Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Barcode-High Resolution Melting Clearly Distinguishes Durum and Bread Wheat Varieties by Novel Primer Sets

Emine Uygur Göçer[a], **Yasemin Kemeç Hürkan**[b], **Kaan Hürkan**[c]*

[a]*Department of Field Crops, Faculty of Agriculture, Iğdır University, Iğdır, TURKIYE*

[b]*Not affiliated with any institution*

[c]*Department of Agricultural Biotechnology, Faculty of Agriculture, Iğdır University, Iğdır, TURKIYE*

ARTICLE INFO

Research Article

Corresponding Author: Kaan Hürkan, E-mail: kaanhurkan@hotmail.com

Received: 02 December 2024 / Revised: 09 February 2025 / Accepted: 24 February 2025 / Online: 29 July 2025

Cite this article

GÖÇER E U, HÜRKAN Y K, HÜRKAN K (2025). Barcode-High Resolution Melting Clearly Distinguishes Durum and Bread Wheat Varieties by Novel Primer Sets. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi)*, 31(3):795-801. DOI: 10.15832/ankutbd.1595264

ABSTRACT

Wheat is a vital global staple, with *Triticum durum* Desf. (durum wheat) primarily used for pasta and couscous, and *Triticum aestivum* L. (bread wheat) widely cultivated for baking. Differentiating these species, especially in processed forms, poses significant challenges due to their genetic similarity and overlapping end-use applications. This study applied Barcode-High-Resolution Melting (Bar-HRM) analysis to distinguish common durum (Cesare, Ovidio, Tiziana, Zivago) and bread wheat (Adana 99, Ceyhan 99, Dinç, Nizar) varieties in Türkiye. DNA was extracted separately from seeds, flours, and fresh leaves to ensure successful recovery, with seed-derived DNA used for HRM analysis. The Internal Transcribed Spacer (ITS) region was amplified via PCR, revealing two polymorphic sites in ITS1 and 20 in ITS2, with 14 sites differentiating the two species. HRM primer sets targeting these regions achieved genotyping confidence levels exceeding 95%. Sequencing validated the HRM results, and phylogenetic analysis confirmed clear separation between durum and bread wheat. This rapid, reliable, and contamination-resistant method enables accurate wheat discrimination from small samples within three hours, offering significant potential for broader applications in agricultural diagnostics and food authenticity testing.

Keywords: *Triticum aestivum*, *Triticum durum*, Bar-HRM, Genotyping, Internal Transcribed Spacer.

1. Introduction

Wheat (*Triticum aestivum* L.) is a globally important staple crop, serving as a primary source of calories and protein for approximately 85% and 82% of the world's population, respectively (Chaves et al. 2013). Its versatility extends beyond human nutrition to the production of diverse food products such as yeast-based and flatbreads, steamed breads, cakes, pasta, biscuits, noodles, couscous, and beer. Additionally, wheat contributes to non-food industries, including biofuel production. Its adaptability allows for cultivation across a range of tropical and subtropical regions under both rain-fed and irrigated conditions. However, despite this adaptability, wheat production is often constrained by environmental stresses (Rahaie et al. 2013).

In Turkey, wheat production holds significant economic and agricultural importance, with the country ranking among the world's leading producers. The Central Anatolia Plateau alone accounts for approximately 40% of Turkey's total wheat output (Yüce et al. 2020). Despite favourable climatic and geographical conditions, Turkish wheat cultivation faces challenges such as fluctuating rainfall patterns, soil degradation, and increasing drought occurrences, all of which threaten crop yields. Climate change is projected to exacerbate these issues, with rising temperatures and water scarcity posing critical risks to wheat productivity in the coming decades (Lv et al. 2019). In response, Turkey has implemented agricultural policies focusing on breeding drought-resistant wheat varieties and promoting sustainable irrigation practices to ensure long-term food security (Karaman et al. 2018; Ceyhan & Akkaya 2020).

Among the different wheat species, durum wheat (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.) are the most significant due to their distinct genetic characteristics and end-use applications. Bread wheat is a hexaploid species (BADD genome) with greater genetic diversity, contributing to its adaptability to diverse environments (Dubcovsky & Dvorak 2007). In contrast, durum wheat is tetraploid (BBAA genome) with a more rigid gluten structure and higher grain hardness, making it particularly suitable for pasta and couscous production. The differences in protein content, gluten composition, and grain hardness influence their distinct uses in food industries (Abdel-Aal et al. 1995; Shewry 2009).

Despite these differences, distinguishing between durum and bread wheat presents practical challenges. Their close genetic relationship, shared ancestry, and overlapping morphological traits complicate accurate identification, especially in hybrid varieties (Peng et al. 2011). Traditional morphological methods, relying on traits like grain size, shape, and color, are often unreliable due to environmental influences (Zeven 1991). Molecular techniques such as Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) offer greater accuracy but can be costly and time-consuming, limiting their routine application in breeding programs (El-Esawi et al. 2018).

High-Resolution Melting (HRM) analysis has emerged as a powerful post-PCR technique for detecting genetic variations, including SNPs, insertions, deletions, and mutations. By monitoring the melting behavior of double-stranded DNA with high precision, HRM enables the identification of subtle genetic differences through distinct melting profiles without requiring additional post-PCR steps such as gel electrophoresis or sequencing (Reed et al. 2007; Wittwer et al. 2003a). Its high sensitivity, cost-effectiveness, and rapid throughput make HRM particularly suitable for applications in genotyping, mutation scanning, and species differentiation (Vossen et al. 2009; Cheng et al. 2014).

While HRM has been widely applied in food authentication and species identification, its potential for differentiating closely related wheat species based on genetic markers offers significant advantages for agricultural practices (Gadaleta et al. 2011; Pastore et al. 2020). By enabling precise, rapid, and cost-effective differentiation between durum and bread wheat, HRM supports breeding programs, quality control, and regulatory compliance in the wheat industry.

The aim of this study is to apply the HRM technique to differentiate between widely cultivated durum and bread wheat varieties using seed, flour, and leaf samples. This approach seeks to address the challenges of traditional identification methods by providing a reliable molecular tool for wheat genotype differentiation.

2. Material and Methods

2.1. Sampling and DNA extraction

We obtained certified seeds of durum wheat (*Triticum durum* Desf.) varieties (Ovidio, Zivago, Cesare, Tiziana) and bread wheat (*Triticum aestivum* L.) varieties (Ceyhan 99, Nizar, Adana 99, Dinç) from certified seed dealers in Türkiye.

We extracted DNA separately from seeds, flours, and fresh leaves of durum and bread wheat samples to ensure the successful recovery of DNA from each source for subsequent analysis. To obtain flour samples, seeds were finely ground using a coffee grinder. Fresh leaves were generated by germinating seeds in a sterilized peat:vermiculite mixture (3:1 w/w) in 15 cm^3 plastic viols for one week. DNA extraction from seeds and flours was performed exactly following the protocols of Aydin et al. (2018), while the protocols of Doyle & Doyle (1987) and Cullings (1992) were used for fresh leaves, as described in the literature. The concentration and integrity of the extracted DNA were assessed using a Qubit 2.0 fluorometer (Invitrogen, USA) and 1% agarose gel electrophoresis. For the HRM analysis, we utilised DNA extracted specifically from seed samples.

2.2. Amplification of the target regions

Since internal transcribed spacer (ITS) region is a good worker for species-level marker, we amplified the partial ITS region including ITS1, 5.8S and ITS2 parts using the primer pairs ITS1A (Rudnóy et al. 2004) and ITS4 (White et al. 1990) by polymerase chain reaction (PCR). The 20µL reaction mix was prepared as 2µL reaction buffer (Thermo Scientific, USA), 4µL dNTP, 1.6µL $MgCl^2$, 0.4µL of 10nM each primer, 0.16µL Taq polymerase (Thermo Scientific, USA) and nuclease free water. The reaction was performed on SimpliAmp thermal cycler instrument (Applied Biosystems, USA) under the conditions 95 °C 3 m initial denaturation followed by 35 cycles of 95 °C 15 s denaturation, 51 °C 30 s annealing, 72 °C 1 m elongation. The reaction was finalised by the 72 °C 10 m final elongation step. The PCR products were checked for specificity using 2% agarose gel electrophoresis. The amplicons were directly sent to Macrogen Inc., (The Netherlands) for purification and both-direction dideoxy sequencing using the ITS1A and ITS4 primers.

2.3. Primer designing and high-resolution melting analysis

The nucleotide sequences of the Internal Transcribed Spacer (ITS) region were imported into Geneious Prime (version 2024.0.7) for analysis. Bidirectional sequencing reads were trimmed and aligned to generate high-quality consensus sequences for each sample. We annotated the ITS1, 5.8S, and ITS2 regions within these sequences to identify polymorphic sites, focusing on regions with potential genetic variation. Polymorphic regions were selected based on sequence variability observed during alignment, with particular attention to single nucleotide polymorphisms (SNPs) and insertions/deletions (indels).

Given that the accuracy of HRM analysis is influenced by the length and guanine-cytosine (GC) content of the DNA fragments, we specifically targeted the non-coding, highly variable regions within ITS1 and ITS2. HRM-specific primers were designed to amplify these regions, optimizing for an expected amplicon length of 80–120 bp and a melting temperature around 60 °C to ensure efficient amplification and clear melting curve differentiation (Table 1). To validate primer specificity and

efficiency, we performed *in silico* analysis using the Primer-BLAST tool available through the National Center for Biotechnology Information (NCBI), ensuring that the primers selectively amplified the intended target regions without non-specific binding.

Table 1- Primers' specifications designed for the high resolution melting

Name	*Sequence*	*Melting Temperature (℃)*	*Amplicon Size (bp)*
whITS1-HRM_19F	GACCACCTCCCCTCCTCG	60.8	97
whITS1-HRM_116R	GACATGCCCCCGGGTTAGG	62.4	
whITS2-HRM_76F	ACCGAAGATTGGGCTGCC	60.0	152
whITS2-HRM_228R	GGTAGTCCCGCCTGACC	58.7	

We normalised the concentrations of DNA extracts to 20 ng μL^{-1} prior to HRM. Twenty microliters of HRM reaction were set up using Luminaris Color HRM Master Mix (Thermo Scientific, USA) including 10µL of HRM mix, 20ng of DNA, 1µL of 10nM each primer and nuclease-free water. The reaction was performed on Quantgene 9600 (Bioer, China) thermal cycler under the conditions of 95 °C 10 m initial denaturation followed by 40 cycles of 95 °C 15 s denaturation, 58.7 °C 30 s annealing, 72 °C 45 s elongation. We added 95 °C 30 s and 50 °C 30 s steps for heteroduplex formation. Melting conditions were 50 °C to 95 °C by 0.1 °C s^{-1} and fluorescent data was acquiesced continuously. Derivative plots were drawn on the Bioer software and the principal component analysis (PCA) with clustering were performed on R studio using the hrm_pca script (Hürkan 2021).

3. Results and Discussion

We successfully extracted DNA from three different sources: seeds, flour, and fresh leaves of wheat samples. The DNA concentrations ranged from 74.70 to 98.04 ng μL^{-1} for seeds, 54.10 to 66.19 ng μL^{-1} for flour, and 144.58 to 219.00 ng μL^{-1} for fresh leaves. The integrity of the extracted DNA was confirmed through agarose gel electrophoresis, which demonstrated that all samples were suitable for downstream applications, including PCR amplification and HRM analysis. Although DNA was extracted from all three sources, HRM analysis was conducted primarily using DNA from fresh leaves due to its higher concentration and superior quality, which ensured consistent and reliable amplification. DNA from seeds and flour was used to validate the consistency of the results, and no significant discrepancies were observed.

The partial ITS region, comprising ITS1, 5.8S, and ITS2, was successfully amplified from all DNA samples (Table 2). The amplicon sizes were 158 bp for ITS1, 63 bp for 5.8S, and 258 bp for ITS2, with no observed length polymorphisms. Polymorphic sites, ranging from two to twenty, were identified within the intron regions of ITS1 and ITS2, while the coding 5.8S region showed no polymorphic variation, as expected. The presence of polymorphic sites contributed to variations in the guanine-cytosine (GC) content among samples, influencing the melting behavior during HRM analysis (Figure 1). The ITS region, located within the 18S–5.8S–26S nuclear ribosomal cistron, is a well-established marker for plant species identification and phylogenetic studies due to its biparental inheritance and high degree of polymorphism (Álvarez & Wendel 2003). These characteristics made ITS1 and ITS2 ideal targets for HRM analysis in distinguishing wheat genotypes.

Table 2- Specification table of internal transcribed spacer region amplified from the studied samples

	ITS1			*5.8S*			*ITS2*		
Sample	***Length***	***G/C***	***SNP***	***Length***	***G/C***	***SNP***	***Length***	***G/C***	***SNP***
Ceyhan99 (BW)	158	60.8	2	63	59.5	0	258	60.5	20
Nizar (BW)	158	60.8	2	63	59.5	0	258	60.9	20
Ovidio (DW)	158	61.4	2	63	59.5	0	258	62.0	20
Zivago (DW)	158	61.4	2	63	59.5	0	258	61.6	20
Cesare (DW)	158	61.4	2	63	59.5	0	258	62.0	20
Tiziana (DW)	158	61.4	2	63	59.5	0	258	61.6	20
Adana99 (BW)	158	60.8	2	63	59.5	0	258	60.9	20
Dinç (BW)	158	60.8	2	63	59.5	0	258	61.6	20

Length: Amplicon length in base pair, G/C: Guanine – cytosine ratio in per cent, SNP: Number of single nucleotide polymorphism

For optimal HRM analysis, amplicons should be less than 300 bp to ensure accurate detection of single nucleotide polymorphisms (SNPs) (Druml & Cichna-Markl 2014). Since the ITS region amplified with ITS1A and ITS4 primers yielded a 479 bp fragment, we designed novel HRM-compatible primer pairs specific to the studied samples. The primer pairs, whITS1-HRM_19F/whITS1-HRM_116R and whITS2-HRM_76F/whITS2-HRM_228R, produced amplicons of 97 bp and 152 bp, respectively. These primers were validated through end-point PCR, confirming specific amplification. The specificity of the primers was further verified using *in silico* analysis with Primer-BLAST from the National Center for Biotechnology Information (NCBI).

The successful implementation of HRM analysis enabled the identification of DNA variants without the need for sequencing (Druml & Cichna-Markl 2014). The GC content significantly influenced the melting temperature (Tm) and denaturation kinetics of the DNA fragments, as GC pairs form three hydrogen bonds compared to the two in adenine-thymine (AT) pairs. This

difference in melting behavior allows HRM to distinguish between DNA variants, with the accuracy of the analysis depending on both the amplicon length and the specificity of the PCR products (van der Stoep et al. 2009).

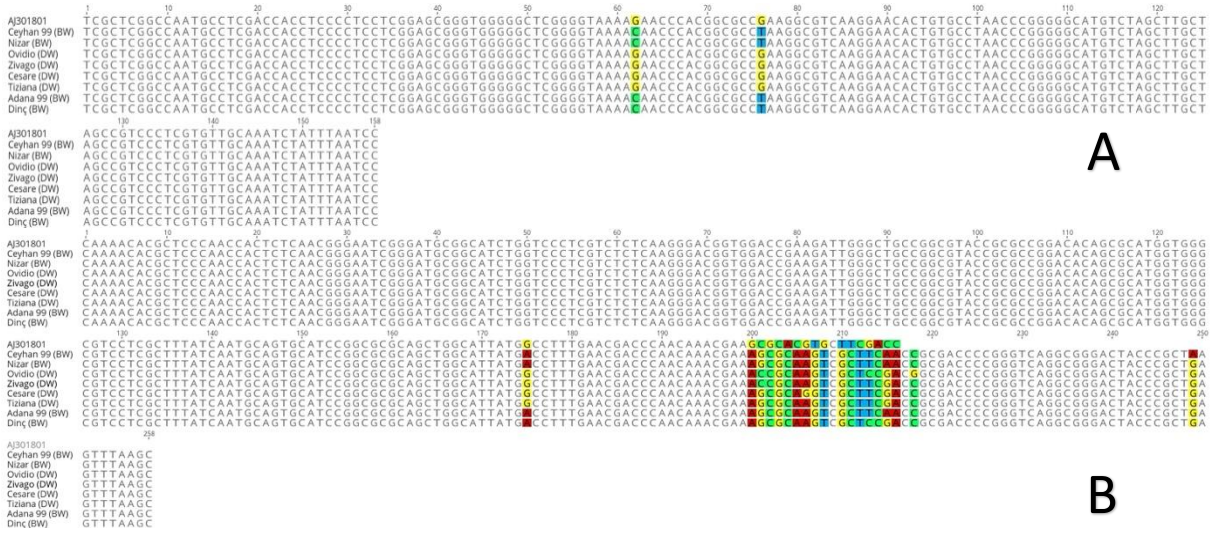
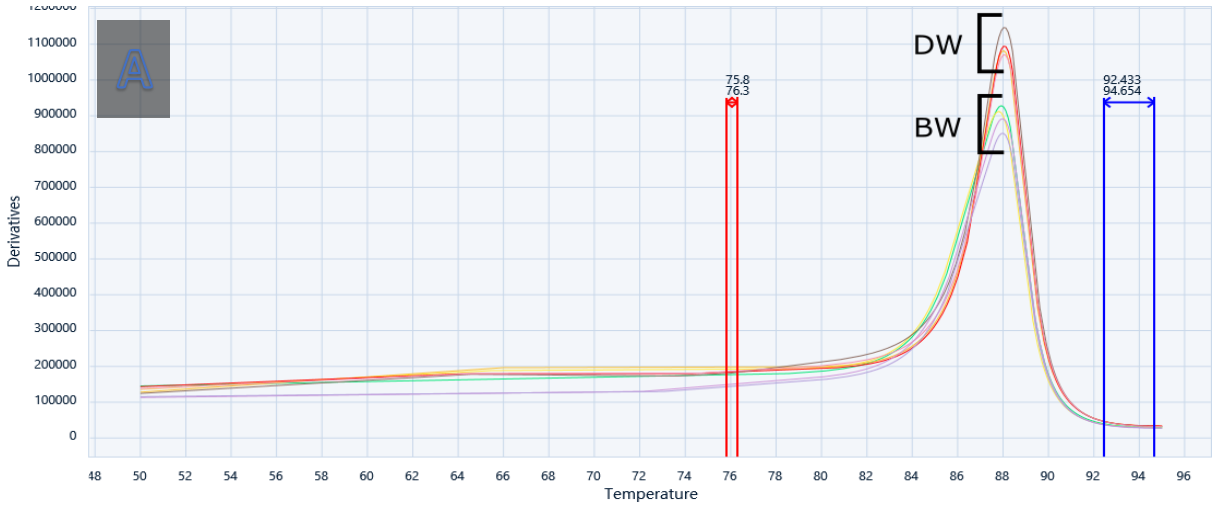


Figure 1- Nucleotide alignments of the ITS1 (A) and ITS2 (B) regions amplified in the present study. The polymorphic sites indicated with colours

HRM derivative plots demonstrated high specificity and sensitivity in distinguishing durum and bread wheat samples based on the ITS1 and ITS2 regions (Figure 2A and 2B). The ITS1 region, despite having only two SNPs, exhibited better resolution in differentiating wheat types compared to the ITS2 region, which contained 20 SNPs. This is attributed to the shorter amplicon length of ITS1, which enhances melting curve resolution (Wittwer et al. 2003b; Liew et al. 2004; Wojdacz & Dobrowolski 2007; Vossen et al. 2009). The genotyping confidence percentage (GCP), calculated by the HRM software, was 97.2% for ITS1 and 95.2% for ITS2, supporting the clear distinction between the wheat types.



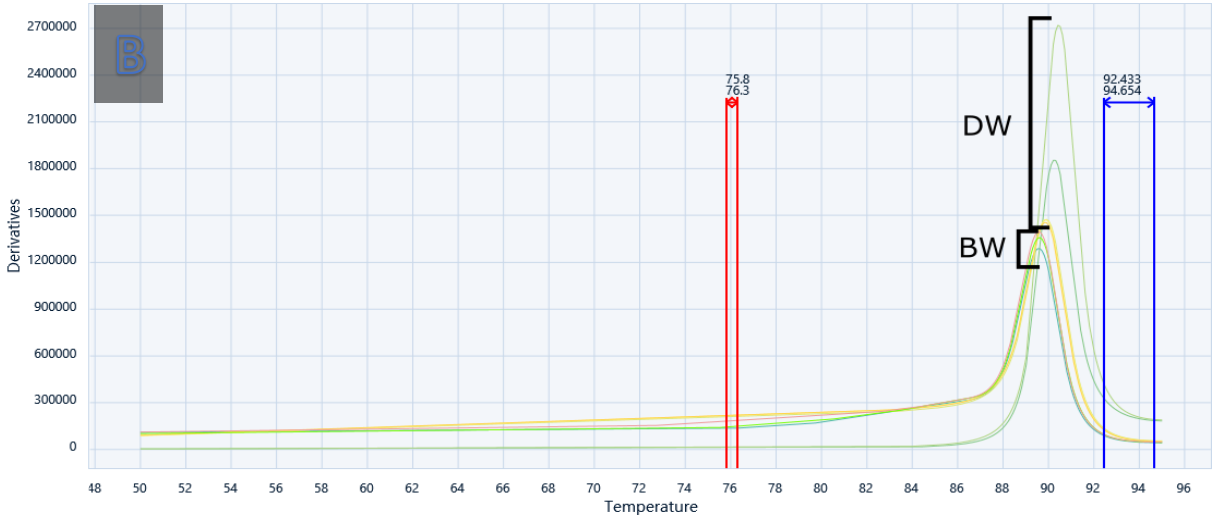


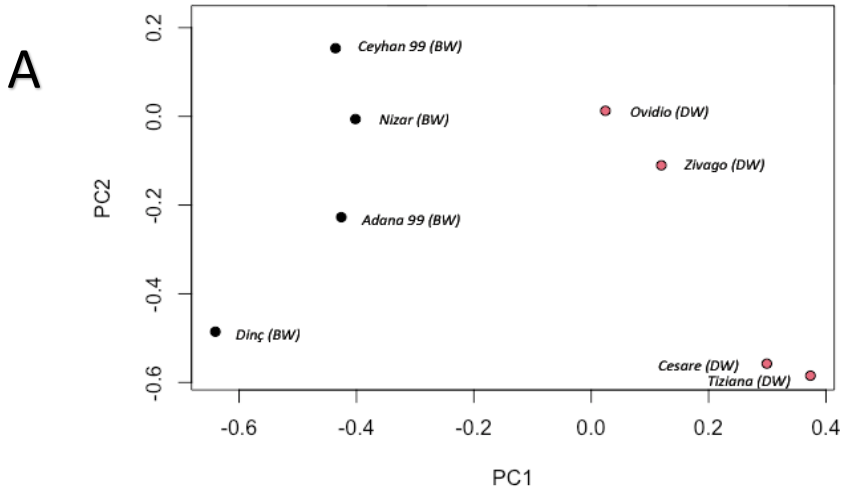
Figure 2- Derivative plots generated by the melting kinetics of the ITS1 (A) and ITS2 (B) amplicons during high resolution melting analysis of the wheat samples. Red and blue zones represent the considered temperature ranges for analysing

To enhance the interpretation of HRM data, we applied principal component analysis (PCA) and clustering techniques. PCA effectively reduced the dimensionality of the melting data, allowing us to identify the most significant variables influencing the melting profiles (Halkidi et al. 2001). Clustering analysis grouped samples based on their melting characteristics, revealing genetic patterns that were not immediately apparent through visual inspection alone (Kaufman & Rousseeuw 1990).

The PCA plots showed clear discrimination between bread wheat (BW) and durum wheat (DW) samples for both ITS1 and ITS2 regions (Figure 3A and 3B). In the ITS1 plot, BW samples clustered on the left with negative PC1 values, while DW samples grouped on the right with positive PC1 values, indicating distinct genetic differences between the groups. The ITS2 plot showed BW samples tightly clustered with negative PC2 values, whereas DW samples were more dispersed, reflecting greater genetic variability within this group. Notably, the DW variety Cesare exhibited the highest PC1 value, suggesting significant genetic divergence from the other samples.

The combination of HRM analysis with PCA and clustering provided a robust framework for differentiating wheat genotypes. This integrated approach enhanced the resolution of genetic differences, improved data interpretation, and confirmed the genetic distinctions between durum and bread wheat. HRM's rapid, cost-effective, and high-throughput capabilities make it a valuable tool for genotyping studies. However, potential limitations include sensitivity to DNA quality, amplicon length, and GC content, which can affect melting curve resolution. Additionally, while HRM is effective for detecting known polymorphisms, it may have reduced sensitivity for identifying rare or complex mutations.

Future research should focus on expanding the range of genetic markers beyond the ITS region to improve discriminatory power, especially for closely related cultivars. Integrating HRM with next-generation sequencing technologies could further enhance genotyping accuracy. Additionally, applying this approach to larger and more diverse wheat populations would provide deeper insights into the genetic diversity and evolutionary relationships within and between wheat species.



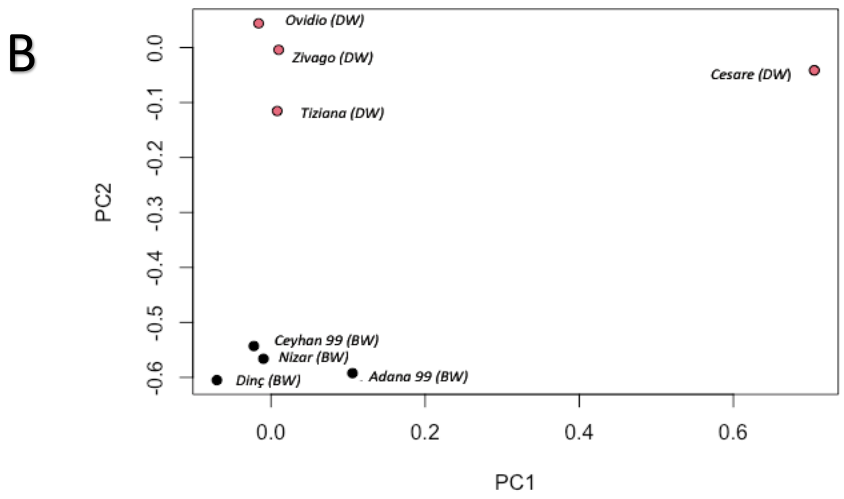


Figure 3- Principal component analysis (PCA) of the raw high-resolution melting (HRM) data from the ITS1 (A) and ITS2 (B) amplicons, derived from the wheat samples. Durum wheat (DW) was colour coded as red, and bread wheat (BW) was as black

4. Conclusions

The newly designed primer pairs targeting the non-coding ITS1 and ITS2 regions of the partial ITS fragment have been shown to unequivocally differentiate between durum and bread wheat varieties. High-resolution melting (HRM) analysis is a precise, accurate, and time-efficient single-tube method that effectively distinguishes these wheat types based on their subtle genetic differences. As no prior study in the literature has employed the HRM method for this purpose, the present research serves as a pioneering effort for researchers and laboratories that need to discriminate between durum and bread wheats from various sampling sources, including seeds, flour, and leaves.

Authors' contributions: EUG: Laboratory experiments, YKH: Statistical analysis and data evaluation, KH: Concept of the study, data analysis and writing the draft manuscript.

Acknowledgements

We would like to thank the anonymous reviewers for their valuable contributions to improving this study.

Confliction of interest

Not applicable.

References

Abdel-Aal E S M, Hucl P & Sosulski F W (1995). Compositional and nutritional characteristics of spring einkorn and spelt wheats. https://doi.org/10.1006/jcrs.1997.0139

Álvarez I J F W& Wendel J F (2003). Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution 29: 417–434. https://doi.org/10.1016/s1055-7903(03)00208-2

Ay N & Hürkan K (2023). A novel high-resolution melting method for detection of adulteration on pistachio (Pistacia vera L.). Brazilian Archives of Biology and Technology 66. https://doi.org/10.1590/1678-4324-2023220550

Ceyhan V & Akkaya A (2020). Wheat production and sustainability in Turkey. *Journal of Agricultural Sciences*

Chaves M S, Martinelli J A, Wesp-Guterres C, Graichen FAS, Brammer S P, Scagliusi S M & Chaves, A L S (2013). The importance for food security of maintaining rust resistance in wheat. *Food Security* 5:157–176. https://doi.org/10.1007/s12571-013-0248-x

Cheng J, Ma X, Zeng C, Zhang J & Li Z (2014). Application of high-resolution melting (HRM) for genotyping and species identification. *Molecular Plant Breeding* 5(2):89–95

Druml B & Cichna-Markl M (2014). High-resolution melting (HRM) analysis of DNA: a new molecular tool in food authentication. *Food Chemistry* 158:245–254. https://doi.org/10.1016/j.foodchem.2014.02.111

Dubcovsky J, & Dvorak J (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, 316(5833), 1862-1866. https://doi.org/10.1126/science.1143986

El-Esawi M A, Germaine K, Bourke P, Malone R (2018). Genetic diversity and population structure of wheat species. *Plant Biotechnology Journal* 16(5): 1089–1100

Gadaleta A, Nigro D, Giancaspro A & Blanco A (2011). The glutamine synthetase (GS2) genes in relation to grain protein content of durum wheat. *Functional & Integrative Genomics*, 11: 665-670. https://doi.org/10.1007/s10142-011-0235-2

Hürkan K (2021). Distinguishing the protected designation of origin apricot (*Prunus armeniaca* L. cv. Şalak) from closely related cultivars by high resolution melting. *Commagene Journal Biology* 5: 136–142. https://doi.org/10.31594/commagene.891932

Karaman H, Ozkan B & Celik A (2018). Enhancing wheat resilience through sustainable practices in Turkey. *Sustainable Agriculture Reviews*.
Kaufman L & Rousseeuw P J (1990). Finding groups in data: an introduction to cluster analysis. Wiley.
Liew M, Pryor R, Palais R, Meadows C, Erali M, Lyon E & Wittwer C T (2004). Genotyping by amplicon melting analysis using LCGreen. *Clin Chemistry* 50:1156–1164.. https://doi.org/10.1373/clinchem.2004.032136
Lv R, Han L, Xiao B, Xiao C, Yang Z, Wang H & Yang C (2019). An extracted tetraploid wheat harbouring the BBAA component of common wheat shows anomalous shikimate and sucrose metabolism. *BMC Plant Biology* 19: 1–11. https://doi.org/10.1186/s12870-019-1796-9
Pastore G, Kernchen S, & Spohn M (2020). Microbial solubilization of silicon and phosphorus from bedrock in relation to abundance of phosphorus-solubilizing bacteria in temperate forest soils. Soil Biology and Biochemistry 151: 108050. https://doi.org/10.1016/j.soilbio.2020.108050
Peng J H, Sun D & Nevo E (2011). Domestication evolution, genetics, and genomics in wheat. *Molecular Biology and Evolution* 28(5):1471–1492. https://doi.org/10.1007/s11032-011-9608-4
Rahaie M, Xue G P & Schenk, P M (2013). The role of transcription factors in wheat under different abiotic stresses. Abiotic stress-plant responses and applications in agriculture, 2: 367-385. https://doi.org/10.5772/54795
Reed K A, Wittwer C T & Oetjen J (2007). High-resolution melting analysis for mutation scanning and genotyping. *Nature Protocols* 2: 1761–1772. https://doi.org/10.1038/nprot.2007.247. https://doi.org/10.1038/nprot.2007.10
Rudnóy S, Páldi E, Bratek Z, Szegő D, Rácz I & Lásztity D (2004). ITS regions in hexaploid bread wheat and its supposed progenitors. *Cereal Research Communications* 32: 423–428. https://doi.org/10.1007/bf03543330
Shewry P R (2009). Wheat. JXB 60(6): 1537-1553
van der Stoep N, van Paridon C D, Janssens T, Krenkova P, Stambergova A, Macek M & Bakker E (2009). Diagnostic guidelines for high-resolution melting curve (HRM) analysis: An interlaboratory validation of BRCA1 mutation scanning using the 96-well LightScanne. Human. Mutant. 30(6): 899-909. https://doi.org/10.1002/humu.21004
Vossen R H A M, Aten E, Roos A & den Dunnen J T (2009). High-resolution melting analysis (HRMA): more than just sequence variant screening. *Human Mutant* 30: 860–866. https://doi.org/10.1002/humu.21019. https://doi.org/10.1002/humu.21019
White T J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. *Academic Press, Inc.* https://doi.org/10.1016/b978-0-12-372180-8.50042-1
Wittwer C T, Reed G H, Gundry C N, Vandersteen J G & Pryor R J (2003a). High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chemistry* 49:853–860.. https://doi.org/10.1373/49.6.853
Wittwer C T, Ririe K M, Andrew R E & Heiner C R (2003b). High-resolution DNA melting analysis for mutation scanning and genotyping. *National Biotechnology* 21: 271–275. https://doi.org/10.1038/nbt0303-271
Wojdacz T K & Dobrowolski S F (2007). A new approach to high-resolution melting analysis using amplicons shorter than 100 bp. *Nucleic Acids Research* 35. https://doi.org/10.1093/nar/gkm059
Yan J, Warburton M L & Crouch J H (2013). High-resolution melting analysis of single nucleotide polymorphisms in wheat breeding. *Journal of Cereal Science* 58(3): 405–411
Yüce İ, Başkonuş T, Dokuyucu T, Akkaya A, Güngör H & Dumlupinar Z (2022). Bazı ekmeklik buğday (*Triticum aestivum* L.) çeşitleri ve ileri hatlarının Kahramanmaraş ekolojik şartlarında tarımsal özellikler ve kalite bakımından değerlendirilmesi. YYU *Journal of Agricultural Sciences* 32(2)
Zeven A C (1991). Wheats with purple and blue grains: A review. *Euphytica*, 56(3): 243–258. https://doi.org/10.1007/BF00042371