Genome-wide association study in Chickpea (*Cicer arietinum* L.) for yield and nutritional components

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Abstract The increase in the global human population and the accompanying challenges in meeting nutritional needs amidst climate change are a worldwide concern. Widespread protein and micronutrient deficiencies contribute to a significant number of individuals experiencing malnutrition, leading to severe health repercussions. This issue can be addressed through genomics-assisted breeding, particularly in enhancing the nutritional profile of vital staple crops like chickpea (*Cicer arietinum* L.). Chickpea, beyond being a rich source of protein, provides a diverse nutritional spectrum encompassing carbohydrates, fats, and minerals. To explore and improve the genetic basis of nutritional traits in chickpea, a study was conducted using 93 kabuli-type single plant derived lines and five cultivars in 2018, 2022, and 2023. Genotyping by sequencing revealed a total of 165K single nucleotide polymorphisms (SNPs) within this kabuli chickpea mini-core collection. After filtering for a minor allele frequency greater than 5%, 113,512 SNPs were utilized, distributed across eight chromosomes of the chickpea genome. Marker-trait associations were analyzed using genome wide association study, leading to the identification of 27 significantly associated SNPs from across all eight chromosomes linked to three seed nutritional concentrations and 100-seed weight. To unravel the molecular mechanisms governing seed protein, fiber, fat concentrations, and 100-seed weight, 31 candidate genes were determined within a 30 kb window size. This comprehensive approach holds promise for advancing crop breeding strategies to combat malnutrition and improve global food security.

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Introduction

Chickpea (*Cicer arietinum* L., $2n = 16$) is one of the most consumed food legumes globally, primarily used for human consumption but also as an animal feed. Chickpea was harvested from 15 million hectares with a production of 15.9 million tons in the world (FAO 2023). It offers a rich source of protein, starch and dietary fiber (Jukanti et al. 2012; Mugabe et al. 2023). Cultivated chickpea is categorized into two groups, desi and kabuli, based on various characteristics such as flower color, seed shape, and seed size (van der Maesen 1972). Desi types have small green, brown, and black seeds and pink flower colors, and display good stress resistance, while kabuli types are favored for their larger seed size, higher yield, and good seed quality parameters (Anbessa et al. 2006; Eker et al. 2022). Kabuli types are also known to have higher protein digestibility for human nutrition (Sanchez-Vioque et al. 1999; Wang et al. 2010).

The rapid increase in the global human population and the associated challenges in meeting nutritional demands amidst climate change have raised concerns worldwide (FAO 2020; FAO 2023). Protein and micronutrient deficiencies have resulted in a significant number of individuals suffering from malnutrition, leading to severe health consequences (WHO 2021; Beermann 2022). Protein deficiency is known to cause muscle deterioration, weakened immune system, growth retardation, and developmental issues (Rytter et al. 2014; Wu 2016). A balanced diet adhering to the Recommended Dietary Allowance (RDA) suggests a daily protein intake of 0.8 g per kilogram of body weight (Wu 2016). This means that females over 14 years of age should aim for 46 g of daily protein, while males over 19 years should consume 56 g (Meyers et al. 2006). According to the USDA (2019), a 100 g serving of chickpeas provides around 20.2 g of protein. Chickpeas, recognized as an economical and highly nutritious legume, have gained popularity as a dietary staple, particularly in developing regions and among individuals following a vegetarian diet (Iqbal et al. 2006; Vandemark et al. 2018).

Chickpea offers a diverse nutritional profile other than protein, including carbohydrates, fat, and minerals. The composition of chickpea seeds varies based on environmental factors, agronomic practices, variety, and type (desi vs. kabuli). Chickpea has higher fat content than other food legumes and some cereals but a lower fat content than oilseed legumes like soybeans and groundnuts. In desi chickpeas, fat content ranges from 3.10 to 4.93%, while kabuli varies from 4.60 to 5.67% (Singh 1985; Jukanti et al. 2012); higher fat kabuli are preferred for the making of the popular dish hummus. The main carbohydrate in chickpea is starch, comprising around 47.4–66.9% of the carbohydrate fraction (Singh 1985); soluble sugars, crude fiber, and dietary fiber contribute to the remaining carbohydrates. Chickpea is rich in dietary fiber, with insoluble and soluble fiber levels of approximately 10–18/100 g and 4–8/100 g, respectively (Tosh and Yada 2010). The presence of dietary fiber offers numerous health benefits, including improved digestion, a reduced risk of certain chronic diseases, and better weight management (Liu et al. 1999; Birketvedt et al. 2005; Petruzziello et al. 2006). Understanding the genetic basis of nutritional traits in chickpea will help in the selection and breeding of varieties with improved protein, fiber, and fat concentrations, thus contributing to the reduction of malnutrition and enhancing global food and nutritional security.

Recent advancements in chickpea genomics have facilitated the production of numerous genetic markers, linkage maps, and genome sequences (Jain et al. 2013; Leonforte et al. 2013; Stephens et al. 2014; Gaur et al. 2020). These developments have aided the use of Genome-wide association studies (GWAS) in chickpea. Genome-wide association study is a powerful tool for mapping complex traits that allows for the screening of diverse crop accessions with high-density markers, enabling the identification of genes associated with phenotypic traits. Identification of genes and the development of molecular markers in or near these genes can aid breeding programs worldwide in the improvement of various important traits in the crop. To date, these efforts have primarily focused on yield, drought resistance, and resistance to diseases such as Ascochyta blight and Fusarium wilt (Anbessa et al. 2009; Cobos et al. 2009). Some studies have explored the genetic architecture underlying chickpea's nutritional traits, but only of a few traits (iron and zinc concentrations and protein content; Jadhav et al. 2015; Upadhyaya et al. 2016; Sab et al. 2020; Mugabe et al. 2023). Therefore, the goals of this research are to study a wider range of nutritional traits to enable enhancement of kabuli chickpea's nutritional value.

The aim of this study is to determine the genetic factors affecting protein, fat, fiber concentrations, and 100-seed weight through GWAS. The findings from this research will not only contribute to the development of chickpea varieties with enhanced nutritional profiles but will also identify genotypes with high 100-seed weight and nutritional quantity. These identified genotypes can serve as breeding lines for the development of improved cultivars. By addressing malnutrition and promoting global food and nutritional security, this research aims to meet the nutritional demands of a growing population in a sustainable manner. Additionally, it strives to make a substantial contribution to the field, offering insights that support the creation of more nutritious chickpea varieties and sustainable agricultural practices.

Materials and methods

Plant material

In this study, 88 kabuli-type single plant derived lines were selected from the USDA Chickpea Core Collection (Kumar et al. 2014; Simon and Hannan 1995; GRIN-Global (ars-grin.gov). Plots of the selected 88 lines were grown with five check cultivars ('Dwelley', 'Frontier', 'Sierra', 'Spanish White', and 'UC5') in an irrigated field study at the Central Ferry farm, Washington (46°39′5.1″ N; 117°45′45.4″ W, elevation 198 m above sea level), in 2018, 2022, and 2023. The experimental design adopted was a randomized complete block design (RCBD), featuring single plots with four replications. Thirty seeds were planted in double rows with 30 cm center spacing, keeping 152 cm between rows and plots, in each of the 152 cm long rows. At maturity, the plots were hand harvested, followed by a standardized drying process to achieve uniform moisture content. Threshing was carried out using a Vogel thresher and the final seed cleaning phase was performed by use of a seed blower.

Phenotyping and descriptive statistics

Seed protein concentration was calculated for three years and fiber and fat analyses were calculated for seed for the first two years. Whole seed analyses were performed by NIR (Bruker Matrix-1). The spectrometer was calibrated by grinding 120 samples from 2020 plots. Total N concentration was determined using the LECO C/N analyzer. Nitrogen concentrations were converted to protein concentration using a 6.25 conversion factor (Jones 1931) (Table S1). The OPUS calibration software was used to calibrate protein. Fat and fiber calibrations were performed using the Bruker NIR according to the manufacturer's instructions. A total of 200 g of harvested seeds per sample was used for each accession for NIR for 2018 and 2022. Three NIR scans were performed for each sample, and protein, fiber, and fat concentrations were estimated using an average of the three scans. The 2023 seed yields were low with insufficient seed for NIR analysis, so 2023 plot samples were ground and analyzed for total N using the LECO C/N analyzer and 6.25 N to protein conversion factor (Jones 1931).

Hundred-seed weight (100-SW) was measured for each accession. The four replications were measured individually by weighing 100 randomly selected seeds that had been dried to an average of 15% moisture after harvest, and the weight was recorded in grams and averaged over replications (Table S1). All analyses were expressed on a dry weight basis. For descriptive statistics, the range, mean, and standard error were calculated, and ANOVA and Pearson correlation between traits were conducted using SPSS 26.

Genotyping and haplotype analysis

DNA was extracted from young leaves of the accessions under controlled greenhouse conditions, employing the DNeasy Plant 96 kit (Qiagen Corp., Valencia, CA, USA). Single-nucleotide polymorphisms (SNPs) were discovered following single-enzyme (ApeKI) genotyping-by-sequencing (Elshire et al. 2011), conducted by a commercial company (LGC Bioserach Technologies, Berlin, Germany). The FreeBayes software (Garrison and Marth 2012) was utilized to call the identification of genetic variants, using the reference genome of kabuli chickpea, 'CDC Frontier' (Varshney et al. 2013). This process implemented with BamTools (Barnett et al. 2011) and the FreeBayes variant caller (Garrison and Marth 2012). The accessions, USDA Chickpea Kabuli Mini-Core Collection used were previously genotyped by Mugabe et al. (2023). The SNPs were filtered with minor allele frequency (MAF) > 5% SNP data. A final total of 113,645 markers across the eight

chromosomes of the chickpea genome were obtained for genetic analysis.

Marker trait association analysis

A genome-wide marker-trait analyses were conducted to identify SNP markers associated with 100-SW and seed protein, fiber, and oil concentrations. Marker-trait associations (MTAs) were performed with the BLINK (Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway) model using the R/GAPIT 3.0 package (Wang and Zhang 2021; Huang et al. 2019). In the BLINK model, a fixed effect model is used to correct false negatives and false positives using Bayesian information. It also uses linkage disequilibrium information to eliminate the need for markers to be evenly distributed throughout the genome (Huang et al. 2019). Bonferroni correction was used to evaluate the significance of the SNP marker-trait association. To establish a significance of association between SNPs and the phenotypic traits, a threshold value of $P \leq 0.05$ was applied.

Analysis of Linkage Disequilibrium

Linkage Disequilibrium (LD) analysis was performed using TASSEL v5.0. Significantly associated SNPs within an LD window were BLASTed against the chickpea genome (https://www.pulsedb.org), and potential candidate genes were determined for in the Arabidopsis homolog (https://www.gramene.org). LD decay was calculated in R using the following formula (Remington et al. 2001).

$$E\left(r^2\right) = \left[\frac{10 + C}{(2 + C)(11 + C)}\right]\left[1 + \frac{(3 + C)\left(12 + 12C + C^2\right)}{n(2 + C)(11 + C)}\right]$$

the expected value of r^2 under drift-recombination equilibrium is denoted as E(r2) and is calculated as $1/(1 + C)$, where N represents the effective population size, c is the recombination fraction between sites, and C is determined by the equation 4Nc.

Results

Phenotypic traits and correlations

Protein, fiber, fat concentrations and 100-SW in 2018, 2022, and 2023 years of USDA Chickpea Core Collection and check cultivars is presented in Table S1. Seed protein concentrations were determined for the years 2018, 2022, and 2023 for 88 chickpea accessions and five check cultivars, while seed fiber and fat concentrations were determined only in the years 2018 and 2022. The seed protein concentrations varied between 16.3−21.4%, 17.4−23% and 19.9−25.2% for the years 2018, 2022, and 2023, respectively (Table 1). The fiber concentrations ranged between 13.3–20.8% in 2018 and between 14.6–18.2% in 2022. Fat concentrations ranged between 3.3–5.9% and 3.0–5.5% in 2018 and 2022, respectively. The 100-SW obtained in 2022 and 2023 showed a wide range in both years, recorded between 15–52.7 g, and 16.4−56.2 g, respectively (Table 1). The averages and standard errors with years and across years can also be seen in Table 1. According to analysis of variance, statistically significant ($P < 0.01$) differences were found between the accessions for each trait. Significant differences were found between environments at $P < 0.01$ for protein and fat traits, and significant differences at $P < 0.05$ for fiber and 100-SW traits. Genotype × Environment interaction was found to be significant in all traits except fat (Table 2).

Table 1 Descriptive statistics for seed nutritional concentrations and 100-SW in the kabuli chickpea mini-core collection

Traits	2018		2022		2023		Mean of years	
	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
Protein (%)	16.3–21.4	18.8±0.13	17.4–23.0	20.1±0.13	19.9–25.2	22.3±0.14	18.3–22.5	20.5±0.11
Fiber (%)	13.3–20.8	15.5±0.17	14.6–18.2	16.2±0.07	–	–	14.1–19.5	15.9±0.11
Fat (%)	3.3–5.9	5.1±0.06	3.0–5.5	4.8±0.06	–	–	3.2–5.7	5.0±0.06
100-SW (g)	-	-	15.0–52.7	34.6±1.00	16.4–56.2	37.6±1.03	15.7–54.4	36.1±1.01

SE: Standard error

Table 2 Analysis of variance for seed nutritional concentrations and 100-SW in the kabuli chickpea mini-core collection

Traits	VS	DF	SS	MS	F	Sig
Protein	Genotype (G)	91	969.403	10.653	9.268	.001[**]
	Environment (E)	2	2029.856	1014.928	43.898	.001[**]
	Block	3	128.044	42.681	1.882	.235
	G × E	182	399.720	2.233	1.713	.001[**]
Fiber	Genotype	91	751.208	8.255	17.795	.001[**]
	Environment	1	70.359	70.359	30.760	.012[*]
	Block	3	8.621	2.874	1.242	.438
	G × E	91	260.887	2.899	5.618	.001[**]
Fat	Genotype	91	210.488	2.313	47.163	.001[**]
	Environment	1	18.348	18.348	224.971	.001[**]
	Block	3	0.653	0.218	3.055	.240
	G × E	91	4.836	0.054	0.887	.743
100-SW	Genotype	91	63929.716	702.524	97.857	.001[**]
	Environment	1	1539.676	1539.676	11.710	.042[*]
	Block	3	538.930	179.643	1.372	.403
	G × E	91	1310.541	14.402	1.642	.001[**]

VS: Variation Source, DF: Degrees of freedom, MS: Mean squares, SS: Sum of squares, F: F statistic

*$P < 0.05$, **$P < 0.01$

Table 3 Analysis of Pearson correlations for seed nutritional concentrations and 100-SW in the kabuli chickpea mini-core collection

	Protein	Fiber	Fat	100-SW
Protein	1			
Fiber	.084	1		
Fat	.160	− .747[**]	1	
100-SW	.352[**]	.187	.327[**]	1

[**]correlated at $P < 0.01$

Pearson correlations were calculated between seed nutritional concentrations and 100-SW traits in the kabuli chickpea mini-core collection. There was a significant but not high positive relationship ($r = .352$[**]) between protein concentration and 100-SW. A high and significant negative relationship ($r = − .747$[**]) was observed between fat and fiber concentrations, and a positive but much lower significant relationship ($r = .327$[**]) was found between fat concentration and 100-SW (Table 3).

Principal component analysis

A Principal Components analysis (PCA) was run to determine population substructure in the panel and remove the effect from the GWAS. The PCA of the 88 USDA Chickpea Core Collection illustrated that the lines were divided into four clusters. One was composed exclusively of accessions from Iran; the second cluster contained only accessions from the Middle East; the third cluster consisted of two accessions from the Americas and several more from the Middle East; and the last and largest cluster was comprised of accessions from nearly every country in the study. Further structure within this fourth subpopulation was lacking, and the grouping of these lines into one cluster is likely the result of germplasm exchange between nations (Fig. S1).

Genome-wide association analysis

Utilizing genotyping by sequencing (GBS), a total of 165K SNPs were identified within the kabuli chickpea mini-core collection. Following the filtration of minor allele frequency to > 5%, this study utilized 113,512 SNPs distributed across eight chromosomes of the chickpea genome. The analysis of marker-trait associations employed the BLINK model, which was also compared with two alternative models: Mixed Linear Model (MLM) (Yu et al. 2006), and the Fixed and random model Circulating Probability Unification (FarmCPU) within the GAPIT package Version 3 (Huang et al. 2019). Notably, the BLINK model demonstrated a good fit of test statistics on *Q-Q* plots. Consequently, considering this favorable performance, BLINK was selected as the optimal model for association analysis in this study. Genome-wide

association analysis identified 27 SNPs significantly associated with three seed nutritional concentrations and 100-SW across all eight chromosomes (Fig. 1; Table 4).

Three marker-trait associations (MTAs) were discovered for seed protein concentration on chromosomes 1, 5, and 7, explaining a phenotypic variation range of 10.4–29.3% in 2018. Two MTAs were identified for protein concentration on chromosomes 4 and 7 based on the three-year average (multiyear). The MTA on chromosome 7 was common to both (Fig. 2).

Six MTAs were identified for fiber concentration for 2018, and four for the average over multiple years. A total of 10 MTAs associated to fiber concentration were distributed to all chromosomes except 7th chromosome explaining a phenotypic variation range of 2.9–38.6% (Fig. 3; Table 4). None of the MTAs were found in common between 2018 and the average over years.

A total of seven MTAs associated with fat concentration were identified on chromosomes 1, 2 and 4. Two MTAs located on chromosomes 1 and 2 were common to all three environments (2018, 2022, and multiyear). The MTAs explained 9.3–54.8% of the phenotypic variation within the three environments (Fig. 4; Table 4).

A total of five MTAs associated with 100-SW were found: three MTAs on chromosomes 1, 2, and 4 in 2018, one MTA on chromosome 1 in 2023, and one MTA on chromosome 2 in the multiyear analysis. The five MTAs identified for 100-SW explained between 16.1–45.4% of phenotypic variation (Table 4; Fig. 5). These MTAs were not found in common between years or the average over years.

Discussion

Chickpeas have gained attention as a versatile and nutritious food source. Rich in protein, fiber, vitamins, and minerals, chickpeas offer a range of health benefits. Chickpeas are an excellent source of plant-based protein, essential for muscle growth and overall health (Jukanti et al. 2012). Chickpeas are low in saturated fat and high in unsaturated fats and high in dietary fiber and contribute to heart and digestive health and can help prevent conditions such as colon diseases from constipation to cancer (Gill et al. 2021; Gupta et al. 2017; Mugabe et al. 2023). Chickpeas contain essential vitamins and minerals, including folate, iron, phosphorus, and manganese, contributing to overall well-being (Derbyshire and Delange
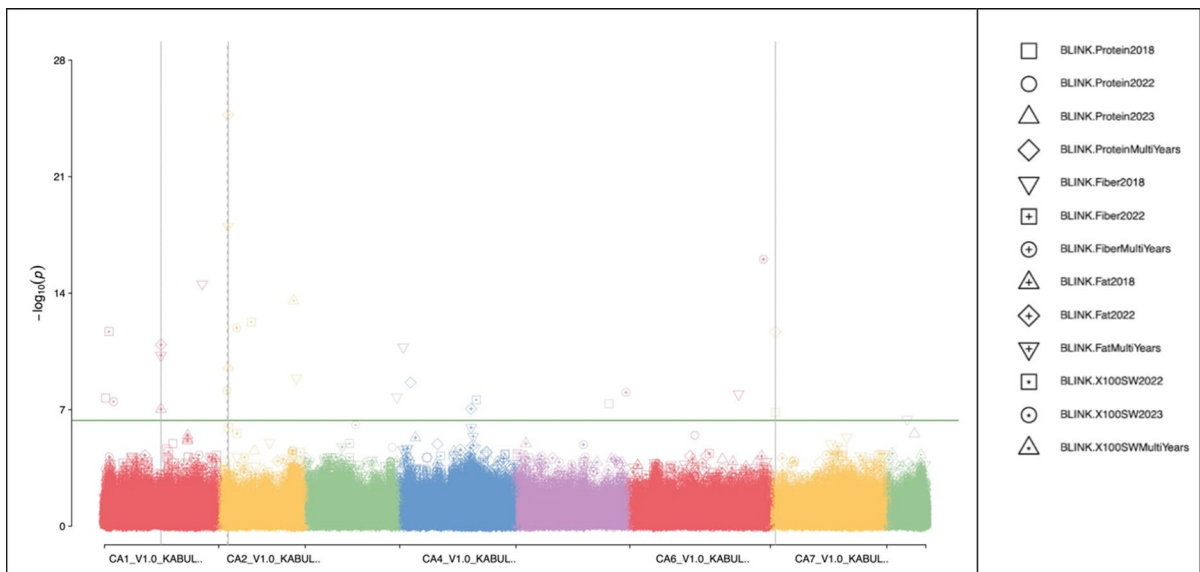
Fig. 1 Joint Manhattan plot for the GWAS analysis of three seed nutritional traits (protein, fiber, and fat in 3, 2, and 2 years, respectively, and averaged over years) and 100 seed weight (100-SW) in 2 years and averaged over years using the BLINK model. Statistical significance threshold is shown with the green horizontal line; multiple associations to the same SNP are shown with vertical lines

Table 4 The single nucleotide polymorphisms (SNPs) significantly associated with seed protein, fiber, and fat concentrations, and 100 seed weight (100-SW) in chickpea identified using the BLINK GWAS model in the kabuli chickpea mini-core collection

| Traits	Years	SNP ID	Chr	Positions	P.value	Effect	MAF	R (%)
Protein	2018	SCA1_V1.0_KABULI_455050	1	455050	2.00E-08	−0.642	0.430	11.34
		SCA5_V1.0_KABULI_39352536	5	39352536	4.41E-08	0.637	0.180	10.42
		SCA7_V1.0_KABULI_2145652	7	2145652	1.47E-07	0.677	0.244	29.32
	Multi years	SCA4_V1.0_KABULI_4583239	4	4583239	2.35E-09	−0.636	0.259	13.86
		SCA7_V1.0_KABULI_2145652	7	2145652	2.11E-12	0.794	0.253	41.65
Fiber	2018	SCA1_V1.0_KABULI_41348657	1	41348657	2.84E-15	−0.681	0.419	11.67
		SCA2_V1.0_KABULI_32939482	2	32939482	1.30E-09	−0.620	0.355	17.90
		SCA3_V1.0_KABULI_38800056	3	38800056	1.73E-08	−0.458	0.238	6.55
		SCA4_V1.0_KABULI_1475346	4	1475346	1.75E-11	−0.777	0.128	22.69
		SCA6_V1.0_KABULI_46039020	6	46039020	1.16E-08	0.557	0.343	2.86
		SCA8_V1.0_KABULI_8553804	8	8553804	3.80E-07	0.328	0.151	6.34
	Multi years	SCA2_V1.0_KABULI_3622279	2	3622279	6.86E-09	−0.466	0.402	5.50
		SCA2_V1.0_KABULI_7717032	2	7717032	1.20E-12	−0.724	0.230	18.77
		SCA5_V1.0_KABULI_46593279	5	46593279	9.13E-09	−0.347	0.477	5.55
		SCA6_V1.0_KABULI_56607658	6	56607658	9.17E-17	1.139	0.092	38.64
Fat	2018	SCA1_V1.0_KABULI_23894664	1	23894664	9.47E-08	−0.266	0.081	36.07
		SCA2_V1.0_KABULI_4047823	2	4047823	2.68E-10	−0.281	0.198	49.67
	2022	SCA1_V1.0_KABULI_23894664	1	23894664	1.26E-11	−0.377	0.080	31.56
		SCA2_V1.0_KABULI_4047823	2	4047823	1.95E-25	−0.512	0.195	48.85
		SCA4_V1.0_KABULI_30217860	4	30217860	8.88E-08	−0.367	0.305	9.26
	Multi years	SCA1_V1.0_KABULI_23894664	1	23894664	5.46E-11	−0.362	0.081	33.78
		SCA2_V1.0_KABULI_4047823	2	4047823	9.85E-19	−0.439	0.198	54.79
100-SW	2022	SCA1_V1.0_KABULI_1915412	1	1915412	2.03E-12	4.278	0.408	22.15
		SCA2_V1.0_KABULI_13779408	2	13779408	5.45E-13	−4.218	0.259	23.94
		SCA4_V1.0_KABULI_32380371	4	32380371	2.56E-08	4.021	0.236	16.05
	2023	SCA1_V1.0_KABULI_3823365	1	3823365	3.29E-08	3.675	0.121	44.07
	Multi years	SCA2_V1.0_KABULI_31697291	2	31697291	2.83E-14	3.448	0.356	45.36

The traits, the year the trait was measured in (or the average over years, (Multi)), chromosome the SNP is on (Chr), position in base pairs of the SNP along the chromosome, significance of the association (P.value), phenotypic effect of the association on the trait, the minor allele frequency of the SNP in this population (MAF) and the percent of the phenotypic variance explained by the SNP (R (%)) are also shown

2021). Moreover, they are affordable and have a long shelf life, making them a viable option for improving food security. As a cost-effective source of nutrition, chickpeas can play a crucial role in providing accessible and nutritious food to vulnerable populations. Governments, non-governmental organizations, breeders, and international agencies can play a pivotal role in promoting chickpea cultivation and consumption. This includes investing in breeding programs and agricultural practices that enhance chickpea production, educating communities about the nutritional benefits of chickpeas, and integrating chickpeas into food aid programs.

This study conducted on a kabuli chickpea mini-core collection revealed substantial variation in seed nutritional concentrations and 100-seed weight (100-SW) across multiple years. Notably, the seed protein concentration showed a wide range from 16.3 to 25.2% over the years 2018, 2022, and 2023. This variability, also identified in other studies (Cobos et al. 2009; Farida Traore et al. 2022), underscores the potential for selective breeding to enhance protein concentration in chickpea accessions. We observed a negative correlation between fat and fiber concentrations, a positive relationship of protein and fat concentrations, and a positive correlation between
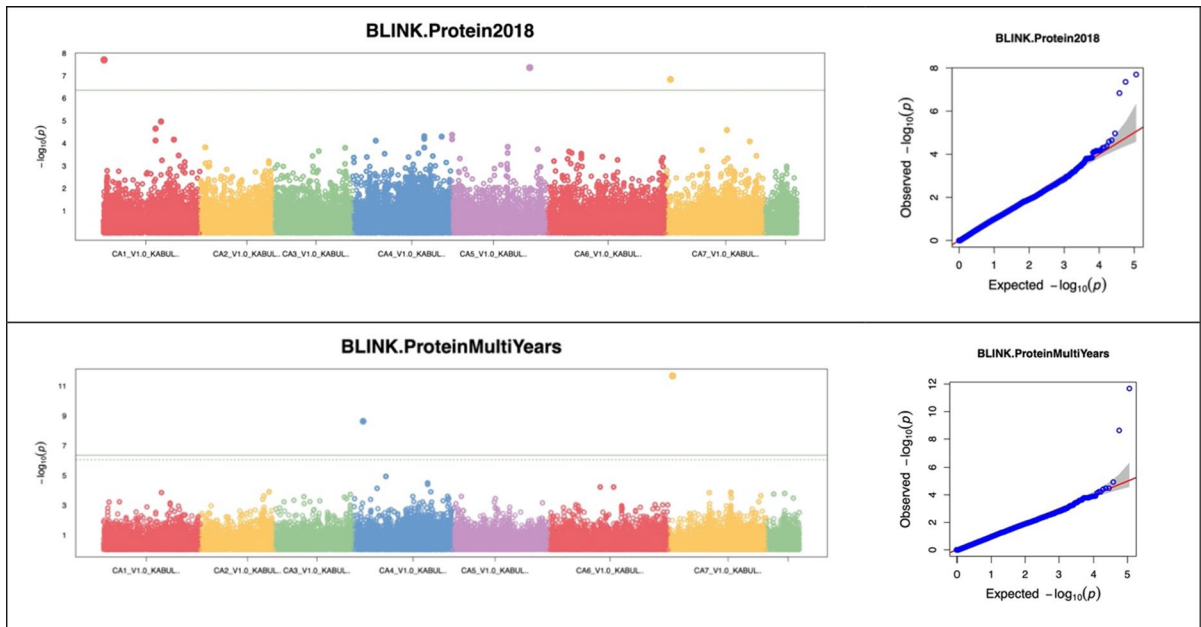
Fig. 2 Manhattan and Q-Q plots for protein concentration GWAS, showing traits with significant marker-trait associations only
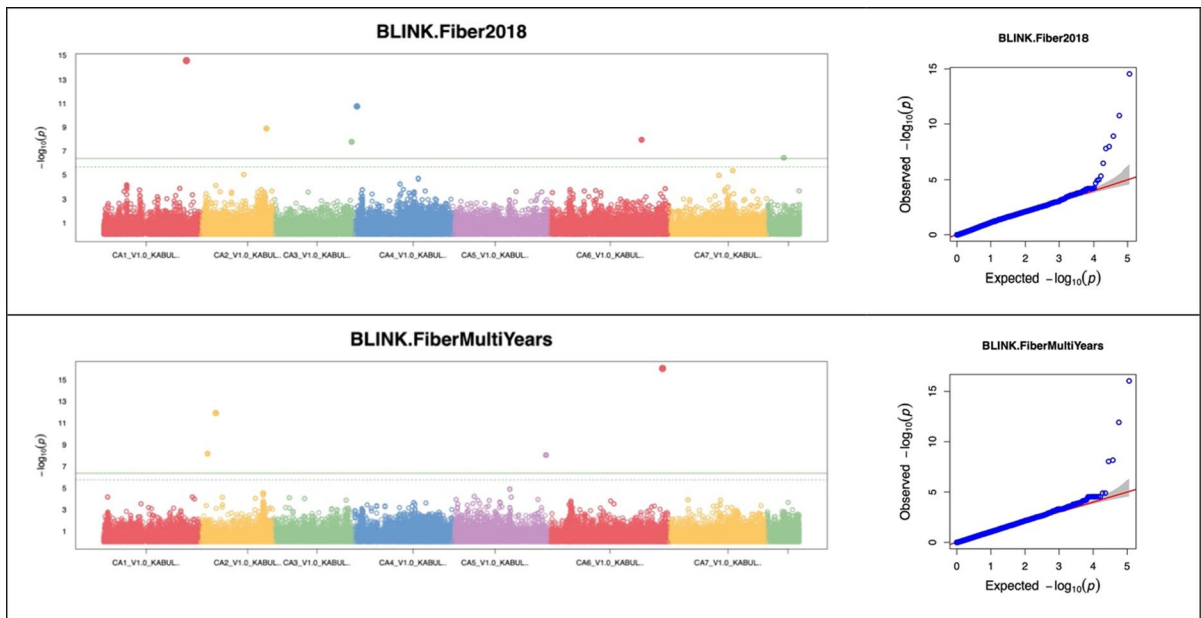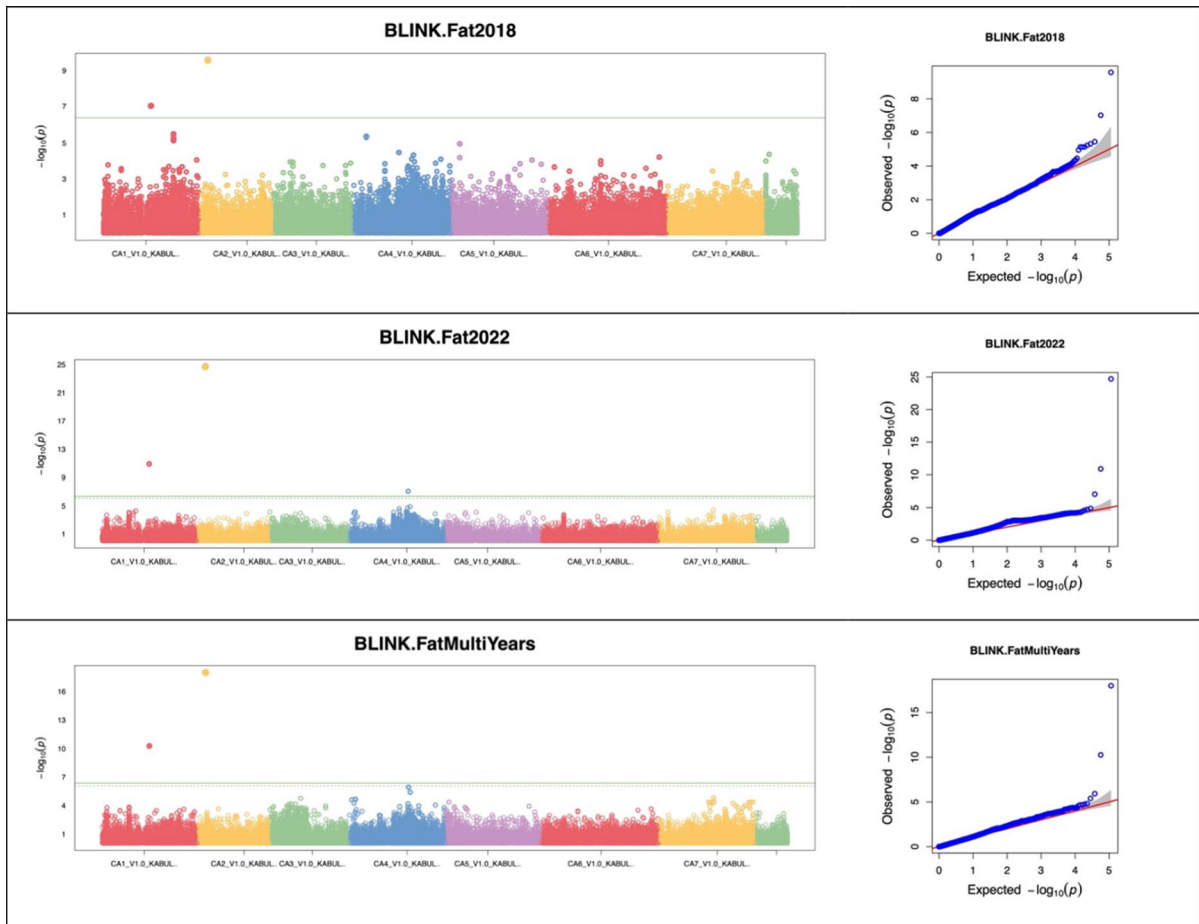

Fig. 3 Manhattan and Q-Q plots for fiber concentration GWAS, showing traits with significant marker-trait associations only

protein concentration and 100-SW. This all indicates that the seed has finite storage capacity for nutrients, and an increase in one may lead to a decrease in others, unless the seed size is increased. In addition,

the source-sink relationship of photosynthates and upstream metabolites that lead to the creation of these seed nutrients is often competitive, thus resulting in an intricate interplay of these traits in chickpeas
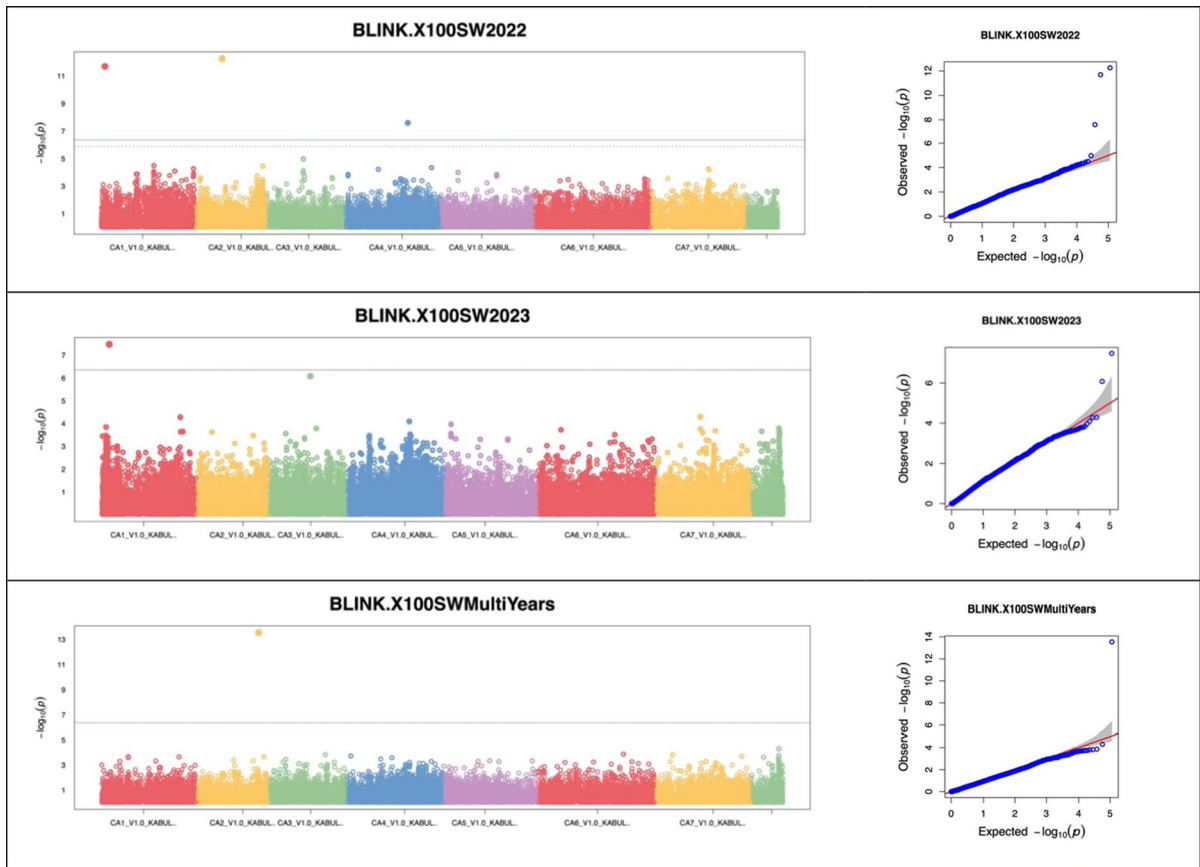
Fig. 4 Manhattan and Q-Q plots for fat concentration GWAS, showing traits with significant marker-trait associations only

(Ereifej et al. 2001; Özer et al. 2010). This will affect the breeding of these traits, as an increase in one may lead to a decrease in another.

The GBS used in this study identified a sufficient number of SNPs to enable GWAS analysis, and the BLINK model demonstrated a good fit of test statistics on *Q-Q* plots in identifying marker-trait associations (MTAs). The identification of marker-trait associations (MTAs) for seed protein, fiber and fat concentration, and 100-SW in kabuli chickpeas provides useful insights into the genetic regulation of these important nutritional traits. The identification of 27 significantly associated SNPs linked to seed nutritional concentrations and 100-SW across all eight chromosomes reaffirms the polygenic nature of these traits in chickpeas (Upadhyaya et al. 2016; Karaca et al. 2019; Srungarapu et al. 2022). In other

studies, in chickpea, Samineni et al. (2022) reported 46 MTAs for protein concentration, Upadhyaya et al. (2016) found seven MTAs, and Srungarapu et al. (2022) identified four. The MTA identified on chromosome 4 by Srungarapu et al. (2022) was consistent in both years they studied and appeared to be close to the MTA on chromosome 4 (4,583,239 bp) identified in our study, within the linkage disequilibrium (LD) decay distance reported in chickpea (Srungarapu et al. 2022). The variation in numbers of associated SNPs and the genomic regions identified across studies may reflect the diverse germplasm used, different environmental conditions and the genotyping methods employed.

Looking at other traits in the current study, the 10 MTAs discovered for fiber concentration do not seem to be in common with the only other GWAS of

Fig. 5 Manhattan and Q-Q plots for 100-SW GWAS, showing traits with significant marker-trait associations only

this trait published to date, who reported two MTAs (Mugabe et al. 2023). The current study identified seven MTAs for fat concentration, two of which were very consistent across both years and the average over years and explained a considerable proportion of the phenotypic variation (9.3–54.8%). These two MTAs thus show a substantial genetic impact on fat concentration that is stable across environments. Multiple genomic regions were also found to be associated with seed fat concentration by Mugabe et al. (2023) but not in the same genomic locations as those reported here, and not of such large effect. Finally, multiple MTAs were identified for 100-SW in the current study, as well as other GWAS (Srungarapu et al. 2022; Thudi et al. 2023) and QTL studies (Kujur et al. 2014; Bajaj et al. 2015; Das et al. 2015; Verma et al. 2015; Roorkiwal et al. 2016; Wang et al. 2019). Co-localization of associated genomic regions between studies is rare, emphasizing the quantitative nature of

the trait, but fairly high phenotypic effect and stability across environments within a study suggest that these QTL are all potentially useful to increase the trait in a breeding study, possibly by marker assisted selection to pyramid them into one genetic background.

To understand potential mechanisms of action of the MTAs identified in this study, candidate genes within a window of 30 kb were sought for the 22 MTA for seed protein, fiber, fat concentrations and 100-SW. Detailed information on the 31 candidate genes thus identified is presented in Table 5. The SNP (SCA1_V1.0_KABULI_455050) on chromosome 1 associated with protein concentration is 1586 bp away from a potential candidate gene whose homolog, AT1G22940 (*TH1*), has biological functions in the thiamine biosynthetic process, metabolic process, and phosphorylation (Strobbe et al. 2021). In a study reported by Rohi et al. (2013) determining B vitamins and protein in wheat flour, a strong

Table 5 The putative candidate genes for seed nutritional concentration and 100-seed weight in kabuli chickpea mini-core collection

	SNPs	Gene name*	Gene distance (bp)	Gene model	Gene position	Biological function
Protein	SCA1_V1.0_KABULI_455050	AT4G30960 (SIP3)	584	Ca_00052	Ca1:455636..455824	Protein phosphorylation; response to water deprivation, salt stress, stimulus, calcium ion
		AT1G10930 (RECQ4A)	818	Ca_00051	Ca1:445935..454232	DNA replication, repair, recombination; cellular response to cold
		AT1G22940 (THI)	1,586	Ca_00053	Ca1:456636..472917	Thiamine biosynthetic process, metabolic process, phosphorylation
	SCA5_V1.0_KABULI_39352536	AT5G56480 (END2)	0 (in gene)	Ca_07477	Ca5:39352504..39353911	Bifunctional inhibitor/lipid-transfer protein/ seed storage 2S albumin superfamily protein; lipid binding
		AT1G24490 (ALB4)	681	Ca_07476	Ca5:39344430..39351855	OxaA/YidC-like membrane insertion protein; protein insertion into Membrane; protein localization to chloroplast; establishment of protein localization to membrane
	SCA7_V1.0_KABULI_2145652	AT4G23850 (LACS4)	957	Ca_03256	Ca7:2146609..2152816	Long-chain fatty acid metabolic process; lipid metabolic process; fatty acid metabolic process
		ATG1G59990 (Heavy seed 3)	5,228	Ca_03257	Ca7:2139954..2140424	DEA(D/H)-box RNA helicase family protein; EMBRYO DEFECTIVE 3108, HEAVY SEED 3, HS3, RH22, RNA helicase 22; ATP binding; nucleotide binding.
		AT1G53320 (TLP7)	13,114	Ca_03255	Ca7:2158766..2159155	Tubby like protein 7; pollen development; regulation of transcription, DNA-templated; response to fungus.
	SCA4_V1.0_KABULI_4583239	AT1G61290 (SYP124)	0 (in gene)	Ca_03799	Ca4:4582894..4586052	Intracellular protein transport; pollen tube growth; protein transport;
		AT2G05990 (MOD1)	2,264	Ca_03800	Ca4:4577748..4580975	Lipid metabolic process; fatty acid metabolic process; fatty acid biosynthetic process; riboflavin biosynthetic process

Table 5 (continued)

	SNPs	Gene name*	Gene distance (bp)	Gene model	Gene position	Biological function
Fiber	SCA1_V1.0_KABULI_41348657	AT3G14400 (UBP25)	0 (in gene)	Ca_23109	Ca1:41346647..41351323	Ubiquitin-dependent protein catabolic process; proteolysis; protein deubiquitination
		AT3G14410	4,799	Ca_23108	Ca1:41338909..41343858	Nucleotide/sugar transporter family protein; carbohydrate transport; transmembrane transport
	SCA2_V1.0_KABULI_32939482	AT5G21160 (LARP1a)	1,811	Ca_10230	Ca2:32930505..32937671	mRNA catabolic process; heat acclimation; cellular heat acclimation
	SCA3_V1.0_KABULI_38800056	AT4G18550 (DSEL)	1,183	Ca_01239	Ca3:38797311..38798873	Lipid metabolic process; negative regulation of seed germination; lipid catabolic process; lipid storage.
	SCA4_V1.0_KABULI_1475346	AT2G30620	187	Ca_07777	Ca4:1474645..1475533	Nucleosome assembly; chromosome condensation; negative regulation of DNA recombination.
		AT2G41680 (NTRC)	1,615	Ca_07776	Ca4:1469782..1473731	NADPH-dependent thioredoxin reductase C; regulation of starch biosynthetic process; hydrogen peroxide catabolic process; regulation of chlorophyll biosynthetic process.
	SCA6_V1.0_KABULI_46039020	AT4G16340 (SPK1)	2,618	Ca_13872	Ca6:46033635..46036402	Regulation of actin polymerization or depolymerization, cell shape, auxin mediated signaling pathway; small GTPase mediated signal transduction.
	SCA8_V1.0_KABULI_8553804	AT1G32340 (NHL8)	1,372	Ca_11489	Ca8:8555176..8559891	Protein polyubiquitination; positive regulation of proteasomal ubiquitin-dependent protein catabolic process; protein ubiquitination.
	SCA2_V1.0_KABULI_3622279	AT5G15330 (SPX4)	0 (in gene)	Ca_10502	Ca2:3606809..3635051	Cellular response to phosphate starvation; phosphate ion homeostasis; cellular response to cold.
	SCA2_V1.0_KABULI_7717032	AT1G51580	554	Ca_18124	Ca2:7714232..7716478	RNA-binding KH domain-containing protein; regulation of gene expression.
	SCA5_V1.0_KABULI_46593279	AT1G64610	907	Ca_04116	Ca5:46589094..46592372	Transducin/WD40 repeat-like superfamily protein.
	SCA6_V1.0_KABULI_56607658	AT4G21534 (SPHK2)	14,485	Ca_17397	Ca6:56590954..56593173	Sphingolipid metabolic process; response to abscisic acid; lipid phosphorylation; cellular response to abscisic acid stimulus.

Table 5 (continued)

	SNPs	Gene name*	Gene model	Gene position	Gene distance (bp)	Biological function
Fat	SCA1_V1.0_KABULI_23894664	AT4G32770 (VTE1)	Ca_19294	Ca1:23912409..23917818	17,745	Fatty acid metabolic process; response to high light intensity, temperature stimulus, oxidative stress; vitamin E biosynthetic process.
	SCA2_V1.0_KABULI_4047823	AT3G02170 (LNG2)	Ca_10454	Ca2:4041210..4045923	1,900	Longifolia2; unidimensional cell growth; regulation of monopolar cell growth.
	SCA4_V1.0_KABULI_30217860	AT3G12120 (FAD2)	Ca_14188	Ca4:30231028..30232182	13,168	Fatty acid desaturase 2; lipid metabolic process; unsaturated fatty acid biosynthetic process; fatty acid biosynthetic process, omega-6 fatty acid desaturase activity.
100-SW	SCA1_V1.0_KABULI_1915412	AT1G61590	Ca_00239	Ca1:1908444..1910481	4,931	Protein kinase superfamily protein; protein phosphorylation; defense response; regulation of lignin biosynthetic process; phosphorylation.
		AT4G04920 (SFR6)	Ca_00238	Ca1:1900469..1905255	10,157	DNA-templated; response to osmotic stress; auxin-activated signaling pathway; abscisic acid-activated signaling pathway; root development; regulation of long-day photoperiodism, flowering.
	SCA2_V1.0_KABULI_13779408	AT1G69330	Ca_11639	Ca2:13771126..13802725	0 (in gene)	RING/U-box superfamily protein; metal ion binding; transferase activity; catalytic activity, acting on a protein.
	SCA4_V1.0_KABULI_32380371	AT3G50870 (MNP)	Ca_15475	Ca4:32390542..32391749	10,171	GATA type zinc finger transcription factor family protein; embryo development ending in seed dormancy; flower development; regulation of flower development; cotyledon development.
	SCA1_V1.0_KABULI_3823365	AT4G02020 (SWN)	Ca_00463	Ca1:3825197..3827551	1,832	SET domain-containing protein; regulation of gene expression by genomic imprinting; response to abscisic acid; vernalization response; regulation of short-day photoperiodism, flowering.
	SCA2_V1.0_KABULI_31697291	AT4G13940 (HOG1)	Ca_17848	Ca2:31670385..31671080	26,211	MATERNAL EFFECT EMBRYO ARREST 58; embryo development ending in seed dormancy; post-transcriptional gene silencing; DNA methylation-dependent heterochromatin assembly

* Arabidopsis homolog

positive correlation ($r = 0.56$) was found between thiamine and protein concentration in whole wheat flour, although this was not found in a smaller study of chickpea seed components (Roorkiwal et al. 2016). The same SNP on chromosome 1 for protein concentration is linked to two other genes (Table 5), but how they may be involved in seed protein concentration is unclear. The SNP associated with protein on chromosome 5 (SCA5_V1.0_KABULI_39352536) is in the potential candidate gene (0 bp) whose homolog, AT5G56480 (*END2*), functions in lipid-transfer and binding and is part of the seed storage 2S albumin superfamily, which has involvement in protein localization. In a GWAS analysis of pea (*Pisum sativum*), a homolog of the same gene *END2* was found to be significantly associated with seed fat concentration (unpublished data). Thus, this gene may influence protein concentration directly or indirectly by influencing a potentially competing seed component, lipid, and fat concentration. The SNP (SCA7_V1.0_KABULI_2145652) on chromosome 7 for protein concentration is 5 kb away from a potential candidate gene whose homolog, AT1G59990 (*HEAVY SEED3-HS3*), is responsible for seed size in Arabidopsis and tends to be highly expressed in developing seeds (Kanai et al. 2013). This gene may consequently help to explain some of the significant correlation between protein concentration and 100-SW in the current study (Table 3) and suggests the potential for developing genotypes with high protein concentration and larger seed size simultaneously (Panthee et al. 2005; Kulwal and Mhase 2017; Samineni et al. 2022). Two additional genes are associated with the SNP on chromosome 7 (Table 5) and the homolog of one, AT4G23850 (*LACS4*) is known to affect lipid and fatty acid metabolism, suggesting another mechanism affecting protein levels indirectly. Finally, the SNP (SCA4_V1.0_KABULI_4583239) on chromosome 4 for protein concentration is in the potential candidate gene (0 bp) whose homolog, AT1G61290 (*SYP124*), regulates protein transport and pollen tube growth, which may not indicate involvement in seed protein concentration. This SNP is also linked (2264) to a gene whose homolog AT2G05990 again involves lipid and fatty acid metabolism. The potential interplay of these two seed components should be more closely studied.

Altogether, 10 MTAs related to fiber concentration and accounted for a fairly high phenotypic variation range of 2.9% to 38.6%. Dietary fibers are mostly indigestible complex starches and carbohydrates, often components of plant cell walls (cellulose, hemicellulose, and pectin), and polysaccharides. The fiber-associated SNP (SCA1_V1.0_KABULI_41348657) on chromosome 1 with a particularly significant p value (2.84E-15) is linked (4799 bp) to a gene whose homolog (AT3G14410) is involved in nucleotide, carbohydrate, and sugar transport, and has been related to glycosylation and polysaccharide biosynthesis (Reyes and Orellana 2008). The fiber SNP (SCA4_V1.0_KABULI_1475346) located on chromosome 4 is within 1615 bp of a regulation of starch biosynthetic gene model (AT2G41680-*NTRC*). Overexpression of *NTRC* led to an increase in the accumulation of starch in leaves exposed to light (Toivola et al. 2013). For other fiber-related SNPs, candidate genes were identified within a range of 0 bp–15 kb (Table 5) that were involved with various functions, including mRNA catabolic processes, lipid storage, chromosome condensation, auxin-mediated signaling pathways, regulation of gene expression, sphingolipid metabolic processes, and response to abscisic acid.

The three MTAs identified for fat concentration were particularly significant, and those that were identified in multiple years (on chromosomes 1 and 2) explained between one third and one half of the phenotypic variance (Table 4). The SNP on chromosome 1 (SCA1_V1.0_KABULI_23894664) was 18 kb distant from a gene whose homolog AT4G32770 (*VTE1*), regulates fatty acid metabolic process and vitamin E biosynthesis in Arabidopsis (Porfirova et al. 2002). The SNP on chromosome 4 (SCA4_V1.0_KABULI_23894664) is 13,168 bp away from a potential candidate gene whose homolog, AT3G12120 (*FAD2*), is known to be involved in a lipid metabolic process, unsaturated fatty acid biosynthesis, fatty acid metabolic process, and omega-6 fatty acid biosynthesis (Lakhssassi et al. 2017). In a study on the genome-wide identification of genes encoding *FAD* (fatty acid desaturase) proteins in chickpea, Saini and Kumar (2019) identified a total of 18 *FAD* genes in both desi and kabuli chickpea genomes, including the same *FAD2* found in the current study (Ca_14188). This gene is crucial for lipid and fatty acid-related processes, and an excellent candidate for marker-assisted selection.

The five SNPs associated with 100-SW were linked to six genes primarily involved with embryo

development and stress responses. Of particular interest, SNP (SCA1_V1.0_KABULI_1915412) on chromosome 1 for 100-SW is 4931 bp away from the gene whose homolog, AT1G61590, is a protein kinase family protein, having biological function on protein phosphorylation, defense response, and regulation of lignin biosynthetic process. AT1G61590 is a DELLA gene, and these genes were found to regulate seed size in Arabidopsis (Gomez et al. 2023). The SNP SCA4_V1.0_KABULI_32380371 on chromosome 4 is linked to a gene whose homolog, AT3G50870 (*MNP*) is a GATA type zinc finger transcription factor family protein involved in embryo development and seed dormancy; SNP SCA2_V1.0_KABULI_31697291 on chromosome 2 is linked to a potential candidate gene whose homolog, AT4G13940 (*HOG1*), is also associated with embryo development and seed dormancy. Godge et al. (2008) reported that *HOG1* had a significant influence on plant and seed yield parameters in petunia and identified *HOG1* as a key gene with potential in regulating seed and plant development and cytokinin signaling, proposing a promising strategy for improving yield in various crop species through a combination of genetic manipulation and conventional breeding.

Conclusions

Chickpea, a valuable and nutritious food source with diverse health benefits, may be further improved with the genetic information presented in this study. Four phenotypically promising chickpea accessions (CSP-52, CSP-59, CSP-73, and CSP-74) have been identified. These selected accessions exhibit a protein concentration of 22% or higher, a fiber concentration of 15% or higher, and a 100-seed weight of 45 g or more. The GWAS revealed 27 SNPs across all eight chromosomes significantly associated with protein, fiber, fat concentrations and 100-seed weight, with varying phenotypic effects, across different chromosomes and environments. These were linked to 31 candidate genes that may help explain molecular mechanisms underlying these important seed traits. These genes and linked SNPs may also offer valuable tools once validated for breeders in optimizing crop nutritional profiles through marker-assisted selection.

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Declarations

Competing interest The authors declare no competing interests.

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