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Durum Wheat Breeding for Enhanced Safety and Nutritional Value: A GWAS Approach to Tackling Aluminum Uptake

Gelişmiş Güvenlik ve Besin Değeri için Makarnalık Buğday Islahı: Alüminyum Alımıyla Mücadelede Bir GWAS Yaklaşımı

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Abstract: The importance of producing safe and high-quality food is on the rise, and developing durum wheat varieties with low aluminum content is crucial in meeting this demand. Breeders can achieve this goal by developing new varieties that are more resistant to aluminum uptake. To reach this purpose, aluminum levels in a diverse collection of durum wheat genotypes were evaluated, including Turkish-released cultivars and local landraces, by using inductively coupled plasma mass spectrometry was used. The results revealed that genotypes ranged from 0.9 to 24.6 mg kg-1, with an average of 3.31 mg kg-1, while 93.1% of them had a low content of ≤ 5 mg kg-1. A genome-wide association study is a robust method for uncovering genetic variations linked to specific traits. In this study, two marker-trait associations were identified on chromosomes 2A and 3A, which explained a phenotypic variation of 14 and 71%. These findings highlight the need for continued monitoring to ensure safe and healthy food for consumers and suggest that collaborative genome-wide association studies and marker-assisted selection can accelerate the development of new durum wheat varieties with reduced aluminum levels. However, further research is necessary to confirm and validate the genetic factors contributing to aluminum content variation among different durum wheat genotypes, although the study's methodology was robust.

Keywords: Durum wheat, Aluminum, SSR, GWAS, MTA.

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Öz: Güvenli ve kaliteli gıda üretmenin önemi her geçen gün artmakta ve bu talebin karşılanmasında alüminyum içeriği düşük durum buğdayı çeşitlerinin geliştirilmesi büyük önem taşımaktadır. Islahçılar, alüminyum alımına daha dirençli yeni çeşitler geliştirerek bu hedefe ulaşabilmeyi amaçlamaktadır. Bu amaca ulaşmak için, Türkiye'de piyasaya sürülen modern çeşitler ve eski yerel çeşitler de dahil olmak üzere çeşitli makarnalık buğday genotiplerinin bulunduğu bir koleksiyonda genotiplerin alüminyum seviyeleri, endüktif olarak eşleştirilmiş plazma kütle spektrometresi kullanılarak değerlendirilmiştir. Sonuçlar, genotiplerin 0.9 ila 24.6 mg kg^{-1} arasında değiştiğini, ortalama 3.31 mg kg^{-1} olduğunu, bunların %93.1'inin ≤ 5 mg kg^{-1} gibi düşük bir içeriğe sahip olduğunu ortaya koymaktadır. Genom çapında ilişkilendirme çalışması önemli özelliklerle ilişkili genetik varyasyou açığa çıkarmada çok güçlü bir tekniktir. Bu çalışmada, 2A ve 3A kromozomları üzerinde bulunan ve %14 ve %71'lik bir fenotipik varyasyonu açıklayan iki markör-özellik ilişkisi tanımlamıştır. Bu bulgular, tüketiciler için güvenli ve sağlıklı gıda sağlamak için sürekli takip ihtiyacını vurgulamakta ve genom çapında ilişkilendirme çalışmalarının ve markör destekli seleksiyon yardımıyla, alüminyum seviyeleri azaltılmış yeni durum buğdayı çeşitlerinin geliştirilmesini hızlandırabileceğini öne sürmektedir. Bununla birlikte, çalışmanın metodolojisi sağlam olmasına rağmen, farklı makarnalık buğday genotipleri arasındaki alüminyum içeriği varyasyonuna katkıda bulunan genetik faktörlerin doğrulanması için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Makarnalık buğday, Alüminyum, SSR, GWAS, MTA.

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INTRODUCTION

Durum wheat (*Triticum turgidum*, L.) is a crucial cereal crop and a rich source of dietary fiber, protein, vitamins, and minerals. Its high protein content makes it suitable for a wide range of end-products of traditional food items (Zingale et al., 2023). Durum wheat accounts for approximately 8% of global wheat production, with the majority (75%) grown in the Mediterranean region, as noted by Alsaleh et al. (2019). According to Mulugeta et al. (2023), Canada and Türkiye are the largest producers of Durum wheat. However, *Triticum turgidum* has a genetic propensity to accumulate elements from the soil in its grains, which can exceed international safety standards as indicated by Delhaize et al. (2012), Garcia-Oliveira et al. (2016), and Liu et al. (2018) and they reported that genetic factors play a significant role in the extent of aluminum accumulation in the grains of various durum wheat cultivars (Mello et al., 2023). Aluminum (Al) is a common element in the earth's crust and is often found in high concentrations in acidic soils (Mello et al., 2023). While aluminum is an essential micronutrient for durum wheat growth in small amounts, as it can improve root growth, enhance nutrient uptake, and raise plant resistance to biotic and abiotic stresses, it can become harmful to plants when present in excess (Ofoe et al., 2023). High levels of aluminum in the soil can limit root growth, reduce nutrient uptake in plants, and lead to stunted growth, chlorosis, and reduced crop yields and quality (Maksimović et al., 2020). Aluminum can be taken up by plant roots and transported to the aboveground tissues of plants, including durum wheat seeds. According to Szabó et al. (2015), wheat roots contain higher concentrations of aluminum compared to the aerial parts, indicating limited translocation of the element. However, the accumulation of aluminum in durum wheat grains can vary depending on various factors, such as the concentration of aluminum in the soil, the duration of exposure, and the plant's genetic makeup (Mello et al., 2023). Some genotypes exhibit higher levels of aluminum accumulation than others. For instance, Gupta et al. (2013), Maksimović et al. (2020), and Rahman et al. (2018) found that the amount of aluminum accumulated in durum wheat seeds varied considerably depending on the genotype of the plant. The presence of high levels of aluminum in durum wheat seeds can have significant implications for the health of humans and animals. Studies have linked increased aluminum intake to several health problems, including Alzheimer's disease, kidney disease, and bone disorders (Mello et al., 2023). Nevertheless, access to sufficient quantities of well-balanced food is a fundamental right of every individual on the planet (Yeken et al., 2019). Breeding crops with essential traits like productivity, seed quality, elemental content, biotic and abiotic stress resistance, among others, is required to meet the rising global food demand. To achieve this, crop improvement programs aim to utilize natural genetic diversity to the fullest extent possible, thus improving selection efficiency (Yeken et al., 2018). Although the soils in southern Türkiye, the center of durum wheat cultivation, are not acidic, as no research or reports were received in this regard. However, ideally, limiting the buildup of aluminum in durum wheat grains is advisable, and cultivating strains of durum wheat may be a viable approach to mitigate the adverse consequences of aluminum toxicity on the grains. These strains can cultivate and yield grains with minimal levels of aluminum. Therefore, it is necessary to survey aluminum levels in durum wheat grains and use biotechnology tools such as DNA molecular markers to detect the loci of genes associated with high or low aluminum concentrations. By harnessing the power of molecular markers, such as Simple Sequence Repeats (SSR), durum wheat breeders can develop new cultivars that thrive in diverse environments at higher speeds. Unlike conventional breeding methods, molecular markers enable breeders to quickly and accurately identify plants that exhibit the desired traits (Alsaleh et al., 2019). SSRs are especially useful in detecting genetic variation in individuals (Nadeem et al., 2018) and can be harnessed for genome-wide association studies (GWAS) and marker-assisted selection (MAS), as indicated by Vieira et al. (2016). A study by (Baloch et al., 2017) and Frouin et al. (2019 has shown that GWAS can be a powerful tool for identifying genetic markers associated with crucial traits in durum wheat. This approach is not only more precise and cost-effective than traditional breeding methods but can also more quickly pinpoint genetic markers linked to aluminum accumulation (Tam et al., 2019). Armed with this information, durum wheat breeders can use MAS to develop a more effective program and evaluate desirable traits in the early generations. Despite the important role that durum wheat plays in Türkiye, there is a deficiency of systematic studies assessing the aluminum content in durum wheat germplasms from this vital region. To address this issue,

a study seems urgent using various durum wheat genotypes to evaluate the variability in phenotypic and genetic aluminum content. Firstly, the phenotypic variation of Al content will be measured and evaluated. Secondly, DNA markers will be employed to screen for genetic polymorphisms. Finally, a GWAS will be conducted to identify the marker-trait associations (MTAs) responsible for the diversity in aluminum content. Once identified, the relevant markers will be thoroughly investigated to locate potential candidate gene locations. These locations can then be incorporated into MAS programs, which aim to cultivate durum wheat varieties with minimal or no levels of aluminum, making them excellent candidates as breeding parents in breeding programs.

MATERIAL AND METHOD

Plant Material

For this study, 130 genotypes of durum wheat (*Triticum turgidum* L.) were used as plant material to explore genetic variation (Alsaleh et al., 2022a) (Supplementary Table 1). The genotypes were grown during the 2019-2020 growing season at the research field of Çukurova University, Adana, Türkiye, to get fresh and healthy seeds for the study. Standard agricultural practices were followed, such as irrigation, fertilization, and pest and disease management. The harvested grains samples were stored in dry and cool storage until they were used for elemental composition analysis at Yozgat Bozok University, Yozgat, Türkiye. These grains were also subjected to several toxic element analyses reported by Alsaleh et al. (2022b) and Alsaleh (2022c).

Isolation of Genomic DNA and SSR Analysis

In February 2020, fresh leaves were obtained for the purpose of isolating genomic DNA and performing SSR analysis. The CTAB protocol was employed at the Laboratory of BİLTEM, Yozgat Bozok University, Yozgat-Türkiye (Alsaleh, 2022c). The consequent DNA was evaluated for both quantity and quality using 8% agarose gel electrophoresis. Following isolation, the DNA was diluted to a concentration of 10 ng μl^{-1} and used for SSR analysis. The same DNA was utilized and reported by Alsaleh et al. (2022b) and Alsaleh (2022c) for Cadmium and Platinum investigations. Microsatellite primers were selected to cover a variety of segments of durum wheat chromosomes (Supplementary Table 2). In Alsaleh's 2022c research, the same set of eighty-two SSR primers was utilized to detect a recently discovered QTL linked with platinum accumulation. Supplementary Table 2 provides an overview of the SSR primers and their relevant information used in the research. The M13-tailed primer approach, based on Alsaleh et al. (2022a), was employed to amplify the SSR region through PCR. The final PCR reaction volume 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 cycle began with primary denaturation at 94° C for 5 minutes, followed by 30 cycles of 94 °C for 1 minute, 55 to 65 °C (depending on the annealing temperature of the primers) for 1 minute, and 72° C for 1 minute. This was followed by eight cycles of 94° C for 30 seconds, 53° C for 45 seconds, and 72° C for 45 seconds. The final extension was 72° C for 10 minutes. The accuracy of the SSR fragments was verified twice using Gene Mapper software v3.7 (Applied Biosystems) in accordance with the manufacturer's instructions. The individual bands of the SSR were analyzed, and the binary scoring method was used to assign a '1' for the presence of bands and a '0' for their absence. This technique facilitates the evaluation and statistical analysis of co-dominant SSR data, as reported by Kaya et al. (2016). Finally, the PCR products were subjected to fragment analysis and loaded onto the ABI 3130xl Genetic Analyzer device (Applied Biosystems).

Aluminum Analysis

The study conducted an aluminum analysis by manually harvesting three spikes of each genotype, one from each replication, and then threshing them by hand to obtain the grains. Soil samples were collected from the experimental field at Çukurova University in Adana. 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^{-1} nitric acid up to 20 ml for analysis of aluminum content 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^{-1} for nebulizer gas, 0.88 L min^{-1} for plasma gas, 3.01 bar for nebulizer pressure, dwell time of 0.01 ms, and a spray chamber temperature of 3.7°C. To ensure accuracy, the entire sample and standards underwent three repeated measurements. The digestion and aluminum measurement (ICP-MS) procedures were conducted at BİLTEM laboratories located at Yozgat Bozok University, Türkiye.

Phenotypic Statistical Analysis

In this study, durum wheat genotypes were analyzed to investigate the variation in aluminum content and distribution of phenotypic frequency among different cultivars and landraces. The panel was divided into four groups based on their origin, including 50 Turkish cultivars (Turkish CVs), 21 foreign cultivars (foreign CVs), 44 Izmir gene bank landraces (ex-situ LDs), and 15 locally grown landraces (in-situ LDs). Statistical analysis was conducted using Microsoft Excel software to perform ANOVA analysis for each group.

Marker-trait Investigation Analysis

The proportion of phenotypic variation explained by aluminum content for each marker was estimated using the R2 value in TASSEL 5 (Glaubitz et al., 2014). To determine significant associations, the Bonferroni threshold for multiple testing and an adjusted corrective threshold were applied (Kaler and Purcell, 2019). Precisely, the 5% Bonferroni threshold for multiple comparisons was used, resulting in 337 markers being included in the current GWAS.

RESULTS AND DISCUSSION

Phenotypic Variations for Aluminum Contents

Although Türkiye, being a primary hub of wheat cultivation, plays a crucial role in facilitating the dissemination of diverse crops across different continents (Arystanbekkyzy et al., 2019), durum wheat is a major staple food crop that has the potential to accumulate aluminum in its grain, leading to negative effects on its yield and quality (Maksimović et al., 2020; Szabó et al. 2015). The degree of aluminum accumulation varies among different genotypes and is influenced by environmental factors such as soil acidity, and safe levels of aluminum in soil vary globally and depend on factors such as soil type and intended use. Various organizations have established guidelines and limits for aluminum in the soil to protect human health and the environment. For instance, the European Union (2012) has appointed a limit of 2000 mg kg^{-1} of soil dry weight for aluminum in agricultural soils. Despite the high concentration of aluminum found in the soil analysis of the experiment site (8312 mg kg^{-1}) (Table 1), which exceeds the established needs of the EU, there is no significant risk due to the soil's neutral pH level of 7.8 (Table 1).

Table 1. Soil analyses of trial area.
Çizelge 1. Deneme Alanının Toprak Analizleri.

----------Structure----------				----------%----------					----------mg kg^{-1} ----------				
pH	EC (dS m^{-1})	Soil class	Texture	Lime	Organic matter	N	P	K	Fe	Zn	Mn	Cu	**Al**
7.6	0.24	C	Silt-loam	29.1	1.3	0.12	0.0011	0.04	2.9	0.5	8.8	1.6	**8312**

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

This neutral pH level does not contribute to increased aluminum toxicity, mitigating the potential negative effects of the high aluminum concentration. As a result, no reports of aluminum toxicity have been observed in the soil of durum wheat cultivation areas in Türkiye. Aluminum is a natural occurrence in food, making it impossible to fully eliminate it from our diet. However, the scientific society is concerned with reducing the average daily consumption of aluminum in food. The absence of established international safe levels of aluminum in durum wheat grains makes it desirable to minimize aluminum accumulation in these grains. The concentration of aluminum in wheat flour varies significantly across

regions, an example: higher values were found in China (Ma et al., 2019) than in Germany (Stahl et al., 2011). On average, cereal products contain 4 mg kg^{-1} of aluminum, but the content can range from 1 to 737 mg kg^{-1} (Stahl et al., 2011). Therefore, breeding varieties that produce grains with low ranks of aluminum is a crucial strategy to address this issue. Therefore, before executing this strategy, it is necessary to measure the aluminum content of different durum germplasms. Regarding our durum wheat genotypes, the "Kümbet-2000" Turkish cultivar had the lowest aluminum content at 0.9 mg kg^{-1}, while the in-situ landrace "İskenderiye" had the highest content at 24.6 mg kg^{-1}. The average aluminum content for all tested genotypes was 3.31 mg kg^{-1}, with 93.1% exhibiting low levels between 0.9 and 5 mg kg^{-1} (Table 2, Figure 1a). Foreign cultivars had aluminum content levels between 1.6 and 3.3 mg kg^{-1}, with an average of 2.11 mg kg^{-1}, while Turkish cultivars had a content range of 0.9 to 5.1 mg kg^{-1}, with an average of 2.38 mg kg^{-1}, and 98% of them had a content of 0.9 to 5 mg kg^{-1} (Table 2, Figures 1b & 2). Regarding our studied landraces, In situ landraces had higher aluminum content levels, ranging from 2.3 to 24.6 mg kg^{-1}, with an average of 6.05 mg kg^{-1}, and 20% of them had a content exceeding 5 mg kg^{-1} (Table 2, Figure 1b & 2). Ex-situ landraces had slightly lower aluminum contents compared to in-situ landraces, ranging from 1.70 to 20.7 mg kg^{-1}, with an average of 3.99 mg kg^{-1}. Roughly 11.4% of ex-situ landraces showed aluminum content over 5 mg kg^{-1} (Table 2, Figures 1b & 2). Out of the entire panel, six genotypes had the highest aluminum content, all of which were landraces. Among these, four were from ex-situ "TR 31902 -Malatya", "TR 81278 -Ankara", "TR 46881 -Erzincan", and "TR 54977 -Yozgat" with aluminum levels of 12.8, 16.1, 17.6, and 20.7 mg kg^{-1}, respectively. The remaining two genotypes with high aluminum content were "Mersiniye" and "İskenderiye" from the in-situ group, showing aluminum levels of 19.4 and 24.6 mg kg^{-1}, respectively (Table 2).

Table 2. Aluminum Content in Cultivars and Landraces Using ICP-MS Analysis.
Çizelge 2. ICP-MS Analizi Kullanılarak Modern ve Yerel Çeşitlerdeki Alüminyum İçeriği.

Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})
1	5.1	**35**	2.8	**69**	1.9	**103**	2.5
2	4.3	**36**	2.2	**70**	2.0	**104**	2.4
3	3.1	**37**	2.7	**71**	3.3	**105**	3.8
4	3.1	**38**	1.8	**72**	2.4	**106**	2.2
5	2.3	**39**	1.9	**73**	9.9	**107**	1.8
6	3.2	**40**	2.1	**74**	1.9	**108**	2.4
7	4.3	**41**	1.7	**75**	1.9	**109**	16.1
8	2.3	**42**	2.1	**76**	2.5	**110**	1.7
9	3.7	**43**	1.7	**77**	2.6	**111**	2.6
10	2.4	**44**	2.3	**78**	2.6	**112**	2.0
11	2.5	**45**	1.4	**79**	2.7	**113**	3.6
12	0.9	**46**	2.3	**80**	3.1	**114**	1.7
13	1.8	**47**	2.8	**81**	2.2	**115**	2.0
14	2.3	**48**	2.0	**82**	17.6	**116**	4.7
15	1.4	**49**	2.0	**83**	2.4	**117**	3.8
16	1.9	**50**	2.8	**84**	2.4	**118**	19.4
17	1.9	**51**	1.7	**85**	2.9	**119**	3.2
18	2.5	**52**	2.5	**86**	3.8	**120**	2.4
19	1.7	**53**	1.6	**87**	2.5	**121**	2.7
20	1.7	**54**	2.4	**88**	2.2	**122**	2.7
21	2.4	**55**	2.2	**89**	2.1	**123**	4.5
22	2.1	**56**	2.0	**90**	2.3	**124**	3.9

Table 2. Aluminum Content in Cultivars and Landraces Using ICP-MS Analysis (continued).
Çizelge 2. ICP-MS Analizi Kullanılarak Modern ve Yerel Çeşitlerdeki Alüminyum İçeriği (devam etti).

Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})	Genotype No	Al content (mg kg^{-1})
23	1.5	**57**	1.6	**91**	20.7	**125**	4.6
24	2.6	**58**	2.0	**92**	2.4	**126**	2.5
25	2.9	**59**	3.0	**93**	2.1	**127**	24.6
26	1.7	**60**	2.4	**94**	2.5	**128**	6.8
27	1.3	**61**	2.3	**95**	1.9	**129**	2.3
28	2.4	**62**	1.6	**96**	4.1	**130**	2.5
29	2.1	**63**	1.9	**97**	3.7	**Min**	**0.9**
30	2.5	**64**	2.2	**98**	3.0	**Max**	**24.6**
31	3.6	**65**	1.7	**99**	2.7	**Average**	**3.31**
32	2.0	**66**	2.7	**100**	12.8	**STDS**	**3.61**
33	2.9	**67**	1.7	**101**	2.2		
34	2.2	**68**	1.7	**102**	2.5		

Compared to a study on unprocessed wheat grain samples from China, our studied genotypes had a lower range and inferior average aluminum content of 3.31 mg kg^{-1}. The researchers found aluminum values ranging from 2.4 to 31.6 mg kg^{-1}, with a mean of 11 ± 6 mg kg^{-1}, and roughly 80% of the samples had values ranging from 5 to 20 mg kg^{-1} (Liang et al., 2019; Nanda et al., 2016; Szabó et al., 2015) while 93.1% of our studied genotypes exhibiting less than 5 mg kg^{-1} (Table 2, Figure 1a). The frequency distribution of aluminum concentrations in the panel's grain was split into four groups according to the source of the genotypes. Notably, both foreign and Turkish cultivars had lower overall aluminum percentages than in-situ or ex-situ landraces, with values of 2.11 and 2.38 mg kg^{-1}, respectively. Conversely, the in-situ and ex-situ landrace groups had the highest average aluminum contents, at 6.05 and 3.99 mg kg^{-1}, respectively (Figure 1b). This indicates that the average aluminum content among the groups can be ranked as follows: in-situ landraces > ex-situ landraces > Turkish cultivars > foreign cultivars. These findings suggest that the geographical origin of genotypes may affect their aluminum levels, with foreign genotypes having lower levels of aluminum than Turkish genotypes.

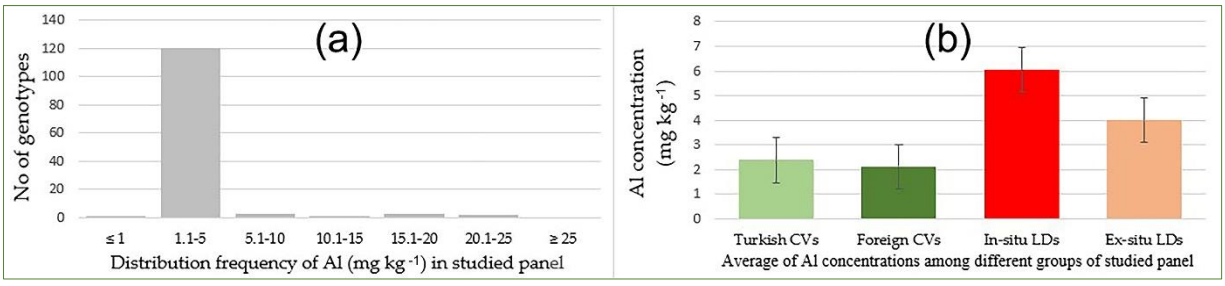

Figure 1.(a) presents the frequency distribution of grain Aluminum concentrations for the entire panel. **(b)**, the frequency distribution of grain Aluminum concentrations is displayed for each group individually. Finally.
Şekil 1(a): Tüm panel için dane Alüminyum konsantrasyonlarının frekans dağılımını göstermektedir. (b), Her grup için ayrı ayrı dane Alüminyum konsantrasyonlarının frekans dağılımı göstermektedir.

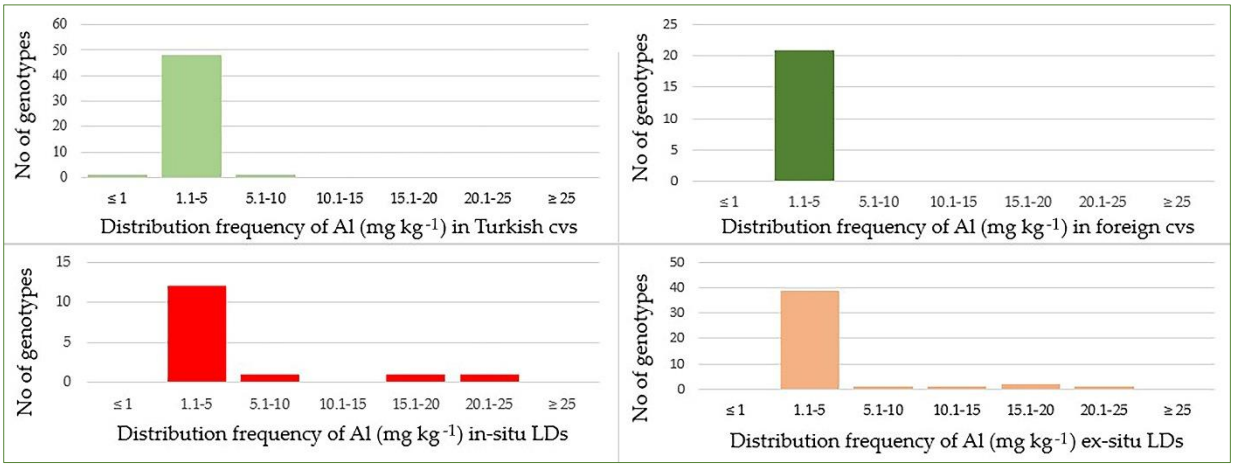

Figure 2. illustrates the frequency distribution of the number of genotypes for each group separately.
Şekil 2. Her grup için genotip sayısının frekans dağılımını ayrı ayrı göstermektedir.

Exploring Genetic Variations and Associations With Markers and Traits

Researchers aim to identify genetic markers and traits linked to aluminum accumulation in durum wheat to develop more resistant varieties. Understanding the genes involved in aluminum accumulation and its impact on the plant can help to develop strategies to mitigate its harmful effects and improve the overall yield and quality of durum wheat crops. As durum wheat is a staple food in many regions, especially in Türkiye and meditation countries, high levels of aluminum in food can pose a risk to human health (Agency for Toxic Substances and Disease Registry– ATSDR., 2008; Mello et al., 2023), therefore, it is crucial to develop methods to reduce aluminum accumulation in durum wheat to ensure food safety and promote human health like using molecular markers for GWAS. Microsatellites have been previously recognized as effective markers in GWAS due to their ability to cover a wider genomic region and offer several advantages, including higher resolution, greater inter-population variability, and significant intrinsic applicability (Alsaleh, 2022c; Väli et al., 2008). Hence, this study employed microsatellite primers. The investigation aimed to expedite the detection of the phenotype and development of new durum wheat varieties with low Al levels. So, in this study, genotyping of 82 SSR primers across genotypes identified 780 polymorphic markers. Markers with allele frequencies below 0.05 were excluded from GWAS, resulting in 337 markers used for analysis. To prevent false positive associations, the study employed an MLM+Q+K model with population structure (Q) and kinship (K) as covariates. The approach enabled the identification of significant MTAs associated with crop aluminum content, as shown in Table 3 and the Manhattan plot (Figure 3).

Table 3. List of Markers Associated with Aluminum Content Using MLM (Q + K) Models.
Çizelge 3. MLM (Q + K) Modelleri Kullanılarak Alüminyum İçeriğiyle İlişkili Markörlerin Listesi.

Marker	Chromosome	p	MarkerR2
wmc522bp238	2A	1.79E-16	0.71
gwm369bp320	3A	9.88E-05	0.14

Two MTAs were identified, "*wmc522bp238*" and "*gwm369bp320*", which were associated with accumulated grain aluminum content and explained a phenotypic variation of 14-71%. The MTA "*wmc522bp238*", located on chromosome 2A, had the highest value in explaining the total phenotypic variance (71%), while "*gwm369bp320*" was lying on 3A. Both MTAs were detected in the A genome, indicating that the A genome may play a critical role in the genetic control of the aluminum accumulation trait.

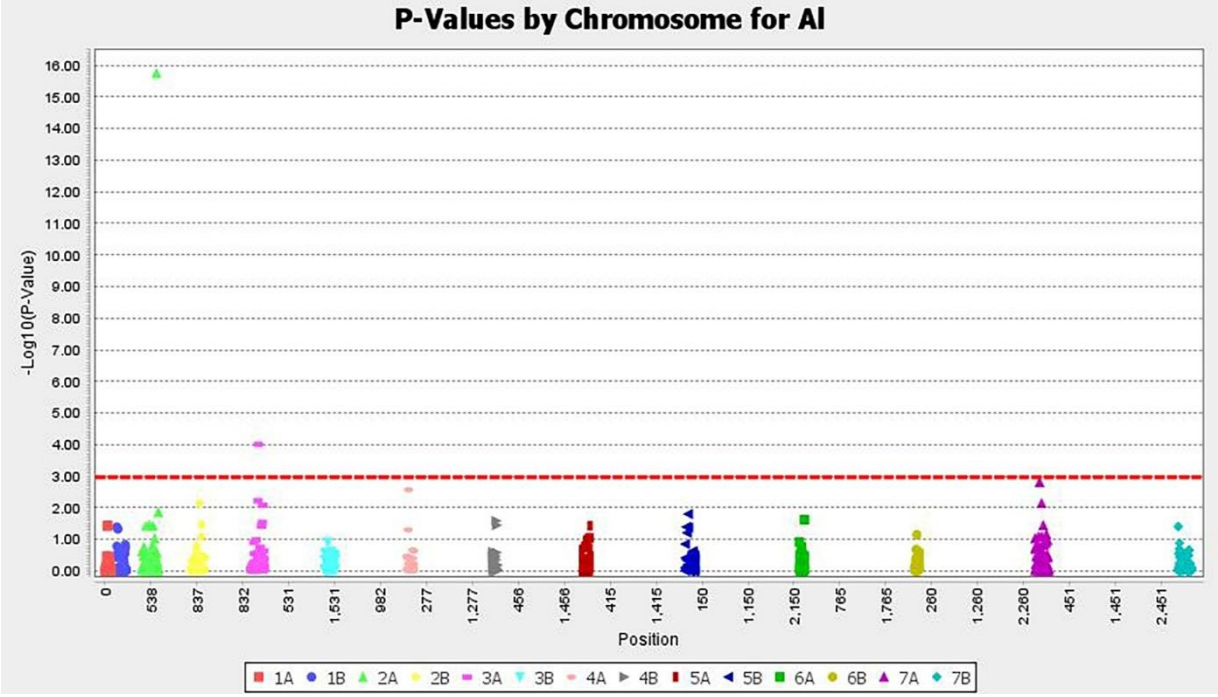

Figure 3. Manhattan plot illustrating the genome-wide scan of SSR markers linked to Aluminum content. The plot features a red horizontal dashed line indicating the significant SSRs associated with Aluminum content.
Şekil 3. Alüminyum içeriğine bağlı SSR markörlerinin genom çapında taramasını gösteren Manhattan grafiği. Alüminyum içeriği ile ilişkili önemli SSR'lar şekil üzerindeki kırmızı yatay kesikli çizgiyle gösterilmektedir.

GWAS here successfully identified the genetic factors responsible for aluminum accumulation in durum wheat. The methodology used in this research was robust and provided valuable insights into the relationship between the identified markers and the trait of interest. The use of GWAS as a tool for MAS in crops will facilitate the identification of these associations. However, ensuing research endeavors are necessary to confirm and validate the genetic elements responsible for the diversity in aluminum levels observed among various types of durum wheat.

CONCLUSION

The purpose of the research was to evaluate the levels of aluminum in various genotypes of Turkish durum wheat germplasm. The findings showed that the durum wheat genotypes investigated generally had low levels of aluminum, which is crucial for ensuring food safety. Additionally, GWAS was employed as a tool to pinpoint genetic factors responsible for aluminum accumulation in durum wheat. The study successfully determined two significant marker-trait associations linked to aluminum contents, which could be utilized in MAS. The robust methodology utilized in the study could enable the development of new durum wheat varieties with low Al levels by identifying alleles associated with Al content, thus reducing the time needed for breeders. However, it is required to perform further investigations to validate the genetic factors contributing to the variation in Al content among diverse durum wheat genotypes.

CONFLICT OF INTEREST

There are no conflicts of interest.

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Supplementary Table 1. outlines 130 genotypes that were evaluated for Aluminum assessments, including the cultivars and landraces selected, their country of origins, release year, group, and pedigree.

Ek çizelge 1*. Alüminyum analizleri için incelenen 130 genotipi, seçilen modern ve yerel çeşitleri, orijin ülkeleri, piyasa çıkma yılı, grubu ve soyağacı dahil olmak üzere özetlemektedir*

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

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No	Name	Country	Year	Group	Pedigree/collection side/ growing locations
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
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;
71	Cham-9	Syria	2010	Foreign CV	PELICANO(SIB)/(SIB)RUFF// 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

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No	Name	Country	Year	Group	Pedigree/collection side/ growing locations
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
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

Supplementary Table 2. Information on the Simple Sequence Repeats primers utilized to screen polymorphic sequences, their chromosomal location, and repeat motif.

Ek çizelge 2. *Basit Dizi Tekrarları Markörlerinin polimorfik dizileri, bunların kromozomal konumlarını ve tekrar motifi bilgileri.*

	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		

Supplementary Table 2. Information on the Simple Sequence Repeats primers utilized to screen polymorphic sequences, their chromosomal location, and repeat motif (continued).

Ek çizelge 2. *Basit Dizi Tekrarları Markörlerinin polimorfik dizileri, bunların kromozomal konumlarını ve tekrar motifi bilgileri (devam etti).*

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
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
	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		

Supplementary Table 2. Information on the Simple Sequence Repeats primers utilized to screen polymorphic sequences, their chromosomal location, and repeat motif (continued).

Ek çizelge 2. *Basit Dizi Tekrarları Markörlerinin polimorfik dizileri, bunların kromozomal konumlarını ve tekrar motifi bilgileri (devam etti).*

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
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		
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		