Molecular characterization of selected Vrn, Lr, and Yr genes in Turkish landraces and modern bread wheat (Triticum aestivum L.) cultivars

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Abstract

Wheat yield is constrained by both biotic and abiotic stress factors. Rust diseases rank among the most important biotic factors, while heading date and root architecture are key traits contributing to yield potential and abiotic stress tolerance. In this study, 104 Turkish landrace and modern bread wheat (*Triticum aestivum L*.) genotypes were molecularly screened for the adult plant resistance genes *Lr34*, *Lr46*, *Lr67*, *Yr26*, and *Yr15* using PCR-based molecular markers, as well as the vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, which influence heading date, root system architecture, and stress tolerance. Vernalization allele analysis revealed that four genotypes carried *Vrn-A1a*, three carried *Vrn-A1c*, and the remaining 97 genotypes harbored *Vrn-A1b*. Regarding rust resistance, *Lr46* was detected in 11, *Lr34* in 7, *Lr67* in 7, *Yr26* in 62, and *Yr15* in 85 genotypes. Genotypes such as Ankara093-44 and Bezostaja were found to harbor multiple resistance gene combinations. Nineteen genotypes carrying the *Vrn-A1b* × *vrn-B1* × *Vrn-D1* combination, associated with narrow root angle, included genotypes also possessing multiple rust resistance genes. These results identify valuable parental genotypes for gene pyramiding strategies and provide genetic resources for marker-assisted selection-based breeding programs targeting rust resistance, drought tolerance, and root system architecture improvement.

Introduction

Wheat (*Triticum aestivum L*.) is a widely cultivated cereal grown on approximately 219 million hectares globally, with an annual production exceeding 789 million metric tons, representing a fundamental component of global food systems (Aydın et al. 2025). Enhancing wheat yield and quality remains among the foremost objectives of breeding programs. However, both biotic and abiotic stress factors substantially constrain wheat production. The development of cultivars resistant to these stress factors is recognized as the most cost-effective and sustainable approach for improving yield and quality (Parzies et al. 2000). Nevertheless, the inherent complexity of the allohexaploid genome architecture represents a significant impediment to achieving breeding objectives within short timeframes. Consequently, the identification and effective utilization of purpose-specific genetic resources constitute the foundation of plant breeding endeavors (Alkan and Kandemir 2015).

Landraces, having evolved under natural selection pressure over millennia, represent particularly valuable genetic resources owing to their possession of tolerance genes for biotic and abiotic stress factors that limit wheat yield and quality. The characterization of genetic diversity accumulated in these genotypes and its transfer to modern breeding programs is of strategic importance for sustainable wheat production. Accordingly, molecular screening studies aimed at identifying genes harbored in landraces are of paramount significance.

Heading date represents one of the fundamental traits contributing to wheat yield potential (Zhang et al. 2014). The principal genetic determinant governing heading date and other phenological traits is the vernalization (*Vrn*) gene system, which controls the cold requirement for the transition from vegetative to reproductive growth (Yan et al. 2003; Zhang et al. 2008; Whittal et al. 2018). Among these, *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, located on homoeologous chromosomes 5A, 5B, and 5D, respectively, exert the most pronounced effects (Yan et al. 2003). *Vrn-A1* is the most potent allele conferring complete insensitivity to vernalization and is epistatic to *Vrn-B1* and *Vrn-D1* (Loukoianov et al. 2005). This vernalization system ensures the survival of winter wheat during the vegetative growth phase under adverse winter conditions, as plants are not tolerant to low temperatures during the reproductive phase (Danyluk et al. 2003). Different combinations of dominant *Vrn* alleles can induce changes in plant height, yield components, and stress tolerance (Stelmakh 1992; Iqbal et al. 2007; Efremova et al. 2016).

Root system architecture, which contributes to yield stability, exerts a substantial influence on plant performance under abiotic stress conditions (Watt et al. 2020; Whittal et al. 2018). Recent studies have demonstrated that *VRN1*, a key regulator of flowering, also modulates root architecture in wheat and barley (Voss-Fels et al. 2018; Güleç et al. 2021; Aydın et al. 2025). ChIP-seq and RNA-seq analyses conducted by Deng et al. (2015) revealed that *VRN1* establishes direct links between the flowering pathway and agronomically critical traits including root development, frost tolerance, and hormone metabolism.

Among biotic stress factors, rust diseases caused by *Puccinia triticina* (leaf rust), *P. striiformis f. sp. tritici* (stripe rust), and *P. graminis f. sp. tritici* (stem rust) can cause yield losses of up to 90% in susceptible cultivars during epidemic years (Aktaş 2001; Mut et al. 2018). To date, more than 150 resistance genes have been identified against these rusts, of which over 100 are associated with leaf rust resistance (Sapkota et al. 2019; Bakhshi et al. 2024; Tong et al. 2024). Non-race-specific adult plant resistance (*APR*) genes, including *Lr34/Yr18/Sr57/Pm38, Lr46/Yr29/Pm39, Lr67/Yr46/Sr55/Pm46*, and *Lr68*, are particularly prominent for durable

resistance (Krattinger et al. 2009; Lagudah et al. 2009; Herrera-Foessel et al. 2014). Among stripe rust resistance genes, *Yr15*, derived from *Triticum dicoccoides*, provides effective all-stage resistance (McIntosh and Silk 1996; Haider et al. 2023), while *Yr26* is predicted to confer resistance against Turkish stripe rust races (Furan et al. 2017). Pyramiding several of these genes provides stronger and more durable resistance (Rani et al. 2019; Tong et al. 2024).

The present study aimed to molecularly screen selected Turkish landrace and modern bread wheat genotypes for the adult plant rust resistance genes *Lr67*, *Lr46*, *Lr34*, *Yr26*, and *Yr15*, as well as the vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, and to evaluate their potential utility as genetic resources in breeding programs.

Materials and methods

Total number of 104 bread wheat genotypes representing diverse ecological regions of Turkey were cultivated under controlled greenhouse conditions. Fresh leaf tissue was collected at the three- to four-leaf stage, and genomic DNA was isolated using a commercial purification kit with quality verification via spectrophotometry. The extracted DNA was subsequently subjected to PCR amplification targeting six vernalization alleles, three leaf rust resistance genes, and two stripe rust resistance genes using allele-specific and SSR-based molecular markers. Amplification products were resolved by agarose gel electrophoresis at appropriate concentrations and scored for the presence or absence of target alleles based on expected fragment sizes. A schematic overview of the entire experimental workflow employed in this study is presented in Fig. 1.

Plant material

A total of 104 landrace and modern bread wheat (*Triticum aestivum L.*) genotypes collected from diverse ecological regions of Turkey (Central Anatolia, Transitional Zones, Southeastern Anatolia, and Eastern Anatolia) were utilized (Table 2). Landraces were collected from Tokat, Eskişehir, Bolu, Kastamonu, Malatya, Karaman, and surrounding provinces. Modern cultivars comprised registered varieties from various Turkish agricultural research institutes. Tosunbey and Tahirova-2000 served as check cultivars for vernalization gene analysis; Chinese Spring and Pavon-76 were used as positive and negative controls for leaf rust (*Lr34*, *Lr46*, *Lr67*) and stripe rust (*Yr26*, *Yr15*) resistance gene analysis.

DNA extraction

DNA extraction

Seeds from each genotype were germinated in Petri dishes containing filter papers moistened with sterile distilled water at room temperature. Germinated seedlings were transplanted into 7-cm-diameter pots and cultivated in a controlled-environment growth chamber (day/night temperature: 22/16°C; 16-h light/8-h dark photoperiod; 60–70% relative humidity). Fresh leaf tissue was harvested from plants at the three- to four-leaf stage (Zadoks GS 13–14) and stored at − 80°C. Genomic DNA was isolated using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. DNA purity and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), and only samples with A260/A280 ratios between 1.8 and 2.0 were used. DNA samples were stored at − 20°C until PCR analysis.

PCR analysis

PCR analyses were performed for six vernalization alleles (*Vrn-A1a*, *Vrn-A1b*, *Vrn-B1*, *vrn-B1*, *Vrn-D1*, *vrn-D1*), three leaf rust APR genes (*Lr34*, *Lr46*, *Lr67*), and two stripe rust resistance genes (*Yr26*, *Yr15*). PCR reactions were prepared in a total volume of 25 µL containing: 12.5 µL 2× PCR Master Mix (Thermo Scientific), 1 µL each primer (10 µM), 2 µL template DNA (~ 50 ng), and 8.5 µL nuclease-free water. Primer sequences and expected fragment sizes are presented in Table 1. Amplification products were resolved on 1.5% (*Vrn* genes) and 2.5% (*Lr* and *Yr* genes) agarose gels in 1× TAE buffer alongside a 100-bp DNA ladder (Thermo Scientific) at 90 V for 1.5–2 h. Gels were stained with ethidium bromide (0.5 µg/mL), visualized under UV transillumination, and documented using a Bio-Rad Gel Doc XR+ system. The presence (+) or absence (−) of each gene was scored by comparison with control genotypes.

Table 1
Primers used in PCR analyses, expected fragment lengths, and references

Locus	Allele	Primer	Primer sequence (5'→3')	Fragment length (bp)	Reference
Vrn-A1	*Vrn-A1a*	VRN1AF/ VRN1-INT1R	F: GAAAAGGAAAAATTCTGCTCG R: GCAGGAAATCGAAATCGAAG	965 + 876	Yan et al. (2004); Zhang et al. (2008)
	Vrn-A1b	VRN1AF/ VRN1-INT1R	F: GAAAAGGAAAAATTCTGCTCG R: GCAGGAAATCGAAATCGAAG	~ 714	Yan et al. (2004); Zhang et al. (2008)
	Vrn-A1c	VRN1AF/ VRN1-INT1R	F: GAAAAGGAAAAATTCTGCTCG R: GCAGGAAATCGAAATCGAAG	~ 734	Yan et al. (2004); Fu et al. (2005)
Vrn-B1	*Vrn-B1*	Intr1/B/F/ Intr1/B/R3	F: CAAGTGGAACGGTTAGGACA R: CTCATGCCAAAAATTGAAGATGA	~ 709	Fu et al. (2005); Zhang et al. (2008)
	vrn-B1	Intr1/B/F/ Intr1/B/R4	F: CAAGTGGAACGGTTAGGACA R: CAAATGAAAAGGAATGAGAGCA	~ 1160	Fu et al. (2005); Zhang et al. (2008)
Vrn-D1	*Vrn-D1*	Intr1/D/F/ Intr1/D/R3	F: GTTGTCTGCCTCATCAAATCC R: GGTCACTGGTGGTCTGTGC	~ 997	Fu et al. (2005); Zhang et al. (2008)
	vrn-D1	Intr1/D/F/ Intr1/D/R4	F: GTTGTCTGCCTCATCAAATCC R: AAATGAAAAGGAACGAGAGCG	~ 1671	Fu et al. (2005); Zhang et al. (2008)
Lr34	*Lr34 (+)*	csLV34	F: GTTGGTTAAGACTGGTGATGG R: TGCTTGCTATTGCTGAATAGT	150 (+) 229 (−)	Lagudah et al. (2006)
Lr46	*Lr46*	Wmc44	F: GGTCTTCTGGGCTTTGATCCTG R: GTTGCTAGGGACCCGTAGTGG	~ 242	William et al. (2003)
Lr67	*Lr67*	CFD71	F: CAATAAGTAGGCCGGGACAA R: TGTGCCAGTTGAGTTTGCTC	SSR, polymorphic	Hiebert et al. (2010)
Yr26	*Yr26*	Xgwm18	F: TTGCTACCATGCATGACCAT R: TTCACCTCGATTGAGGTCCT	~ 198	Röder et al. (1998); Ma et al. (2001)
Yr15	*Yr15*	Xgwm413	F: TGCTTGTCTAGATTGCTTGGG R: GATCGTCTCGTCCTTGGCA	~ 95	Peng et al. (2000)

Results

Distribution of vernalization alleles

Molecular screening for the *Vrn-A1* locus revealed that four genotypes (Ağsünteri, Tahirova-2000, Halbert, and Altındane T.) carried *Vrn-A1a*, three genotypes (Yayla-305, Tir Line-14YBGB-246, and Kaşıkçı Buğdayı) harbored *Vrn-A1c*, and the remaining 97 genotypes possessed *Vrn-A1b*. At *Vrn-B1*, 63 genotypes carried dominant *Vrn-B1* and 42 carried recessive *vrn-B1*. At *Vrn-D1*, 40 genotypes carried dominant *Vrn-D1* and 65 harbored recessive *vrn-D1* (Fig. 2; Table 2).

Assessment of allele combinations revealed that 18 genotypes carried exclusively recessive vrn alleles (winter growth habit). No genotype was identified with the triple-dominant *Vrn-A1a* × *Vrn-B1* × *Vrn-D1* combination. The most frequent combination was *Vrn-A1b* × *Vrn-B1* × *vrn-D1*, detected in 35 genotypes (Table 2).

Table 2
Vrn allele combinations of genotypes and their potential agronomic implications

Allele combination	n	Genotypes
Vrn-A1a × *Vrn-B1* × *vrn-D1*	2	Ağsünteri, Halbert
Vrn-A1a × *vrn-B1* × *Vrn-D1*	2	Altındane T., Tahirova-2000
Vrn-A1c × *vrn-B1* × *vrn-D1*	2	Yayla-305, Kaşıkçı Buğdayı
Vrn-A1c × *vrn-B1* × *Vrn-D1*	1	Tir Line (14YBGB-246)
Vrn-A1b × *vrn-B1* × *vrn-D1*	17	Clark's Cream1, Clark's Cream2, Kırık1, Kırık2, Kırık5, Kırık7, Sönmez-2001, Albostan1, Albostan3, MV18, Altay-2000, Çalıbasan2, Çalıbasan3, Bezostaja, Kabak Buğdayı, Arı Buğday4, Gerek-79
Vrn-A1b × *vrn-B1* × *Vrn-D1*	19	Ankara093-4, Köse220-39, Akbuğday1, 4–11, Domaniç, Kamçı1, K.Kamçı2, Kamçı2, Kamçı3, Gülümbür/Makas, Tir Line (14YBGB-243), Çalıbasan1, Müfitbey, Gülümbür1, Alpu-2001, Topbaş2, Topbaş4, Esperia, No Name2
Vrn-A1b × *Vrn-B1* × *vrn-D1*	35	AK702, Yektay406, HD 2329, Akbuğday2-7, Albostan2, Tosun21, Altındane S, Ankara, Elbistan, Hatipbuğdayı, Göderedi1-2, Kamçı2–3, K.Akbuğday1, Kobak, Kocabuğday, Sarı Buğday, No Name, Topbaş1,5, Kırık4,6, Tosunbey, Sarı Misli, Demir-2000, Gülümbür2, Çalıbasan4-5, Boğruala et al.
Vrn-A1b × *Vrn-B1* × *Vrn-D1*	19	Atay85, Karakılçık, K.Akbuğday2, K.Kamçı1, Şahman1-4, Demir-2000, Adana-99, Göderedi3, Urumeli1-2, Arı Buğday1-3, Buğday, Topbaş3, Kırık3

Association between Vrn allele combinations and root angle

In a previous study (Güleç et al. 2021), genotypes carrying *Vrn-A1b*, *vrn-B1*, and *Vrn-D1* alleles individually exhibited narrower mean root angles, whereas those carrying *Vrn-B1* and *vrn-D1* displayed wider root angles (Fig. 3). At the allele combination level, *Vrn-A1b* × *vrn-B1* × *Vrn-D1* genotypes were classified in the narrow root angle group, while *Vrn-A1a* × *Vrn-B1* × *vrn-D1* genotypes were in the wide root angle group. In the present study, 19 genotypes carried the former and two (Ağsünteri and Halbert) the latter combination (Table 2).

Distribution of rust resistance genes

The presence of *Lr46*, *Lr34*, *Lr67*, *Yr26*, and *Yr15* genes across the 104 screened genotypes is summarized in Table 2, and representative gel electrophoresis images are presented in Fig. 4. The *Lr46* gene was detected in 11 genotypes (Kıraç-66, Demir-2000, Gülümbür/Makas Buğdayı, Adana-99, Tir Line-14YBGB-243, Halbert, Bezostaja, KırmızıBuğday, Kobak2, Kırık2, and Akbuğday7). The *Lr34* gene was identified in seven genotypes: Ankara093-44, 4–11, Altındane S., Bezostaja, Alpu-2001, Esperia, and Müfitbey. Notably, the Bezostaja genotype was found to harbor both *Lr34* and *Lr46* simultaneously. The *Lr67* gene was detected in seven genotypes (Yayla305, Ankara093-44, Yektay406, MV18, Kırmızı Kamçı1, Altay-2000, and Ağsünteri). The Ankara093-44 genotype was found to carry both *Lr34* and *Lr67*.

Regarding stripe rust resistance genes, *Yr26* was detected in 62 genotypes and *Yr15* in 85 genotypes. A total of 49 genotypes were identified carrying both stripe rust resistance genes simultaneously.

Table 3
The genotypes included in the study and their corresponding allelic profiles

1	Genotype	*Lr46* Gene	*Lr34* Gene	*Lr67* Gene	*Yr26* Gene	*Yr15* Gen		Genotype	*Lr46* Gen	*Lr34* Gene	*Lr67* Gene	*Yr26* Gene	*Yr15* Gene
	Yayla305	**-**	**-**	**+**	**+**	**+**	**53**	**Tir line (14BGB-246)**	**-**	**-**	**-**	**-**	**+**
2	Ak702	-	-	-	+	+	54	Sarı buğday 1	-	-	-	+	+
3	Ankara093-44	-	+	+	+	+	55	Halbert	+	-	-	-	+
4	Köse220-39	-	-	-	+	-	56	Tahirova2000	-	-	-	+	-
5	Clark'sCream 1	-	-	-	+	+	57	Çalıbasan 1	-	-	-	-	+
6	Yektay406	-	-	+	-	+	58	Çalıbasan 2	-	-	-	-	+
7	Kırik 1	-	-	-	+	-	59	Kocabuğday 1	-	-	-	-	+
8	HD 2329	-	-	-	-	+	60	No name 3	-	-	-	+	+
9	Kıraç66	+	-	-	+	+	61	Çalıbasan 3	-	-	-	-	+
10	Atay85	-	-	-	-	+	62	Gülümbür 1	-	-	-	+	+
11	Akbuğday 1	-	-	-	+	+	63	Bezostaja1	+	+	-	+	+
12	Sönmez 2001	-	-	-	+	+	64	Kocabuğday 2	-	-	-	-	+
13	Akbuğday 2	-	-	-	-	+	65	Kaşıkçı Buğday	-	-	-	+	+
14	4–11	-	+	-	+	+	66	Müfitbey	-	+	-	+	+
15	Akbuğday 3	-	-	-	-	+	67	Akbuğday 5	-	-	-	-	+
16	Akbuğday 4	-	-	-	-	+	68	Ağsünteri	-	-	+	-	+
17	Albost an 1	-	-	-	+	+	69	Buğday	-	-	-	-	-
18	Albost an 2	-	-	-	+	+	70	Topbaş 1	-	-	-	-	+
19	Tosun21	-	-	-	-	+	71	No name 1	-	-	-	+	+
20	Albost an 3	-	-	-	-	-	72	Topbaş 2	-	-	-	+	+
21	Domaniç	-	-	-	-	+	73	Topbaş 3	-	-	-	-	+
22	Altındane S	-	+	-	-	+	74	Şahman 4	-	-	-	+	+
23	Ankara	-	-	-	+	+	75	Topbaş 4	-	-	-	+	+
24	Elbistan	-	-	-	+	+	76	Akbuğday 6	-	-	-	+	+
25	Göderedi 1	-	-	-	-	+	77	Alpu2001	-	+	-	-	+
26	Göderedi 2	-	-	-	+	+	78	Kırmızbuğday	+	-	-	-	-
27	Altındane T	-	+	-	-	+	79	Clark'sCream 2	-	-	-	+	+
28	Kamçı 1	-	-	-	-	+	80	Topbaş 5	-	-	-	+	+
29	Kamçı 2	-	-	-	+	+	81	Kobak 2	+	-	-	-	+
30	MV18	-	-	+	+	+	82	Kırik 2	+	-	-	+	+

1	Genotype	*Lr46* Gene	*Lr34* Gene	*Lr67* Gene	*Yr26* Gene	*Yr15* Gen		Genotype	*Lr46* Gen	*Lr34* Gene	*Lr67* Gene	*Yr26* Gene	*Yr15* Gene
	Yayla305	**-**	**-**	**+**	**+**	**+**	**53**	**Tir line (14BGB-246)**	**-**	**-**	**-**	**-**	**+**
31	Kamçı 3	-	-	-	+	+	83	Akbuğday 7	+	-	-	-	+
32	Karakılçık	-	-	-	+	-	84	Kırık 3	-	-	-	+	-
33	K.Akbuğday 1	-	-	-	-	+	85	Kırık 4	-	-	-	+	+
34	K.Akbuğday 2	-	-	-	-	-	86	Sarı Misli	-	-	-	+	+
35	K.Kamçı 1	-	-	+	-	-	87	Demir2000	-	-	-	-	+
36	K.Kamçı 2	-	-	-	+	+	88	Kırık 5	-	-	-	+	+
37	K. Kamçı 3	-	-	-	+	+	89	Urumeli 2	-	-	-	+	+
38	Altay2000	-	-	+	+	+	90	Tosunbey	-	-	-	+	+
39	Şahman 1	-	-	-	+	+	91	Gülümbür 2	-	-	-	+	-
40	Şahman 2	-	-	-	+	+	92	Kırık 6	-	-	-	+	-
41	Şahman 3	-	-	-	+	+	93	Sarı buğday 2	-	-	-	+	+
42	Demir 2000	+	-	-	-	+	94	Çalıbasan 4	-	-	-	+	-
43	Ari buğday 1	-	-	-	+	-	95	Kabak buğdayı	-	-	-	+	+
44	Ari buğday 2	-	-	-	+	-	96	Esperia	-	+	-	+	+
45	Kocabuğday 1	-	-	-	-	+	97	Boğruala	-	-	-	-	+
46	Kobak 1	-	-	-	-	+	98	Kırık 7	-	-	-	+	+
47	Adana 99	+	-	-	+	+	99	Ari buğday 4	-	-	-	+	+
48	Gulumbur /Makas	+	-	-	+	+	100	Kobak 3	-	-	-	+	+
49	Göderedi 3	-	-	-	-	+	101	Gerek79	-	-	-	-	+
50	Urumeli 1	-	-	-	+	+	102	No Name 2	-	-	-	-	+
51	Ari buğday 3	-	-	-	+	-	103	Hatip buğdayı	-	-	-	+	-
52	Tir L. (14BGB-243)	+	-	-	-	+	104	Çalıbasan 5	-	-	-	+	-

Discussion

The *Vrn* gene system of winter wheat plays a decisive role in the adaptation of wheat to diverse climatic regions worldwide. The dominant or recessive status of *Vrn* alleles determines different levels of sensitivity to vernalization (Shindo and Sasakuma 2002), whereby winter growth habit is defined by recessive *vrn-B1* and *vrn-D1* alleles at the Vrn-1 locus, while the presence of one or more dominant alleles confers a spring growth habit (Stelmakh 1992). In the present study, 18 genotypes were determined to carry exclusively recessive vrn alleles, indicating a strong winter growth habit with complete vernalization requirement.

Comparison of the *Vrn-A1a* and *Vrn-A1b* alleles has demonstrated that *Vrn-A1a* promotes early maturity, whereas *Vrn-A1b* extends the maturation period (Koval and Goncharov 1998; Kamran et al. 2014). The four genotypes carrying *Vrn-A1a* (Ağsünteri, Tahirova-

2000, Halbert, and Altındane T.) identified in our study therefore possess early-maturation potential. The *Vrn-A1c* allele, characterized by a large deletion in the first intron, is rare in hexaploid wheat (Fu et al. 2005). The identification of three landraces (Yayla-305, Tir Line-14YBGB-246, and Kaşıkçı Buğdayı) carrying this rare allele in our study underscores the richness of genetic diversity present in Turkish landraces.

Efremova et al. (2016) and Goncharov (2004) reported that cultivars carrying both *Vrn-A1a* and dominant *Vrn-B1* alleles matured earlier, were more productive than cultivars possessing only a single dominant allele, and exhibited greater resistance to spring and autumn frost. In light of these findings, the Ağsünteri and Halbert genotypes, which carry both alleles, are considered to possess high potential for deployment as parents in breeding programs targeting early maturity, high yield potential, and frost tolerance. Given that genotypes carrying the dominant *Vrn-D1* allele tend to be more advantageous under drought and high-temperature stress during grain filling (Stelmakh 1998), the identification of 40 genotypes carrying this allele in our study provides a valuable gene pool for drought-resistance breeding.

Although genetic loci affecting root traits have been shown to influence cereal yield and agronomic performance, knowledge regarding the genetic control of root development in major cereal species remains limited. Voss-Fels et al. (2018) demonstrated that *VRN1* modulates root architecture in both wheat and barley, with lines carrying the spring *VRN1* allele developing deeper roots, and confirmed that this effect occurs through pleiotropy. Watt et al. (2020) emphasized that wheat root systems should be considered as a target selection criterion in breeding for climate resilience.

Studies by Güleç et al. (2021) and Aydın et al. (2025) demonstrated that genotypes with the *Vrn-A1b × vrn-B1 × Vrn-D1* allele combination exhibited narrower root angles, while those carrying the *Vrn-A1a × Vrn-B1 × vrn-D1* combination displayed wider root angles. Narrow root angle is associated with root systems penetrating deeper soil horizons, conferring an advantage in water uptake under drought conditions. The identification of 19 genotypes carrying the *Vrn-A1b × vrn-B1 × Vrn-D1* combination in our study suggests that these genotypes potentially exhibit narrow root angle and deep rooting tendencies. Direct phenotyping of these genotypes in drought tolerance studies is of critical importance for validating these molecular-level preliminary findings.

Genes conferring adult plant resistance (*APR*) constitute the foundation of sustainable resistance breeding owing to their non-race-specific, broad-spectrum protection. The *Lr34* gene encodes an ABC transporter protein and represents one of the best-characterized *APR genes* conferring multiple resistance against leaf rust, stripe rust, stem rust, and powdery mildew (Krattinger et al. 2009; Lagudah et al. 2009). The detection of this gene in seven genotypes in our study is valuable for their incorporation into breeding programs as broad-spectrum resistance sources. Particularly, the co-occurrence of both *Lr34* and *Lr46* in the Bezostaja genotype, when evaluated alongside its lodging resistance, cold tolerance, high adaptability, and bread-making quality, demonstrates that this genotype constitutes a multifaceted genetic resource. Tomkowiak et al. (2021) reported that the co-presence of these two genes significantly extends the latent period of the disease and reduces disease severity compared to susceptible cultivars.

The *Lr67* gene operates through a slow-rusting mechanism similar to *Lr34*, albeit conferring lower levels of resistance to leaf rust compared to *Lr34* (Hiebert et al. 2010; Bakhshi et al. 2025). The co-occurrence of both *Lr34* and *Lr67* in the Ankara093-44 genotype suggests that this genotype possesses a resistance level enhanced through additive effects. The additional lodging resistance of this genotype positions it as a prominent parental candidate offering multiple advantages in breeding programs.

The detection of the *Lr46* gene in 11 genotypes indicates a moderate prevalence of this gene in Turkish wheat genetic resources. The *Lr46/Yr29* gene complex is known to serve as a resistance source against leaf rust, stripe rust, and powdery mildew in wheat resistance breeding programs worldwide (Kolmer et al. 2015; Sönmez et al. 2023; Spychała et al. 2024). The non-race-specific, broad-spectrum mode of action of this gene impedes the breakdown of resistance by pathogens.

Regarding stripe rust resistance, the detection of *Yr15* in 81.7% (85/104) of the genotypes in our study indicates high prevalence of this gene in the Turkish wheat population. *Yr26* was detected in 59.6% (62/104) of the genotypes, indicating a substantial frequency. Given that pyramiding of both genes confers stronger resistance (Rani et al. 2019; Yan et al. 2021), the identification of 49 genotypes carrying both genes simultaneously represents a valuable finding for breeding strategies.

Among the most notable findings of this study is the co-occurrence of multiple rust resistance genes and advantageous Vrn allele combinations with respect to root architecture and stress tolerance in certain genotypes. Among the 19 genotypes possessing the *Vrn-A1b × vrn-B1 × Vrn-D1* combination (narrow root angle potential), Ankara093-44, Gülümbür/Makas Buğdayı, Esperia, 4–11, and

Müfitbey also harbor multiple rust resistance genes. These genotypes carry the potential for providing integrated resistance against both biotic and abiotic stress factors and are considered genetic resources that could form the basis for gene pyramiding strategies in breeding programs.

Conclusions

In this study, 104 landrace and modern bread wheat genotypes were comprehensively screened for rust resistance genes (*Lr34, Lr46, Lr67, Yr26, Yr15*) and vernalization genes (*Vrn-A1, Vrn-B1, Vrn-D1*) using molecular markers. Genotypes harboring multiple rust resistance gene combinations are anticipated to exhibit elevated resistance levels. In this regard, Ankara093-44 and Bezostaja (*Lr34 + Lr67/Lr46 + Yr15 + Yr26*), Yayla305, Altay-2000, and MV18 (*Lr67 + Yr15 + Yr26*), Kıraç-66, Gülümbür/Makas Buğdayı, and Adana-99 (*Lr46 + Yr15 + Yr26*), and 4–11, Müfitbey, and Esperia (*Lr34 + Yr15 + Yr26*) represent potential genetic resources for deployment as parents in gene pyramiding strategies.

Based on vernalization allele profiles, Altındane T. and Tahirova-2000 (*vrn-B1 + Vrn-D1 + Vrn-A1a*) possess potential for early heading and terminal stress tolerance; Ağsünteri and Halbert (*Vrn-A1a + Vrn-B1*) display potential for early maturation, high yield, and frost tolerance; and 19 genotypes (*Vrn-A1b × vrn-B1 × Vrn-D1*) exhibit potential for narrow root angle and deep rooting.

For plant breeders, developing genotypes with earlier maturity, superior adaptation to extreme conditions such as frost and drought, narrow root angles, and genetic resistance to mitigate disease-induced losses is recognized as the most cost-effective and sustainable strategy. The results obtained in this study provide valuable contributions toward establishing novel gene pyramiding strategies against racial shifts in rust diseases, interpreting phenotypic studies related to heading time of genetic materials, and accelerating drought-resistance breeding programs.

Future studies should focus on phenotypic evaluation of the identified genotypes in multi-environment field trials, additional screening for supplementary rust resistance genes, direct phenotyping of the effects of *Vrn* allele combinations on root angles, and implementation of gene pyramiding strategies through crossing programs.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by T.G, M.E.S and B.D. The first draft of the manuscript was written by T.G and M.E.S and all authors commented on previous versions of the manuscript. T.G, M.E.S and N.A approved the final manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

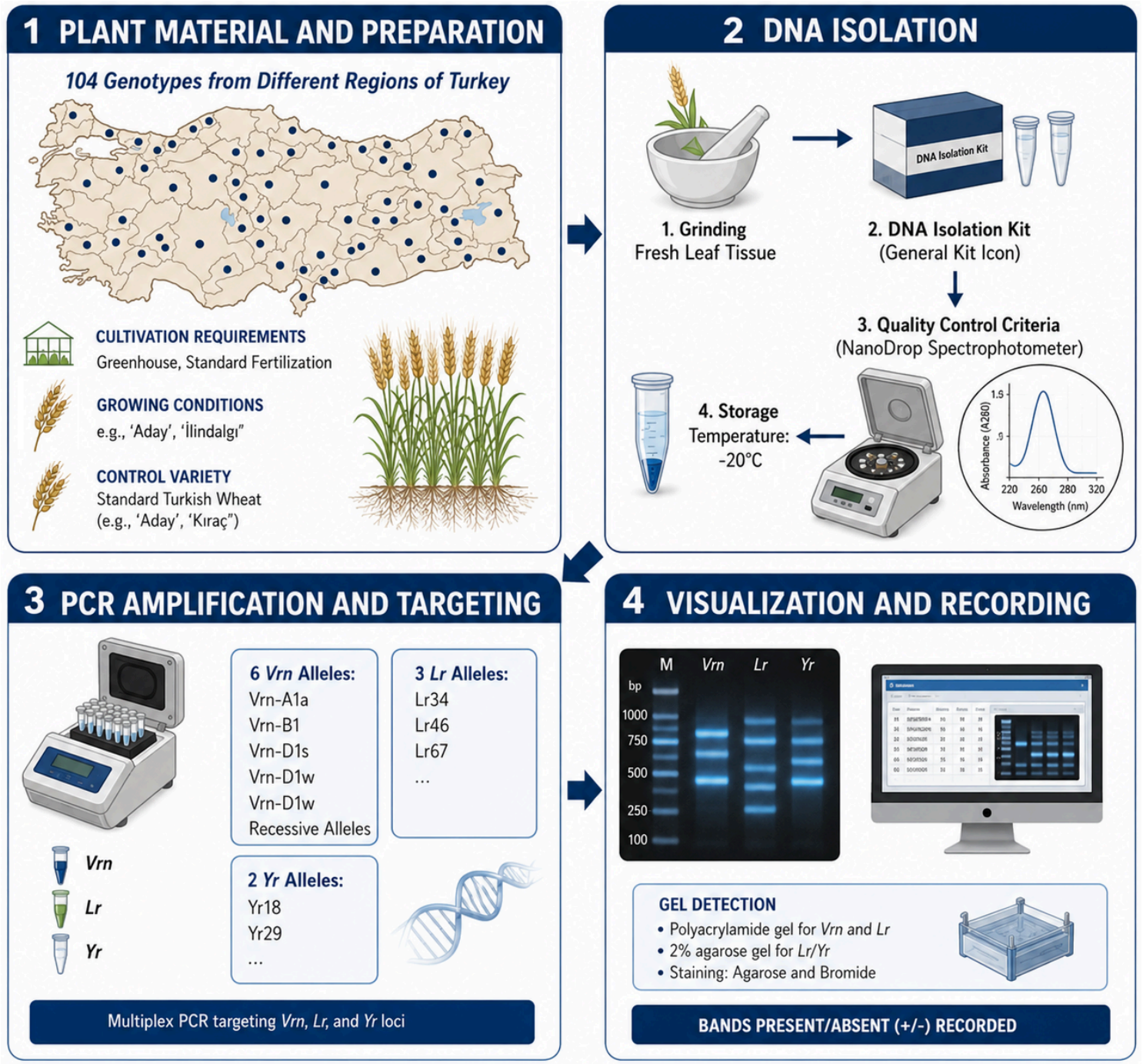

Figure 1

Schematic representation of the experimental workflow comprising four stages: (1) plant material preparation involving 104 genotypes from diverse Turkish regions; (2) DNA isolation from fresh leaf tissue with NanoDrop quality assessment; (3) PCR amplification targeting six *Vrn* alleles, three *Lr*genes, and two *Yr* genes; (4) agarose gel electrophoresis visualization and band scoring

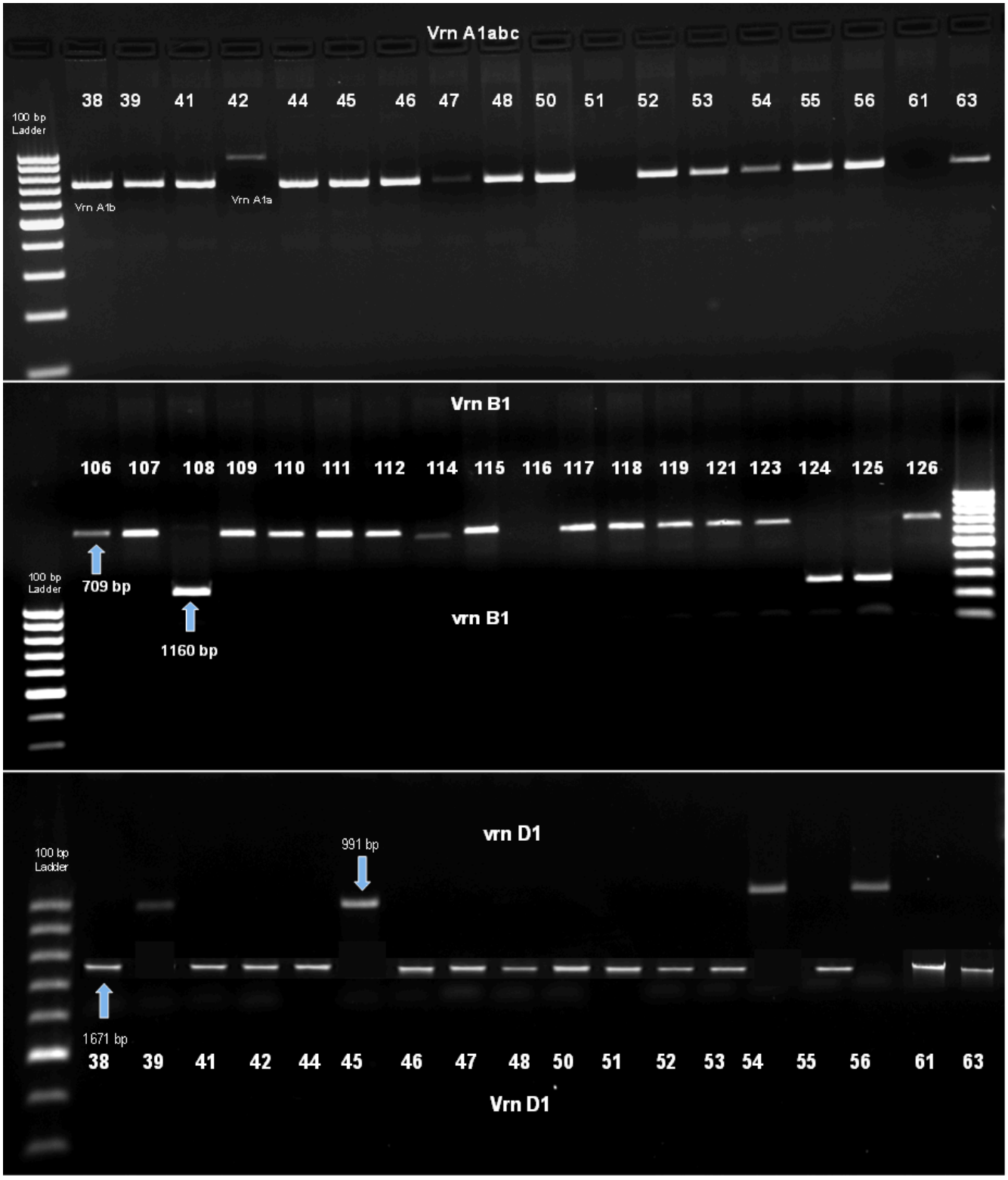

Figure 2

Agarose gel electrophoresis images of representative genotypes screened with *Vrn-A1a/b*, *Vrn-B1/vrn-B1*, and *Vrn-D1/vrn-D1* primers. *M*100-bp DNA ladder

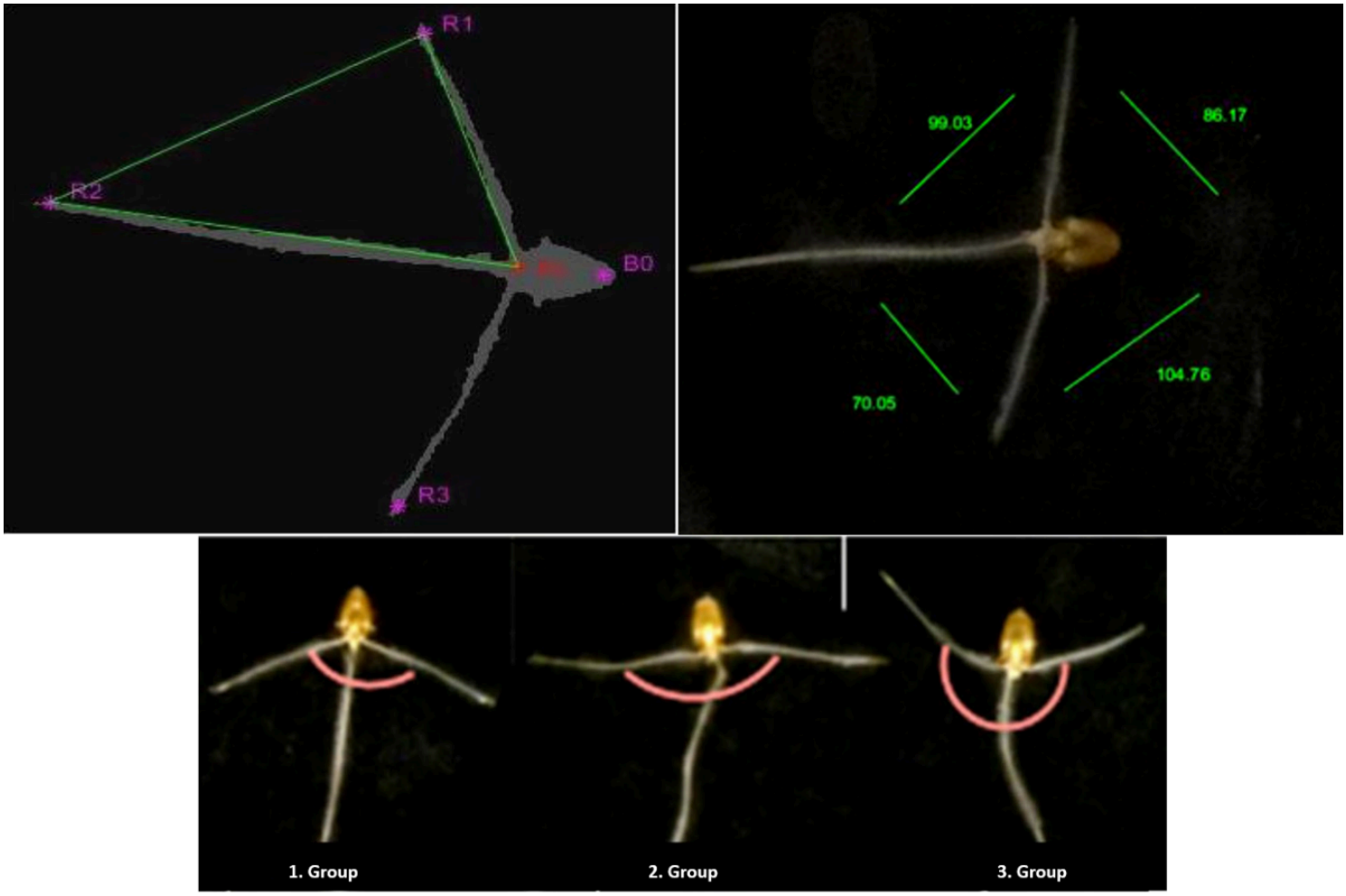

Figure 3

Seminal root angles measured by image processing and root angle groups according to *Vrn* allele combinations. *Group 1* narrow root angle (<90°); *Group 2* intermediate (90–140°); *Group 3* wide (>140°) (Güleç et al. 2021; Aydın et al. 2025)

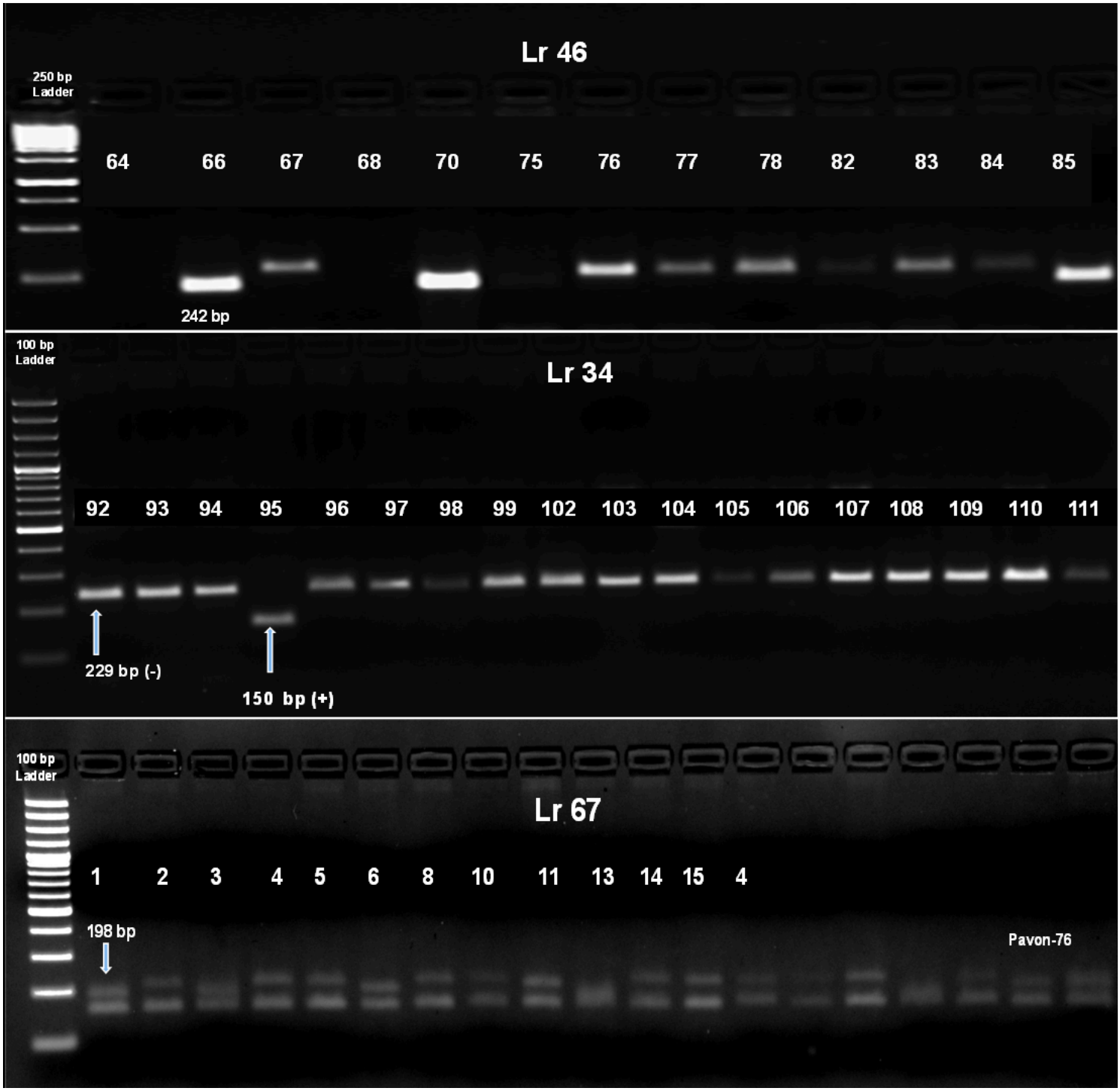

Figure 4

Agarose gel electrophoresis images of PCR amplification products for *Lr46*, *Lr34*, and *Lr67* genes in representative genotypes. *M* 100-bp DNA ladder. Lane codes: 66 Tir Line-14YBGB-243; 70 Halbert; 86 Bezostaja; 95 Alpu-2001; 1 Yayla305; 3 Ankara093-44; 6 Yektay406