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The Quantitative Genetics of Flowering Traits in Wide Crosses of Chickpea

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Abstract: Chickpea (*Cicer arietinum* L.) is one of the most ımportant food legume crops in the world. Chickpea is valued for its nutritive seed composition, which is high in protein content and used increasingly as a substitute for animal protein. Days to fırst flowerıng is an important component of the adaptation and productivity of chickpea in rainfed environments characterized by terminal drought and heat stress. This study aimed to identify the inheritance pattern and identify quantitative trait loci (QTLs) for days to first flowering and flowering color in $F_{2:4}$ generation nested association mapping (NAM) populations of chickpea obtained using wide crosses between Gokce as the cultivated variety and wild accessions of *C. reticulatum* and *C. echinospermum*. A total of ten populations of 113 to 191 individuals each were grown under field conditions near Sanliurfa, Turkey. Two populations were genotyped for 46 single nucleotide polymorphism (SNP) markers, enabling QTL analysis. Flowering time differed between families, with the frequency distributions indicating quantitative inheritance controlled by both genes of major and minor effects. Three significant QTLs for the flowering time were mapped in one mapping family. For flower color, chi-square tests showed that five populations accepted single-gene action, two populations accepted two-gene action, and three populations accepted neither model. Two significant QTLs at three genomic regions were identified across the two genotyped populations. Days to first flowering was positively correlated with flower color for two of the ten populations. The diversity of QTLs identified underscored the potential of crop wild relatives of chickpea as sources of novel alleles for chickpea breeding.

Keywords: chickpea (*Cicer arietinum*); nested association mapping (NAM); flowering time; flower color; genetic control; inheritance

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1. Introduction

Food security is a growing global problem requiring urgent increases in agricultural yields. Plant breeders can target multiple traits to improve yields, such as early maturity, size and other characteristics of seeds, and disease resistance. Among these traits, the timing of flowering is a marker that is directly related to agriculturally important traits, such as yield [1,2]. For example, early flowering is beneficial for crop production because it is often associated with the avoidance of extreme weather conditions later in the growing season, such as drought [3]. A better understanding of the control of flowering time will help to improve legume crops by enabling more rapid breeding for a locally optimal flowering time.

Chickpea is an annual grain legume or "pulse" crop used extensively for human consumption in major chickpea-producing countries, including India, Pakistan, Mexico, Turkey, Canada, Syria, and Australia, accounting for over 20% of world pulse production [4]. Chickpea and soybean are the only two legume crops that provide all essential amino acids

and are important sources of vitamins (B1, B2, B5, B6), minerals (Zn, Ca, Mg, Mn), and carbohydrates [5]. As a consequence, chickpea is an important part of subsistence diets and food security and a tool for reducing childhood malnutrition [6].

Chickpea, like most cultivated crops, has relatively narrow genetic and phenotypic diversity compared to its wild crop relatives [7–9]. This has implications for the breeding of climate-resilient crop varieties because the dearth of adaptive variation significantly limits the adaptability of the crop to changing environments and marginal environments that define many parts of the developing world [10]. Thus, breeding with only cultivated genetic resources will have diminishing returns, raising an urgent need for new sources of diversity. Wild species are a key but underutilized resource for crop improvement and harnessing their potential represents a primary challenge for 21st-century agriculture [11–13]. Chickpea was domesticated from two wild relatives, namely, *C. reticulatum* and *C. echinospermum*, which are still distributed in Northern Syria and Southern Turkey [14]. Wild crop relatives of chickpea have so far had a limited role in chickpea improvement [12,14,15].

Early flowering is a key mechanism to protect chickpea from late-season heat and drought without a significant trade-off in yield with widespread effects on many other traits. Development of even earlier flowering varieties, if yield can be maintained, may both make chickpea more resilient to drought, and also expand the range of environments where it can be grown [16,17]. The transition to flowering in legumes is initiated by environmental signals, including photoperiod and vernalization, and endogenous signals, such as autonomous and circadian clock [16–19]. The multiple genes responsible for flowering in legumes and their regulatory interactions present a great challenge to elucidating their expression. Further, understanding of flowering time needs to be considered against the background of intra- and inter-species genetic variations. We anticipate flowering time differences between natural populations of *C. reticulatum* because the selection of flowering time is common in natural populations adapting to climatic extremes [20–22]. Flower color can be simply measured and has been found to be a useful simple genetic marker to predict other traits of interest, such as seed coat width and wilt resistance, and to identify successful hybridization [23–25].

Several genes scattered across the chickpea genome are genetically similar to genes that are known to regulate flowering time in model plant species, such as *Arabidopsis*, any of which genes might contain natural genetic variation in wild crop relatives [18,26,27]. Alleles of major effect for flowering time were identified in chickpea cultivars by examining the modality of flowering time distributions [16,28–31] or via quantitative trait locus (QTL) mapping [18,26,32–34] or whole-genome sequencing methods [9,27,35,36]. Early flowering time alleles were introduced with success into commercial breeding programs, such as the super early flowering allele *efl-1* discovered in the Indian desi-type cultivar ICCV 96029 [37], which most probably maps to chromosome 5 [18,38]. Differences in the flowering time genes found between studies could be due to different parental varieties and environmental growing conditions, as well as a lack of shared genetic markers.

In recent years, nested association mapping (NAM) designs have shown promise as a means to elucidate the genetic architecture of complex traits, such as flowering time in crops [39–41]. The crossing design includes a reference line crossed with multiple lines of interest, with each family then being developed as a selfing NAM population. Following genotyping of the lines using high throughput markers, such as single nucleotide polymorphisms (SNP), this design allows a wide panel of genetic diversity to be assessed using a combination of quantitative trait locus (QTL) analysis and a genome-wide association study (GWAS). The method is scalable and once homozygous lines have been established, multiple traits, environments, and sites can be tested in separate experiments.

Here, we investigated the quantitative genetics of flowering time in NAM populations of chickpea (*Cicer arietinum* L.) and its wild cicer relatives, *C. reticulatum* Ladiz. (the wild progenitor) and *C. echinospermum* P. H. Davis (also considered part of the primary gene pool that can be crossed naturally with chickpea [14]). These species all have the same chromosome number (2N = 16) and are interfertile [42]. The aim of this study was to

determine and compare the major genetic loci responsible for controlling flowering time and flower color in chickpea nested association mapping (NAM) families representing wide crosses between cultivated *C. arietinum* and wild lines of *C. reticulatum* and *C. echinospermum* using quantitative genetics and trait locus mapping approaches. The hypothesis was that NAM populations would show variation in genetic control of these traits reflecting differences between the different wild parents. Tests were performed to determine and compare the number of major effect genes for these traits that were segregating in each mapping family.

2. Materials and Methods

A total of ten populations consisting of 1700 genetically distinct lines were used in the experiment. To develop the populations, crosses were made between "Gokce," which is a commercial variety of the cultivated species *C. arietinum* [43], and wild accessions belonging to *C. reticulatum* and *C. echinospermum* species recently collected in Turkey (Table 1, [15]). The wild and cultivated varieties show many differences. Wild parents, in general, had the following properties: small-seeded, purple-flowered, prostrate and indeterminate growth habit, and spiny stems, with pod shattering. In terms of flowering time, both early and late accessions were present relative to the parental Gokce cultivar. Each first-generation hybrid (F_1) was grown in the glasshouse to obtain approximately 200 F_2 seeds. Then, F_2s were advanced by selfing, by bulking five seeds per line per generation. After two more generations of selfing, the seeds of the $F_{2:4}$ generation were used for these field experiments.

Table 1. Chickpea NAM populations, their pedigrees, and the number of genotypes for generation advance.

Population	Parent 1 (Female)	Species	Parent 2 (Male)	Species	Total Lines Planted
POP1	Gokce	*C. arietinum*	Bari1-092	*C. reticulatum*	161
POP2	Gokce	*C. arietinum*	Cudi 2-152	*C. reticulatum*	184
POP3	Gokce	*C. arietinum*	Cudi1-022	*C. reticulatum*	155
POP4	Gokce	*C. arietinum*	Egil-073	*C. reticulatum*	185
POP5	Gokce	*C. arietinum*	Egil-065	*C. reticulatum*	169
POP6	Gokce	*C. arietinum*	Oyali-084	*C. reticulatum*	189
POP7	Gokce	*C. arietinum*	Savur-063	*C. reticulatum*	191
POP8	Gokce	*C. arietinum*	Sirna-060	*C. reticulatum*	185
POP9	Gokce	*C. arietinum*	S2Drd-065	*C. echinosphermum*	113
POP10	Gokce	*C. arietinum*	Karab-092	*C. echinosphermum*	168

2.1. Field Experiments

The field experiment took place under local field conditions at the Field Experiment Station of Harran University, near Sanliurfa Turkey (37.10 N 39.06 E, 550 m altitude, "hot dry summer" CSA Köppen climate type). The field site was treated pre-emergence with the herbicide Fluodoxinil 500 g.a.i/ha to control weeds. A 0-20-20 (% N:P:K) fertilizer was applied at 100 kg/ha before planting. Sowing by hand was done on 12th January 2019. The usual chickpea winter planting time in this region is mid-November but a delay was necessary due to heavy rainfall during this period. The 1700 lines and parents of populations were planted in 69 blocks without replication. Each block consisted of 25 unique lines and the two repeated parental lines in an augmented design (a type of incomplete block design) to correct for spatial effects [44]. Five seeds from each line were randomly chosen, nicked using a nail-clipper to promote germination, and planted along a 1 m length with 20 cm intra-row and 50 cm inter-row spacing. The field was monitored every two days from sowing and the flowering date was recorded when the first flowers were seen on each row. Flowering time was measured as the number of days from sowing to the first flowering time. Flower color was recorded as either white or purple for all five individuals in each row and the genotype was called heterozygous if there was a mix of flower colors present. Due to pod shattering and the indeterminate growth habits of

wild phenotypes, rows were harvested manually on a daily basis to prevent seed mixing between genotypes.

2.2. Laboratory Experiments

Genomic DNA was extracted from a seed from each of Gokce, Oyali-084, and Karab-092 using Qiagen Plant Maxi Kit Manchester, UK) and approximately 2500 ng gDNA per sample was sent to Novogene (Cambridge, UK) for Illumina paired-end 150 bp sequencing. Sequence reads were mapped to the reference CDC Frontier chickpea genome from NCBI (BioSample: SAMN02981489) using the BWA MEM function with default parameters [45]. Samtools v1.2 [46] was used to filter aligned BAM output files to regions of interest using a list of 2472 100 bp long sequences containing validated kompetitive allele-specific PCR (KASP) SNP markers from Biosearch Technologies, UK. The HaplotypeCaller tool [47] in GATK v4.1.4.0 [48] was used with default parameters to identify SNP variants between each of the three parents. IGV-WEB [49] was used to view filtered BAM alignments at 496 of the identified variants to confirm a subset of 48 SNPs that were polymorphic in each mapping family and that were approximately evenly distributed throughout the chickpea genome.

Fresh leaves were sampled from each line in the field from the Gokce × Oyali-084 (hereafter GO) and the Gokce × Karab-092 (hereafter GK) families and quickly dried in individual paper envelopes at ambient temperature. Ninety-five samples from each family were sent to Biosearch Technologies, UK, for automated DNA extraction and KASP genotyping of the 48 selected SNPs. Dual-fluorescence KASP data did not form discrete two-dimensional clusters because each sample represented five individuals bulked per generation, therefore contributing a mix of ten SNP copies per sample. SNP genotypes for each line were determined by mapping data to the line of best fit of dual-fluorescence KASP data and visualizing the resulting trimodal distribution to determine fluorescence thresholds to distinguish each distribution peak that corresponded to each homozygous allele and heterozygotes, respectively. Approximately 10 to 20% of the data points falling between distribution peaks were conservatively not genotyped.

2.3. Quantitative Genetics Analysis

Statistical analysis was performed with Microsoft Excel (Segregation tests; Redmond, DC, USA), SPSS v23 (ANOVA and Tukey; IBM Armonk, NY, USA), and R v3.6.2 Figures and spatial effect correction; [50]. Quantitative flowering time data were first corrected for spatial field effects by making use of the augmented random block design with replicated parental lines using the aug.rcb command in the R package "plantbreeding" [51]. The flowering time data was summarized for each NAM population and means were compared using ANOVA (corrected flowering time as the dependent variable, population as the independent variable) followed by Tukey HSD tests to investigate further the differences between populations. Additive inheritance of flowering time in the field was tested using a general linear model of mid-parent values against the mean of each NAM population.

Histograms were constructed to show the frequency distribution of flowering time. Highly continuous trait expression approaching a normal distribution was interpreted to represent complex control with many contributing genes versus discrete modes in the distribution indicative of major gene inheritance.

In order to investigate possible simple genetic inheritance mechanisms, the individuals within populations were classified as early, intermediate, or late flowering. To make these categorizations, we used the least significant difference (LSD) estimated using ANOVA across all populations, assuming that the same criterion would be effective at distinguishing flowering time phenotypes within populations. In each population, lines with days to first flowering less than the early parent minus the LSD were considered as early lines, whilst lines with days to first flowering more than the late parent plus the LSD were considered as late lines. The goodness-of-fit to expected segregation ratios for inheritance models for one, two, and three genes were determined using chi-square (χ^2) analysis with a probability threshold of 0.05. The gene number tests were based on the expected

ratios of parent-like traits in recombinant inbred lines, which were 1:1 for a single gene (AA:aa), 1:3 for two genes (aabb:A-bb, aaB-, A-, B-), or 1:7 for three genes (aabbcc:A-bbcc, aaB-cc, aabbC-, A-B-cc, A-bbC-, aaB-C-, A-, B-C-; [52]). Heterozygous lines were not considered because of not using single seed descent and the relatively few generations since the establishment of the populations. This approach did not affect the expected ratios of parent-like homozygous traits.

Goodness-of-fit chi-square tests were performed on flower color counts to check for expected patterns of segregation, assuming one or two gene inheritance. The expected ratios of parent-like traits under one-, two-, or three-gene models were tested as for flowering time. Spearman ranked correlation tests were performed between flowering time and flower color scored as 1 for white, 2 for heterozygous, and 3 for purple. Significant correlations between these traits would suggest a linkage between the genes responsible for these two traits in NAM populations.

2.4. Quantitative Trait Locus Mapping

A genetic map for each of the GO and GK families (95 genotyped individuals each) was constructed based on the known genomic positions of each of the 46 successfully genotyped SNP in units of 1M bp. Quantitative trait locus analysis of genotype and matching phenotype data was performed with the R/qtl2 package [53]. The mapping population was treated as F_2 to reflect the bulk sampling of five individuals per line per generation from this stage, assuming relatively little loss of alleles within lines during the two generations of five seeds per line descent. The genotype data scored treating the Gokce cultivated allele as the reference (R) and the wild allele as other (O). The analysis applied the linear mixed model genome scan option using genotype probabilities at 1M bp step intervals and kinship values that leave the focal chromosome out. Flowering was treated as a quantitative trait in day units, and flower color was treated as an ordinal trait coding for cultivar-like (white scored as 1), wild-like (purple scored as 3), or heterozygous (both white and purple flowers scored as 2). Logarithm of odds (LOD) thresholds and 95% QTL confidence limits were estimated using 5000 data permutations. Effect sizes and directions for allele and genotypes at each significant QTL were calculated using the coefficient option of R/qtl2. The QTL map was drawn using MapChart [54].

3. Results

3.1. Flowering Time

Flowering time data for the ten NAM populations in the field are provided in Supplementary Data S1. The mean flowering times of the NAM populations were between 97 to 112 days (Gokce × Savur-063 and Gokce × Oyali-084; Table 2, Figure 1). Some lines showed transgressive flowering time phenotypes that were both earlier and later flowering time genotypes than the parents. The earliest flowering line in population Gokce × Karab-092 flowered 86 days after sowing. In contrast, the slowest lines flowered 120 days after sowing. The mean number of days to fırst flowering across all 10 NAM populations was close to the corresponding mid-parent value, which suggested a strong additive effect on flowering time (beta 0.87, $F_{11,8}$ 239.4, $p < 0.0001$).

There were significant differences between populations regarding the mean flowering time (ANOVA F = 125.87, $p < 0.001$) with Tukey's HSD tests indicating six distinct levels (Figure 2). The flowering time variation was mostly continuous, but in no population did the distribution of flowering time conform to a normal distribution (Figure 1). Square root transformations of the data did not significantly increase the normality fit of the data distributions.

Chi-square tests accepted a 1:1 ratio of flowering time segregation for one population, suggesting a single gene of major effect for flowering time (Gokce × S2Drd-065). Five further populations (Gokce × Bari1-092, Gokce × Cudi1-152, Gokce × Egil-073, Gokce × Savur-063, and Gokce × Karab-092) accepted a 1:3 segregation ratio, suggesting two genes of major effect. Furthermore, one populatıon (Gokce × Sirna-060) ac-

cepted a 1:7 segregation ratio, suggesting three genes of major effect. One population (Gokce × Cudi2-022) did not have any significantly early or late lines compared to the parents based on LSD values. Two populations (Gokce × Egil-065 and Gokce × Oyali-084) did not have any lines with days to first flowering later than the late parent (Egil-065 and Oyali-084) (Table 3).

Table 2. Summary of flowering time observations for chickpea NAM populations. The female parent was Gokce (mean = 105, se = 0.09) and the male parent was the wild genotype in all populations. Correlations with flower color are shown. Correlations were insignificant unless followed by asterisks (* means $p < 0.05$, ** means $p < 0.01$).

	Male Parent Mean	Male Parent St. Error	Mid-Parents	Population Mean	Std. Error of Mean	Range	Variance	Correlation
POP1 Gokce × Bari1-092	107	0.38	106	105.63	0.62	93–113	11.23	0.009
POP2 Gokce × Cudi1-152	95	0.27	99.5	102.20	0.75	88–120	58.16	0.049
POP3 Gokce × Cudi2-022	113	0.79	109	108.27	0.24	101–120	8.98	0.223 **
POP4 Gokce × Egil-073	110	0.46	107.5	106.60	0.26	92–116	12.87	0.094
POP5 Gokce × Egil-065	118	0.31	111.5	110.70	0.33	101–120	19.73	−0.019
POP6 Gokce × Oyali-084	119	0.28	112	112.08	0.27	97–120	14.19	0.158 *
POP7 Gokce × Savur-063	90	0.46	97.5	97.13	0.55	87–119	57.19	0.044
POP8 Gokce × Sirna-060	90	0.33	97.5	98.91	0.61	87–116	67.46	0.078
POP9 Gokce × S2Drd-065	97	0.32	101	100.98	0.71	87–119	55.43	0.077
POP10 Gokce × Karab-092	92	0.31	98.5	100.92	0.62	86–119	64.73	0.058

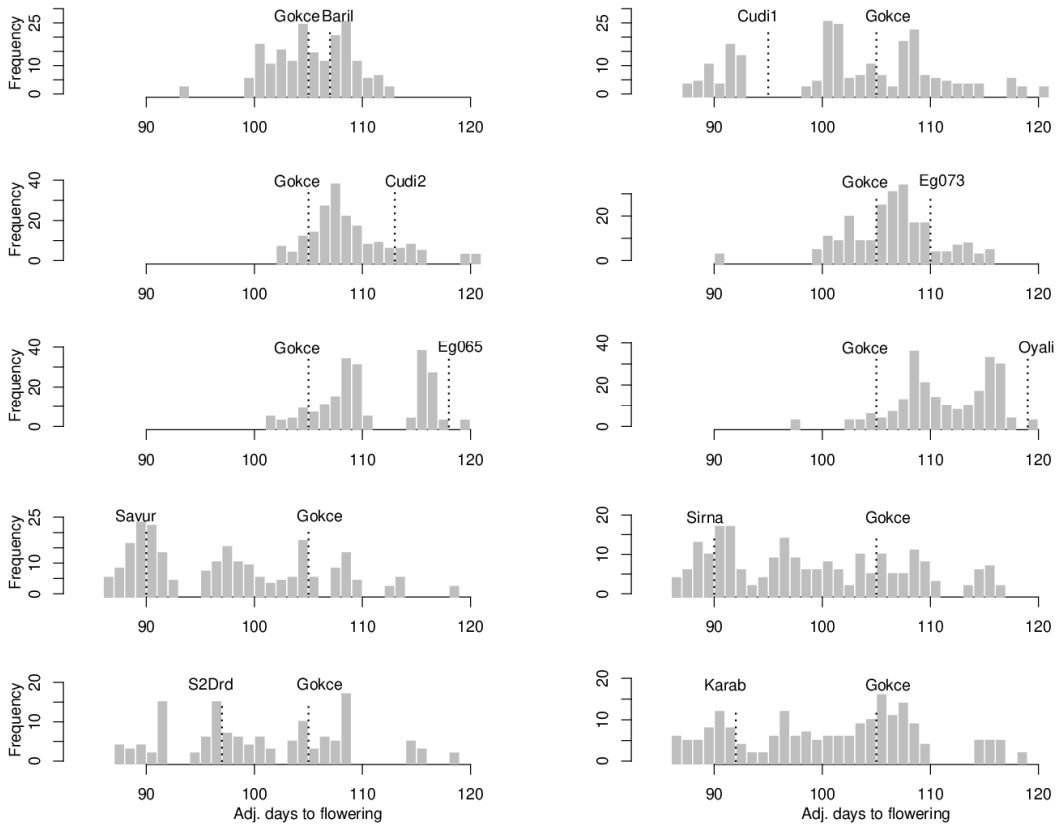

Figure 1. Frequency distribution of days to first flowering in ten chickpea NAM populations. Grey bars show the distribution of progeny values. Parental values are indicated with vertical dotted lines and labels.

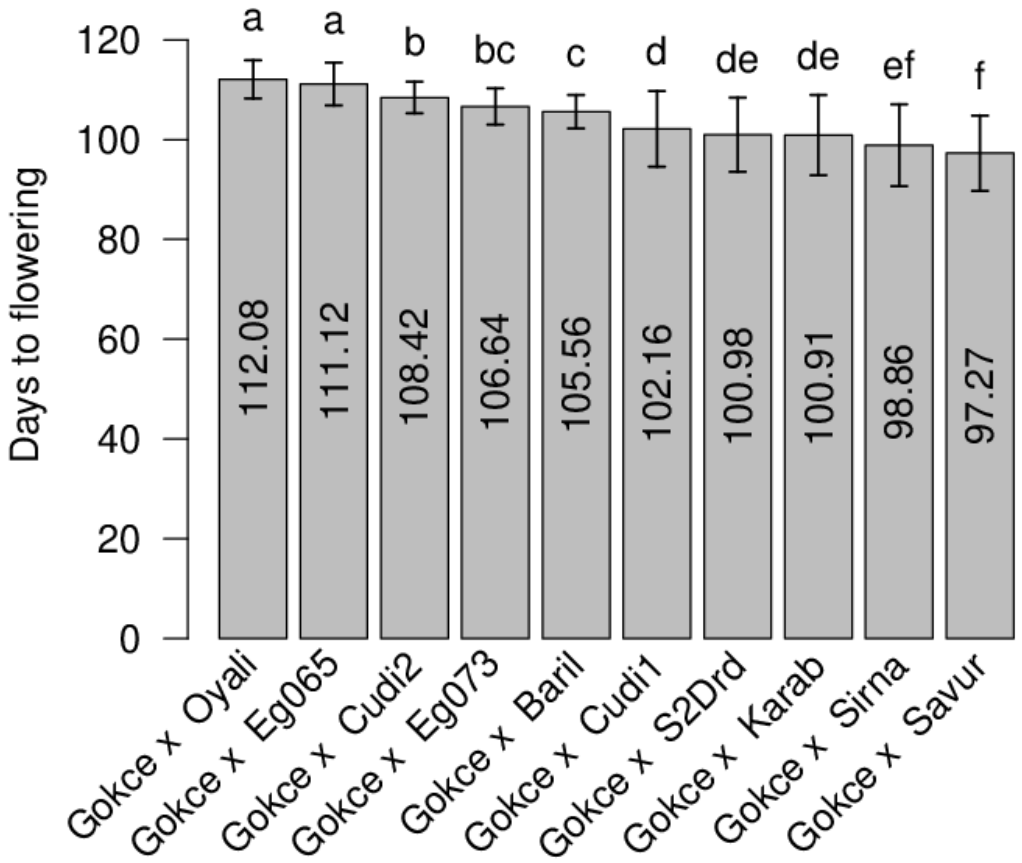

Figure 2. Mean of days to first flowering comparison between ten chickpea NAM populations. Grey bars show the mean values per population. Error bars are the standard deviations. Letters refer to the results of Tukey's HSD tests indicating six distinct levels.

Table 3. Results of goodness-of-fit chi-square tests (χ^2) for best fit to one-, two-, or three-gene models for the flowering time of chickpea NAM populations. NS: non-significant at a 5% significance level. Bold highlighted rows indicate the preferred gene model for each population.

Population	Early Flowering Phenotype	Late Flowering Phenotype	df	Ratio Tested	χ^2	*p*-Value
POP1 Gokce × Bari1-092	38	11	1	1:1 **1:3** 1:7	14.88 **0.17 NS** 4.43	$p < 0.05$ $p > 0.05$ $p < 0.05$
POP2 Gokce × Cudi1-152	16	61	1	1:1 **1:3** 1:7	26.30 **0.73 NS** 4.82	$p < 0.05$ $p > 0.05$ $p < 0.05$
POP3 Gokce × Cudi2-022	0	0	-	-	-	-
POP4 Gokce × Egil-073	32	15	1	1:1 **1:3** 1:7	16.15 **1.20 NS** 16.20	$p < 0.05$ $p > 0.05$ $p < 0.05$
POP5 Gokce × Egil-065	3	0	-	-	-	-
POP6 Gokce × Oyali-084	1	0	-	-	-	-
POP7 Gokce × Savur-063	9	28	1	1:1 **1:3** 1:7	9.76 **0.01 NS** 4.7	$p < 0.05$ $p > 0.05$ $p < 0.05$
POP8 Gokce × Sirna-060	2	35	1	1:1 1:3 **1:7**	29.43 7.57 **1.70 NS**	$p < 0.05$ $p < 0.05$ $p > 0.05$
POP9 Gokce × S 2Drd-065	23	27	1	**1:1** 1:3 1:7	**0.32 NS** 11.76 51.30	$p > 0.05$ $p < 0.05$ $p < 0.05$
POP10 Gokce × Karab-092	14	32	1	1:1 **1:3** 1:7	7.04 **0.72 NS** 13.53	$p < 0.05$ $p > 0.05$ $p < 0.05$

Information about genotyped SNPs are provided in Supplementary Data S2 and genotype and phenotype data for the two mapping populations are provived in Supplementary Data S3. Three significant flowering time QTLs were identified on chromosomes 3, 6, and 7 for the Gokce × Oyali-084 mapping population while no significant flowering time QTLs were found for the Gokce × Karab-092 population (Table 4, Figure 3). For each flowering time QTL, homozygosity at the reference cultivated allele (RR) led to a later flowering time with an effect size of 1 to 2 days per QTL (Table 4, Figure 4). Interestingly, homozygous wild alleles (OO) also had the effect of slightly later flowering time of 0 to 1 days per QTL, although these were smaller effect sizes that were less than that of cultivated alleles. Heterozygous genotypes had a transgressively earlier flowering time of 2 to 3 days per QTL.

Table 4. Summary of quantitative trait loci for flowering time and flower color for two chickpea NAM populations. Locations are described by chromosome number, position in 1M bp units, and 95% confidence limits in parentheses. LOD is the logarithm of the odds score. SNP is the marker nearest to the QTL peak. SNPs named c#.loc# are genotype probabilities at 1M bp map intervals. Mu is the model-predicted mean trait value. Units are days for flowering time or a score of 1 for cultivated white, 2 heterozygous white and purple, and 3 for wild purple for flower color. 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 (RR − OO)/2.

Trait	**Flowering Time**			**Flower Color**			
Family	**Gokce × Oyali**			**Gokce × Oyali**		**Gokce × Karab**	
Location	3, 14.1 (10.8–26.5)	6, 11.0 (2.0–20.9)	7, 0.2 (0.2–10.7)	4, 5.9 (5.9–11.3)	8, 1.3 (0.3–11.6)	4, 8.9 (5.9–18.9)	5, 21.9 (7.9–29.9)
LOD	4.24	3.19	3.7	10.78	3.70	3.64	6.60
SNP	CaSc40395	c6.loc11	TC86258	Ca_TOG896936	c8.loc1	c4.loc9	c5.loc22
Mu	111.24	110.43	111.98	2.14	2.08	2.12	1.93
RR	1.53	2.31	1.04	0.69	−0.48	0.41	0.25
RO	−2.73	−2.45	−2.00	0.05	0.09	0.12	0.39
OO	1.20	0.13	0.95	−0.74	0.39	−0.53	−0.65
Add. effect	−0.17	−1.09	−0.05	−0.72	0.44	−0.47	−0.45

3.2. Flower Color

All of the populations evaluated showed segregation for both purple and white flower color (Table 5, Figure 5). Two of the ten NAM populations had white flowers on the Gokce parent for most lines (Gokce × Bari-092, and Gokce × Egil-073), while the other NAM populations were mostly purple. Chi-square tests accepted a 1:1 ratio of flower color segregation among homozygous genotypes for five populations suggesting a single gene of major effect for flower color. Two further populations (Gokce × Bari-092, and Gokce × Egil-073) accepted a 1:3 segregation ratio, suggesting two genes of major effect. Finally, three populations (Gokce × Oyali-084, Gokce × S2drd-065, and Gokce × Karap-092) did not accept any Mendelian gene model. White was the most common flower color in these populations.

Two significant flower color QTLs were identified for each of the Gokçe × Oyali-084 and Gokce × Karab-092 mapping populations, representing three distinct genome locations in total (Table 4). The analysis of flower color as a binary trait with either white or purple color scored as dominant was also explored and identified a subset of the same QTLs as the quantitative analysis in similar positions. The common QTL was at the top of chromosome 4. The flower color alleles of the two Gokce × Oyali-084 QTLs had opposing directions of effects, while the presence of a wild-like allele had a similar effect for both Gokce × Karab-092 QTLs (Figure 6).

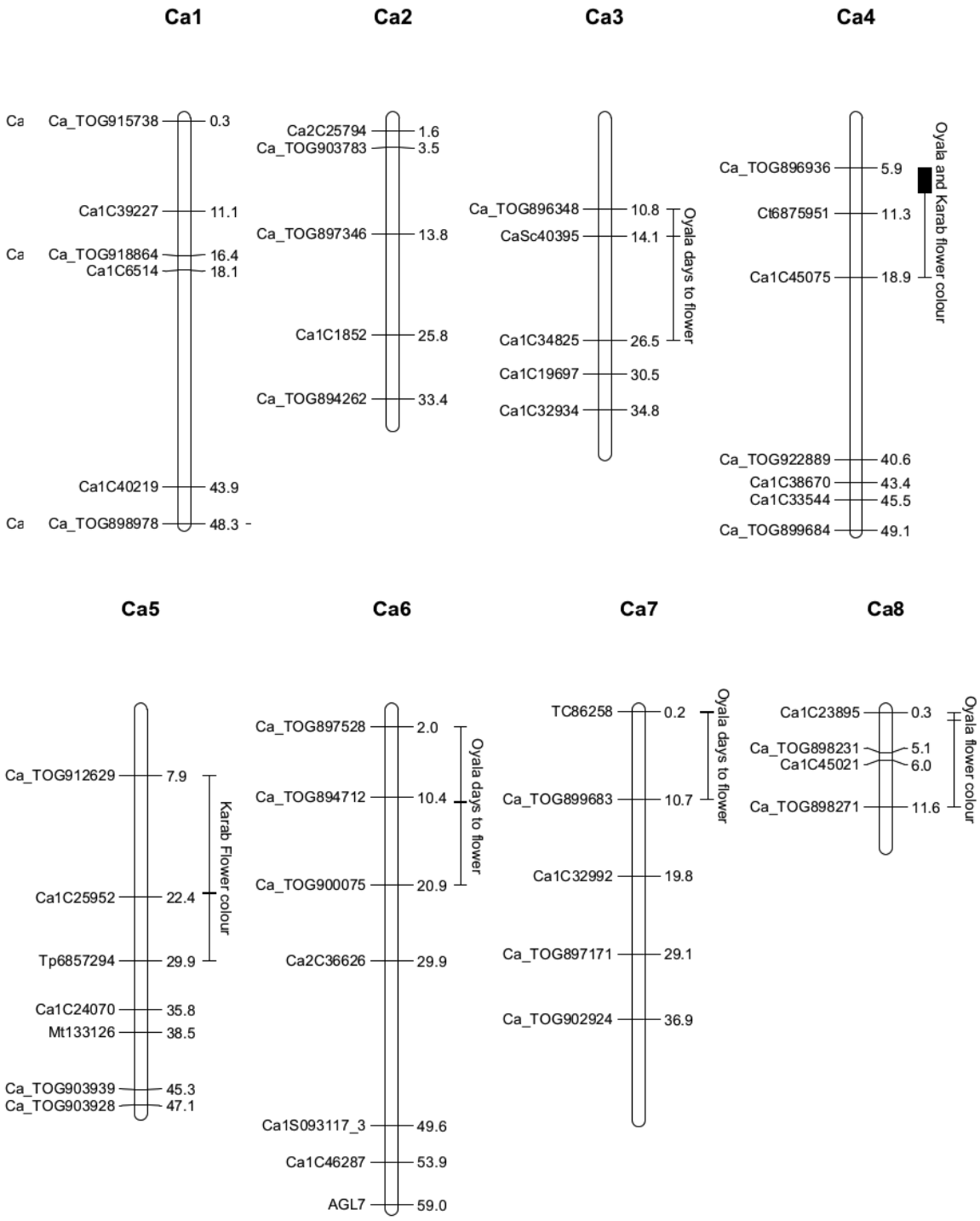

Figure 3. Genetic map showing quantitative trait loci for flowering time and flower color identified in the Gokce × Oyali and Gokce × Karab families. Chromosomes (linkage groups) are shown as white vertical bars with genotyped SNP positions marked with horizontal dashes and labels to the left of chromosomes. QTLs for each family and trait are shown as vertical lines to the right of the corresponding chromosome with a horizontal dash or bar showing the QTL LOD peak or peak range between the two families.

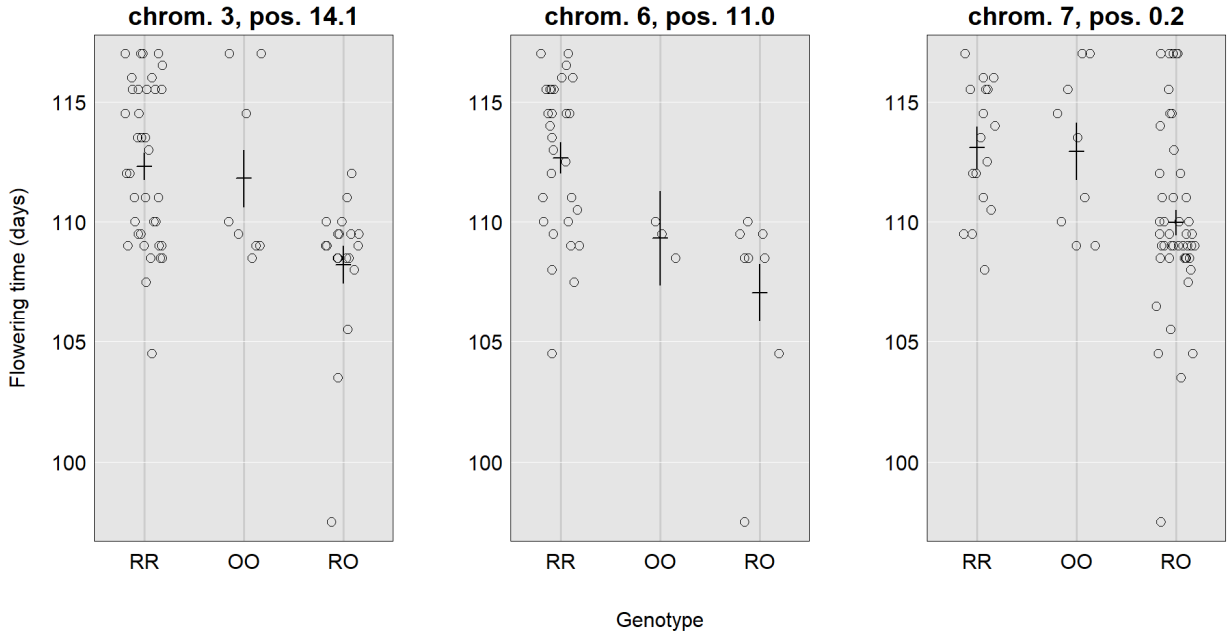

Figure 4. Flowering time phenotypes by genotype at the QTLs identified in the Gokce × Oyali-084 mapping population. The *x*-axis shows the genotypes made up of cultivated reference (R) or wild other (O) alleles. Circles are individual measures. Crosses are the mean and standard error per genotype.

Table 5. Results of goodness-of-fit chi-square tests (χ^2) for the best fit to one-, two-, or three-gene models for the flower color of chickpea NAM populations. NS: non-significant at a 5% χ^2 significance level.

Population	Purple Flowering Phenotype	White Flowering Phenotype	df	Ratio Tested	χ^2	*p*-Value
POP1 Gokce × Bari1-092	37	91	1	1:1	22.78	$p < 0.05$
				1:3	**1.04 NS**	$p > 0.05$
				1:7	31.50	$p < 0.05$
POP2 Gokce × Cudi1-152	77	61	1	**1:1**	**1.86 NS**	$p > 0.05$
				1:3	27.14	$p < 0.05$
				1:7	126.81	$p < 0.05$
POP3 Gokce × Cudi2-022	78	56	1	**1:1**	**3.61 NS**	$p > 0.05$
				1:3	20.15	$p < 0.05$
				1:7	105.11	$p < 0.05$
POP4 Gokce × Egil-073	28	84	1	1:1	28.00	$p < 0.05$
				1:3	**0.00 NS**	$p > 0.05$
				1:7	16.00	$p < 0.05$
POP5 Gokce × Egil-065	84	62	1	**1:1**	**3.27 NS**	$p > 0.05$
				1:3	24.36	$p < 0.05$
				1:7	119.86	$p < 0.05$
POP6 Gokce × Oyali-084	73	39	1	1:1	10.32	$p < 0.05$
				1:3	5.76	$p < 0.05$
				1:7	51.02	$p < 0.05$
POP7 Gokce × Savur-063	73	53	1	**1:1**	**3.17 NS**	$p > 0.05$
				1:3	19.57	$p < 0.05$
				1:7	100.68	$p < 0.05$
POP8 Gokce × Sirna-060	62	52	1	**1:1**	**0.88 NS**	$p > 0.05$
				1:3	25.84	$p < 0.05$
				1:7	114.29	$p < 0.05$
POP9 Gokce × S2Drd-065	49	22	1	1:1	10.27	$p < 0.05$
				1:3	4.84	$p < 0.05$
				1:7	22.18	$p < 0.05$
POP10 Gokce × Karab-092	47	28	1	1:1	4.81	$p < 0.05$
				1:3	6.08	$p < 0.05$
				1:7	42.29	$p < 0.05$

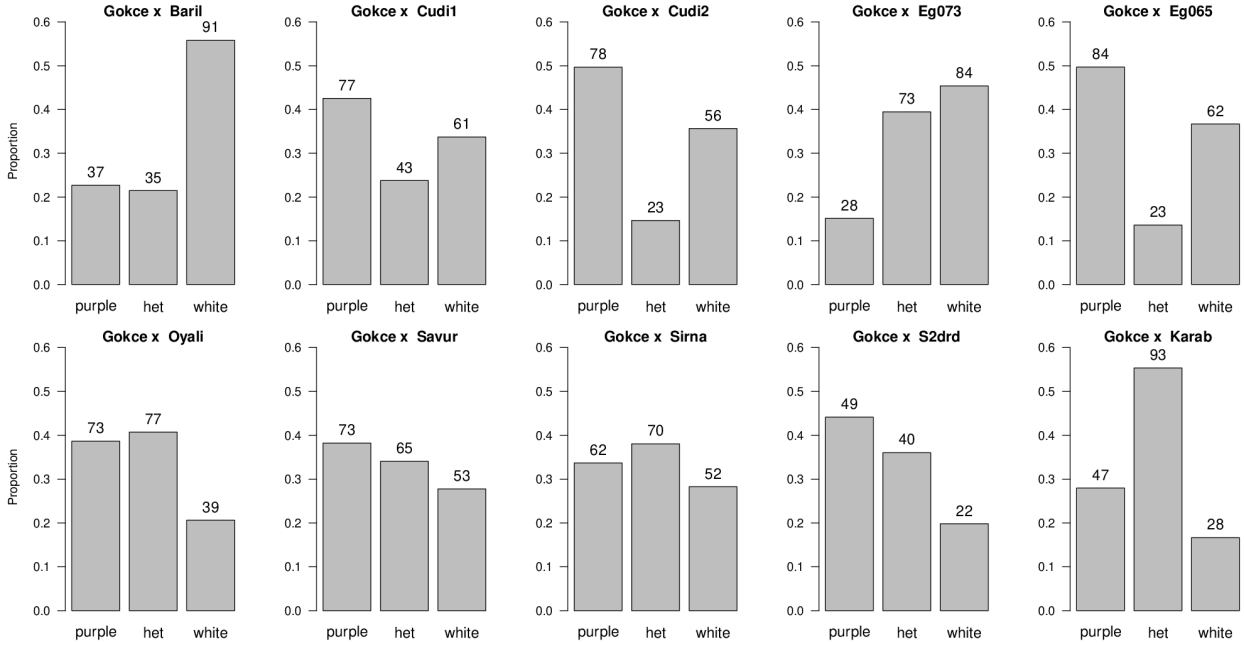

Figure 5. Frequency distribution of flower color in ten chickpea NAM populations. The het category means heterozygous mix of white and purple flowers. Bar heights are proportions and values above bars are counts.

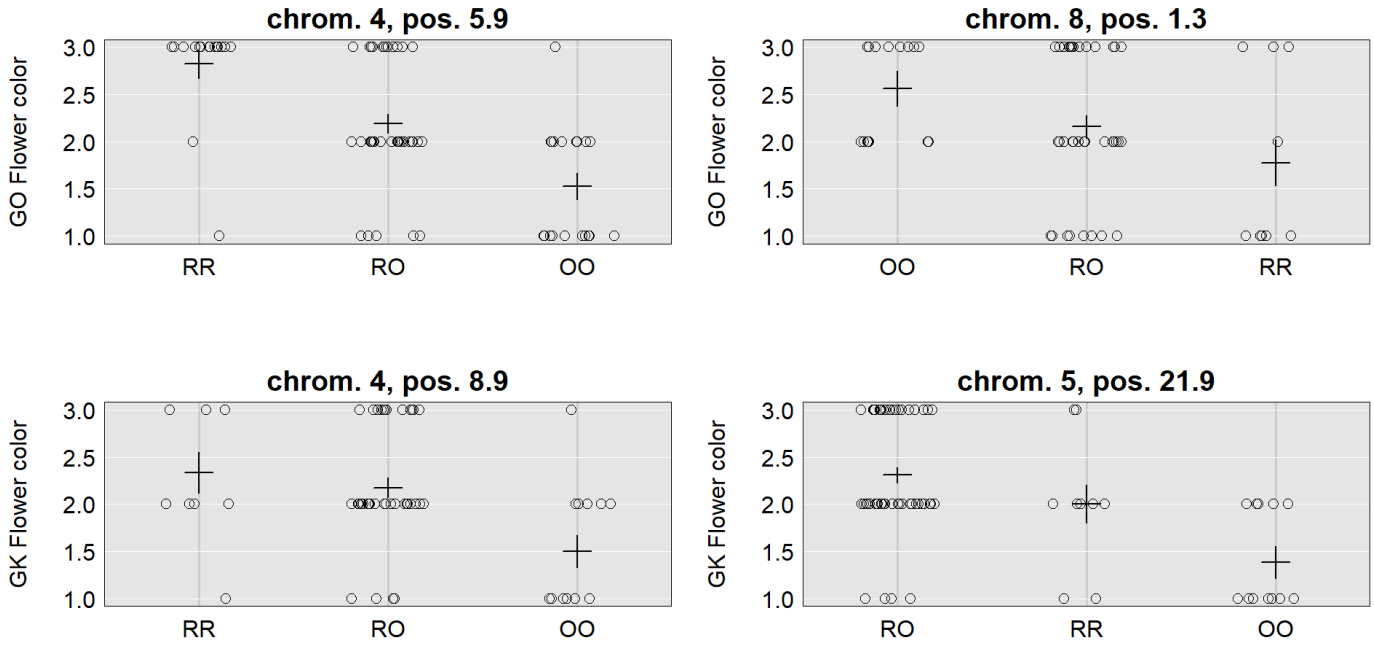

Figure 6. Flower color phenotypes by genotype at the QTLs identified in the Gokce × Oyali-084 (GO) and Gokce × Karab-092 (GK) mapping populations. The *x*-axis shows genotypes made up of cultivated reference (R) or wild other (O) alleles. The *y*-axis shows phenotypes scored ordinally as cultivar-like white (1), wild-like purple (3), or a mix of colors (2). Circles are per individual measures. Crosses are the mean and standard error per genotype.

3.3. Flowering Trait Correlations

Flowering time and flower color were found to be significantly correlated in just two of the ten populations (Gokce × Cudi2-022 and Gokce × Oyali-084) (Table 2). This means that, in general, these traits were not linked and flower color could not be used as a marker

for flowering time. However, the correlation in the two populations needs verification to confirm that these results were not simply due to chance.

4. Discussion

The main aim of this study was to perform a quantitative genetic analysis of wide crosses between chickpea and its wild crop relatives to provide new insights into the genetic control of traits of agricultural interest. In particular, this study investigated the genetic control of flowering traits in ten chickpea NAM populations derived from wide crosses with multiple accessions of two wild relatives. Flowering time is an important agricultural trait, as earliness allows crops to avail of short suitable growing periods and to avoid unfavorable environmental conditions at other times [3,16]. It is important that earliness does not come at the cost of reduced yield due to individuals not having accumulated sufficient resources for seed formation and filling by the time of flowering. Contrasting genetic control was found for flowering time and flower color between different NAM populations highlighting the genetic diversity across wild accessions that is available for introgression into crops. New early flowering alleles would be of particular value for plant breeding. While no lines with such extreme early flowering were identified in this NAM panel, alleles of major effect that cause intermediate flowering time were found to be common in the chickpea NAM populations. Stacking of these alleles, as well as alleles identified in other studies, could enable further gains in early flowering [31]. Alternatively, later-maturing varieties could be developed to suit alternative cultivation environments that capitalize on longer growing seasons that might occur regionally under future climate change scenarios [10].

In common with the findings of other quantitative genetic studies of chickpea [16,29,30], flowering time was found to be highly heritable and influenced by multiple genes in each population. Local genotype x environmental effects from the field experiment are also likely to have contributed additional sources of variation in flowering time. The involvement of several genetic systems responding to day length and/or temperature, their possible interaction, and the genotype × environment interaction cause a typical continuous frequency distribution of flowering time in many hybrid progeny analyses [1,16–18]. In our experiment, the ten NAM populations were grown under the same conditions; therefore, significant differences in flowering time between populations suggest that these populations were segregating for allelic variation of different subsets of flowering time genes contributed by the various wild parental lines.

Although the frequency distributions of days to first flowerıng for 10 NAM populations appeared to be somewhat continuous, two or more peaks in the distributions were observed for most NAM populations, which suggested simple genetic control due to a few genes with major effects. The one exception was population 3 (Gokce × Cudi2-022), which showed more continuous intermediate flowering time variation (Figure 1). The quantitatively inherited genes might have also been overlaid by several smaller contributing qualitative genes as in most cases, major as well as minor gene effects are involved in determining flowering time [16,55].

Goodness-of-fit tests provided additional evidence that genes with major effects were important in some of the families, as five out of seven populations tested were best explained by a two-gene model. The QTL mapping analysis found three significant flowering time QTLs in one of the two study populations, located on chromosomes 3, 5, and 6. In each case, the wild allele showed earlier flowering, but there was also an interactive heterozygous effect. Several other studies of flowering time in chickpea have identified QTLs in the central region of chromosome 3 [26,29,33,34,56]. Several genes similar to key flower regulation genes in the model plant *Arabidopsis* are found in this genomic region, including three copies of flowering locus T (FT), two cycling DOF factors (CDF), CO-like (COLh), and LUX-like [18]. Among these, the FT ortholog was shown to be the major determinant for shifts in flowering time control that accompanied chickpea domestication [56]. This FT cluster colocalizes to within the QTL interval we identified on chromosome 3 to between

markers CaSc40395 and Ca1C34825. More detailed genetic studies, such as complementation test crosses involving parental lines from different studies, are needed to better understand how many of these candidate genes contribute to flowering time variation in chickpea [31]. A few previous studies detected flowering time QTLs on chromosome 6 [34], while, to our knowledge, no QTLs have been found on chromosome 7. These QTLs might represent additional genes regulating flowering time in our survey of new wild chickpea germplasm.

The qualitative trait flower color was confirmed in most cases to be under one- or two-gene control but across families, where changes in the direction of dominance indicated that multiple alleles might be present in the parental lines. In agreement with other studies, two or three complementary flower color genes, usually with dominant effects, were reported for chickpea [25,57]. Of these, the genetically defined "B" locus for flower color was identified as a bHLH transcription factor that interactively regulates pigmentation in both flowers and seed coats [58]. This bHLH gene localizes to within the flower color QTL we identified on chromosome 4, where it falls in proximity to the nearest QTL marker TOG896936. It will be interesting to determine in further studies whether other QTLs for flower color that were identified in our current study may also be governed by genes from the flavonoid pathway.

Two out of the ten populations showed non-independence in the expression of flowering time and flower color indicative of genetic linkage between some of the genes responsible for both traits. Correlations between flowering time and several other traits that affect yield, such as a significantly negative association with ascochyta blight resistance, were observed in chickpea [16,33]. The associations present in these NAM populations could be very useful for chickpea breeding programs to optimize the trade-offs between flowering time and final yield.

It would be important to perform further genetic tests to identify the different genes and flowering response pathways that are represented by these major effect alleles in different NAM populations and to ascertain how these wild-derived genes may relate to those identified from within the cultivated germplasm of chickpea where several genetic loci have been characterized [31,34]. These tests could be done by crossing lines in different families and measuring progeny flowering time phenotypes. The effects of alleles at different genes could then be stacked within breeding lines to generate varieties with flowering time responses tailored to varied agricultural production environments [31].

5. Conclusions

The use of wild species in breeding programs provides new opportunities to transfer new alleles for yield-related traits and to improve chickpea adaptation to local environments. This study found evidence for genes of both major and minor effects contributing to the important flowering time trait. In different cross populations, models of gene inheritance and QTL mapping supported one, two, or three major genes controlling flowering time and flower color. Together, these findings highlight the great variation present in wild cicer genetic resources with the potential to enhance chickpea plant breeding for climate resilience. More research is needed to determine new alleles/genes that contribute to yield increase and also resistance to biotic and abiotic stress factors. Future detailed studies of these NAM populations would permit the identification of genes underlying quantitative control of flowering time and color providing new alleles and molecular markers to use in chickpea crop improvement.

Supplementary Materials: The following supporting information can be downloaded from https://www.mdpi.com/article/10.3390/agriculture12040486/s1. Supplementary Data S1: Chickpea field phenotypes. Flowering time data for ten chickpea NAM families and their founding parents. Explanation of the table columns: "ID" gives names of NAM individuals or parents (P1: Gokce, P2: Oyali-082, P3: Karab-094); "Blk" gives field block number (repeated for each population); columns "DFF_POP1" to "DFF_POP10" give days to first flowering values for each NAM family in the same number order as Table 1 in the main text. "NA" indicates missing measures. Supplementary Data S2:

Chickpea markers. Summary genetic marker information. Explanation of table columns: "Marker" gives SNP marker names; "Chrom" gives chromosome location; "Position" gives M base pair position on the reference chickpea genome; "Refallele" is the reference base; "Altallele" is the alternative base; "Sequence" is the unique sequence surrounding the SNP (the SNP itself is indicated in square brackets within the sequence). Supplementary Data S3: Chickpea map phenotypes and genotypes. Genotype and phenotype information for two chickpea mapping families. Explanation of table columns: "Fieldid" gives sample names used in the field experiments; "DFFmod" is corrected days to first flowering; "FC" is flower color (coded as white cultivar-like = 1, purple wild-like = 3, and mixed colors = 2); "Genoid" are sample names for genotyping (names starting with 1 belong to the Gokce × Oyala-084 mapping family and names starting with 2 belong to the Gokce × Karab-092 family); subsequent columns list genotypes for each SNP listed in map order. In the genotype rows; "R" denotes reference (cultivated) alleles, "O" denotes other (wild) alleles, and "-" are uncalled genotypes.

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