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From Grain to Genome: Investigating Arsenic Levels in Triticum turgidum ssp durum Desf. Using GWAS

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ABSTRACT

Producing safe and high-quality food is increasingly important, and developing durum wheat varieties with low toxicity is crucial to meeting this demand. Durum wheat breeders can achieve this goal by developing new varieties that are either more resistant to arsenic uptake or better adapted to grow in areas with high arsenic levels. High levels of arsenic can pose serious health hazards, which makes it critical to evaluate the arsenic levels. Therefore, this study evaluated the arsenic levels in diverse durum wheat genotypes, including Turkish-released cultivars and local landraces. The results showed that all genotypes had significantly low and non-toxic levels of arsenic, with an average concentration of 5.24 µg/kg. These concentrations were much lower than the minimum reported in numerous published research studies and well below the risky international standard limits for durum wheat grain (0.1 mg/kg). The study also identified two significant marker-trait associations linked to arsenic contents located on chromosomes 4A and 7B, which explained 11-17% of the phenotypic variation. These findings provide valuable insights into the arsenic levels in durum wheat genotypes and highlight the need for ongoing monitoring to ensure safe and healthy food for consumers. By conducting collaborative genome-wide association studies and employing marker-assisted selection, durum wheat breeders can accelerate the creation of new varieties that have reduced arsenic levels by identifying alleles linked to arsenic content. This study emphasizes the importance of developing low-toxicity durum wheat varieties to ensure the safety and quality of our food supply. The findings can inform breeding programs to develop such varieties and contribute to sustainable agriculture. While the study's methodology was robust, further research is necessary to confirm and validate the genetic factors contributing to variation in arsenic content among different durum wheat genotypes.

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Introduction

Durum wheat (*Triticum turgidum* ssp. *durum* Desf.) is one of the primary grains used for making pasta, couscous, bulgur, and other traditional foods (Nachit et al., 2001; Alsaleh et al., 2015; Giraldo et al., 2016; Baloch et al., 2017). It is a rich dietary fiber, protein, vitamins, and minerals source, and the high protein content of durum wheat also makes it an important crop for livestock feed, particularly in areas where others are limited (Shewry and Sandra, 2015; Iqbal et al., 2022). The Food and Agriculture Organization (FAO, 2020) reports that Türkiye is the world's 9th largest wheat producer. Nonetheless, durum wheat typically possesses the genetic propensity to accumulate toxic elements from the soil in its seeds, which may exceed the safety thresholds of international standards (Vergine et al., 2017). The World Health Organization (WHO, 2022) states that Arsenic (As) is a naturally occurring element in soil, water, and air. It is a toxic substance that can harm human health and the environment, and its presence in agricultural soils can pose a risk to crop production. Arsenic can be introduced through human activities such as mining, smelting, and using pesticides and fertilizers (Abedin et al., 2002). Arsenic is not considered an essential nutrient for plant growth, and its presence in the soil can adversely affect durum wheat growth and yield. Durum wheat plants may exhibit a range of symptoms when exposed to elevated levels of Arsenic, including stunted growth, reduced yield, and yellowing of the leaves; it can also affect the quality of the grain, reducing its protein content and making it unsuitable for human consumption (Hossain et al., 2012). The uptake of Arsenic by durum wheat is influenced by various factors, such as soil properties, irrigation water quality, and farming practices, as noted by Zhang et al. (2009). In areas where the soil naturally has Arsenic, durum wheat may accumulate high levels in its grain, leading to potential health risks for humans who consume wheat-based products (Corguinha et al., 2015). Prolonged intake of high levels of Arsenic through food can result in various health problems, including cancer, skin lesions, neurological disorders, and cardiovascular disease (IARC, 2004; Moon et al., 2012; Pompa et al., 2021). Numerous global studies have

revealed the buildup of Arsenic in wheat grains, which includes investigations carried out by (Zhao et al., 2010; Shahid et al., 2017; and Hirzel et al., 2019). Thus, monitoring As levels in durum wheat grain and taking appropriate measures to minimize As exposure through the dietary intake is crucial. The extent of As accumulation in the grain is also influenced by genetic factors among durum wheat cultivars (Shi et al., 2019). However, the contribution of genetic versus environmental factors to As accumulation in durum wheat remains a topic of ongoing research and debate. Therefore, continued research is necessary to develop new durum wheat cultivars with low As contents and greater resilience to these stresses while improving yield potential, nutritional quality, and processing characteristics using new technologies such as Molecular Markers. Utilizing molecular markers, such as Simple Sequence Repeats (SSR), in breeding programs can expedite the creation of new cultivars that thrive in diverse environments (Nadeem et al., 2018). Unlike traditional breeding methods, molecular markers enable durum wheat breeders to quickly and accurately identify plants with desired traits, such as low As contents, stress tolerance, improved yield potential, and nutritional quality. SSR markers are especially useful in detecting genetic variation in closely related individuals and can be utilized for mapping and marker-assisted selection (MAS) (Ellegren, 2004; Alsaleh et al., 2016; Vieira et al., 2016). Frouin et al. (2019) suggested that a genome-wide association study (GWAS) could be a valuable approach to discovering genetic markers linked to significant traits, including arsenic accumulation. This approach is more precise and cost-effective than traditional breeding methods and can quickly identify genetic markers related to arsenic accumulation (Tam et al., 2019). Using molecular markers like SSRs in GWAS, durum wheat breeders can pinpoint the location of genes controlling vital traits like arsenic accumulation. Having this information can aid in creating more effective breeding techniques, like MAS, which can evaluate desirable traits in the initial generations. Despite the significant role that Türkiye plays in wheat domestication and diversity, there is a lack of systematic studies assessing the arsenic content in durum wheat germplasm from this region. Aiming to address this deficiency, a study utilizing a variety of durum wheat genotypes seeks to evaluate the variability in phenotypic arsenic content. The study involves a three-step process: first, the phenotypic variation of As content was measured and evaluated. Second, SSR markers were used to screen for genetic polymorphisms. Finally, the marker-trait association analysis was carried out to pinpoint the alleles accountable for the diversity in the arsenic content characteristic. After identification, the relevant markers were investigated in more detail to pinpoint potential candidate gene locations. These locations can then be integrated into MAS programs, which aim to cultivate durum wheat varieties with minimal or no levels of arsenic, making them excellent candidates as breeding parents in breeding programs.

Materials and Methods

Plant Material

The durum wheat genotypes tested in this study was obtained from Professor Dr. Hakan Özkan at Çukurova University, Adana, Türkiye. The panel consisted of four sets, including 50 released cultivars from Türkiye (referred to as Turkish CVs), 21 foreign cultivars from various countries (referred to as foreign CVs), 44 gene bank landraces (referred to as ex-situ LDs) from the National Genebank in İzmir/Türkiye, and 15 landraces (referred to as in situ LDs) commonly grown by domestic farmers, particularly in southeastern Türkiye. This same panel has been used in previous studies, including research on diversity structure (Alsaleh et al., 2022a) and assessments of cadmium and platinum in Turkish durum wheat diversity (Alsaleh et al., 2022b,c). For additional information on these genotypes, please refer to Table 1a, 1b, and 1c. During the growing season of 2019/2020, genotypes were grown at the research area of Field Crops Department of Agricultural Faculty, Çukurova University in Adana, Türkiye. The field experiment was set up in a randomized block design with three replications and 30 cm spacing between rows. Throughout the experiment, herbicides and fungicides were used to control weed growth and disease, respectively, and regular agronomic and plant protection assessments were conducted as necessary.

Arsenic Analysis

In June 2020, manual harvesting was carried out by randomly selecting three spikes of each genotype, one from each replication. The harvested spikes were then threshed by hand, and the resulting grains were stored in a dry storeroom. A soil sample was collected from the same experimental field. However, to reduce analytical investigation costs, the seeds from three replications of each genotype were combined, milled, and dried in an oven. The resulting mixed flour was then dissolved in an acidic solution using the "HPR-FO-52" procedure for wheat flour by the SK-10 high-pressure rotor microwave digestion system (ETHOS EASY Milestone, Italy) at a concentration of 0.5 g. After digestion, the samples were cooled to room temperature and diluted with 10% v/v nitric acid up to 20 ml for As content analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific ICAPQC, USA). The ICP-MS settings were 1550 W for radiofrequency power, 0.96 L/min for nebulizer gas, 0.88 L/min for a plasma gas, 3.01 bar for nebulizer pressure, dwell time of 0.01 ms and a spray chamber temperature of 3.7oC. To guarantee precision, the entire sample and standards underwent three repeated measurements. The Digest and As measurement (ICP-MS) procedures were carried out at BİLTEM laboratories at Yozgat Bozok University, Türkiye, as stated by Alsaleh et al. (2022b) and Alsaleh (2022c).

Isolation of Genomic DNA

In order to identify molecular markers, a single plant was randomly selected from each genotype. In February 2020, young leaves were collected, and the DNA isolation process was performed using the CTAB protocol (Doyle and Doyle, 1987) at the Laboratory of BİLTEM. After isolation, the DNA was assessed for both quantity and quality using 8% agarose gel electrophoresis. The DNA was subsequently thinned to 10 ng/µl for SSR analysis. This DNA was also utilized in the Cadmium and Platinum investigation by Alsaleh et al. (2022b) and Alsaleh (2022c), respectively.

Table 1a. Country of origins, release years, groups and pedigrees of 130 durum wheat genotypes evaluated for Arsenic assessments

No	Name	Country	year	Group	Pedigree/collection side/ growing locations
1	Kunduru-1149	Türkiye	1967	Turkish CV	(S)LV-TUR
2	Çeşit-1252	Türkiye	1999	Turkish CV	61-130/KUNDURU-414-44//377-2
3	Yılmaz-98	Türkiye	1998	Turkish CV	DF-9-71/3/V-2466//ND-61-130/414-44/4/ERGENE
4	Yelken-2000	Türkiye	2000	Turkish CV	ZF/LEEDS//FORAT/3/ND-61-130/LEEDS/4/(TR.SE)AU-107/5/GERARDO
5	Altın	Türkiye	1998	Turkish CV	BARRIGON-YAQUI-ENANO/2*TEHUACAN-60//2B//LONGSHANKS/3/BERKMEN-469
6	Meram-2002	Türkiye	2002	Turkish CV	ND-61-130/414-44//CAKMAK-79
7	Dumlupınar	Türkiye	2006	Turkish CV	BERKMEN/G-75-T-181
8	Şölen-2002	Türkiye	2002	Turkish CV	STERNA,MEX/ALTAR-84/3/GANSO/FLAMINGO,MEX//CANDO
9	Altıntoprak-98	Türkiye	1998	Turkish CV	ALTAR-84/ARAOS
10	Çakmak-79	Türkiye	1979	Turkish CV	UVEYIK-162/ND-61-130
11	Eminbey	Türkiye	2007	Turkish CV	CMK79//14-44/OVIACHIC-65/3/BERKMEN/OVIACHIC-65/4/KUNDURU-1149/5/LEEDS//DWARF-MUTANT/SARIBASAK
12	Kümbet-2000	Türkiye	2000	Turkish CV	ND-61-130//414-44/377-2/3/DF-15-72
13	İmren	Türkiye	2009	Turkish CV	DF-21-72/GERARDO-VZ-466//ND-61-130/414-44/3/ERGENE/4/DF-21-72//ND-61-130/UVEYIK-162/3/128-3
14	Balcalı-2000	Türkiye	2000	Turkish CV	MAGHREBI-72/(SIB)FLAMINGO,MEX//CRANE(SIB)/ND-USA-2299/3/(SIB)YAVAROS-79/4/DACKIYE/(SIB)RABICORNO//(SIB)WINGET; (SIB)STERNA,MEX
15	Sham-1	Türkiye	1984	Turkish CV	PELICANO/RUFF//GAVIOTA/ROLETTE; PELICANO(SIB)/(SIB)RUFF//GAVIOTA(SIB)/(SIB)ROLETTE
16	Ankara-98	Türkiye	1998	Turkish CV	KOBAK-2916/LEEDS//6783/3/BERKMEN-469/7/CRANE/GANSO//APULICUM/3/DF-17-72/4/DI-165137/GEDIZ-
17	Balcalı-85	Türkiye	1985	Turkish CV	JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO,MEX
18	Fuatbey-2000	Türkiye	2000	Turkish CV	---
19	Akbaşak-073144	Türkiye	1970	Turkish CV	(S)LV-TUR
20	Artuklu	Türkiye	2008	Turkish CV	LAHN//GANSO/STORK
21	Mirzabey-2000	Türkiye	2000	Turkish CV	GD-2/D-1184528
22	Aydın-93	Türkiye	1993	Turkish CV	JORI-69/HAURANI
23	Diyarbakır-81	Türkiye	1981	Turkish CV	LD-393//BELADI-116-E/2*TEHUACAN-60/3/COCORIT-71
24	Eyyubi	Türkiye	2008	Turkish CV	MORUS//ALTAR-84/ALONDRA
25	Selçuklu-97	Türkiye	1997	Turkish CV	073-44*2/OVI/3/DF-21-72//ND-61-130/UVEYIK-162
26	Fatasel-185/1	Türkiye	1964	Turkish CV	Selected from FATA bring from Burdur in 1952
27	Altınbaç-95	Türkiye	1995	Turkish CV	KUNDURU//D-68111/WARD
28	Harran-95	Türkiye	1995	Turkish CV	KORIFLA//DS-15/GEIGER ; DURUM-DWARF-S-15/CRANE//GEIER
29	Sarıçanak-98	Türkiye	1998	Turkish CV	DACKIYE/GEDIZ-75//USDA-575
30	Tüten-2002	Türkiye	2002	Turkish CV	ALTAR/AVETORO/3/GANSO/FLAMINGO,MEX//CANDO
31	Turabi	Türkiye	2004	Turkish CV	CRESO/CRANE
32	Ege-88	Türkiye	1988	Turkish CV	JORI-C-69/ANHINGA//FLAMINGO,MEX
33	Güney yıldızı	Türkiye	2010	Turkish CV	RASCON-39/TILD-1
34	Fırat-93	Türkiye	2002	Turkish CV	SNIPE/3/JORI-C-69/CRANE/GANSO/ANHINGA; ANHINGA(SIB)/(SIB)VOL//(SIB)FLAMINGO,MEX/3/SHAW
35	Şahinbey	Türkiye	2008	Turkish CV	Lagost-2 ICD.86-0471-ABL-OTR-8AP-0TR-20AP-OTR
36	Zühre	Türkiye	2011	Turkish CV	SN-TURK-M-183-84-375/(SIB)NIGRIS//TANTLO-1
37	Gündaş	Türkiye	2012	Turkish CV	LGT3/4/BICRE/3/CHAM-1//GAVIOTA/STARKE
38	Akçakale-2000	Türkiye	2002	Turkish CV	SCHELLENTE//CORMORANT/RUFFOUS/3/AJAIA
39	Gökgöl-79	Türkiye	1979	Turkish CV	BUCK-BALCARCE//BARRIGON-YAQUI-ENANO*2/TEHUACAN-60
40	Amanos 97	Türkiye	1997	Turkish CV	OSTRERO//CELTA/YAVAROS,AUS
41	Kızıltan-91	Türkiye	1991	Turkish CV	UVEYIK-162/61-130//BARRIGON-YAQUI-ENANO*2/TE
42	Özberk	Türkiye	2005	Turkish CV	FLAMINGO,MEX/GARZA//CANDEAL-1/GREBE/3/CENTRIFEN/FLAMINGO,MEX/PETREL/5/AKBASAK-073-44/YERLI/6/CAR
43	Urfa-2005	Türkiye	2005	Turkish CV	Fg'S'/Gr'S'//CandeaI I/4/Grebe 'S'/3/Ctfn/Fg'S'//Ptl 'S'/5/Akb.073.44/ye rli/6/Carc'S
44	Ceylan-95	Türkiye	1995	Turkish CV	STORK(SIB)/(SIB)RABICORNO
45	Salihli-92	Türkiye	1992	Turkish CV	SHWA//21563/ANHINGA/3/EGE-88; B.BAL//BARRIGON-YAQUI-ENANO*2/TEHUACAN-60
46	Gap	Türkiye	2004	Turkish CV	GEDIZ-75(SIB)/(SIB)FLAMINGO,MEX//(SIB)TEAL,MEX
47	Soylu	Türkiye	2012	Turkish CV	----
48	Ali baba	Türkiye	2010	Turkish CV	AWALI-2/BITTERN
49	Tunca-79	Türkiye	1979	Turkish CV	FATA(SEL.181-1)/ND-61-130//LEEDS

Table 1b. Country of origins, release years, groups and pedigrees of 130 durum wheat genotypes evaluated for Arsenic assessments

No	Name	Country	year	Group	Pedigree/collection side/ growing locations
50	Saribasak	Türkiye	1970	Turkish CV	LV-TUR
51	Vatan	Tadjikistan	1978	Foreign CV	TADZHIKSKAYA-CHERNOKOLOSAYA/KHORANKA-46
52	Zenit	Italy	1992	Foreign CV	VALRICCARDO/VIC
53	Saragolıa	Italy	2004	Foreign CV	IRIDE/LINEA-PSB-0114
54	Svevo	Italy	1996	Foreign CV	CIMMYT-SELECTION/ZENIT
55	Claudio	Italy	2011	Foreign CV	Sel.CIMMYT-35/Durango/ISEA-1938/Grazia
56	Baio	Italy	1998	Foreign CV	DUILLO/F-21//G-76
57	UI-Darwin	USA	2006	Foreign CV	IDO-445/MANNING
58	UC1113	USA	2005	Foreign CV	KIFS//RSS/BD-1419/3/MEXIS-CP/4/WAHAS/5/YAVAROS-79
59	AC-Pathifinder	Canada	1999	Foreign CV	WESTBRED-881/DT-367; DT-367/WESTBRED-881
60	AC-Navigator	Canada	1999	Foreign CV	KYLE/WESTBRED-881
61	Floradur	Austria	2003	Foreign CV	HELIDUR/CIMMYT-4833
62	C9	West bank	---	Foreign CV	---
63	C43	West bank	---	Foreign CV	---
64	Inbar	West bank	1978	Foreign CV	D-27534/3/JORI(SIB)//LD-357-E/2*TEHUACAN-60; LD-357-E/2*TEHUACAN-60//JORI-69; D-27534-13-M-4-Y-1-M/3/JORI(SIB)//LD-357-E/2*TEHUACAN-60
65	Creso	Italy	1974	Foreign CV	60/4/CPB-144; CAPELLI-B-144/5/YAKTANA-54//(SELECTION-14)NORIN-10/BREVOR/3/CAPELLI-63/4/3*TEHUACAN-60; MARINGA/ZENATI/CPB-144
66	Simeto	Italy	1988	Foreign CV	CAPEITI-8/VALNOVA
67	Irıde	Italy	1996	Foreign CV	ALTAR-84/IONIO; ALTAR-84/(SIB)ARES
68	Dylan	Italy	2002	Foreign CV	NEUDUR/ULISSE
69	Ofanto	Italy	1990	Foreign CV	ADAMELLO/APPULO
70	Cham-1	Syria	1984	Foreign CV	PELICANO/RUFF//GAVIOTA/ROLETTE; PELICANO(SIB)/(SIB)RUFF//
71	Cham-9	Syria	2010	Foreign CV	STJ3//BICRE/LOUKOS-4
72	TR 32090	Türkiye	---	Ex-situ	Ankara
73	TR 53861	Türkiye	---	Ex-situ	Yozgat
74	TR 80984	Türkiye	---	Ex-situ	Eskişehir
75	TR 72025	Türkiye	---	Ex-situ	Konya
76	TR 81249	Türkiye	---	Ex-situ	Elaziğ
77	TR 81371	Türkiye	---	Ex-situ	Niğde
78	TR 71914	Türkiye	---	Ex-situ	Konya
79	TR 81356	Türkiye	---	Ex-situ	Konya
80	TR 81381	Türkiye	---	Ex-situ	Sivas
81	TR 45305	Türkiye	---	Ex-situ	Yozgat
82	TR 46881	Türkiye	---	Ex-situ	Erzincan
83	TR 81259	Türkiye	---	Ex-situ	Malatya
84	TR 81273	Türkiye	---	Ex-situ	Ankara
85	TR 47949	Türkiye	---	Ex-situ	Kars
86	TR 54969	Türkiye	---	Ex-situ	Yozgat
87	TR 63315	Türkiye	---	Ex-situ	Konya
88	TR 81238	Türkiye	---	Ex-situ	Erzincan
89	TR 56206	Türkiye	---	Ex-situ	Eskişehir
90	TR 56128	Türkiye	---	Ex-situ	Eskişehir
91	TR 54977	Türkiye	---	Ex-situ	Yozgat
92	TR 54973	Türkiye	---	Ex-situ	Yozgat
93	TR 53860	Türkiye	---	Ex-situ	Yozgat
94	TR 56135	Türkiye	---	Ex-situ	Eskişehir
95	TR 32015	Türkiye	---	Ex-situ	Malatya
96	TR 31930	Türkiye	---	Ex-situ	Malatya
97	TR 32167	Türkiye	---	Ex-situ	Yozgat
98	TR 35150	Türkiye	---	Ex-situ	Yozgat
99	TR 31887	Türkiye	---	Ex-situ	Elaziğ
100	TR 31902	Türkiye	---	Ex-situ	Malatya
101	TR 31893	Türkiye	---	Ex-situ	Malatya
102	TR 35148	Türkiye	---	Ex-situ	Yozgat
103	TR 81277	Türkiye	---	Ex-situ	Ankara
104	TR 81283	Türkiye	---	Ex-situ	Ankara
105	TR 81284	Türkiye	---	Ex-situ	Ankara
106	TR 81367	Türkiye	---	Ex-situ	Konya
107	TR 81374	Türkiye	---	Ex-situ	Konya
108	TR 81258	Türkiye	---	Ex-situ	Malatya
109	TR 81278	Türkiye	---	Ex-situ	Ankara
110	TR 81323	Türkiye	---	Ex-situ	Ankara
111	TR 81304	Türkiye	---	Ex-situ	Malatya

Table 1c. Country of origins, release years, groups and pedigrees of 130 durum wheat genotypes evaluated for Arsenic assessments

No	Name	Country	year	Group	Pedigree/collection side/ growing locations
112	TR 81369	Türkiye	---	Ex-situ	Niğde
113	TR 81550	Türkiye	---	Ex-situ	Niğde
114	TR 81544	Türkiye	---	Ex-situ	Niğde
115	TR 81338	Türkiye	---	Ex-situ	Ankara
116	Bağacak	Türkiye	---	In-situ	Southeast of Türkiye
117	Menceki	Türkiye	---	In-situ	Southeast of Türkiye
118	Mersiniye	Türkiye	---	In-situ	Southeast of Türkiye
119	Sivaslan	Türkiye	---	In-situ	Southeast of Türkiye
120	Şırnak Alkaya	Türkiye	---	In-situ	Southeast of Türkiye
121	Kurtulan	Türkiye	---	In-situ	Southeast of Türkiye
122	Karadere	Türkiye	---	In-situ	Southeast of Türkiye
123	Hacıhalil	Türkiye	---	In-situ	Southeast of Türkiye
124	Hevidi	Türkiye	---	In-situ	Southeast of Türkiye
125	Beyaziye	Türkiye	---	In-situ	Southeast of Türkiye
126	Mısrı	Türkiye	---	In-situ	Southeast of Türkiye
127	İskenderiye	Türkiye	---	In-situ	Southeast of Türkiye
128	Karakılçık	Türkiye	---	In-situ	Southeast of Türkiye
129	Havrani	Türkiye	---	In-situ	Southeast of Türkiye
130	Levante	Türkiye	---	In-situ	Southeast of Türkiye

Analysis of Simple Sequence Repeats

Diverse microsatellite primers were chosen to cover various segments of durum wheat chromosomes. In a study conducted by Alsaleh (2022c), the same set of eighty-two SSR primers was utilized to detect a recently discovered QTL linked with platinum accumulation. Table 2a, 2b, and 2c furnishes a concise overview of the SSR primers and their associated information utilized in the research. The M13-tailed primer approach, following the technique of Schuelke (2000), was employed to amplify the SSR region through PCR. The final volume of the PCR reaction was 12 µl, containing 1X buffer, 0.125 mM dNTPs, 0.4 pmol "M13" forward primer, 0.3 pmol reverse primers, 3.0 pmol universal M13 primer labeled with one of four fluorescent dyes (6-FAM, VIC, NED, or PET), 0.12U Taq DNA polymerase, and approximately 25 ng genomic DNA. The PCR amplification process started with a primary denaturation at 94°C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 to 65 °C (depending on the annealing temperature of the primers) for 1 min, and 72°C for 1 min. Eight cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s were then carried out. The final extension was 72°C for 10 min. The accuracy of the SSR fragments was assessed twice using Gene Mapper software v3.7 (Applied Biosystems) following the manufacturer's guidelines. The individual bands of the SSR were analyzed, and the binary scoring method was employed to assign a '1' for the presence of bands and a '0' for their absence. This technique facilitates the assessment and statistical analysis of co-dominant SSR data, as Kaya et al. (2016) reported. Finally, the PCR products were loaded onto the ABI 3130xl Genetic Analyzer device (Applied Biosystems) for fragment analysis.

Statistical Analysis

The genotype panel was divided into four groups based on their origin as explained above to perform ANOVA analysis. Variance analysis for arsenic content and the distribution of phenotypic frequency were conducted by using Microsoft Excel software. The proportion of phenotypic variation explained by arsenic content for each marker was estimated using the R2 value in TASSEL 5 (Bradbury et al., 2007). The Bonferroni threshold for multiple testing and an adjusted corrective threshold was applied to determine significant associations (Kaler and Purcell, 2019). Specifically, the 5% Bonferroni threshold for multiple comparisons was used, resulting in 337 markers being included in the current GWAS.

Results and Discussion

Phenotypic Variation for Arsenic Contents

Based on the ICP-MC analysis, the genotypes demonstrated low concentrations and non-toxic As contents. The As content ranged from 0.175 µg/kg in the Turkish cultivar "Eminbey" to 43.81 µg/kg in the "Mısrı" genotype, which was the landrace of in-situ LDs, with an average concentration of 5.24 µg/kg. Nevertheless, the As content in all genotypes remained significantly below the hazardous threshold of 0.1 mg/kg, as illustrated in Table 3. Figure 1a showed the frequency distribution of grain As contents for the entire panel. When the genotypes were divided into four groups, the foreign and Turkish CVs had lower average As contents at 3.33 and 4.82 µg/kg, respectively. On the other hand, the in-situ and ex-situ LDs groups had the highest average As contents at 8.78 and 5.42 µg/kg, respectively, as illustrated in Figure 1b.

Table 4 depicted the soil properties of the experimental area at Çukurova University, Adana, Türkiye, where the field experiment was conducted.

Variation in genetics and associations with markers and traits

The MLM+Q+K approach identified two marker-trait associations (MTA) significantly associated with arsenic contents after a Bonferroni correction at a significance level of $P<0.05$ was applied (as shown in Table 5). The significant SSR markers associated with As content were displayed in the Manhattan plot (Figure 2), with a 5% Bonferroni correction threshold.

Table 2a. Chromosomal locations and repeat motifs of the Simple Sequence Repeats primers utilized to screen polymorphic sequences

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
1	WMC120F	GGAGATGAGAAGGGGGTCAGGA	1A	(CA), (GA), (GT)
	WMC120R	CCAGGAGACCAGGTTGCAGAAG		
2	WMC231F	CATGGCGAGGAGCTCGGTGGTC	3B	GA)10 , (GT)8
	WMC231R	GTGGAGCACAGGCGGAGCAAGG		
3	WMC406F	TATGAGGGTCGGATCAATACAA	1B	(CA)16
	WMC406R	CGAGTTTACTGCAAACAAATGG		
4	WMC477F	CGTCGAAAACCGTACACTCTCC	2B	(GT)16
	WMC477R	GCGAAACAGAATAGCCCTGATG		
5	WMC1F	ACTGGGTGTTTGCTCGTTGA	3B/6A	(CT)(CA)
	WMC1R	CAATGCTTAAGCGCTCTGTG		
6	WMC361F	AATGAAGATGCAAATCGACGGC	2B	(CA)10
	WMC361R	ATTCTCGCACTGAAAACAGGGG		
7	WMC107F	GAATTCAGGCCCTTCTCGGA	7A	(GT)15
	WMC107R	CATTGAACCTCGCATAACGG		
8	CFA2147F	TCATCCCCTACATAACCCGA	1B/1D	(CATC)4
	CFA2147R	ATCGTGCACCAAGCAATACA		
9	GWM156F	CCAACCGTGCTATTAGTCATTC	3B/5AL/5BS	(GT)14
	GWM156R	CAATGCAGGCCCTCCTAAC		
10	WMC296F	GAATCTCATCTTCCCTTGCCAC	2A	(GA)11 & , (GT)28
	WMC296R	ATGGAGGGGTATAAAGACAGCG		
11	GWM304F	AGGAAACAGAAATATCGCGG	2A/5A	(CT)22
	GWM304R	AGGACTGTGGGGAATGAATG		
12	WMC218F	TCTCCTGTCGGCTGAAAGTGTT	7B	(TG)7CGTGC(GT)7
	WMC218R	CCATGGAGGTTCACCTAGCAAA		
13	WMC128F	CGGACAGCTACTGCTCTCCTTA	1B	(GA)10 &, (GT)16
	WMC128R	CTGTTGCTTGCTCTGCACCCTT		
14	WMC262F	GCTTTAACAAAGATCCAAGTGGCAT	4AL	GA)29
	WMC262R	GTAAACATCCAAACAAAGTCGAACG		
15	WMC307F	GTTTGAAGACCAAGCTCCTCCT	3B	GT)8 (GA)13
	WMC307R	ACCATAACCTCTCAAGAACCCA		
16	WMC312F	TGTGCCCGCTGGTGCGAAG	1A	(GA)14
	WMC312R	CCGACGCAGGTGAGCGAAG		
17	WMC317F	TGCTAGCAATGCTCCGGGTAAC	2BL	(GT)23
	WMC317R	TCACGAAACCTTTTCCTCCTCC		
18	WMC31F	GTTCACACGGTGATGACTCCCA	1B	(GA)11, (GT)19
	WMC31R	CTGTTGCTTGCTCTGCACCCTT		
19	WMC327F	TGCGGTACAGGCAAGGCT	5AL	(GT)25
	WMC327R	TAGAACGCCCTCGTCGGA		
20	GWM369F	CTGCAGGCCATGATGATG	3A/4B/7B	(CT)11(T)2(CT)21
	GWM369R	ACCGTGGGTGTTGTGAGC		
21	WMC476F	TACCAACCACACCTGCGAGT	7B	(GT)7 118, (GT)25
	WMC476R	CTAGATGAACCTTCGTGCGG		
22	WMC511F	CGCACTCGCATGATTTTCCT	4BS	(GT)7, CGTG
	WMC511R	ATGCCCGGAAACGAGACTGT		
23	WMC612F	GAGGTCAGTACCCGGAGA	3B	
	WMC612R	CCACCCCAATTCAAAAAG		
24	WMC626F	AGCCCATAAACATCCAACACGG	1B	
	WMC626R	AGGTGGGCTTGGTTACGCTCTC		
25	WMC657F	CGGGCTGCGGGGGTAT	4B	
	WMC657R	CGGTTGGGTCATTTGTCTCA		
26	WMC662F	AGTGGAGCCATGGTACTGATTT	7B	
	WMC662R	TGTGTACTATTCCCGTCGGTCT		
27	WMC727F	CATAATCAGGACAGCCGCAC	5AL	
	WMC727R	TAGTGGCCTGATGTATCTAGTTGG		
28	WMC75F	GTCCGCCGCACACATCTTACTA	5B	(GT)13
	WMC75R	GTTTGATCCTGCGACTCCCTTG		
29	BARC354F	CGTTGTTTGCGTAGAAGGAGGTT	6B	
	BARC354R	GCGAATGCGGGCGATAAAGTGG		
30	CFA2191F	AGAGCAGGAGGTTGGGTTCT	3B	(TCCC)4
	CFA2191R	CCGGAATTTCACTACCAGGA		
31	BARC85F	GCGAACGCTGCCCGGAGGAATCA	7B	(CAT)8
	BARC85R	GCGTCGCAGATGAGATGGTGGAGCAAT		
32	CFA2114F	ATTGGAAGGCCACGATACAC	6A	(CA)32
	CFA2114R	CCCGTCGGGTTTTATCTAGC		
33	CFD238F	GTTGAGGAGGACAAAGAGGC	2B	(GGGA)3
	CFD238R	GATACGAGCGAGCCCATAAA		
34	CFD242F	CCAGTTTGCAGCAGTCACAT	7A	(GTT)15(AGC)5

Table 2b. Chromosomal locations and repeat motifs of the Simple Sequence Repeats primers utilized to screen polymorphic sequences

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
	CFD242R	CAGACCTTAACGGGGTTGAA		
35	GWM456F	TCTGAACATTACACAACCCTGA	1B/3D	(GA)21
	GWM456R	TGCTCTCTCTGAACCTGAAGC		
36	GWM375F	ATTGGCGACTCTAGCATATACG	4B	
	GWM375R	GGGATGTCTGTTCCATCTTAGC		
37	GWM513F	ATCCGTAGCACCTACTGGTCA	4BL/5B/7BS	(CA)12
	GWM513R	GGTCTGTTCATGCCACATTG		
38	GWM77F	ACCCTCTTGCCCGTGTTG	3BS	(CA)10 (GA)40
	GWM77R	ACAAAGGTAAGCAGCACCTG		
39	WMC553F	CGGAGCATGCAGCTAGTAA	6A	(CA)24
	WMC553R	CGCCTGCAGAATTCAACAC		
40	BARC77F	GCGTATTCTCCCTCGTTTCCAAGTCTG	3B	(ATCT)6
	BARC77R	GTGGGAATTTCTTGGGAGTCTGTA		
41	BARC78F	CTCCCCGGTCAAGTTTAATCTCT	4A	(TC)27(TATC)43
	BARC78R	GCGACATGGGAATTTCAGAAGTGCCTAA		
42	CFA2141F	GAATGGAAGGCGGACATAGA	5A/5D	(GA)18
	CFA2141R	GCCTCCACAACAGCCATAAT		
43	CFD7F	AGCTACCAGCCTAGCAGCAG	5B/5DL	(TC)27
	CFD7R	TCAGACACGTCTCCTGACAAA		
44	CFD168F	CTTCGCAAATCGAGGATGAT	2A/2D	(CTG)20
	CFD168R	TTCACGCCCAGTATTAAGGC		
45	CFD71F	CAATAAGTAGGCCGGGACAA	4A/4D	(CA)10(GA)30
	CFD71R	TGTGCCAGTTGAGTTTGCTC		
46	GWM293F	TACTGGTTCACATTGGTGCG	5AL/5B/5D/7B	(CA)24
	GWM293R	TCGCCATCACTCGTTCAAG		
47	WMC407F	GGTAATTCTAGGCTGACATATGCTC	2A	(GA)16
	WMC407R	CATATTTCCAAATCCCCAACTC		
48	WMC486F	CCGGTAGTGGGATGCATTTT	6B	(GT)28
	WMC486R	ATGCATGCTGAATCCGGTAA		
49	WMC517F	ATCCTGACGTTACACGCACC	7B	(CA)
	WMC517R	ACCTGGAACACCACGACAAA		
50	WMC522F	AAAAATCTCACGAGTCGGGC	2A	(CT)
	WMC522R	CCCGAGCAGGAGCTACAAAT		
51	WMC524F	TAGTCCACCGGACGGAAAGTAT	5A	(GT)
	WMC524R	GTACCACCGATTGATGCTTGAG		
52	WMC532F	GATACATCAAGATCGTGCCAAA	3A	(GA)
	WMC532R	GGGAGAAATCATTAACGAAGGG		
53	WMC592F	GGTGGCATGAACTTTCACCTGT	2B	
	WMC592R	TGTGTGGTGCCCATTAGGTAGA		
54	WMC596F	TCAGCAACAAACATGCTCGG	7A	
	WMC596R	CCCGTGTAGGCGGTAGCTCTT		
55	WMC616F	TAAAGCTAGGAGATCAGAGGCG	5B	(XX)
	WMC616R	TAATCCCATCTTGAGAAGCGTC		
56	WMC633F	ACACCAGCGGGGATATTTGTTAC	7A	(XX)
	WMC633R	GTGCACAAGACATGAGGTGGATT		
57	GWM124F	GCCATGGCTATCACCCAG	1B	(CT)27(GT)18
	GWM124R	ACTGTTCGGTGCAATTTGAG		
58	WMC335F	TGCGGAGTAGTTCTTCCCCC	7B	(CA)5G(CA)12
	WMC335R	ACATCTTGGTGAGATGCCCT		
59	WMC364F	ATCACAATGCTGGCCCTAAAAC	7B	(CA)18
	WMC364R	CAGTGCCAAAATGTCGAAAGTC		
60	WMC658F	CTCATCGTCCTCCTCCACTTTG	2A	(XX)
	WMC658R	GCCATCCGTTGACTTGAGGTTA		
61	WMC73F	TTGTGCACCGCACTTACGTCTC	5B	(CA)9
	WMC73R	ACACCCGGTCTCCGATCCTTAG		
62	WMC83F	TGGAGGAAACACAATGGATGCC	7A	(GT)28
	WMC83R	GAGTATCGCCGACGAAAGGGAA		
63	BARC89F	GGGCGCGGCACCAGCACTACC	5B	(TCA)11
	BARC89R	CTCCGAGGCCACCGAAGACAAGATG		
64	BARC74F	GCGCTTGCCCCTTCAGGCGAG	5B	(GA)13(GATA)7(GA)9
	BARC74R	CGCGGGAGAACCACCAGTGACAGAGC		
65	CFA2028F	TGGGTATGAAAGGCTGAAGG	7A	(CA)21
	CFA2028R	ATCGCGACTATTCAACGCTT		
66	GWM130F	AGCTCTGCTTCACGAGGAAG	2B/7A/7D	(GT)22
	GWM130R	CTCCTCTTTATATCGCGTCCC		
67	CFA2183F	TCTTGGATGGATTTGTGAGC	3A	(CA)26
	CFA2183R	TTCCTTCTCCTTCATTAGCTGC		

Table 2c. Chromosomal locations and repeat motifs of the Simple Sequence Repeats primers utilized to screen polymorphic sequences

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
68	CFA2234F	AATCTGACCGAACAAAATCACA	3A	(CA)17
	CFA2234R	TCGGAGAGTATTAGAACAGTGCC		
69	CFA2263F	GGCCATGTAATTAAGGCACA	2AL	(CA)24
	CFA2263R	CTCCCAGGAGTACAGAAGAGGA		
70	WMC397F	AGTCGTGCACCTCCATTTTG	6B	(CA)
	WMC397R	CATTGGACATCGGAGACCTG		
71	BARC181F	CGCTGGAGGGGGTAAGTCATCAC	1B	(CT)17
	BARC181R	CGCAAATCAAGAACACGGGAGAAAGAA		
72	WMC311F	GGGCCTGCATTTCTCCTTTCTT	7B	(GT)12
	WMC311R	CTGAACTTGCTAGACGTTCCGA		
73	WMC181F	TCCTTGACCCCTTGCACTAACT	2A	(GT)19, (GT)10
	WMC181R	ATGGTTGGGAGCACTAGCTTGG		
74	WMC11F	TTGTGATCCTGGTTGTGTTGTGA	3A/3D	(CT)
	WMC11R	CACCCAGCCGTTATATATGTTGA		
75	GWM388F	CTACAATTCGAAGGAGAGGGG	2B	(CT)4(CA)11(CA)12
	GWM388R	CACCGCGTCAACTACTTAAGC		
76	WMC76F	CTTCAGAGCCTCTTTCTCTACA	7B	(GT)
	WMC76R	CTGCTTCACTTGCTGATCTTTG		
77	GWM333F	GCCCGGTCATGTAAAACG	7B	(GA)19
	GWM333R	TTTCAGTTTGCGTTAAGCTTTG		
78	GWM335F	CGTACTCCACTCCACACGG	5B	(GA)14(GCGT)3
	GWM335R	CGGTCCAAGTGCTACCTTTC		
79	GWM294F	GGATTGGAGTTAAGAGAGAACCG	2AL	(GA)9TA(GA)15
	GWM294R	GCAGAGTGATCAATGCCAGA		
80	GWM630F	GTGCCTGTGCCATCGTC	2A/2B	(GT)16
	GWM630R	CGAAAGTAACAGCGCAGTGA		
81	CFD60F	TGACCGGCATTCAGTATCAA	5B/6D	(CA)25
	CFD60R	TGGTCACTTTGATGAGCAGG		
82	CFD73F	GATAGATCAATGTGGGCCGT	2B/2D	(CT)19
	CFD73R	AACTGTTCTGCCATCTGAGC		

Table 3. Evaluation of durum wheat cultivars and landraces in terms of Arsenic content, using analytical analysis conducted via ICP-MS.

Genotype No	As content (µg/kg)	Genotype No	As content (µg/kg)	Genotype No	As content (µg/kg)	Genotype No	As content (µg/kg)
1	3.33	35	15.67	69	1.22	103	3.03
2	3.62	36	1.65	70	4.47	104	2.94
3	3.02	37	4.91	71	1.63	105	5.84
4	3.54	38	0.38	72	4.41	106	4.63
5	2.52	39	3.85	73	3.84	107	5.74
6	14.07	40	2.42	74	5.22	108	4.56
7	13.07	41	2.56	75	3.95	109	5.94
8	12.89	42	2.73	76	6.46	110	4.94
9	2.22	43	0.36	77	2.76	111	6.15
10	4.44	44	1.04	78	4.86	112	3.01
11	0.18	45	4.40	79	5.58	113	7.67
12	1.80	46	1.40	80	25.35	114	4.60
13	9.19	47	4.96	81	2.11	115	5.05
14	0.36	48	1.69	82	3.63	116	6.25
15	2.73	49	3.64	83	3.82	117	5.16
16	2.80	50	3.72	84	2.37	118	3.87
17	25.66	51	2.60	85	3.65	119	6.35
18	3.05	52	6.43	86	6.87	120	4.78
19	2.19	53	5.10	87	3.60	121	3.60
20	2.22	54	2.13	88	4.29	122	7.74
21	2.97	55	3.87	89	2.46	123	3.40
22	3.12	56	2.82	90	5.24	124	4.55
23	1.07	57	3.49	91	3.15	125	3.73
24	2.65	58	2.81	92	4.81	126	43.81
25	1.30	59	1.21	93	6.31	127	6.60
26	16.92	60	2.68	94	4.03	128	25.83
27	2.96	61	1.71	95	5.84	129	2.53
28	4.25	62	3.69	96	8.64	130	3.48
29	10.06	63	3.15	97	14.18	Min	0.175
30	12.14	64	2.65	98	5.82	Max	43.81
31	3.38	65	3.61	99	3.51	Average	5.24
32	4.08	66	4.11	100	3.56	STDS	5.53
33	4.24	67	7.50	101	5.96		
34	3.42	68	3.03	102	8.17		

Table 4. Results of soil analyses of experimental area.

Structure				%					mg kg^{-1}				
pH	EC (dS m^{-1})	Soil class	Texture	Lime	Organic matter	N	P	K	Fe	Zn	Mn	Cu	As
7.6	0.241	C	Silt-loam	29.08	1.29	0.124	0.00106	0.036	2.93	0.54	8.81	1.59	9.56

Reference: Laboratory analyses results of Soil Science and Plant Nutrition Department of Çukurova University

Table 5. List of markers that show a significant association with Arsenic content using MLM (Q + K) models.

Marker name	Lucus	P	Marker R2
wmc262-bp236	4A	1.11E-05	0.17
wmc517-bp224	7B	4.11-04	0.11

p: The values of the association effect and significance. R^2: phenotypic variance imparted by each marker.

Discussion

Improving end-use quality is a significant goal of durum wheat breeding programs. In recent years, enhancing the quality of durum wheat genotypes concerning common toxic elements has become a crucial objective, in addition to developing desirable agronomic traits. There is a growing emphasis on improving low-toxicity varieties in crop breeding. Therefore, monitoring the levels of harmful ingredients in food and setting limits and regulations to ensure our food is safe is crucial. Moreover, it is also essential to provide accurate information about the levels of toxic elements in food, such as the results of studies on the arsenic content in durum wheat genotypes. Therefore, farmers and food producers need to prioritize producing safe food that meets consumers' expectations regarding taste, nutritional value, and sustainability. While it may be challenging to eliminate arsenic from durum wheat, durum wheat breeders can play a crucial role in reducing the toxicity of toxic elements, especially arsenic. One way they can do this is to develop new durum wheat varieties with low arsenic levels by selecting varieties that are naturally low in arsenic uptake. In addition, breeders can work towards ensuring that the durum wheat varieties they develop meet the highest safety and quality standards. Different countries have set different standards for arsenic levels in durum wheat. For example, The European Union (2015) has set maximum levels of 0.1 mg/Kg of inorganic arsenic in foodstuffs, including durum wheat, as specified in Annex II of Commission Regulation (EU) No 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 (European Union, 2015). CODEX Alimentarius Commission (FAO and WHO, 2018) identified the maximum Level of Arsenic in Salt, food grade: 0.5, polished rice: 0.2, and husked rice: 0.35 mg/kg. The United States has no specific limit for inorganic arsenic in durum wheat. Still, it has set a limit of 0.5 mg/kg of total arsenic in rice, which is also a crop that can accumulate arsenic, as reported by the U.S. Food and Drug Administration (2016). Durum wheat in Canada must not exceed 0.35 mg/kg of total arsenic, according to the Canadian Food Inspection Agency's 2020 regulations. The natural levels of arsenic in soil typically range from 1 to 40 mg/kg, with an average of 5 mg/kg, according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2007). It is important to note that these standards are subject to change and may vary depending on the country, region, or regulatory agency. However, despite the significance of testing and monitoring arsenic levels in durum wheat to ensure they meet the established safety standards, prior research has yet to be conducted on As accumulation in Turkish durum wheat germplasm. Therefore, In light of this, the present study aimed to evaluate the levels of Arsenic in diverse genotypes of durum wheat. The results, evaluated against international standards for As levels, demonstrated that the entire board of durum wheat genotypes tested had significantly low and non-toxic levels of Arsenic, with an average concentration of 5.24 µg/kg (as shown in Table 5). These values are exemplary results, as high levels of As can risk human health. 50% of studied genotypes showed low "As" contents, ranging between 3 and 6 µg/kg (Table 1 and Figure 1a). In Turkish cultivars, 46% exhibited a range between 0.175 and 3 µg/kg, while 36% fell between 3.001 and 6 µg/kg. For foreign cultivars, 48% ranged between 0.175 and 3 µg/kg, and 43% varied between 3.001 and 6 µg/kg. Arsenic levels for the "ex-situ" and "in situ" LDs, 68% and 53%, fell within the 3.001-6 µg/kg range (Figure 1c). In contrast, four genotypes (TR 81381-Sivas from ex-situ LDs, Balcalı-85 from Turkish CVs, Karakılçık and Mısırı from in-situ LDs) showed the highest As contents 25.3, 25.7, 25.8, 43.8 µg/kg respectively (Table 3). However, it is still far below the risky limit of 0.1 mg/kg, suggesting that the durum wheat genotypes tested in this study were safe for human consumption regarding As contamination. The panel's grain As concentrations frequency distribution was categorized into four groups based on the genotypes' origin. The in-situ and ex-situ landrace groups had the highest average As contents, at 8.78 and 5.42 µg/kg, respectively. On the other hand, the average As ranges were lower for foreign and Turkish CVs, at 3.33 and 4.82 µg/kg, respectively. Thus, the average As content among the groups investigated can be ranked as follows: in-situ LDs > ex-situ LDs > Turkish cultivars > foreign cultivars (Figure 1b); this suggests the genotypes' geographical origin may impact their As levels, with foreign genotypes having lower levels of As than Turkish genotypes. Additionally, it is necessary to emphasize that foreign and Turkish cultivars exhibited lower arsenic percentages overall than in-situ or ex-situ landraces. Compared to other researches, our studied genotypes showed lower levels of Arsenic; in Serbia, the average concentration of As in wheat grains was 83 µg/kg, according to (Skrbic and Onjia, 2007). In a study by Huang et al. (2008), trace element levels in wheat grains from multiple regions in China were evaluated, revealing As concentrations ranging from 29 to 86 µg/kg. While in Zhengzhou, China, Liu et al. (2009) noticed higher concentrations of Arsenic varied from 110 to 160 µg/kg.

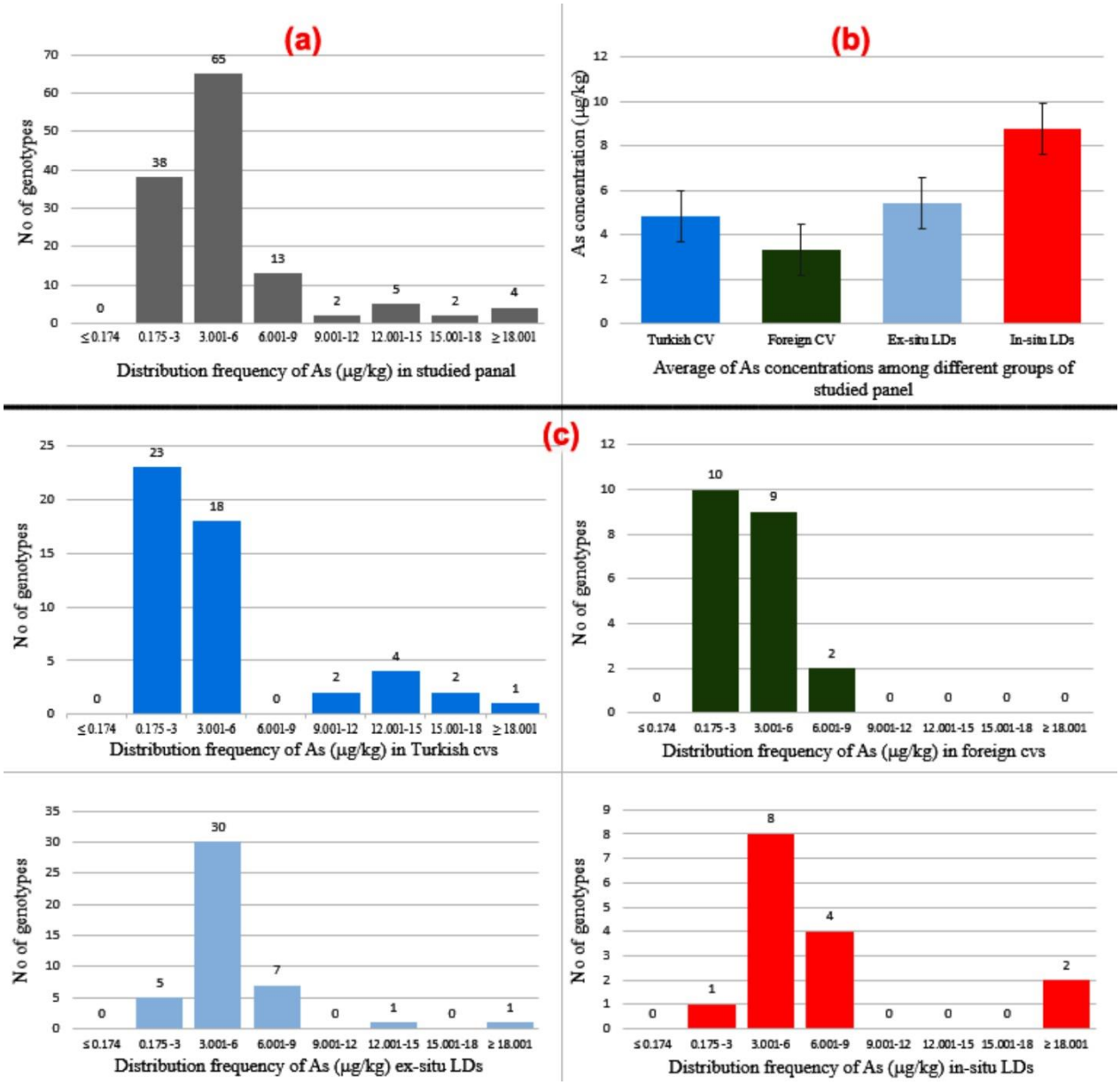

Figure 1-(a) displayed the frequency distribution of grain As concentrations for the entire panel. Figure 1-(b) showed the average of grain As concentrations among different groups of studied panel. Figure 1-(c) illustrated the frequency distribution of the number of genotypes for each group separately.

Italy's highest As concentration in wheat grains, discovered by Cubadda et al. (2010), was 60 µg/kg, exceeded by our study's highest observed value of 43.81 µg/kg. Additionally, our study's average As concentration (5.24 µg/kg) was significantly lower than the lowest reported average arsenic concentration of 19 µg/kg in wheat grains by Corguinha et al. (2015). The soil in the experimental field has an arsenic level of 9.56 mg/kg, which falls within the typical range of arsenic levels found naturally in soil. Typically, natural levels of Arsenic in soil range from 1 to 40 mg/kg, with an average of 5 mg/kg (ATSDR, 2007) (Table 4). It is worth noting that even though the As levels detected in the durum wheat genotypes tested were significantly low and non-toxic, it is crucial to continually monitor As levels in durum wheat to ensure they remain within safe limits. In this study, 780 polymorphic markers were identified from 82 SSR primers that were genotyped across genotypes. Markers with low allele frequencies (<0.05) were not helpful for further analysis and were excluded from GWAS. After removing these markers, 337 markers were used for analysis. In the study, population structure (Q) and kinship (K) were incorporated as covariates in an MLM+Q+K model to prevent false positive associations. This approach was used to identify significant MTAs associated with arsenic content in crops. The analysis results are shown in Table 3 and the Manhattan plot (Figure 2), which identified two significant MTAs. The use of GWAS as a tool for MAS in crops enabled the identification of these significant associations. The markers "*wmc262bp236*" and "*wmc517bp224*" were significantly associated with accumulated grain As content and explained a phenotypic variation of 11-17%. The MTA "*wmc262bp236*", which was located on chromosome 4A, had the highest value in explaining the total phenotypic variance (17%), while "*wmc517bp224*" was situated on 7B (Table 5 and Figure 2).

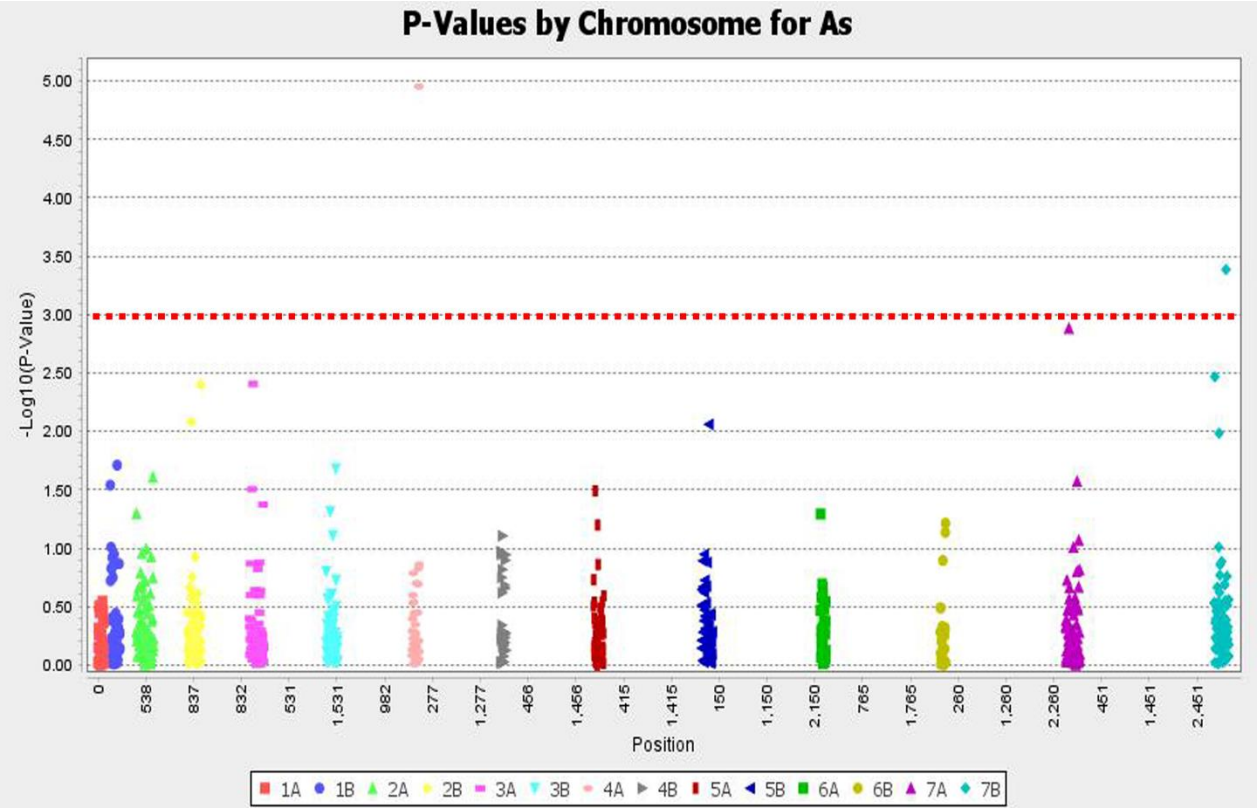

Figure 2. Manhattan plot illustrating the genome-wide scan of SSR markers linked to Arsenic content. The plot features a red horizontal dashed line indicating the significant SSRs associated with Arsenic content.

Overall, this study successfully identified genetic factors responsible for arsenic accumulation in durum wheat through the application of GWAS as a tool for MAS in crops. The research aimed to reduce the time required for durum wheat breeders to detect the phenotype and develop new varieties with low As levels by identifying alleles associated with As content. Previous research has recognized the use of microsatellites in Genome-Wide Association studies because they can cover a wider genomic region and offer various advantages. These advantages include higher resolution, greater inter-population variability, and significant intrinsic applicability. Consequently, the present study employed microsatellite primers. The methodology used in this research was robust and provided valuable insights into the relationship between the identified markers and the trait of interest. However, future studies could validate the genetic factors contributing to the variation in As content among different durum wheat genotypes.

Conclusions

In this research, the levels of arsenic in various genotypes of a durum wheat germplasm panel were evaluated. The results showed that all the durum wheat genotypes in the study had low and non-toxic levels of arsenic, which is critical for maintaining food safety. By utilizing GWAS as a MAS tool in crops, the study identified genetic factors accountable for arsenic accumulation in durum wheat. Two significant marker-trait associations linked to arsenic contents were successfully identified. The robust methodology employed in the study could reduce the time required for durum wheat breeders to develop new varieties with low As levels by identifying alleles related to As content. However, future studies should confirm the genetic factors contributing to the variation in As content among different durum wheat genotypes.

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