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Identifying Loci Associated with Grain Quality Traits of Landraces and Modern Varieties of Spring Bread Wheat under Conditions of Western Siberia

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ABSTRACT

Landraces of wheat represent valuable genetic resources for enhancing the grain quality of modern wheat varieties. In this study, a panel comprising 94 landraces sourced from the VIR collection (Russia) and 142 wheat varieties from breeding programs of Russia and Kazakhstan was subjected to genome-wide association study (GWAS) to pinpoint genomic regions and identify candidate genes associated with grain quality traits in wheat breeding. Genotyping of the germplasm panel was conducted using the Affymetrix 25K platform, while phenotypic data for agronomic traits were collected over two growing seasons (2022 and 2023) in Western Siberia. Investigation of the genotypic structure revealed seven distinct clusters within the landraces and breeding varieties population, indicating a clear genetic differentiation among the landraces. Notably, the B genome of the landrace germplasm exhibited the highest proportion of SNP markers (41%). Based on experiment data among 294 MTAs seven unique loci were identified with a −log10(p) value >6 in the landrace collection that had significant associations with grain protein content, grain gluten, strong gluten content, gluten index, and vitreousness (AX-158572632, wsnp_Ex_c10084_16572374, BobWhite_c31129_60, AX-158586137, AX-94949506, BobWhite_c46257_130, wsnp_JG_c5646_2148382). These markers were distributed on chromosomes A, 2B, 5B, 6B, and 6D with ranged of phenotypic variation 9–26%.

1. INTRODUCTION

The reduction of genetic diversity in modern bread wheat breeding programs presents a challenge amidst climate change and evolving pests and pathogens (Kose et al., 2023). Primitive cultivars and landraces offer a rich source of genetic variability, cultivated over millennia under diverse environmental conditions with minimal inputs (Adhikari et al., 2022; Demirel et al., 2023). Despite their potential, the genotypic richness of this germplasm remains largely untapped in breeding efforts (Mitrofanova et al., 2009). Landraces from the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) harbor valuable genetic diversity for adapting to adverse environmental conditions and ensuring grain quality (Mitrofanova, 2012; Riaz et al., 2017; Dinglasan et al., 2019). The VIR collection of spring bread wheat comprises 15,048 accessions from 97 countries, representing genetically diverse landraces and breeding lines registered between 1920 and 1990 (Phan et al., 2018; Tyryshkin et al., 2022). The Kazakhstan-Siberia Spring Wheat Improvement Network (KASIB) was established in 2000 and involves 20 institutions from Russia and Kazakhstan. Over two decades, the network has conducted 12 joint experiments and evaluated over 500 spring bread wheat varieties (Gomez-Becerra et al., 2006; Morgounov et al., 2020). The core set of 142 spring bread wheat varieties (ON-KASIB) reflects the genetic diversity of the entire KASIB germplasm.

Genome-wide association studies (GWAS) offer a genetic tool to identify new markers associated with grain yield and quality by comparing wheat varieties from different breeding periods, including landraces. Understanding past selection processes and developing strategies for future breeding efforts are essential (Rasheed et al., 2016; Shvachko et al., 2024). Landraces can contribute to improving modern wheat varieties and countering genetic erosion, albeit with limited capacity to cope with spatially heterogeneous and temporally unpredictable environments (Jaradat, 2013). Numerous studies have demonstrated broader genetic diversity in landraces compared to commercial wheat varieties (Lopes et al., 2015; Pascual et al., 2020; Jambuthenne et al., 2022). At present, genomic selection and the discovery of untapped beneficial quantitative trait loci (QTL) play a significant role in incorporating genetic material from landraces into modern varieties through marker-assisted selection (MAS), pyramiding, or biotechnological tools, thereby enhancing their potential for deployment in breeding programs (Kroupin et al., 2019; Ma et al., 2020; Marone et al., 2021). Reif et al. (2005) investigated 253 CIMMYT or CIMMYT-related modern wheat varieties and landraces using 90 simple sequence repeat (SSR) markers distributed across the wheat genome. The study revealed numerous unique alleles in landraces absent in modern spring bread wheat varieties, representing valuable sources for broadening the genetic base of elite wheat breeding germplasm. Analysis of 687 wheat accessions, including 148 landraces and 539 cultivars mainly from China and Pakistan, through genotyping of 477 unique genome regions using a wheat 90K single nucleotide polymorphism array, identified signatures of selection associated with disease resistance, vernalization, quality, adaptability, and yield-related traits (Liu et al., 2019). Two core collections comprising 94 wheat landraces as sources of new alleles and 142 promising adapted wheat varieties from breeding programs in Russia and Kazakhstan, developed between 2000 and 2020, offer a broad spectrum of variation for searching useful agronomic traits in wheat breeding (Morgounov et al., 2024). Association mapping, conducted using a multi-locus GWAS-GLM panel on quality-related traits over two growing seasons, identified candidate genes for selection of target loci.

The objective of this study was to perform association mapping on landraces from the VIR collection and germplasm from the international KASIB program to detect genomic regions and identify candidate genes for enhancing grain quality in wheat varieties.

2. MATERIALS AND METHODS

Germplasm Panel

The germplasm panel comprised 94 accessions of wheat landraces sourced from various geographical locations (Supplementary Table 1). These studied landraces belonged to nine botanical varieties: *aureum* (Link) Mansf. (*albidum* Alef.), *graecum* (Koern.) Mansf., *lutescens* (Alef.) Mansf., *erythrospermum* (Koern.) Mansf., *ferrugineum* (Alef.) Mansf., *milturum* (Alef.) Mansf, *caesium* (Alef.) Mansf., *turcicum* (Koern.) Mansf., and *erythroleucum* (Koern.) Mansf. The collection of landraces from Russia encompassed diverse regions including Krasnodar Krai, the Volga region, the North-Western region, Western and Eastern Siberia, as well as the Far East. The collection of spring bread wheat from breeding programs of Russia and Kazakhstan (KASIB) consists of 142 accessions developed for the period from 2000 to 2020 (Supplementary Table 2).

Field and laboratory experimentation

The experiments were conducted over two growing seasons (2022 and 2023) and were planted in the field following black fallow. Accessions were distributed in plots measuring 1.4 m^2 each, spaced 0.15 m apart, with a sowing rate of 25 g of seeds per plot. In 2022 and 2023 there were four replications. Standard varieties included the early maturity variety Pamyati Azieva and the late maturity variety Element 22. Agronomic traits were recorded following standard agronomy practices. Phenotypic evaluations of two core collections were conducted, covering 8quality grain traits and the main yield components. Protein, gluten, ash content, and vitreousness of the tested accessions were analyzed using NIR-spectroscopy according to standard ISO 12099:2017(E). Wet gluten content and gluten index were evaluated using the Gluten Index Device-Y08, while SDS sedimentation volume was measured using the Sedimentation Device-15 (manufactured by YUCEBAS MACHINERY, Türkiye) according to GOST ISO 21415-2-2019 and GOST 34702-2020. The thousand kernel weight was determined as two parallels, 500 grains were counted and weighed with an accuracy of 0.01 g. The yield components were recorded following methodical instructions for exploring the wheat collection (Methodical instructions,1985).

Experimental Sites and Weather

The experimental field's soil type is thin meadow chernozem, with an organic matter content ranging from 3.5% to 4%. The region experiences a sharply continental climate, with an average annual rainfall of approximately 300–380 mm and a sum of effective temperatures ranging from 2000°C to 2100°C. Factors limiting wheat grain yield and quality include insufficient rainfall, the threat of soil erosion by wind, and low phosphorus content. The average duration of the winter period is 164 days, with soil freezing to depths of 165–220 cm and snow cover varying between 20–25 cm. Growing seasons are characterized by average temperatures. In 2022, the rainfall for the growing season was 227 mm, with uneven distribution in May; and in 2023, it was 160 mm, with excess rainfall in August to early September.

Genotyping and Statistical Analysis

Genomic DNA was isolated from the leaves of 94 landraces and 142 spring bread wheat accessions using a protocol with modifications described by Delaporta et al. (1983) at a rate of 3 fold. Landrace and KASIB program accessions were genotyped using the Affymetrix 25K platform (Germany, www.traitgenetics.de). Markers with a minor allele frequency (MAF) lower than 5% and markers with not certain chromosome localization were excluded from the analysis. A total of 17,190 markers were including in GWAS. Marker-trait associations (MTAs), which was identified using the TASSEL v. 5.0 software with a method GLM (Bradbury et al., 2007) considered population structure (Q-matrix) and genetic kinship (K-matrix) using a Bayesian algorithm in the STRUCTURE v. 2.3.4software (Pritchard et al., 2000). The number of subgroups (K) was calculated based on the highest value of ΔK, with K values ranging from 1 to 10, and 10 independent runs for each K value (Evanno et al., 2005) in the Structure Harvester web program (Earl & von Holdt, 2012). K-matrix was calculated using TASSEL v. 5.0 software. MTAs were considered for significance level of $-\log10(p)$ value of >6. The chromosomal localization of SNP markers was preserved within the T3/Wheat Association Mapping Panel, accessible at https://wheat.triticeaetoolbox.org; to hypothesize candidate genes utilizing the IWGSC RefSeq v. 1.0 reference genome. Conversion of SNP markers into locus-specific KASP-markers was determined as per https://wheat.triticeaetoolbox.org (Supplementary Table 3).

Results were analyzed using a two-way analysis of variance (ANOVA); the significance of mean differences was assessed using Tukey' test ($P < 0.05$) in SPSS (PASW) Statistics 20.0 («IBM», USA).

3. RESULTS AND DISCUSSION

Phenotypic characterization

Phenotyping of wheat landraces and varieties originating from diverse breeding programs for agronomic traits during two growing seasons showed that landraces and modern varieties differed above all on yield-related and grain quality traits (Table 1). The superiority of landraces over modern wheat varieties is evident in their higher levels of protein, gluten content, and grain vitreousness. These characteristics exhibit variations among different ecological groups: landraces from Russia displayed levels of 15.9–23.1%, 33.9–57.7%, and 57.9–67.8%, respectively; those from Kazakhstan showed levels of 15.1–20.7%, 31.7–50.7%, and 57.9–62.2%; and landraces from Central Asia demonstrated levels of 17.7–20.5%, 39.9–49.6%, and 59.1–61.7%.

Table 1. The yield-related and grain quality traits of landraces and program KASIB germplasm in 2022 and 2023.

Trait	Pamyati Azieva, St	Element 22, St	Landraces			KASIB
			Russia	Kazakhstan	Central Asia*	
Protein content, %	16.7	16.6	18.2^{b}	18.2^{b}	18.1^{b}	16.9^{a}
Gluten content, %	36.2	36.7	41.9^{b}	41.0^{b}	40.8^{b}	37.1^{a}
Ash content, %	1.82	1.81	1.81^{c}	1.77^{a}	1.82^{c}	1.78^{a}
Wet gluten content, %	46.2	48.1	47.0^{d}	44.5^{b}	44.9^{b}	41.4^{a}
Gluten index, %	86.0	70.1	75.1^{a}	79.2^{b}	79.9^{b}	95.5^{d}
Flour output, %	57.5	55.2	55.0^{a}	54.7^{a}	55.3^{a}	56.2^{c}
Vitreousness, %	56.8	56.7	57.9^{b}	57.9^{b}	59.1^{c}	56.4^{a}
Sedimentation volume, ml	61.3	40.0	33.1^{a}	32.9^{a}	33.3^{a}	39.7^{d}
No. spike grains, pcs.	29.5	38.0	24.6^{a}	24.6^{a}	23.2^{a}	30.9^{d}
Grain weight of spike, g	1.13	1.36	0.84^{a}	0.85^{a}	0.85^{a}	1.29^{d}
Thousand kernel weight, g	41.6	40.0	34.1^{a}	34.2^{a}	36.5^{c}	42.1^{d}
Yield, g/m^{2}	288	361	186^{a}	169^{a}	183^{a}	341^{d}

Note. Means with different letters within each column are significantly different ($p < 0.05$);
* Kyrgyzstan, Tajikistan, Uzbekistan

In comparison, the protein and gluten content of standard varieties ranged from 16.6–16.7% and 36.2–36.7%, respectively. Notably, modern wheat varieties exhibited reduced grain vitreousness at 56.4%, a key indicator associated with superior bread-making quality. However, landraces, particularly those from Central Asia, displayed a higher value of 59.1% for this trait (Table 1). Advancements in breeding techniques have led to improvements in grain technological characteristics, resulting in increased kernel size and yield in breeding wheat varieties. The superior bread-making quality of modern varieties was indirectly affirmed by SDS sedimentation (39.7 ml) and gluten index (95.5%), whereas landraces showed values of 32.9–33.3 ml and 75.1–79.9% for these traits, respectively. Modern varieties boasted a thousand kernel weight of 42.1 g, and flour output of 56.2%, whereas landraces exhibited indicators ranging from 34.1–36.5 g and 54.7–55.3%, respectively. The yield components of modern varieties differed from landraces: increased number of grains per spike, grain weight per spike (30.9 pcs. and 1.29 g). The grain yield of breeding varieties was also larger compared to that of landraces (341 g/m^{2} vs.169–186 g/m^{2}).

Molecular Characterization

The differences in genomes polymorphism between landraces and modern varieties were detected during comparison of genotyping results of two collections (Figures 1).

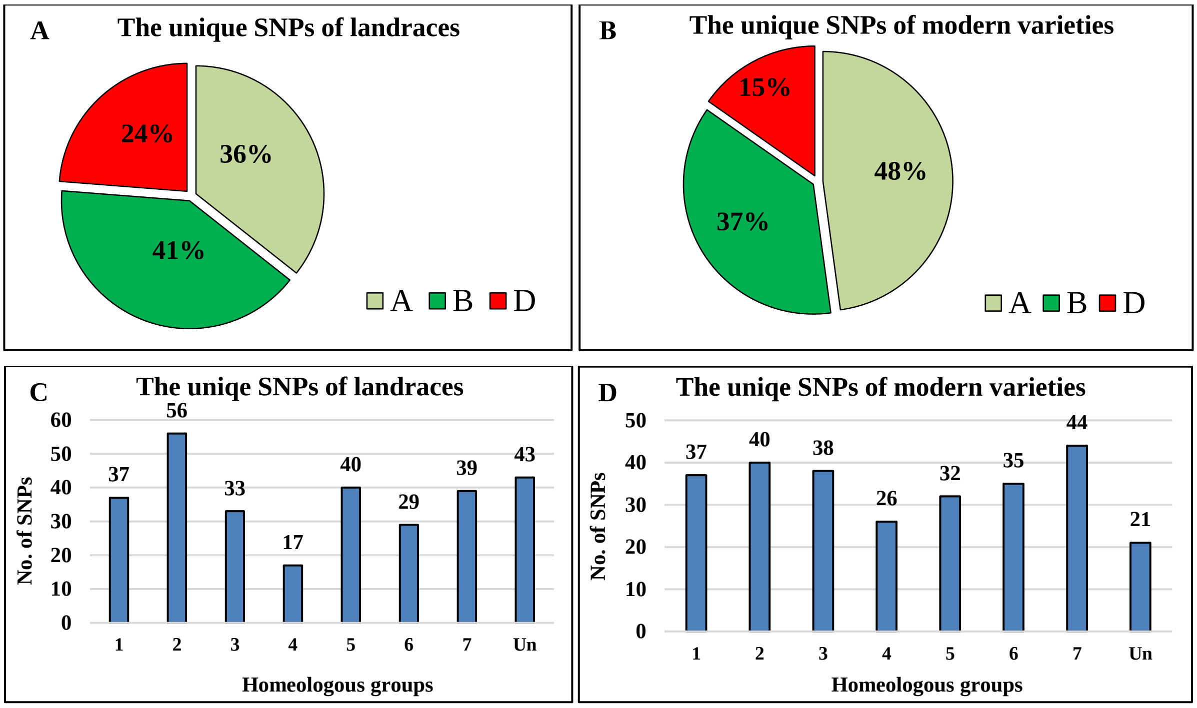

Figure 1. SNP loci distribution in the A, B, D genomes (sub-figures A, B), and homeologous groups of the germplasm of wheat landraces and modern varieties (sub-figures C, D); Note. Un – markers with unknown localization

In the landrace collection, a total of 294 SNPs were identified, distributed as follows: 36% in the A genome, 41% in the B genome, 24% in the D genome, and subsequently in the homeologous groups – 37 SNPs, 56 SNPs, 33 SNPs, 17 SNPs, 40 SNPs, 29 SNPs, and 39 SNPs, with 43 SNPs having an unknown localization. Conversely, in the collection of modern varieties, 273 SNPs were identified, with 48% in the A genome, 37% in the B genome, 15% in the D genome, and subsequently in the homeologous groups – 37 SNPs, 40 SNPs, 38 SNPs, 26 SNPs, 32 SNPs, 35 SNPs, and 44 SNPs, with 21 SNPs having an unknown localization. To dissect the genetic underpinnings influencing grain quality traits, we employed GWAS, unveiling a total of 294 markers in landrace accessions (36% SNPs within the A genome, 41% within the B genome, and 24% within the D genome). Similarly, within the KASIB program collection, 273 markers were identified (comprising 48% SNPs within the A genome, 37% within the B genome, and 15% within the D genome). Notably, the B genome of landrace germplasm exhibited the highest marker count (41%). Previous investigations, such as that conducted by Rahimi et al. (2019), highlighted a greater polymorphism within the B genome of both landraces and developed wheat varieties. Another study by Rufo et al. (2019) assessing genetic diversity across 354 bread wheat genotypes, including Mediterranean landraces and modern varieties, noted the lowest marker count within the D genome, with the B genome showcasing superior coverage. The linkage disequilibrium (LD) was investigated the distance between marker pairs 1,337,792 bp with an average density of 303 SNPs on one chromosome (Figure 2a). A population structure of varieties from both wheat collections was constructed using the STRUCTURE software, based on 17,190 SNP markers evenly distributed across all wheat chromosomes (Figure 2b). The landrace accessions clustered into four distinct groups labeled 1–4, including four varieties of program KASIB in fourth subcluster. An exception was the purple fifth subcluster, which encompassed 49% of the landraces included in the VIR collection between 1909 and 1948, along with 51% of modern wheat varieties from various breeding programs. Subclusters 6–7 were predominantly composed of germplasm from breeding programs in Russia and Kazakhstan, including one individual landrace accession (к-34556, Kitayskaya).

Marker trait associations (MTAs) identified by GWAS.

Association mapping conducted on the landrace population found five SNP markers that were significantly ($p < 0.00001$) associated with protein, gluten content, and gluten index for two years of research: AX-15857 2632, Bobhite_c31129_60, AX-158586137, BobWhite_c46257_130, and wsnp_JG_c5646_2148382 (Table 2).

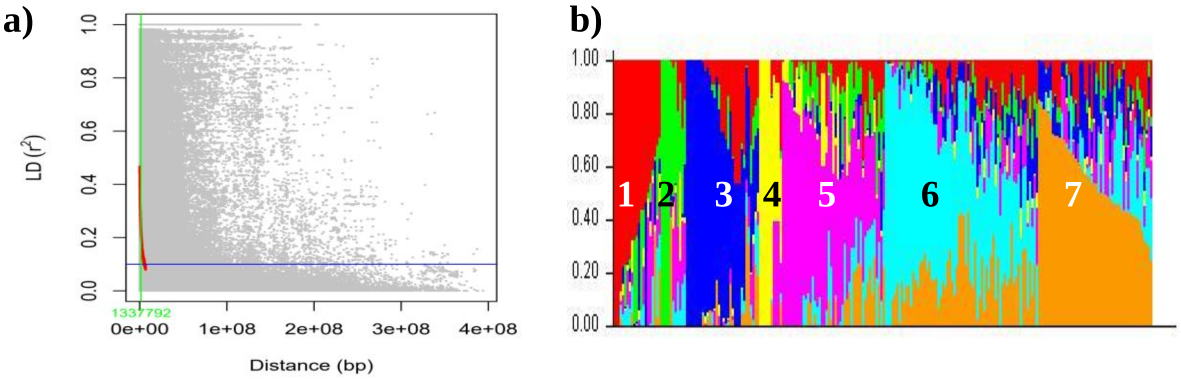

Figure 2. a) The rate of linkage disequilibrium (LD) decay based on 17,190 SNPs (p-value < 0.001); **b)** Population structure of landraces and KASIB germplasms using SNP marker system

Table 2. SNP markers with significant association identified in landraces genotypes.

Trait	Marker Name	Chr.	Position, Mb (RefSeq 1.0)	Target allele	Favorable season	P-value	R^2, %	Phenotypic effect
Protein content, %	BobWhite_c46257_130*	6B	514.675109	A/G	2022	5.19E-06	9	0.83
					2023	1.28E-07	11	0.43
Gluten content, %	AX-15858 6137*	5B	599.619662	C	2022	2.55E-07	26	0.15
					2023	3.55E-06	21	0.36
	BobWhite_c46257_130*	6B	514.675109	A/G	2022	5.14E-06	9	2.60
					2023	1.13E-07	11	1.44
	wsnp_JG_c5646_2148382*	6D	469.248518	C	2022	4.64E-06	22	0.30
					2023	4.64E-07	23	0.36
Strong gluten, %	Bobhite_c31129_60*	2B	68.201577	C	2022	7.73E-11	15	0.71
					2023	1.42E-06	10	0.36
	AX-15857 2632*	2A	677.050366	G	2022	7.51E-09	11	13.7
					2023	4.37E-10	12	10.1
Gluten index, %	wsnp_Ex_c10084_16572374	2A	692.934554	T	2023	4.45E-06	22	13.5
	BobWhite_c31129_60*	2B	68.201577	C	2022	3.04E-12	14	18.7
					2023	3.36E-10	11	11.5
	BobWhite_c46257_130*	6B	514.675109	G	2022	1.17E-08	10	11.6
					2023	8.39E-13	14	11.5
Vitreousness, %	AX-94949506	6A	610930266	A	2023	6.10E-06	19	3.76

Note. A/G – heterozygous alleles; *SNPs showing association during two growing seasons

At the same time, the presence of favorable allele of the marker BobWhite_c46257_130 increased the protein and gluten content but decreased the gluten index in landraces compared to modern varieties. In this study, the favorable allele of the marker wsnp_JG_c5646_2148382 (6D) was also associated with increasing of gluten content by 0.30–0.36%. In the genotypes of wheat landraces two SNP markers Bobhite_c31129_60 and AX-158572632 associated with decreasing of strong gluten content and gluten index were identified. On the contrary, in 2023, the favorable allele of SNP wsnp_Ex_c10084_16572374 was detected in 27% of landrace accessions and increased the gluten index by 13.5%. For the vitreousness trait, in 2023 moderately significant association was discerned for the locus AX-94949506. This SNP was identified in genotypes of landraces Khivinka, Belokoloska, Khozyaystvennyi obrazets # 151, krasnaya, Sandyk, Rasa XXXIII 94 Bar-50.

In this study the extremely significant associations in genotypes of landraces were identified for seven loci localized on chromosomes 2A, 2B, 5B, 6B, and 6D with the range of phenotypic variation 9–26% (Table 2). There are currently no data of previous studies for markers AX-158572632 (chromosome 2A, region 677.05 Mb) and BobWhite_c46257_130 (chromosome 6B, region 514.67 Mb). Notably, these loci were present in 39% and 55% of landrace genotypes, respectively. According to date of Ruud and Lillemo (2018), SNP Bobwhite_c31129_60 linked to gene TraesCS2B02G107000, controlling resistance to *Septoria nodorum blotch*. Previously, the gene TraesCS2A02G443000 associated with duration of period from flowering to ripeness was identified in study of Alemu et al. (2021). SNP AX-158586137 located on chromosome 5B was associated with drought tolerance (Sallam et al., 2024). Christensen and Borgen (2023) investigated locus wsnp_JG_c5646_2148382 linked to gene *Bt9*, controlling resistance to common bunt. In our research, in B genome of landraces were detected SNPs showed a pleiotropic effect on two quality-related traits – BobWhite_c31129_60 (for strong gluten content and gluten index) and BobWhite_c46257_130 (for protein and gluten content). Since there is currently no information about the association of identified loci with quality-related traits, it can be assumed that these loci of wheat landraces are potential novel for breeding programs aimed at improving grain quality. Association mapping conducted on the landrace and KASIB varieties populations performed over two growing seasons, revealed 48 marker-trait associations (MTAs), of which 22 MTAs were significantly linked to protein, gluten, ash content in grain, gluten index, and flour output. Significant associations were identified on chromosomes 1A, 2B, 4A, 5D, 6A, and 7A (Table 3).

Table 3. SNP markers with significant association identified in landraces and modern varieties.

Trait	Marker Name	Chr.	Position, Mb (RefSeq 1.0)	Target allele	Favorable season	P-value	R^2, %	Phenotypic effect
Protein content, %	TA005766-0499*	1A	11.895533	G	2022	2.97E-08	10	0.46
					2023	8.21E-12	15	1.55
	AX-94662379*	2B	74.250224	T	2022	1.42E-07	11	1.40
					2023	7.11E-13	17	1.90
	Excalibur_c30378_344*	4A	615.437273	G/T	2022	2.09E-08	13	1.44
					2023	7.84E-10	14	1.55
	BS00011469_51*	4A	621.848245	C	2022	1.29E-07	9	1.15
					2023	1.72E-08	10	1.27
	AX-94499635	4A	714.436088	A	2023	2.24E-09	13	1.61
	TG0131	5D	546.689237	T	2023	4.19E-08	10	1.03
	wsnp_JD_c4438_5567972	5D	546.689483	A	2023	1.56E-07	10	1.04
	AX-158551598	5D	546.824244	G	2023	3.3E-07	10	1.04
	AX-158586520*	5D	546.839205	C	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	BS00073116_51*	5D	546.864045	T	2022	6.21E-07	10	1.15
					2023	1.1E-07	10	1.27
	Excalibur_c42190_383*	5D	546.906373	A	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	Excalibur_c28592_173*	5D	546.910406	T	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	D_GDS7LZN01CBWNE_99	5D	546.700415	T	2023	3E-07	10	1.02
	CAP8_c145_89*	5D	547.273494	T	2022	6.67E-08	11	1.25
					2023	1.75E-08	11	1.35
	CAP7_rep_c12715_390*	5D	547.273595	C	2022	5.32E-08	11	1.25
					2023	2.38E-08	11	1.34
	wsnp_CAP11_c209_198671	5D	547.273762	T	2022	9.5E-08	11	1.23
					2023	4.55E-08	11	1.32
	AX-158586515*	5D	547.451565	T	2022	1E-07	11	1.24
					2023	2.66E-08	11	1.34
	AX-94626326*	5D	547.709624	A	2022	1.49E-07	11	1.19
					2023	3.12E-08	11	1.29
	Tdurum_contig75814_655*	6A	571.685447	C/T	2022	4.41E-07	10	1.17
					2023	4.71E-09	12	1.25
	AX-110992521	7A	27.107477	A/G	2023	1.45E-09	13	1.65
	AX-58590420*	7A	693.453814	G	2022	8.83E-10	14	1.04
					2023	8.42E-10	13	1.08
Gluten content, %	TA005766-0499	1A	11.895533	G	2023	3.9E-10	13	4.47
	AX-94662379	2B	74.250224	T	2023	9.49E-11	15	5.44
	Excalibur_c30378_344*	4A	615.437273	G/T	2022	2.97E-07	11	4.72
					2023	2.91E-08	13	4.43
	BS00011469_51*	4A	621.848245	C	2022	4.98E-08	10	4.07
					2023	6.45E-08	10	3.82
	AX-94499635	4A	714.436088	A	2023	5.85E-08	12	4.59
	TG0131*	5D	546.689237	T	2022	5.96E-07	9	3.11
					2023	2.07E-07	9	3.06
	wsnp_JD_c4438_5567972	5D	546.689483	A	2023	5.29E-07	10	3.11
	AX-158586520*	5D	546.839205	C	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	BS00073116_51*	5D	546.864045	T	2022	2.15E-07	11	4.07
					2023	4.58E-07	10	3.82
	Excalibur_c42190_383*	5D	546.906373	A	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	Excalibur_c28592_173*	5D	546.910406	T	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	CAP8_c145_89*	5D	547.273494	T	2022	2.26E-08	12	4.43
					2023	7.68E-08	11	4.05
	CAP7_rep_c12715_390	5D	547.273595	C	2022	1.88E-08	12	4.43
					2023	1.09E-07	11	4.01
	AX-158586515*	5D	547.451565	T	2022	4.13E-08	12	4.39
					2023	1.22E-07	11	4.01
	Tdurum_contig75814_655*	6A	571.685447	C/T	2022	2.17E-07	11	4.18
					2023	4.02E-09	13	5.00
	AX-110992521	7A	27.107477	A/G	2023	7.3E-09	13	5.00
	AX-158590420*	7A	693.453814	G	2022	2.97E-09	13	3.47
					2023	5.26E-09	13	3.21
	BS00011469_51	4A	621.848245	T	2023	1.04E-07	10	0.14
	TG0131	5D	546.689237	C	2023	9.58E-07	9	0.11
	BS00073116_51	5D	546.864045	G	2023	7.4E-07	10	0.14

Table 3. (continued)

Ash content, %	Excalibur_c42190_383	5D	546.906373	G	2023	4.44E-07	11	0.14
	Excalibur_c28592_173	5D	546.910406	C	2023	4.44E-07	11	0.14
	CAP8_c145_89	5D	547.273494	C	2023	6.94E-07	10	0.14
	CAP7_rep_c12715_390	5D	547.273595	T	2023	5.76E-07	10	0.14
	wsnp_CAP11_c209_198671	5D	547.273762	C	2023	3.52E-07	11	0.15
	AX-158586515	5D	547.451565	C	2023	5.4E-07	11	0.14
	AX-94626326	5D	547.709624	G	2023	3.88E-07	11	0.14
Flour output, %	AX-94662379	2B	74.250224	T	2022	2.43E-07	12	4.79
	AX-94499635	4A	714.436088	A	2022	2.36E-09	16	5.54
	AX-110992521	7A	27.107477	A/G	2022	3.47E-08	13	5.17
	TA005766-0499*	1A	11.895533	A	2022	2.83E-14	15	16.2
					2023	8.04E-18	18	13.0
Gluten index, %	Tdurum_contig41931_480*	1A	351.882499	A	2022	1.02E-16	25	19.8
					2023	1.56E-13	21	13.2
	AX-94662379*	2B	74250224	C	2022	1.43E-16	18	20.2
					2023	8.58E-20	21	15.7
	Excalibur_c30378_344*	4A	615.437273	G	2022	1.04E-14	17	18.7
					2023	1.61E-15	19	14.0
	BS00011469_ 51	4A	621.848245	T	2022	3.11E-07	7	11.0
	AX-94499635*	4A	714.436088	G	2022	3.15E-14	17	18.4
					2023	4.49E-19	22	14.9
	TG0131	5D	546.689237	C	2022	2.51E-07	7	9.27
	Tdurum_contig75814_655*	6A	571.685447	C	2022	5.26E-10	11	16.7
					2023	1.29E-10	12	4.65
	AX-110992521*	7A	27.107477	A	2022	2.47E-16	18	21.5
					2023	4.38E-18	20	16.4

Note. A/G, C/T, G/T – heterozygous alleles;
* SNPs showing association during two growing seasons

Fourteen markers were associated with high protein and gluten content: on chromosome 2B (AX-94662379), chromosome 4A (BS00011469_51, AX-94499635), chromosome 5D at 546.68–547.45 Mb (TG 0131, wsnp_JD_c4438_556 7972, AX-158586520, Excalibur_c42190_383, Excalibur_c28592_173, CAP8_c145_89, CAP7_rep_c12715_390, AX-158586515), chromosome 6A (Tdurum_contig75814_655), 7A chromosome (AX-110992521, AX-158590420). On average, the presence of favorable alleles of these SNPs increased the protein content by 1.0–1.4% and gluten content by 3–4% depending on a locus. However, favorable alleles of seven loci TA005766-0499 (1A), AX-94662379 (2B), BS00011469_51, AX-94499635 (4A), TG0131 (5D), Tdurum_contig75814_655 (6A) and AX-110992521 (7A) led to reduction of gluten index.

For the loci of chromosome 1A at the region 351.88 Mb (Tdurum_contig41931_480) and chromosome 4A at the region 615.43 (Excalibur_c30378_344), significant associations were found for two years of research on increasing of the gluten index by 13.2–18.8%. In 2022, an association with increased protein, gluten content, and flour output by 4.79–5.54% was found for three loci – AX-94662379 (2B), AX-94499635 (4A), AX-110992521 (7A).

As a result of the conducted studies, we detected the genomic region: 3 SNPs on chromosome 4A at 615.43–714.43 Mb and 12 SNPs on chromosome 5D at 546.68–547.70 Mb (RefSeq v. 1.0), containing a whole cluster of loci responsible for the concentration of protein and gluten (Table 3). The region on chromosome 4A demonstrated colocalization for both protein and gluten content was determined: candidate gene TraesCS4A02G341700 (621.84 Mb), which identified in study of Hu et al. (2020) like locus of resistance to Fusarium head blight; gene TraesCS4A01G330800 was previously described in study of Leonova and Ageeva (2022) to control the upper internode diameter; and new locus AX-94499635.

In our research, the protein and gluten content in landrace genotypes containing the heterozygous alleles G/T in Excalibur_c30378_344 (candidate gene TraesCS4A01G330800) was higher ($p < 0.0000002$) than that G/G haplotype of modern varieties, which was associated with high gluten index and excellent bread making quality. In this study the region of 5D chromosome was also characterized by colocalization for protein and gluten content. Numerous studies have confirmed the close relationship between these traits (Hao et al., 2022; Leonova et al., 2022). Nine candidate genes were annotated within at 1.02 Mb of 5D chromosome, of which six were previously described as candidate genes for resistance to Soil-borne wheat mosaic virus (Liu et al. 2020): TraesCS5D01G530600, TraesCS5D01G530700, TraesCS5D01G530900, TraesCS5D01G531100, TraesCS5D02G531200, TraesCS5D01G532100 genes. Among genes of this region, two were also linked to highly associated SNPs – TraesCS5D01G532400, TraesCS5D01G532800, and one new SNP (AX-158586520). The novel MTAs associated with grain protein content, grain gluten content, and gluten index were identified: Tdurum_contig41931_480 (1A), AX-158572632 (2A), AX-94499635 (4A), AX-158586137 (5B), AX-

158586520 (5D), AX-11099252, AX-158590420 (7A), which needs the evaluation of their effects and subsequent use in marker-assisted selection of wheat genotypes.

4. CONCLUSION

A comparative analysis between landraces and the promising wheat varieties from breeding programs of Russia and Kazakhstan for quality-related traits and genotypic difference showed the prospects of landraces as starting material for extending the genetic diversity of modern wheat varieties. Based on experiment data among 294 MTAs seven unique loci were identified with a −log10(p) value >6 in the landrace collection that had significant associations with grain protein content, grain gluten, strong gluten content, gluten index, and vitreousness. 22 candidate genes in landraces and modern wheat varieties, and also seven new MTAs were identified, which can be used for improvement of the wheat grain quality in breeding programs. Genomic regions in chromosomes 4A and 5D were determined, demonstrating colocalization for protein and gluten content. The practical interest for breeding are landrace genes absent in the genotype of modern wheat varieties, the introgression of which allows preserving the gene pools of varieties lost in the twentieth century and extending the genetic diversity of wheat. Landraces as sources of valuable allelic variants of fourteen loci, associated with high protein and gluten content can be used for breeding to improve grain quality traits.

AUTHOR CONTRIBUTIONS

AM and VS conceptualized the idea for research. AC, SE and MK conducted the experiment and collected data. SS and IP collected reviews of literature and prepared the original draft of the manuscript. All the authors participated in the review and editing process to refine the paper before submission.

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