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Inheritance of seed weight and growth habit in 10 intercross chickpea (*Cicer arietinum*) nested association mapping populations

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Abstract

Objective of investigation: Chickpea is a major global food legume for which seed weight and plant growth habit are important yield and harvestability components for plant breeding. This study tested seed weight and plant growth habit inheritance and identified quantitative trait loci (QTL).

Experimental material: A 10 nested association mapping (NAM) populations of chickpea were created from crosses between 'Gokce', a cultivar and wild crop relative accessions of *Cicer reticulatum* and *Cicer echinospermum*. Families were then developed to the F2:4 generation.

Method of investigation: A 10 families were grown at the Field Experiment Station, Harran University near Şanlıurfa, Turkey during 2019.

Data collection: A 100-seed weight and prostrate or erect growth habit was scored in the field. Two families were genotyped for 60 single-nucleotide polymorphisms (SNP).

Result and conclusions: A 100-seed weight showed polygenic control, and three QTLs were found. Growth habit was controlled by one or two QTLs. The two traits were significantly correlated for five populations. The crop wild relatives of chickpea contain variations at novel loci affecting seed weight compared to the literature.

KEYWORDS

100-seed weight, chickpea (*Cicer arietinum*), crop wild relative, growth habit, nested association mapping (NAM) family, quantitative trait locus (QTL)

1 | INTRODUCTION

Chickpea is the most produced legume after common beans in the world. Major chickpea producing countries include India, Pakistan, Mexico, Turkey, Canada, Syria and Australia, altogether accounting for over 20% of world pulse production (FAO, 2022). Chickpea is one of only two legume crops that provide all essential amino acids and are important sources of B vitamins (B1, B2, B5 and B6), several minerals and energy (Thavarajah, 2012). As a consequence, chickpea is an important part of subsistence diets and food security, for example, in Ethiopia (Woolf et al., 2011; Young & Pellett, 1994), and chickpea cultivation has been proposed as a tool for reducing childhood malnutrition (Malunga et al., 2014). The protein content nutritive seed composition of chickpea is also used

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increasingly as a substitute for animal protein (Bampidis & Christodoulou, 2011).

Selection for increased 100-seed weight are important for yield, meeting consumer demand and improving processability (Hossain et al., 2010; Sundaram et al., 2019). Similarly, erect growth habit is important for mechanized harvesting to improve production (Gaur, 2018). Indeed, the Western Asia region has typically achieved yields of chickpea greater than the world mean yield between 2011 and 2020 (Western Asia 12,361 hg/ha versus World 9831 hg/ha, FAO, 2022) in part due to the large-seeded kabuli varieties that are grown in this region. Although some countries in the region, such as Turkey, perform well in terms of mean yield between 2011 and 2020, other countries with high dependency on chickpea, such as Syria, require improvement (Turkey 12,305 hg/ha versus Syria 6833 hg/ha). In addition, large seeds can provide associated benefits of improved germinability and growth at early growth stages (Dahiya et al., 1985; Gan et al., 2011; Narayanan et al., 1981).

Divergent types of chickpea show large differences in seed size ranging from large-seeded kabuli types and smaller seeded desi types that are gown in different regions. Therefore, it is important to understand the inheritance of these traits as a tool for their further improvement. Previous studies investigating cultivated chickpea variation have identified several major QTLs for seed size (Abbo et al., 2005; Cho et al., 2002; Cobos et al., 2007; Jamalabadi et al., 2013). These studies have also noted an association between seed weight and growth-habit characteristics such as plant height. These trait associations could reflect pleiotropic or epistatic genetic effects, physical linkage of contributing genes or linkage disequilibrium left over from parental associations (e.g., wild x cultivar crosses). Wild chickpea shows a typically prostrate growth habit compared with the erect growth habit of cultivars, which has major impacts on harvestability and yield (Kumar et al., 2003).

Modern chickpea, like many crops, shows limited genetic and phenotypic diversity (Abbo et al., 2003; Singh et al., 2021). Wild crop relatives have potential for crop improvement but integrating their variation into crop varieties can be a difficult plant-breeding challenge (McCouch, 2012; Warschefsky et al., 2014). Cultivated chickpea was domesticated from two wild relatives, *Cicer reticulatum* Ladiz and *Cicer echinospermum* P.H. Davis, from Northern Syria and Southern Turkey (Von Wettberg et al., 2018). There is scope to introduce alleles for favourable traits such as improved tolerance to environmental conditions into chickpea by hybridizing these primary and secondary genepools with chickpea cultivars (Kahraman et al., 2017; Maphosa et al., 2020; Singh et al., 2021; Singh & Ocampo, 1997).

1.1 | Project aims

We performed wide crosses between a cultivated chickpea variety (kabuli type) and multiple wild *C. reticulatum* and *C. echinospermum* genotypes and developed advance generation nested association mapping populations to test the inheritance of chickpea traits of interest, including 100-seed weight and growth habit for future plant-breeding programmes. We examined the distribution of 100-seed weight in nine families and performed tests for the number of major genes and correlations with growth habit. Finally, we performed SNP genotyping and QTL mapping of 100-seed weight and growth habit for two of these families.

2 | MATERIALS AND METHODS

2.1 | Field experiments

This research was carried out at the Field Experiment Station of Harran University, Turkey (37.10 N 39.06E, 550 m altitude, 'hot dry summer' CSA Köppen climate type) between January and June 2019 and 2020. The 10 nested association mapping (NAM) populations used in this experiment have been described previously (Lakmes et al., 2022). Briefly, the samples consist of approximately 1700 distinct lines from 10 families, each derived from an original cross between the chickpea (*Cicer arietinum*) kabuli-type cultivar 'Gokce' and a wild accession of either *C. reticulatum* or *C. echinospermum* (described in von Wettberg et al., 2018) that were then progressed by spontaneous self-pollination for four generations. Different to the classic NAM design, five seeds were sampled from each line at each generation from the F_2 stage to prevent excessive loss of lines. Therefore, each line maintains the heterozygosity present at the F_2 stage and was treated as such for the following quantitative genetic analyses. The development of these NAM populations allows the genetic basis of many complex quantitative traits to be investigated.

In each year, the five seeds per line were planted in the field with 20 cm intra-row and 50 cm inter-row spacing into 68 blocks of 25 lines each. Parental lines were included in each block as part of an augmented design to account for block effects (Federer, 1961). Phenotypic measures were made on the $F_{2:4}$ generation during the 2019 growing season only as plants were infected by *Ascochyta* blight during 2020. Pods were harvested daily by hand as they turned from green to yellow to prevent mixing between lines as many lines retained the pod-shattering trait of their wild progenitor (Ladizinsky, 1979). The 100-seed weight was recorded on a balance for manually cleaned seed during the summer following harvest. Growth habit per line was recorded as either erect (cultivar-like), prostrate (wild-like) or a mixture (heterozygous) at time of flowering by comparing with the growth-habit parental lines in the same block. Phenotype data are provided in Supporting Information

2.2 | Quantitative genetics analysis

Statistical analysis was performed with Microsoft Excel (Redmond, USA), SPSS v23 (IBM Armonk, USA) and R v3.6.2 (https://www.r-project.org). Augmented design was used to estimate differences between blocks based on replicated samples, which were then used to adjust the seed weight data controlling for field block effects using the R package 'plant breeding' (Rosyara, 2012). The adjustment was

done in two steps: first, for replicates of the two parents and second, for replicates of the 'Gokce' parent across all blocks and families. Summary descriptors for each family were calculated, and the seed weight distribution in each family was tested for normality using Shapiro–Wilk's W test in R.

Goodness of fit chi-square tests were applied to growth-habit frequencies per family, to check for expected patterns of segregation, assuming one- or two-gene inheritance. The expected ratio for a single gene was 1:1 and for two genes 1:3 (Hossain et al., 2010). To account for the experimental design of sampling five seeds per line over two generations, only counts of fully homozygous rows were included in this test, as the ratio of each homozygote will remain unchanged as heterozygotes lines are equally likely to fix for either allele (Lakmes et al., 2022).

In order to test the extent to which traits were inherited independently, Spearman's rank correlation tests were performed between 100-seed weight and growth habit scored as 1 for cultivar-like erect, 2 for heterozygous and 3 for wild-like prostrate for each family. Spearman's rank correlation tests are appropriate for categorical data, but ordinal values allow to identify the direction of associations. Because the cultivar parent 'Gokce' has large seeds and an erect growth habit, then significant correlations would suggest linkage between the major genes controlling these traits.

2.3 | Genotyping

The 'Gokce' × Oyali-084 (GO) and 'Gokce' × Karab-092 (GK) families were prioritized for genotyping as they represented a *C. arietinum* × *C. reticulatum* cross with light seed weight and a *C. arietinum* × *C. echinospermum* cross with heavy seed weight, respectively, and could be expected to uncover distinctive seed weight QTLs. During both the 2019 $F_{2:4}$ and 2020 $F_{2:5}$ growing seasons, leaf samples were collected from each line of the two families in the field and dried in separate paper envelopes. Genotyping of samples from 2019 was described previously (Lakmes et al., 2022) Briefly, Illumina HiSeq 150 basepair paired end whole genome sequencing of each parent was performed by Novogene (Cambridge, UK). Reads were mapped to the NCBI chickpea reference genome CDC Frontier (BioSample: SAMN02981489). Next, 48 Kompetitive Allele Specific PCR (KASP) markers from the list maintained by Biosearch Technologies (Hoddesdon, UK) that were polymorphic between 'Gokce' and the wild accessions and distributed across the genome were selected for genotyping. A 95 samples from each family were then KASP genotyped by Biosearch Technologies. Because each sample was a pool of five individuals from each line and did not generate discrete homozygous and heterozygous KASP fluorescence signals, the distribution of KASP fluorescence signals for each SNP were checked manually to determine suitable thresholds to call genotypes.

In 2020, a mostly overlapping set of 113 GO lines and 104 GK lines were KASP genotyped by Biosearch Technologies. The 48 SNPs genotyped in 2020 mostly overlapped with those of 2019, but the 12 worst performing SNPs were replaced with others to ensure good coverage. The SNPs genotyped in 2019 are presented in previous studies (Lakmes et al., 2022), and the SNPs genotyped in 2020 are provided in Supporting Information. The genotype data across the two years were compared and combined, excluding genotypes that were different across the two years. The final dataset consisted of 160 GO lines and 152 GK lines genotyped for 60 SNPs (74.6% and 75.1% coverage, respectively). The combined genotypes are provided in Supporting Information. The genotypes from 2019 are presented in previous studies (Lakmes et al., 2022), and the genotypes from 2020 are presented in Supporting Information.

2.4 | Quantitative trait loci (QTL) analysis

A physical genetic map of the SNP genomic locations on the reference CDC Frontier chickpea genome from NCBI (BioSample: SAMN02981489) in 1 Mbp units was used. Quantitative trait locus analysis was performed with the R package qtl2 (Broman et al., 2018) as described previously (Lakmes et al., 2022). Briefly, genotype and map data were transformed to a matrix of genotype probabilities at 1 Mbp step intervals and used to calculate a kinship matrix between samples, leaving out the focal chromosome ('loco' option). Then linear mixed models with trait covariates were performed to identify QTL regions with logarithm of odds (LOD) scores greater than the 95% confidence interval of the 5000-step permutation threshold.

The QTL regions were plotted using MapChart (Voorrips, 2002). The QTL effect sizes for each genotype, additive and dominant effects and genotype × phenotype plots were extracted for the marker closest to each QTL peak using R package qtl2. Percentage variance explained (PVE) was calculated from LOD scores according to $1–10^{(-2\ LOD/n)}$, where n is the number of measured phenotypes.

3 | RESULTS

3.1 | 100-seed weight

The mean adjusted 100-seed weight of the NAM families was between 19.54 and 27.41 g ('Gokce' × Oyali-084 and 'Gokce' × S2Drd-065, Table 1). The adjusted 100-seed weight of majority of the individual lines fell between the two parents, but a few lines in some populations had transgressive 100-seed weight greater or less than the most extreme parent (Figure 1). Lines with high 100-seed weight would be of interest to progress for breeding. Also, the frequency distribution of 100-seed weight showed that some NAM families, such as 'Gokce' × S2Drd-065, were of potential use for breeding with relatively heavy mean 100-seed weight.

There were significant differences between populations in mean 100-seed weight (ANOVA $F = 27.79$, $p < 2E10^{-16}$). Within populations, the 100-seed weight variation was mostly continuous, and it conformed to a normal distribution in half of the populations (Table 1), with the remaining populations showing broader tails to their distributions. These results are indicative of polygenic control of 100-seed weight.

TABLE 1 Summary of 100-seed weight observations for chickpea NAM families and Spearman's rank correlation with plant growth habit

Family male parent	Number of family lines	Parent 1female (Std. dev.)	Parent 2male (Std. dev.)	Mid parent value	Family mean (Std. dev.)	Correlation
Baril-092 (r)	161	40.13(.51)	10.60(0.37)	25.36	20.33(5.34**)	−.22**
Cudi1−152 (r)	184	39.94(.46)	12.73(.22)	26.33	23.89(6.90)	−.12
Cudi2−022 (r)	155	40.13(.51)	12.70(.42)	26.41	22.94(5.64)	−.22**
Egil-073 (r)	185	40.04(.32)	13.25(3.48)	26.64	24.63(5.34**)	−.26**
Egil-065 (r)	169	40.13(.45)	1.36(.17)	25.24	20.22(4.72)	−.11
Oyali-084 (r)	189	40.16(.48)	12.41(2.91)	26.29	19.60(4.46**)	−.08
Savur-063 (r)	191	40.10(.61)	10.58(0.23)	25.34	21.48(4.68)	−.14
Sirna-060 (r)	185	40.29(.36)	8.65(0.13)	24.47	21.67(5.95**)	−.18*
S2Drd-065 (e)	113	40.38(.26)	13.32(.32)	26.85	27.40(6.23)	.00
Karab-092 (e)	168	4.40 (.77)	12.11(0.33)	26.26	24.76(6.11**)	−.31**

Notes: The female parent was *Cicer arietinum* cultivar 'Gokce', and the male parent was the wild accession listed in the left column. '(r)' and '(e)' after male parent names indicate the species as *Cicer reticulatum* or *C. echinospermum*, respectively. Values are derived from augmented design adjusted seed weights. Asterisks in standard error column indicate if the population does not show a normal distribution. Correlations are between 100-seed weight and growth habit and are nonsignificant unless followed by asterisks.
*indicates $p < .05$.
**indicates $p < .01$.

3.2 | Growth habit

All of the families showed segregation for both erect and prostrate growth habit (Figure 2). Eight of the 10 NAM families had a higher frequency of erect growth habit than for prostrate growth habit except for 'Gokce' × Baril-092 and 'Gokce' × Cudi-52 suggesting mostly dominant expression of alleles for erect growth habit. Chi-square tests (Table 2) accepted a 1:1 ratio of growth habit segregation among homozygous genotypes for four families, suggesting a single gene of major effect for growth habit ('Gokce' × Bari-092, 'Gokce' × Cudi2−022, 'Gokce' × Egil-073 and 'Gokce' × Sirna-060). Four further populations ('Gokce' x Cudi1−152, 'Gokce' × Egil-065, 'Gokce' × Savur-063 and 'Gokce' × S2drd-065) accepted a 1:3 segregation ratio among homozygous genotypes, suggesting two genes of major effect. Only two populations ('Gokce' × Oyali-084 and 'Gokce' × Karab-092) did not accept either gene model, suggestive of more complex genetic control.

3.3 | Correlations

The 100-seed weight data was significantly negatively correlated with prostrate growth habit for five populations ('Gokce' × Bari1−092, 'Gokce' × Cudi2−022, 'Gokce' × Egil-073, 'Gokce' × Sirna-060 and 'Gokce' × Karab-092) (Table 1) meaning that some of the genes for these traits might be genetically linked.

3.4 | QTL

Three significant QTLs each were detected for 100-seed weight and growth habit (Table 3, Figure 3). A seed weight QTL was found on chromosome 1 in the GO family and two different seed-weight QTLs were found on chromosomes 4 and 7 of GK family. These seed weight QTLs had PVE ranging from 8.72 to 11.39% and negative additive effect sizes ranging from −2.16 to 2.94 g in the direction of the wild allele (O). However, phenotype × genotype plots of these QTLs showed that just one homozygous wild genotype (OO) was present at the GK chromosome 7 genetic map location, unlike the other two QTLs (Figure 4). Two growth-habit QTLs on chromosomes 1 and 6 were detected for the GO family and one QTL on chromosome 1 for the GK family. Although both families had a growth-habit QTL on chromosome 1, the 95% confidence range in locations did not overlap between these QTLs, suggesting that they might represent different genetic loci. These QTLs had PVE ranging from 11.24 to 26.45% and positive additive effects in the direction of the wild allele (O) with the wild-type score of three (Table 3, Figure 5).

4 | DISCUSSION

This study measured the inheritance of the important agricultural traits, 100-seed weight and growth habit in wide cultivar × wild cross chickpea NAM families. The two traits showed contrasting continuous distribution for 100-seed weight and discrete distribution for growth habit, but their QTL architecture was similar. The 100-seed weight showed continuous, broadly distributed variation, and three QTLs of major effect >8% PVE were detected across the two genotyped families. Growth habit was inherited as a discrete trait with phenotypes conforming to one or two single gene control, but three QTLs of major effect >11% PVE were also detected across the two mapping families. Prostrate growth habit and 100-seed weight showed significant correlations in half of the NAM families, suggesting genetic

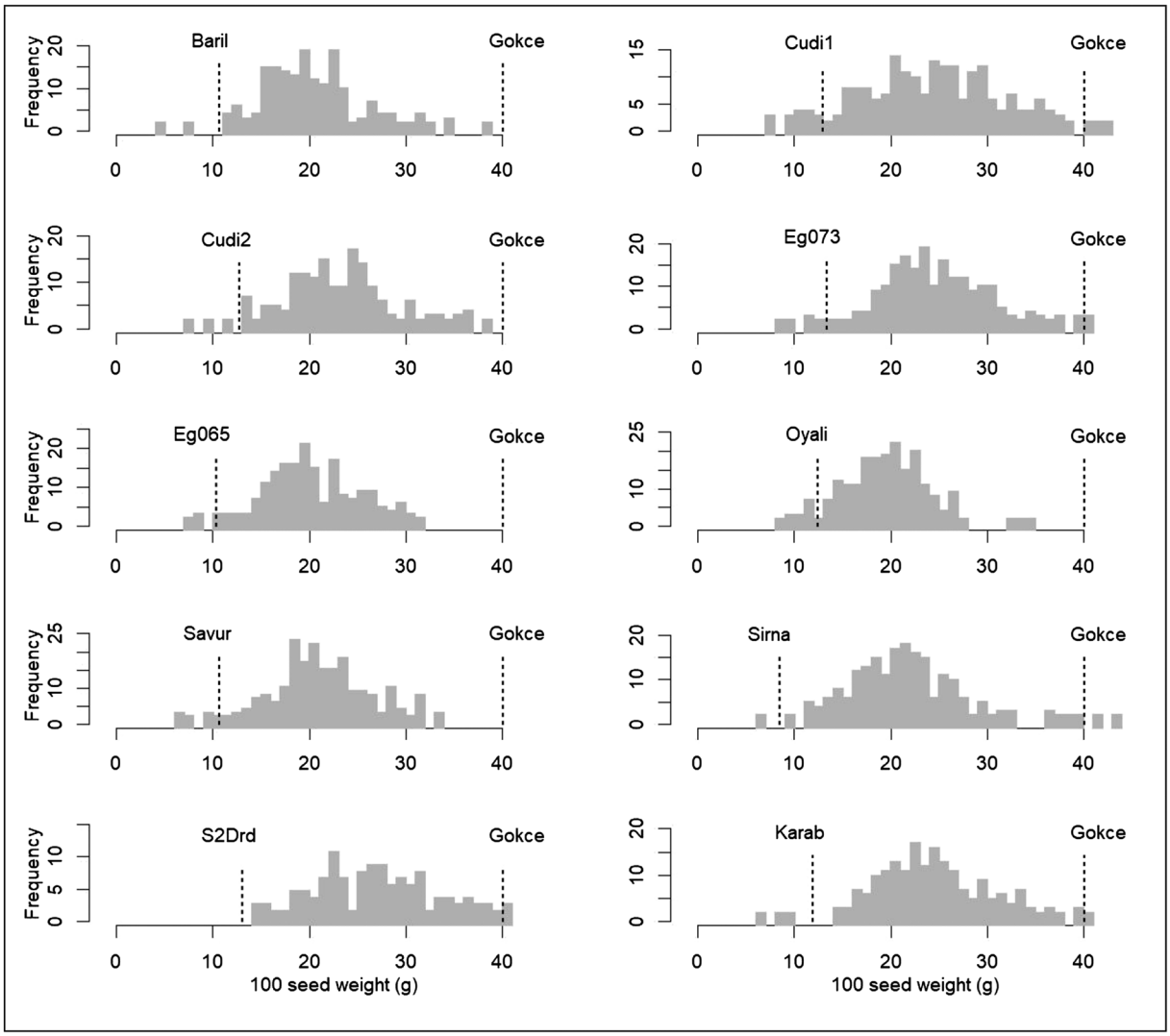


FIGURE 1 Frequency distributions of 100-seed weight for chickpea 10 NAM families. Labelled vertical dotted lines indicate parental 100-seed weight values.

linkage. A QTL region at the start of chromosome 1 for each trait could be contributing to the linkage between traits.

This study observed some genotypes with transgressively light or heavy seeds within each of the intercross NAM families and three different QTL regions contributing to the variation. Transgressive inheritance of seed weight has previously been observed in crosses between distinct chickpea cultivars (Kivrak et al., 2020). Past QTL studies of chickpea 100-seed weight have found different patterns of inheritance ranging from one or two major gene controls to multigenic quantitative inheritance (Cobos et al., 2007; Hossain et al., 2010; Singh et al., 2016; Sohail et al., 2018). These differences depend possibly on the cross parents used in these experiments.

Some dominance or complementary gene interactions towards lighter seed weight were found in nine mapping populations, with mean population values being typically smaller than midparent values, except for one population ('Gokce' × S2drd-065). The literature presents contrasting results in this regard with reports of dominance of either large seed alleles (Niknejad et al., 1971; Upadhyaya et al., 2006) or small seed alleles (Hossain et al., 2010; Kumar & Singh, 1995; Malhotra et al., 1997). Alternatively, these results could reflect some hybrid breakdown effects in these wide population crosses, caused by genetic incompatibilities between greatly diverged parents, as has previously been observed in chickpea (Kahraman et al., 2017). Seed weight heritability has generally been found to be high in the literature (broad and narrow sense heritability estimates between .81 and 95; Cobos et al., 2009; Hossain et al., 2010; Malhotra et al., 1997; Niknejad et al., 1971). In this experiment, the large difference between parents in these wide crosses might also have contributed to the observed standard deviations. More research, including replication of genotypes across years, is required to estimate heritability in these mapping populations and control for environmental variation.

Growth-habit differences between wild and cultivated chickpea appear to be under simple genetic control in agreement with other reports from the literature (Aryamanesh et al., 2010; Kumar

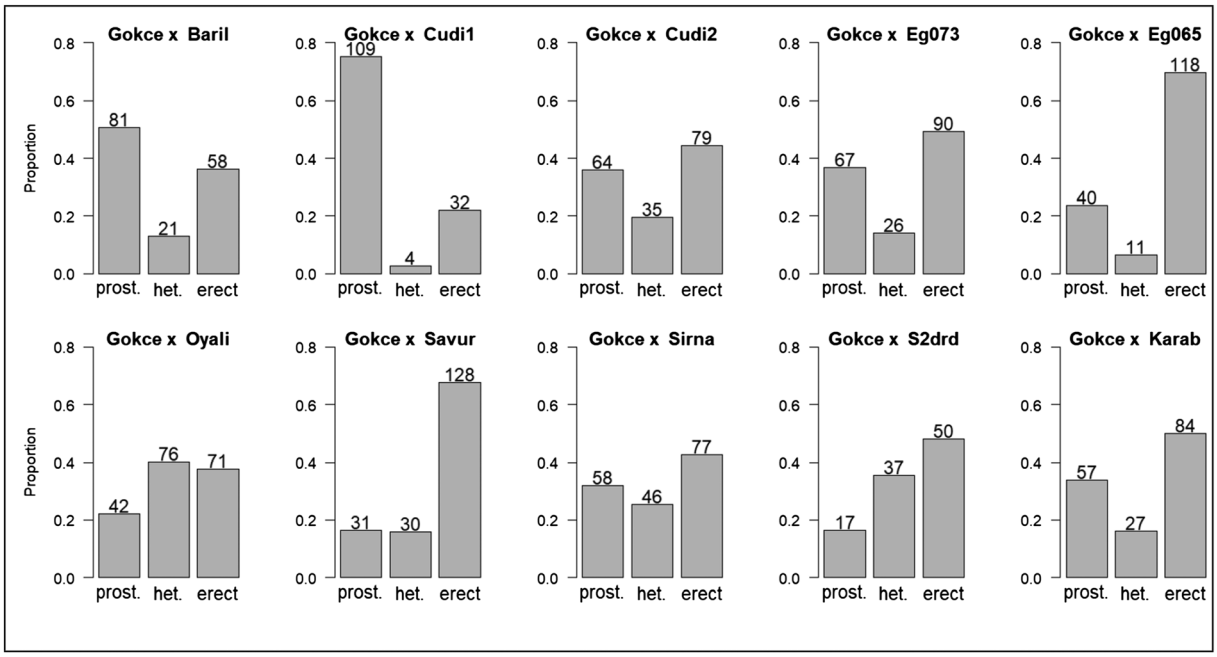


FIGURE 2 Growth habit frequencies for 10 chickpea NAM families. Numbers above bars are counts. 'Prost.' indicates prostrate growth habit, and 'het.' indicates a mix of erect and prostrate growth habits within a line.

TABLE 2 Tests of one or two gene inheritance of growth habit frequencies of chickpea NAM families

Family male parent	Erect (cultivar) phenotype	Prostrate (wild) phenotype	Df	Ratio tested	X^2 (sig.)
Bari1–092	58	81	1	1:1 1:3	3.8120.74**
Cudi1–152	32	109	1	1:1 1:3	42.05** 0.40
Cudi2–022	79	64	1	1:1 1:3	1.5729.21**
Egil-073	90	67	1	1:1 1:3	3.3726.16**
Egil-065	118	40	1	1:1 1:3	38.51**0.01
Oyali-084	71	42	1	1:1 1:3	7.44* 8.92*
Savur-063	128	31	1	1:1 1:3	59.18**2.57
Sirna-060	77	58	1	1:1 1:3	2.6723.23**
S2Drd-065	50	17	1	1:1 1:3	16.25**0.01
Karab-092	84	57	1	1:1 1:3	5.17*17.89**

Note: Asterisks indicate the significant deviations from goodness of fit chi-square tests. Accepted gene models are indicated with bold text ratios and chi test results.
*indicating significant $p < .05$.
**indicating $p < .001$.

et al., 2003; Singh & Shyam, 1959), although up to eight genomic regions were found to be associated with this trait using genome-wide association study (Upadhyaya et al., 2017). Prostrate growth habit was significantly correlated with 100-seed weight in five of the 10 NAM families, suggestive of genetic linkage. The presence of QTLs for both traits on chromosome 1 also supports this finding. Alleles for

TABLE 3 Summary of quantitative trait loci for 100-seed weight and growth habit for two chickpea NAM families. Locations are described by chromosome number, position in 1 M bp units and 95% confidence limits in parentheses. LOD is logarithm of odds score. PVE is the percentage variance explained. SNP is marker nearest to the QTL peak. SNPs named c#.loc# were not directly genotyped but are genotype probabilities at 1 Mbp map intervals. Mu is the model predicted mean trait value. Growth habit units were a score of 1 for cultivated erect, 2 heterozygous erect and prostrate and 3 for wild prostrate growth habit. Genotype effect sizes on the mean phenotype are summarized as R for the reference cultivated allele and O is the other wild allele. Genotype RO is a measure of the dominance effect. The additive effect is calculated as (OO−RR)/2

Trait	100-seed weight (g)			Growth habit		
Family	Gokce × Oyali	Gokce × Karab		Gokce × Oyali		Gokce × Karab
Location	114.3 (0.3–48.3)	410.9 (5.9–21.9)	728.2 (10.7–36.9)	148.3 (17.6–48.3)	656.0 (49.6–59.0)	111.1 (5.7–16.4)
LOD	3.09	3.81	3.97	10.68	4.14	7.41
PVE	8.73	10.97	11.39	26.45	11.24	20.11
SNP	c1.loc14	c4.loc11	c7.loc28	Ca_TOG898978	c6.loc56	Ca1C39227
Mu	20.13	22.63	23.96	2.07	2.07	2.10
RR	2.53	2.33	3.37	−.64	−0.37	−.75
RO	−.73	1.22	−1.59	0.21	.34	−.07
OO	−1.79	−3.55	−1.78	.43	.03	.83
Add. Effect	−2.16	−2.94	−2.57	.54	0.20	.79

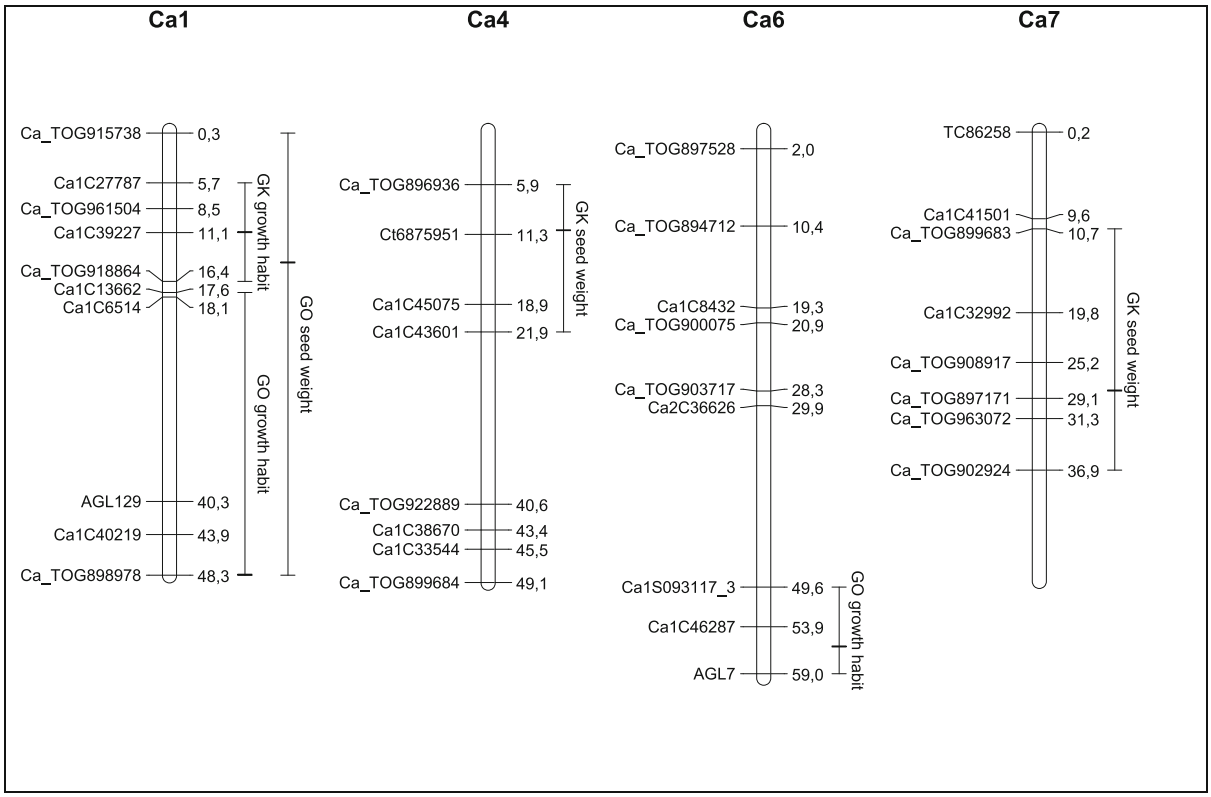


FIGURE 3 Genetic map of chickpea chromosomes 1, 4, 6 and 7 showing quantitative trait loci for 100-seed weight and growth habit in the mapping families, 'Gokce' × Oyali-084 (GO) and 'Gokce' × Karab-092 (GK). Chromosomes (linkage groups) are shown as white vertical bars with genotyped SNP positions marked with horizontal dashes and labels to the left of chromosomes. QTLs for each family and trait are shown as vertical lines to the right of the corresponding chromosome with a horizontal dash or wider bar showing the QTL LOD peak or peak range between the two families.

greater seed weight are currently associated with alleles for erect growth habit in these mapping populations, which will facilitate selection of favourable phenotypic combinations of these two traits, but genetic linkage should be considered as part of future plant-breeding efforts. Erect growth habit has also been found to be highly correlated with early flowering time in another wide cross between *C. arietinum*

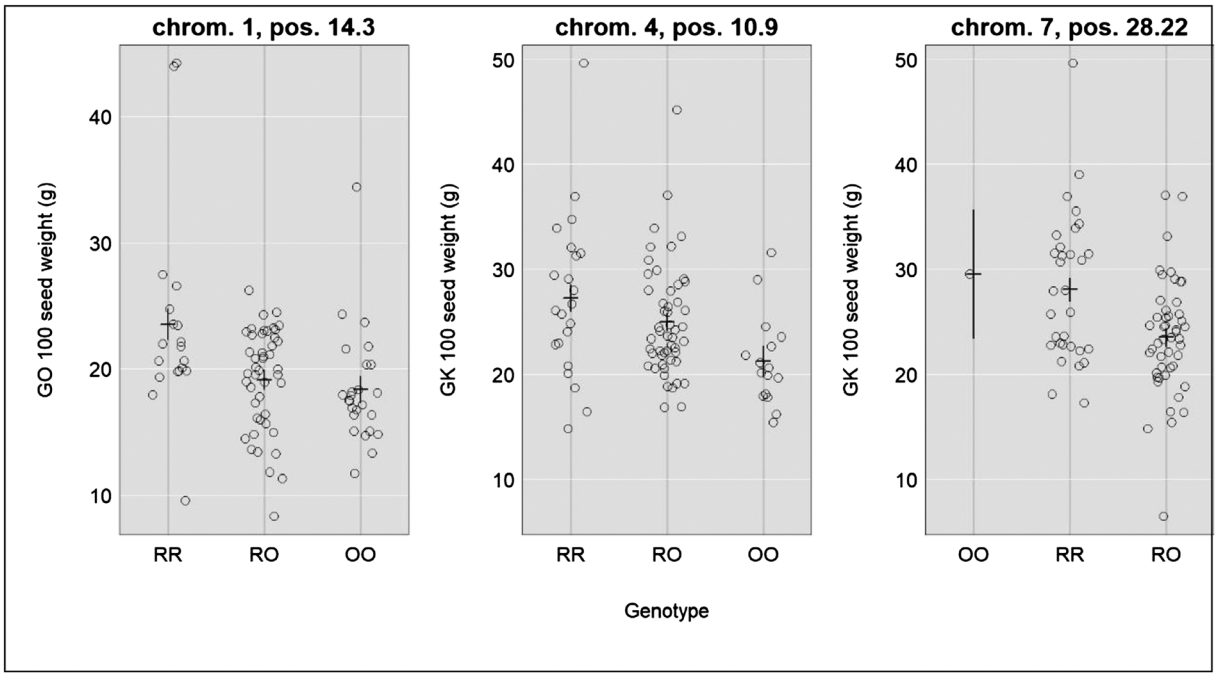


FIGURE 4 Phenotype × genotype plot for 100-seed weight at three QTLs in two chickpea mapping families 'Gokce' × Oyali-084 (GO) and 'Gokce' × Kara-092 (GK). The x-axis shows genotypes made up of cultivated reference (R) or wild other (O) alleles. Circles are per individual measures. Crosses are the mean and standard error per genotype.

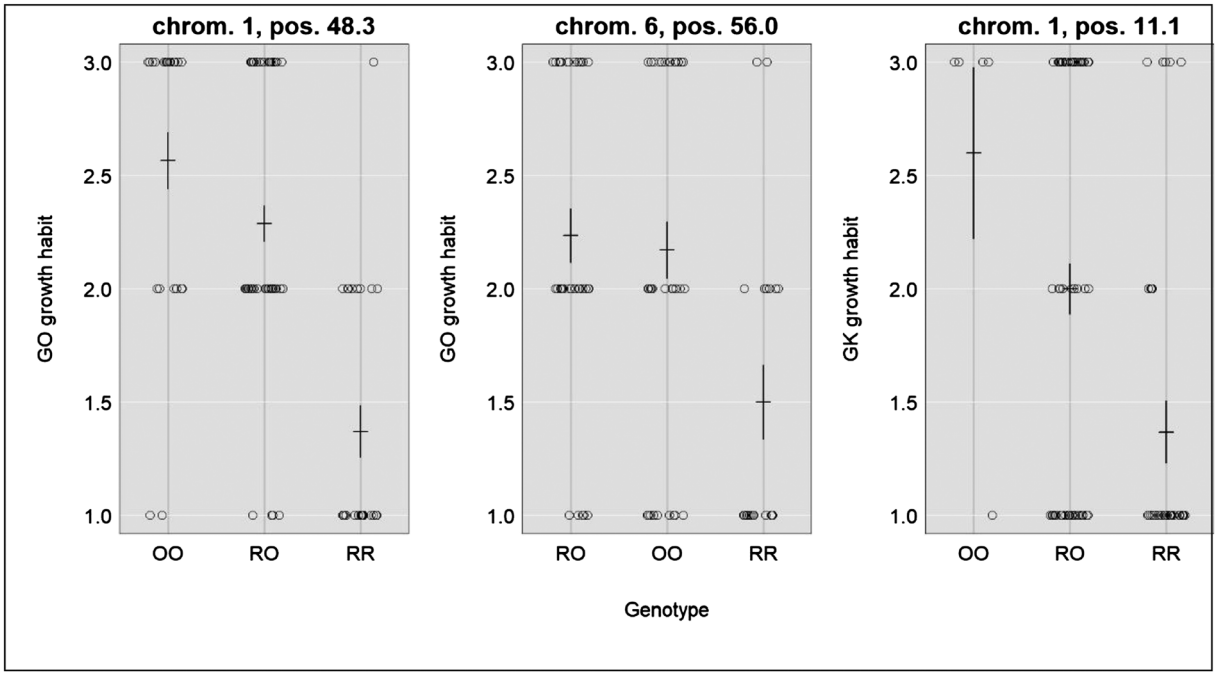


FIGURE 5 Phenotype × genotype plot for growth habit at three QTLs in two chickpea mapping families 'Gokce' × Oyali-084 (GO) and 'Gokce' × Kara-092 (GK). The x-axis shows genotypes made up of cultivated reference (R) or wild other (O) alleles. The y-axis shows phenotypes scored ordinally as cultivar-like erect (1), wild-like prostrate (3) or a mix of growth habits (2). Circles are per individual measures. Crosses are the mean and standard error per genotype.

and *C. reticulatum*, potentially due to pleiotropic effect of flowering-time candidate genes (Ortega et al., 2019) showing the importance of investigating multiple traits as part of the same study.

Multiple studies have aimed to map QTLs for the important chickpea yield traits of seed weight and size in a variety of cross types in the past. Prior to genome sequencing of chickpea (Jain et al., 2013;

Varshney et al., 2013), different genotyping methods make it difficult to directly compare the QTLs found in different studies. In studies that can be compared, one to four QTLs have been found in manually genotyped studies (Abbo et al., 2005; Cho et al., 2002; Cobos et al., 2007; Hossain et al., 2010; Jamalabadi et al., 2013; Jingade & Ravikumar, 2019) and one to eight QTLs in high-throughput genotyped and bulk QTL-seq studies (Bajaj et al., 2015; Das et al., 2015; Singh et al., 2016; Verma et al., 2015). In all these studies, QTLs for seed weight and size are frequently found on chromosome 1, as in this study. However, some studies have found multiple distinct QTLs for seed weight that have been identified on chromosome 1 emphasizing how different mapping families harbour many different alleles for 100-seed weight (Bajaj et al., 2015; Das et al., 2015). Some progress has been made towards identifying the candidate genes contributing to this complex trait. A high-density genotyping by sequencing map and gene expression study of an intraspecific chickpea population identified 101 candidate genes for seed traits (Verma et al., 2015). A bulked QTL-seq approach identified four candidate genes on chromosome 4; Ca_04364, Ca_04600, Ca_04602 and Ca_04607 for 100-seed weight in chickpea that lie within the GK QTL identified in our study (Singh et al., 2016). The functions of these genes are cell division, protein kinase, unknown, random slug protein and transmembrane protein, respectively. A 90 SNP marker trait associations were identified as part of a resequencing study of 429 chickpea cultivars, several of which fall within our identified QTLs on chromosomes 1, 4 and 7. Another resequencing study found 23 seed-size candidates that function in cell growth, division and transcription (Rajkumar et al., 2020). In addition, a role for RNA-dependent DNA methylation during seed development resulting in greater methylation and differential gene expression at many loci in large-seeded chickpea has been reported (Rajkumar et al., 2020). The SNP marker closest to the QTL for seed weight on chromosome 7 of the GK mapping family shows strong segregation distortion against the wild (O) allele (Figure 4). This result might reflect the nearby presence of a hybrid incompatibility in the form of negative epistatic interactions between particular alleles derived from widely diverged parents. Previous wide-cross studies of chickpea (usually *C. arietinum* × *C. reticulatum*) have found extensive segregation distortion of marker alleles typically, favouring the wild parent (Aryamanesh et al., 2010; Cobos et al., 2007, 2009). Studies of wide crosses involving *C. arietinum* and *C. echinospermum* have found even greater hybrid incompatibility that have restricted their use in chickpea breeding (Kahraman et al., 2017; Ladizinsky & Adler, 1976).

Erect chickpea varieties are desirable for agriculture because they improve yield though their associations with determinate flowering, resistance to lodging and early flowering (Ortega et al., 2019). Studies that have mapped chickpea growth habit have found between one and two QTLs on chromosomes 1,3, 4 and 7 (Ali et al., 2015; Aryamanesh et al., 2010; Cobos et al., 2009; Hegde et al., 2021; Ortega et al., 2019) and found that alleles for erect growth habit are typically dominant to those for prostrate habit. In addition to being a useful trait in its own right, the major growth-habit QTL on chromosome 3 is associated with *Ascochyta* blight resistance (Aryamanesh et al., 2010) and a flowering-time gene cluster (Ortega et al., 2019).

Fine-mapping studies of some of these growth-habit QTLs have identified the gene candidates to two zinc finger genes: Ca_06999 and Ca_07000 and the chromosome 1 QTL (Ali et al., 2015) or the pleiotropic effect of three flowering-time genes in the phosphatidylethanolamine-binding protein (PEBP) family (Ortega et al., 2019).

A previous study mapping flowering time in these interspecific families found a flowering-time QTL at 14.1 Mbp (95% CI 10.8 to 26.5) on chromosome 3 but no QTLs for growth habit. Our mapping family appears to have identified another distinct QTL on chromosome 6. A genome-wide association study of chickpea genebank accessions found eight SNPs on six chromosomes to be significantly associated with growth habit (Upadhyaya et al., 2017) that might correspond to the QTLs of this study on chromosomes 1 and 6. The wide QTLs found for this study with limited genotyping resolution could be improved with future genotyping investment to make progress towards identifying new candidate genes in these mapping families.

In conclusion, we show how the use of wild crop relatives of chickpea in breeding programmes can offer opportunities to transfer new alleles to expand the trait diversity of chickpea cultivars. Wild crop relatives of chickpea do not show favourable seed weight and growth-habit characteristics, but wide-cross studies such as this can generate variations to understand the genetic basis of these traits as targets for plant breeding. Wide crossing studies are also informative about between-trait associations, allowing favourable wild alleles for other traits, such as disease or stress resistance, to be introduced to cultivated chickpea without impacting desired traits such as seed weight and growth habit. Inheritance patterns and QTLs varied between different families derived from different wild accessions, highlighting the importance of studying multiple sources of genetic variation. Through careful analysis of segregating wide-cross families, we identified hybrid lines with heavy 100-seed weight and favourable growth habit correlations that will facilitate further breeding through exploration of other useful introduced traits.

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CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest associated with this paper.

AUTHOR CONTRIBUTIONS

AL, AJ and AK performed the experiments. Abdulkarim Lakmes, Adrian C Brennan, R Varma Penmetsa and Wenbin Wei analysed the results. All authors contributed to writing the paper.

DATA AVAILABILITY STATEMENT

The data associated with this paper are available in the supporting information.

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