass (BYHA) at KATC; while the ICCV 05017 expressed more at KALRO, Perkerra. The contribution of ICCV 05107 was similar to phenotypic observation under irrigated or rainfed conditions. However, the contribution of ICCV 94954 to 100-seed weight (SDWT) was mostly reductive as observed from phenotypic values.

DISCUSSION

Phenotypic evaluation of $F_{3:5}$ genotypes. The existence of strong genotypic behavior for the trait days to maturity, 100-seed weight, above-ground biomass, grain yield, and harvest index under rainfed and irrigated environments (Tables 1 and 2) could be attributed to genotype x environment interaction. This is an indication that genetic variability existed among the genotypes and that the genotype x environment (G x E) interaction plays a critical role in gene expression. The G x E interaction is also crucial in the identification of QTLs. This is in agreement with results from other authors (Soresa and Nayagam 2019; Tsehaye *et al.,* 2020; Danakumara *et al.,* 2023) and is important in determining yield stability and QTL mapping (Kushwah *et al.,* 2022; David *et al.,* 2023; Danakumara *et al.,* 2023). The genotype x environment interactions are important in identifying different QTLs between environments (Beugnot *et al.,* 2023). This led to the successful mapping of eight QTLs for grain yield and its associated traits and these are important in marker-assisted breeding to improve chickpea productivity.

Genetic Linkage Map. The low polymorphism of approximately 62.5% (Table 3) has been attributed to low genetic variability in chickpea varieties (Gaur *et al.,* 2012; Afza *et al.,* 2018, Varshney *et al.,* 2021). In related findings, Rehman (2009) found a low polymorphism of 42% and 307 SSR markers, out of 2,409 (approx. 12.7%) were polymorphic between chickpea parents screened (Nayak *et al.,* 2010). On the other hand, Lal and Ravikumar (2018) reported that only 60 out of 400 (15%) SSR markers were polymorphic.

The low polymorphism has been linked to deleterious mutations that can be overcome by genomics-assisted breeding and/or gene editing (Varshney *et al.,* 2021).

The mapping of the 45 SSRs on the linkage groups was not different from what was mapped by others with similar markers (Tar'an *et al.,* 2007; Rehman, 2009; Hiremath *et al.,* 2012), though, the orientation and distances differed. This could be due to the type and size of the population used and number of markers. This, therefore, resulted in less coverage of the map and consequently large intervals between markers of detected QTL. However, it was reported that with markers spaced about 10 cM to 15 cM apart, it is possible to identify a few markers associated with the trait of interest if phenotypic data and QTL analysis were done well (Bernardo, 2008). However, according to Yadava *et al.* (2023), large QTL intervals mapped using SSR markers may lead to the introgression of undesirable linkage drag during MAS. Thus,

there is a need for more SSR markers and the use of SNP that are abundant in the genome.

Mapping of QTLs

Rainfed condition. Quantitative trait loci for above-ground biomass (BYHA) on LG 3 and two on LG 4 by different markers, representing major-effect and minor-effect QTLs (Figure 1), could be one QTL placed in a different location within the same LG due to marker recombination. According to Varshney *et al.* (2014), a QTL with a PVE of more than 10% was considered a major-effect QTL while a PVE<10% was a minor-effect QTL. Similar research showed that a QTL for biomass was mapped on LG 3 with a PVE of 13.50% and reported as a major contributing character to seed yield in chickpeas (Jayalakshmi *et al.,* 2020). Therefore, this view was adopted in the current study.

One above-ground biomass (BHYA) QTL on LG 4 (*qbyha-4-1)* was mapped on the same location with QTL for grain yield (SYHA) (8.2% PVE) by the same markers H1H15 - ICCM68, but a minor-effect QTL, indication that transferring this region will lead to varieties with the two traits. Plant biomass may indicate more grain due to its role in better growth, adaptation to water stress, and the emergence of pods. In sorghum, stay green QTLs were responsible for modulated canopy development through reduced pre-flowering water requirements thus increasing availability during grain filling resulting in high grain yield (Borrell *et al.,* 2014).

QTL for grain yield identified on LG 4 agrees with the findings of Varshney *et al.* (2014) that the LG 4 referred to as *CaLG04* has been identified as a '*QTL-hotspot'* region that harbors QTLs for drought tolerant traits including several yield traits. Thudi *et al.* (2014) reported 32 marker-trait associations (MTAs) for yield with a phenotypic variation of between 11.43 - 20.03%; while Thakro *et al.* (2023) identified two major genomic regions harboring 7 QTLs for yield and drought yield index on LG 1 and 4 with PVE varying from 21.8 - 41.3%. Another QTL for grain yield (SYHA) in LG 6 (11.08% PVE) was mapped under rainfed and irrigated conditions and this was treated as a major effect QTL. This indicates that grain yield QTLs could be located in other linkage groups, but majorly on LG 4, and thus a need to identify specific genes governing this trait for an efficient breeding process.

QTL for days to maturity (DM) was on LG 4 (13.3% PVE) and shared one marker with grain yield (SYHA) on the same linkage group; and could mean that the two traits are linked. Days to maturity (DM) is an important trait determining yield and yield stability; and drought tolerance mechanisms. Under drought stress, plants shorten the cycle to escape terminal drought, though, delayed maturity contributes to high yield under optimum conditions. In addition, Rehman (2009) mapped DM on LG 7 which was also associated with the reproductive period. Five MTAs were identified for DM with one marker TA14 explaining 79.31% phenotypic variation (Thudi *et al.,* 2014). Five QTLs were identified on LGs 2 and 8 explaining phenotypic variation between 1.9 and 20.68% (Thudi *et al.,* 2024). Markers linked with DM and have negative effects are important for selecting early mature genotypes, which is a drought tolerance mechanism for crops under limited moisture conditions

Irrigated condition. The distances that were slightly different for HI QTL on LG 8 in irrigated and rainfed conditions (Table 4), could be due to the recombination of the markers. This QTL was treated as one, and there is a likelihood that the QTL for HI is in LG 8. Similar findings mapped HI on LG 8 with a QTL for drought tolerance score (DTS) as well as stomatal conductance, canopy temperature, and various phenological traits (Rehman, 2009). Cobos *et al.* (2007) reported that the same LG 8 was associated with seed weight, indicating that there is a correlation between

HI with seed weight and probably with drought tolerance traits. However, LGs 4 and 6 were reported for this trait by Kushwah *et al.* (2022). The HI is important because genotypes with high HI have a better ability to partition the photosynthates into grain development during drought, which can result in better yield. This will be key in breeding chickpea varieties adapted to drought due to climate change.

Seed weight is an important trait in determining not only the growth habits of the crop but also consumer demand especially for large seeds and processability (Sundaram *et al.,* 2019; Lakmes *et al.,* 2022). Additionally, it correlated with yield and hence useful in indirect selection (Gulwane *et al.,* 2022; Kandwal *et al.,* 2022), and has a high heritability and genetic advancement (Jayalakshmi *et al.,* 2020), hence important in genetic improvement for phenotypic selection of grain yield trait.

QTL for 100-seed weight on LG 1, under irrigated and rainfed, was mapped in different locations by other authors (Figure 1). Verma *et al.* (2015) mapped 7 QTLs on LGs 1, 2, 5, 6 and 7; while Lal and Ravikumar (2018) mapped two QTLs on LGs 1 and 2. In marker-trait associations (MTAs), 26 markers were identified falling in '*QTL-hotspot'* region reported on LG 4 (CaLG04) (Thudi *et al.,* 2014). Kushwah *et al.* (2022) mapped the QTL for 100-seed weight on LG 6 and 7, Yadava *et al.* (2023) mapped two QTLs on LGs 5, and 7 with a PVE ranging between 44.15 to 49.58%. In comparison with other findings, this study found that there is a likelihood the QTL for seed weight is located in LG 1 and other LGs with varying expressions and this is an indication that several genes govern the trait.

Yield and yield-associated traits are important in improving chickpea productivity, especially with climate change and the need to meet food security and nutrition for the increasing global population. This has led to continuous improvement in technologies such as the use of genomics which have led to novel, precise, and efficient breeding processes (Roorkiwal *et al.,* 2020; Jain *et al.,* 2023; Singh *et al.,* 2023). Genomics has been useful in identifying QTLs associated with traits important in crop improvement (Barmukh *et al.,* 2021; Kushwah *et al.,* 2022; Istanbuli *et al.*, 2024). The mapping of the QTLs for yield and associated traits differed in location on LGs with various authors including the results from the present study (Table 4). This indicates that traits are controlled by different genes. Therefore, there is a need to identify the different putative genes for application in MAS breeding to hasten the variety release process for farmers.

CONCLUSION

Eight QTLs were mapped on a linkage map spanning a total length of 335.04 cM, with a marker density of 7.21 cM. Three QTL for above-ground biomass (BYHA) were identified, one on LG 3 (8.67% PVE) and two on LG 4 (13.5 - 32.4% PVE), two for SYHA on LGs 4 and 6 (8.24 - 11.08% PVE); and one each for SDWT on LG 1 (12.19% PVE), HI on LG 8 (9.9% PVE) and DM on LG 4 (13.31%). Four QTLs were mapped on LG 4; two QTLs for above-ground biomass (BYHA), one for grain yield (SYHA), and one for DM supporting the research that reported this region as a '*QTL hotspot'*. The location of these QTLs for the studied traits differed with various authors, indicating several genes controlling the traits. There is a need for more markers to be mapped in these regions and the identification of putative genes for these traits. A highly saturated linkage map is valuable in genetic studies, mapping quantitative trait loci (QTLs), facilitating marker-assisted breeding, and identifying putative genes. Marker trait associations and genes associated with QTL for yield and yield-associated traits will be useful for molecular breeding in chickpea improvement. In addition, the selection of genotypes with high genetic value,

based on identified QTL will be useful in chickpea improvement through gene pyramiding by marker-assisted recurrent selection (MARS) and marker-assisted backcrossing (MABC) breeding methods. Furthermore, the 188 genotypes responded differently to environmental conditions and therefore, there is a need for further evaluation across different environments to identify stable yields in a given agroecological zone.

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