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# DETERMINING APHID RESISTANCE GENES IN BREAD WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS USING DNA MARKERS

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## SUMMARY

The Russian wheat aphid (RWA; *Diuraphis noxia* [Kurdjumov]) is one of the world's most economically important and invasive pests of wheat, barley, and other cereals and has a crucial economic impact on autumnal wheat worldwide. The development of resistant cultivars may cause the continuous emergence of new RWA biotypes that are virulent for RWA control, emphasizing the need to determine new sources of resistance. Controlling RWA with systemic insecticides is economically expensive and hazardous to the environment and human health. Therefore, the most efficient way to control RWA is to ascertain and develop wheat cultivars with resistant genes. The presented study sought to determine the *Dn* genes in 25 wheat cultivars, including 19 cultivars from Uzbekistan's wheat breeding program and six cultivars from Russian breeding. The PCR screening proceeded with six (Xgwm44, Xgwm111, Xgwm635, Xgwm337, Xgwm642, and Xgwm473) SSR markers associated with *Dn* genes to recognize the genetic polymorphisms among the wheat cultivars. The results helped researchers in breeding programs, genetic improvement, and pest management, contributing to the economic viability of wheat farming. In turn, it enhances food security and promotes financial stability at both regional and national levels by increasing wheat yields and minimizing losses.

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**Keywords:** Bread wheat, *Triticum aestivum* L., cultivars, Russian wheat aphid (*Diuraphis noxia*), RWA, *Dn* genes, DNA markers, resistance

**Key findings:** According to the acquired results, seven *Dn* (RWA-resistant) genes, including *Dn2*, *Dn4*, *Dn6*, *Dn8*, *Dn9*, *Dn626580,* and *Dn2401,* gained identification in bread wheat cultivars. These tolerant wheat cultivars would be beneficial in future gene pyramiding-like molecular approaches for breeding communities.

## INTRODUCTION

Aphid is one of the chief factors that negatively affect the wheat yield. The grain aphids belong to the *Aphididae* family of the order *Homoptera*. It is native to Southern Russia, the Middle East, and Central Asia; however, since the late 1970s and early 1980s, it has rapidly spread to other major grain-producing regions in Europe, Africa, and North and South America. Russian wheat aphid injects salivary toxins during feeding that cause rapid, systemic phytotoxic effects on plants, resulting in acute plant symptoms and potentially significant yield losses. Aphids cause yield losses directly (35%–40%) by sucking the plant sap and indirectly (20%–80%) by infecting them with viral and fungal diseases (Aslam *et al.*, 2005; Kinley *et al*., 2021; Buronov *et al*., 2023). Therefore, effective aphid pest management requires better knowledge of the pest's biology, reproduction, ecology, and distribution.

Like some other biotic and abiotic stresses, the sap-sucking in plants activates the signal system and increases the concentration of jasmine and salicylic acids, ethylene, and other compounds (Smith *et al*., 2009; Costarelli *et al*., 2020; Sapakhova *et al*., 2022). However, when two biotypes of *D. noxia* fell on a plant with the *Dn7* resistance gene to the aphid, the $Ca^{2+}$ signaling systems - phosphoinositol, lipoxygenase, and NADPH-oxidase became activated. Past studies also observed that the expression of a large number of genes increased in the barley plant infected with the RWA1 biotype of the aphid compared with the plant infected with the RWA2 biotype, which also has a wide range of virulence (Botha *et al*., 2010).

In advanced breeding, marker-assisted selection (MAS) could replace the conventional plants' phenotypic screening approaches with precise and higher throughput technologies (Kushanov *et al*., 2021). MAS studies have progressed, aiming to genetically pyramid genes into one wheat genotype using DNA markers to achieve wheat plants' resistance to the different aphid biotypes (Paux *et al*., 2010; Mundt, 2018). The DNA markers associated with the genes of interest should first require identification, and then, the successful implementation of MAS can carry the desired traits (Ma *et al*., 1998; Xing *et al*., 2006; Tocho *et al*., 2012; Kushanov *et al*., 2022).

For genetic mapping of reasonably valuable traits in crop plants, utilization of several varied molecular markers is necessary (Kushanov *et al*., 2017; Adylova *et al*., 2018). The marker types used in mapping approaches influence the consistency of the research findings (Tolmay *et al*., 2020). Li and Peng (2014) evaluated the pest resistance in 70 wheat genotypes imported from Central Asia to identify the loci genetically associated with wheat resistance to English grain aphid (EGA). The said studies further revealed that wheat accessions bore genotyping with 51 SSR markers, and three genotypes showed high and medium resistance, while 17 genotypes showed high and moderate resistance to EGA.

The genetic diversity of these genotypes also incurred investigation using DNA markers. PCR analysis continued to study the association of 97 SSR loci, which covered all the wheat chromosomes homogeneously; four SSR loci showed genetic association with EGA resistance and four with EGA flexibility. After association analysis with aphid dynamic density, four loci, i.e., Xgwm192b, Xgwm391,

Xbarc98, and Xgwm613b, were consistently evident at different stages of wheat. In addition, the said study also compared loci for resistance to EGA, adaptability, and resistance to Russian wheat aphids. The SNP markers linked to Russian wheat aphid (RWA) resistance in Dn4-derived wheat lines were in distinction, unveiling novel chromosomal regions that influence RWA resistance (Kisten *et al*., 2020). Bapela and Tolmay (2022) extensively reviewed breeding wheat for RWA resistance. The assessment of 80 donor lines revealed 25 genotypes resistant to all four South African RWA biotypes, emphasizing the crucial role of selecting resistant lines for genetic improvement.

The results gathered in this study will benefit future breeding programs and in developing mapping populations by using the resistance in the germplasm. The presented research helped determine the genes responsible for resistance to RWA in widely cultivated Uzbekistan bread wheat cultivars.

## MATERIALS AND METHODS

### Plant material

In the latest study, 25 bread wheat (*T. aestivum* L.) cultivars served as samples to determine the *Dn* genes, which are resistant to aphids (*Diuraphis noxia*) (Table 1). Molecular analysis transpired at the Institute of Genetics and Plant Experimental Biology (IGPEB), the Academy of Sciences of the Republic of Uzbekistan.

**Table 1.** Characteristics of bread wheat cultivars.

| Cultivars | Origin | Originator/Organization | Characteristics |
|---|---|---|---|
| Krasnodarskaya-99 | Krasnodar, Russia | KRIA[1] | Susceptible (Control) |
| Oq marvarid | Tashkent, Uzbekistan | IGBEP[2] | Moderately resistant |
| Ilg'or | Tashkent, Uzbekistan | IGBEP[2] | Susceptible |
| E'zoz | Tashkent, Uzbekistan | IGBEP[2] | Moderately resistant |
| Zomin-1 | Kashkadarya, Uzbekistan | SARI[3] | Moderately resistant |
| Jasmina | Samarkand, Uzbekistan | SamAI[4] | Susceptible |
| Forboma | Samarkand, Uzbekistan | SamAI[4] | Moderately resistant |
| Asr | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Semrug' | Jizzakh, Uzbekistan | SRIRA[6] | Susceptible |
| Istiqlal | Jizzakh, Uzbekistan | SRIRA[6] | Susceptible |
| Do'stlik | Jizzakh, Uzbekistan | SRIRA[6] | Moderately resistant |
| Antonina | Krasnodar, Russia | KRIA[1] | Moderately resistant |
| Yesaul | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Mars | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Nodir | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Yog'du | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Qadr | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Navbakhor | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Aleksevich | Krasnodar, Russia | KRIA[1] | Resistant (Control) |
| Uzbekistan-25 | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Druzhba | Krasnodar, Russia | KRIA[1] | Moderately resistant |
| Yuka | Krasnodar, Russia | KRIA[1] | Moderately resistant |
| Andijan-4 | Andijan, Uzbekistan | ASRICL[5] | Susceptible |
| Andijan-2 | Andijan, Uzbekistan | ASRICL[5] | Moderately resistant |
| Vassa | Krasnodar, Russia | KRIA[1] | Resistant |

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**Table 2.** The panel of SSR markers associated with RWA resistant *Dn* genes.

| DNA markers | Primer Forward/Reverse (5'-3') | Chromosome position | Associated gene | Associated allele (Reference) |
|---|---|---|---|---|
| Xgwm44 | GTTGAGCTTTTCAGTTCGGC<br>ACTGGCATCCACTGAGCTG | 7DS | *Dn6* | 180 bp (Liu *et al*., 2002) |
| Xgwm111 | TCTGTAGGCTCTCTCCGACTG<br>ACCTGATCAGATCCCACTCG | 7DS | *Dn2, Dn6* | 200 bp (Liu *et al*., 2001; 2002) |
| Xgwm635 | TTCCTCACTGTAAGGGCGTT<br>CAGCCTTAGCCTTGGCG | 7DS | *Dn8* | 100 bp (Liu *et al*., 2001) |
| Xgwm337 | CCTCTTCCTCCCTCACTTAGC<br>TGCTAACTGGCCTTTGCC | 1DL | *Dn4* | 175 bp (Liu *et al*., 2002) |
| Xgwm642 | ACGGCGAGAAGGTGCTC<br>CATGAAAGGCAAGTTCGTCA | 1DL | *Dn9* | 180 bp (Liu *et al*., 2001) |
| Xgwm473 | TCATACGGGTATGGTTGGAC<br>CACCCCCTTGTTGGTCAC | 7DS | *Dn626580, Dn2401* | 244 bp (Valdez *et al*., 2012; Fazel-Najafabadi *et al*., 2015) |

**DNA isolation**

From the fresh wheat plant leaves, genomic DNA isolation used the CTAB (cetyltrimethylammonium bromide) method with minor modifications using liquid nitrogen (Yu *et al*., 2017). NanoDrop Eight (Thermo Fisher Scientific, USA) employment helped measure the DNA quality and quantity. DNA samples with higher amounts reached further diluting up to 25 ng/µL working concentration.

**SSR analysis and gel-electrophoresis**

Polymerase chain reaction (PCR) based SSR genotyping continued as previously described by Kushanov *et al*. (2022). A total of six SSR markers associated with aphid resistance genes attained selection from the database (Table 2). The PCR ran in the T100 thermal cycler (Bio-Rad, USA) instrument with a volume of 10 µL mixture, including 2.0 µL 5xScreenMix (Evrogen, Russia), 1.0 µL (25 ng/µL) genomic DNA, 1.0 µL primer pairs, and 6.0 µL DNase-free distilled water. The PCR used in a hot-start program had 35 cycles, including the denaturation step for 20 s at 94 °C, the annealing temperature at 55 °C–60 °C for 30 s, and the elongation step for 50 s at 72 °C.

Using gel electrophoresis in a 2.5% agarose gel determined the molecular weight of the PCR products. Agarose gels stained with ethidium bromide helped capture and photo-documentation using GelDoc Go Gel Imaging System (Bio-Rad, USA). Using the AmpliSize Molecular Ruler (Bio-Rad, USA) in a gel, measured the amplicon sizes. The construction and visualization of the phylogenetic tree used the NCSS 12.

**RESULTS AND DISCUSSION**

According to the PCR assay, the 25 wheat genotypes generated 31 alleles from six SSR markers associated with *Dn* genes. The average number of alleles per locus was 5.2, ranging from two (observed in Xgwm473) to seven (observed in Xgwm337 and Xgwm642) (Table 3). The polymorphism information content (PIC) varied from 0.3726 to 0.8366, averaging 0.7291. Similarly, heterozygosity (He) ranged from 0.4953 to 0.8545, with an average of 0.7577. According to Tolmay *et al*. (2020), each DNA marker, viz., Xgwm44 and Xgwm111, had eight alleles with PIC values of 0.9812 and 0.9833, respectively. Additionally, Liu *et al.* (2001) reported that amplifying Xgwm111 resulted in five alleles.

In the latest study, the DNA marker Xgwm44 had four alleles, while Xgwm111 revealed six. Furthermore, in the previous research by Fazel-Najafabadi *et al*. (2015), the marker Xgwm473 was found to amplify the four DNA bands. Liu *et al*. (2001, 2002) also reported that the three markers, i.e., Xgwm635, Xgwm642, and Xgwm337, had four, six, and two alleles, respectively. In the studied 25 wheat cultivars, the presence of

**Table 3.** SSR markers and their polymorphism information content (PIC) and heterozygosity (He) values.

| DNA markers | Molecular weight (pair bases, pb) | Number of alleles | PIC | HE |
|---|---|---|---|---|
| Xgwm44 | 147-424 | 4 | 0.7581 | 0.7191 |
| Xgwm111 | 153-272 | 6 | 0.8045 | 0.8284 |
| Xgwm635 | 95-156 | 5 | 0.7684 | 0.7961 |
| Xgwm337 | 147-689 | 7 | 0.8366 | 0.8545 |
| Xgwm642 | 116-268 | 7 | 0.8348 | 0.8530 |
| Xgwm473 | 244-307 | 2 | 0.3726 | 0.4953 |

**Table 4.** Identification of aphid resistance genes in wheat cultivars using DNA markers.

| Plant samples | Xgwm44 (180 bp) | Xgwm111 (200 bp) | Xgwm635 (100 bp) | Xgwm337 (175 bp) | Xgwm642 (180 bp) | Xgwm473 (244 bp) |
|---|---|---|---|---|---|---|
| | *Dn6* | *Dn2, Dn6* | *Dn8* | *Dn4* | *Dn9* | *Dn626580, Dn2401* |
| Krasnodarskaya-99 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oq marvarid | 0 | 1 | 0 | 1 | 0 | 1 |
| Ilg'or | 0 | 1 | 0 | 0 | 0 | 0 |
| E'zoz | 0 | 0 | 0 | 0 | 1 | 1 |
| Zomin-1 | 0 | 0 | 0 | 1 | 1 | 0 |
| Jasmina | 0 | 0 | 0 | 0 | 0 | 0 |
| Forboma | 0 | 0 | 0 | 0 | 0 | 0 |
| Asr | 0 | 0 | 0 | 0 | 0 | 0 |
| Semurg' | 0 | 0 | 0 | 0 | 0 | 0 |
| Istiqlal | 0 | 0 | 0 | 1 | 0 | 0 |
| Do'stlik | 0 | 1 | 1 | 0 | 0 | 0 |
| Antonina | 0 | 0 | 1 | 0 | 0 | 0 |
| Yesaul | 0 | 0 | 0 | 0 | 1 | 1 |
| Mars | 0 | 0 | 1 | 0 | 0 | 0 |
| Nodir | 0 | 1 | 0 | 1 | 1 | 0 |
| Yog'du | 0 | 1 | 0 | 0 | 0 | 0 |
| Qadr | 0 | 0 | 0 | 0 | 0 | 0 |
| Navbakhor | 0 | 1 | 0 | 0 | 0 | 0 |
| Alekseevich | 1 | 1 | 0 | 0 | 1 | 1 |
| Uzbekistan-25 | 1 | 0 | 0 | 0 | 1 | 0 |
| Druzhba | 0 | 0 | 0 | 0 | 0 | 0 |
| Yuka | 1 | 0 | 0 | 0 | 0 | 0 |
| Andijan-4 | 1 | 1 | 1 | 1 | 0 | 0 |
| Andijan-2 | 1 | 1 | 0 | 1 | 0 | 0 |
| Vassa | 0 | 1 | 0 | 1 | 0 | 1 |

Note* 1—Amplification for molecular markers; 0—no amplification.

known *Dn* genes is available in Table 4. Molecular screening of these wheat genotypes revealed significant differences in the frequencies of these genes. The studied wheat breeding material showed the most prevalent *Dn* gene was *Dn6*. The DNA marker Xgwm111 fragments (200 bp) appeared in association with the *Dn2* and *Dn6* genes, occurring in 10 out of the 25 wheat genotypes, such as Oq marvarid, Ilg'or, Do'stlik, Nodir, Yog'du, Navbahor, Alekseevich, Andijan-4, Andijan-2, and Vassa (Figure 1). According to Liu *et al*. (2001), the DNA marker Xgwm111 amplified RWA resistance DNA bands at 210, 200, 220, and 225 bp, which were in association with four *Dn* genes, i.e., *Dn1*, *Dn2*, *Dn5*, and *Dnx*, respectively. In the succeeding study of Liu *et al.* (2002), the DNA marker Xgwm111 amplified a 200 bp DNA band specific to the resistant cultivar PI-243781 and a 220 bp band

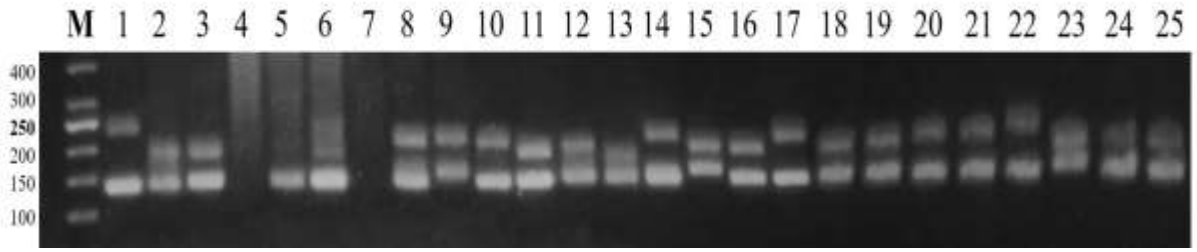


**Figure 1.** Electropherogram of the primers for Xgwm111 associated with *Dn2* and *Dn6* resistance genes. M – Molecular weight size marker (base pairs, bp). 1 - Krasnadar-99 (Control, S), 2- Oq marvarid, 3 - Ilg'or, 4 - E'zoz, 5 - Zomin-1, 6 - Jasmina, 7 - Forboma, 8 - Asr, 9 - Semrug', 10 - Istiqlal, 11 - Do'stlik, 12 - Yesaul, 13 - Mars, 14 - Nodir, 15 - Yog'du, 16 - Qadr, 17 - Navbakhor, 18 - Aleksevich, (Control, R), 19 - Antonina, 20 - Uzbekistan-25, 21 - Druzhba, 22 - Yuka, 23 - Andijan-4, 24 - Andijan-2, 25 - Vassa.

from the DNA of the susceptible Russian wheat cultivar Wichita.

Another marker, Xgwm44, with a resistance band of 180 bp, represented the *Dn6* gene in five wheat cultivars, i.e., Alekseevich, Uzbekistan-25, Yuka, Andijan-4, and Andijan-2. In a past study (Liu *et al.*, 2002), the DNA marker Xgwm44 manifested amplification of the 180 bp band in the resistant cultivar and a 190 bp band in the susceptible cultivar. In this promising research, the DNA marker Xgwm337 allele (175 bp) for the *Dn4* gene was evident in seven wheat cultivars, viz., Oq marvarid, Zomin-1, Istiqlal, Nodir, Andijan-4, Andijon-2, and Vassa. Liu *et al.* (2002) also amplified this marker at 175 bp in the wheat-resistant cultivar PI-372129. The maker Xgwm642 (180 bp) manifested a connection with the *Dn9* gene that emerged in six wheat cultivars, i.e., E'zoz, Zomin-1, Yesaul, Nodir, Alekseevich, and Uzbekistan-25. The *Dn8* gene surfaced through the Xgwm635 marker allele (100 bp) in four wheat cultivars, including Do'stlik, Antonina, Mars, and Andijan-4. According to Liu *et al.* (2001), the 180 bp band of the marker Xgwm642 and 100 bp band of the marker Xgwm635 amplified in the wheat genotype PI-294994 as resistant sources, were found to link with the *Dn8* and *Dn9* genes, respectively.

The Xgwm473 marker with 244 bp allele indicated a correlation with both the *Dn626580* and *Dn2401* genes, which was also evident in five wheat cultivars, i.e., Oq marvarid, E'zoz, Yesaul, Alekseevich, and Vassa. Past studies revealed that the 244 bp DNA band of the marker Xgwm473 was notably a marker for the *Dn626580* gene in Russian wheat populations (Valdez *et al*., 2012). However, markedly, this marker also appeared to associate with the *Dn2401* gene in the wheat-resistant cultivar CI2401 (Fazel-Najafabadi *et al*., 2015).

Two wheat cultivars, viz., Alekseevich and Andijan-4, exhibited the highest number of resistance genes, and cultivar Alekseevich had *Dn6* (Xgwm44), *Dn2/Dn6* (Xgwm111), *Dn9* (Xgwm642), and *Dn626580/Dn2401* (Xgwm473), while cultivar Andijan-4 carried *Dn6* (Xgwm44), *Dn2/Dn6* (Xgwm111), *Dn8* (Xgwm635), and *Dn4* (Xgwm337). Six wheat cultivars, such as Ilg'or (Dn2/*Dn6*), Istiqlal (*Dn4*), Antonina (*Dn8*), Mars (*Dn8*), Yog'du (*Dn2/Dn6*), Navbahor (*Dn2/Dn6*), and Yuka (*Dn6*) have only one/two *Dn* genes in their genome. However, according to the DNA markers, the wheat cultivars, viz., Jasmina, Forboma, Asr, Semurg, Qadr, Druzhba, and the susceptible cultivar Krasnodarskaya-99 did not possess the *Dn* genes. It is worth mentioning that the current study presented the outcomes of PCR screening for the *Dn* genes in the new cultivars. Past studies revealed that according to the DNA marker-based screening of 80 wheat accessions for RWA resistance, the bread wheat cultivars were distinct with *Dn* genes (Turaev *et al*., 2023a, b).

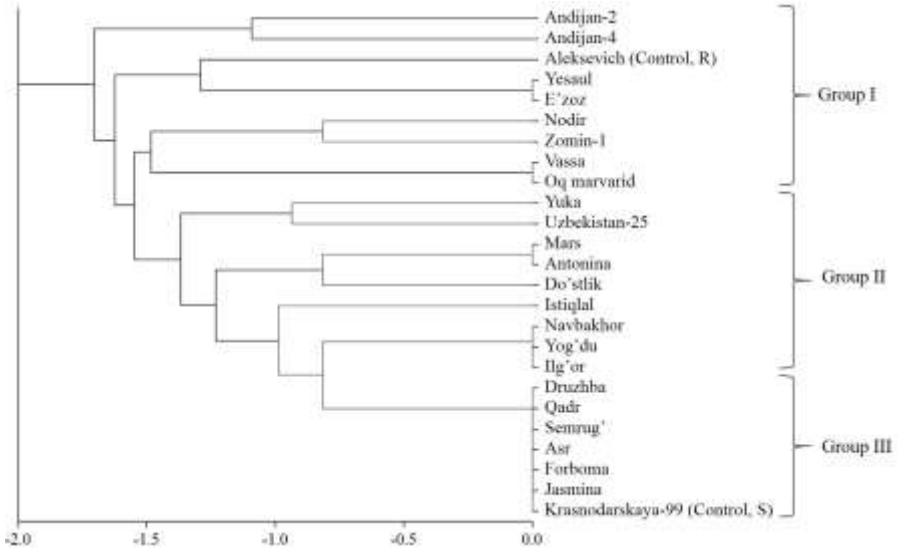


**Figure 2.** A phylogenetic tree of wheat cultivars constructed based on the alleles of DNA markers linked to aphid resistance *Dn* genes.

The phylogenetic tree of 25 wheat cultivars' construction used specific alleles of particular DNA markers linked to *Dn* genes (Figure 2). According to the phylogenetic study, the present wheat cultivars became categorized into three groups based on their aphid resistance alleles. Group I comprises the cultivars Andijoan-2, Andijan-4, Alekseevich (resistant control), Yesaul, E'zoz, Nodir, Zomin, Vassa, and Oq marvarid. It was visible that these cultivars possessed two to four resistance genes. Group II consists of nine wheat cultivars (Yuka, Uzbekistan-25, Mars, Antonina, Do'stlik, Istiqlal, Navbakhor, Yog'du, and Ilg'or), with each carrying one or two resistance genes. Group III consists of seven cultivars, including Druzhba, Qadr, Semrug', Asr, Forboma, Jasmina, and Krasnodarskaya-99 (susceptible control), which showed no resistance allele.

The wheat cultivars in this present-day study have not previously undergone molecular screening studies for RWA resistance *Dn* genes. However, some cultivars underwent studies for abiotic stress tolerance. Baboeva *et al.* (2023) observed that the wheat cultivars Andijon-4 and Oq marvarid were tolerant to climate impacts, while E'zoz and Vassa cultivars were remarkably susceptible. Interestingly, all these cultivars emerged to possess the *Dn* genes in the presented study.

## CONCLUSIONS

Based on the molecular screening of 25 bread wheat cultivars using six DNA markers associated with seven aphid resistance *Dn* genes (*Dn2, Dn4*, *Dn6*, *Dn8*, *Dn9*, *Dn2401,* and *Dn626580*), significant differences occurred in the frequencies of these significant *Dn* genes. It has also surfaced that out of the 25 wheat cultivars, 18 genotypes possess RWA resistance genes, with seven cultivars lacking any resistance genes. Wheat cultivars Alekseevich and Andijan-4 revealed the highest number of resistance genes, such as *Dn2, Dn6*, *Dn9*, *Dn626580,* and *Dn2401*, and *Dn2, Dn4*, *Dn6*, and *Dn8*, respectively. Cluster analysis based on genetic polymorphisms among these wheat cultivars categorized wheat genotypes according to the presence and absence of resistant *Dn* genes. Given the genetic complexity of the resistance to RWA, wheat genotypes with multiple *Dn* genes demonstrating such resistance will be a valuable source for molecular breeding techniques, such as marker-assisted selection and gene pyramiding.

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