www.incda-fundulea.ro; doi.org/10.59665/rar4258 First Online: May, 2025. DII 2067-5720 RAR 2025-154

Investigation of the Presence of *Lr24* Gene Providing Tolerance to Brown Rust Disease Using SCAR Marker in the Spring Bread Wheat Gene Pool

Yavuz Balmuk[1], Orhan Kurt[2], Hüseyin Uysal[3*], Safa Hacikamiloğlu[2], Cuma Karaoğlu[4], Cemal Şermet[1]

[1]Black Sea Agricultural Research Institute, Samsun, Türkiye
[2]Ondokuz Mayıs University, Department of Field Crops, Samsun, Türkiye
[3]Akdeniz University, Department of Agricultural Biotechnology, Antalya, Türkiye
[4]Field Crops Central Research Institute, Ankara, Türkiye
[*]Corresponding author. E-mail: huseyinuysal@akdeniz.edu.tr

ABSTRACT

Leaf rust is an important fungal disease in wheat and causes significant yield losses worldwide. Previous studies have reported that *Lr9, Lr19, Lr24, Lr41,* and *Lr47* genes provide resistance to leaf rust in wheat. This study was conducted in 2022 to determine whether Altındane, Kirve, Pandas, Kate-A1 and Gönen-98, spring bread wheat varieties registered in Türkiye, have the *Lr24* gene that provides tolerance to brown rust disease. In the study, Sunco and Torres spring bread wheat varieties, which have the brown rust disease tolerance gene *Lr24,* were used. In order to detect the presence of the *Lr24* gene in the wheat varieties evaluated, a SCAR (Sequence-Characterized Amplified Region) marker was used in 3 replications.

As a result of the research, it was determined that none of the 5 spring wheat varieties registered in Türkiye had the brown rust disease tolerance gene *Lr24,* while Torres and Sunco spring wheat varieties examined had the tolerance gene *Lr24*. Finally, it was concluded that one or both Torres and Sunco wheat varieties with the wheat brown rust tolerance gene *Lr24* could be used as a wheat brown rust tolerance gene donor parent in a hybridization breeding program to develop new wheat varieties tolerant to brown rust disease.

Keywords: *Lr24*, Sunco, Torres, leaf rust, SCAR.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an annual herbaceous plant in the Poaceae family, among the cool climate cereals. It is the most widely cultivated plant in the world. It is also used as a source of protein, especially carbohydrates, in human nutrition. Anatolia is one of the important gene centers of wheat and one of the first settlements where wheat farming was carried out (Davis, 1965-1985). Of the worldwide total wheat production, Turkey ranks 11th, with 22 million tons (FAO, 2023).

One of the most important problems in wheat cultivation is rust diseases. These diseases cause significant losses in yield and quality. Brown rust is the most important disease of wheat plants after yellow rust. The cause of brown rust is a fungus called *Puccinia recondita* f. sp. *tritici*, which appears as brown pustules on the leaves of wheat plants, and the infected leaves turning yellow and dry over time. Due to the reduced leaf surface, photosynthesis decreases, and grain filling is negatively affected. As a result, serious yield and quality losses occur.

Yield and quality losses can be prevented to a great extent by early diagnosis of the disease and implementation of appropriate management strategies. A study on the genetics of resistance to brown rust emphasized the negative effects of this disease on wheat plants and the importance of developing resistant varieties against the disease (Ren et al., 2023). The most effective, cost-effective, and environmentally friendly method of controlling this disease is the development of tolerant varieties (Singh et al., 2004). Before working towards this goal, it is extremely important to determine the genetic similarities and differences of

Received 25 March 2025; accepted 15 May 2025.

existing gene sources in terms of this disease. This determination is important in terms of labour and time planning in the breeding program to be initiated in order to develop a tolerant variety.

Molecular markers are used as an auxiliary tool in shortening the breeding period and transferring the desired characters to plants. In recent years, molecular marker methods have also been frequently used in wheat breeding studies. Molecular markers play a key role in transferring the desired characteristics to plants. One of the most widely used techniques today is SCAR molecular markers. SCAR markers were first developed from the RAPD technique (Yang et al., 2013) and are used as reliable markers because they are reproducible and co-dominant (Kumla et al., 2012).

The use of genes tolerant to brown rust in wheat breeding is a preferred method to reduce losses caused by brown rust. There are many races of brown rust, and varieties are not tolerant to all races. Sensitivity may occur in tolerant varieties with the emergence of new disease races (Başer, 2020). Indeed, it has been reported that a new breed of brown rust has been discovered in Western Australia, causing problems in many varieties (Salam et al., 2013). More than 80 *Lr* genes, distributed across all 21 wheat chromosomes, have been identified, and most of these genes are known to be located in the short arms of individual wheat chromosomes (Bilgen et al., 2023). Wheat samples with the genes *Lr9*, *Lr19*, *Lr24*, Lr41, and *Lr47* are resistant to the leaf rust (Zuev et al., 2024). In brown rust studies, *Lr9* and *Lr24* genes should be taken into consideration primarily in terms of resistance (Örs, 2018). Indeed, it has been reported that the Magenta variety carrying the *Lr24* gene retains its resistance to both rust pathotypes found in Western Australia (Anonymous, 2014).

This research was carried out to detect the presence of brown rust tolerance gene *Lr24* using SCAR marker in the gene pool consisting of 2 CIMMYT origin (Sunco and Torres) reported carrying tolerance genes, and 5 local (Altındane, Kirve, Pandas, Kate-A1 and Gönen-98) spring bread wheat varieties.

MATERIAL AND METHODS

In the research, 2 CIMMYT-originated (Sunco and Torres) and spring-characteristic bread wheat varieties of 5 local (Altındane, Kirve, Pandas, Kate-A1, and Gönen-98) were used.

Each variety was planted in multiple pots containing peat and transferred to a plant growth unit with a 22-hour light and 2-hour dark photoperiod. When the plants reached the 2-3 leaf stage, leaf samples were taken for DNA isolation and stored at -80°C. Genomic DNA isolation was performed using the FavorPrep Plant Genomic DNA Extraction Mini Kit (FAPGK001, Favorgen, Australia) according to the protocol recommended by the company. For this purpose, during the genomic DNA isolation stage, 100 mg of plant sample was crushed in a mortar using liquid nitrogen, turned into powder and placed in 1.5 ml Eppendorf tubes. The concentration of DNA obtained after genomic DNA isolation was measured using the SPECTRO Star Nano (BMG Labtech) device. Then, the quality and quantity of the total DNA obtained were controlled by running on 0.8% agarose gel. The isolated DNAs were stored at -20°C.

PCR was performed on the isolated DNAs. The primers required for PCR were obtained from the study of (Gupta et al., 2006) (Table 1).

Table 1. Primer Sequences and Properties Required for PCR (Gupta et al., 2006)

Lr gene	Primer Name	Primer Sequence	Marker Tip	Size (bp)	Anneling temperature (ºC)
Lr24	SCS1302	F:CGCAGGTTCCAAATACTTTTC R:CGCAGGTTCTACCTAATGCAA	SCAR	607	50

PCR conditions were optimized using Dream Taq DNA Polymerase (5 U/µL), EP0705, 20 ng/µl DNA was used for the reaction. PCR reaction was prepared using 2,5 µl 10X Dream Taq Reaction Buffer, 2 µl Template DNA 20 ng/ul, 1 µl forward primer, 1 µl reverse primer, 0,2 µl Dream Taq DNA Polymerase, 0,5 µl 10 mM dNTPs and 17,8 µl sterile nuclease-free ddH_2O in a total volume of 25 µl. The components of the PCR are given in Table 2, and the PCR conditions are given in Table 3.

As a result of PCR amplification, PCR products were separated by running on 0.8% (W/V) agarose gel at 90 volts for 45 minutes. In order to visualize DNA bands, 0.5 µg/ml ethidium bromide was added and visualized on the Biorad Gel Doc XR+ Gel imaging device.

Table 2. PCR components [DreamTaq DNA Polymerase (5 U/µL), EP0705]

Components	Quantity (µl)
10X Dream Taq Reaction Buffer	2.5
Primer F	1
Primer R	1
10 mM dNTPs	0.5
Template DNA 20 ng/ul	2
Dream Taq DNA Polymerase	0.2
Nuclease free water	17.8
Total	**25**

Table 3. PCR conditions [DreamTaq DNA Polymerase (5 U/µL), EP0705, NEB]

Temperature (°C)	Time	Numbers of cycle
95	3 min	1
95	30 sec	34
50	30 sec	
72	1 min	
72	5 min	1
4	Indefinite	1

RESULTS AND DISCUSSION

In this study, PCR was performed to identify varieties resistant to brown rust disease using SCAR markers of 7 wheat genotypes. According to the results, resistant and susceptible varieties containing the brown rust tolerance gene *Lr24* were identified. The gel image of the results is given in Figure 1.

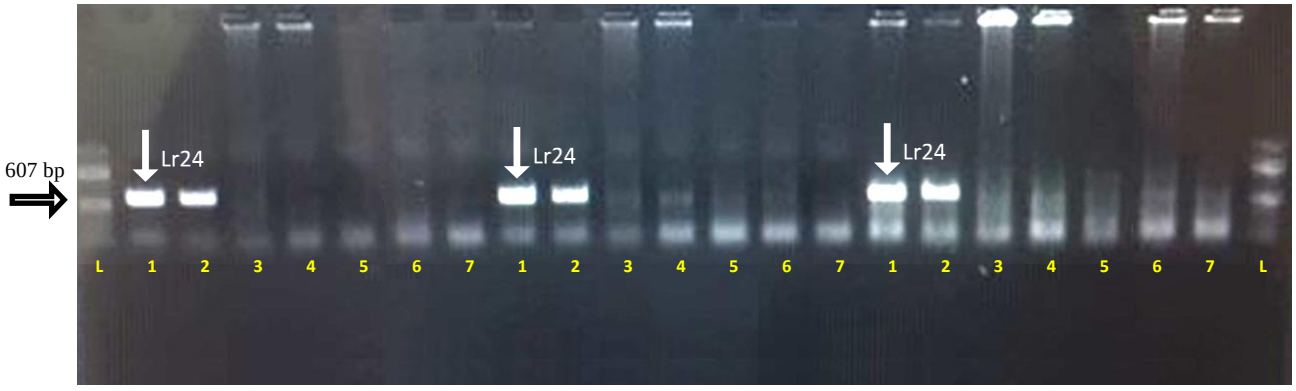

Figure 1. Lane L: Leader Lane 1-2: Tolerant Wheat varieties (Torres and Sunco),
Lane 3-4-5-6-7: Sensitive Wheat varieties (Kirve, Altındane, Gönen-98, Kate-A1, Pandas)

According to the results of molecular analysis, in the evaluation between varieties; 2 control varieties (Torres and Sunco) and 5 (Pandas, Altındane, Kate-A1, Gönen-98 and Kirve) bread wheat varieties with spring characteristics were used. The analysis was performed with 3 replications. As a result of molecular screening, it was determined that the results were similar in all 3 replications and that Torres and Sunco varieties had the *Lr24* gene content. In addition, it was determined that none of the local varieties (Pandas, Altındane, Kate-A1, Gönen-98 and Kirve) used in the study carried the *Lr24* gene.

Today, the effects of biotic and abiotic stress factors on plants are increasing. Fungi, which are among the biotic stress factors, cause great damage to agricultural products that are important for humans. Therefore, economic losses are experienced and people's access to food is becoming more and more difficult. Today, efforts are made to minimize the effects of fungi by using agricultural pesticides. However, the negative effects of pesticides on human health restrict the use of these control methods (Nicolopoulou-Stamati et al., 2016). Instead, the importance of growing and breeding plant varieties that are highly tolerant or resistant to fungi is increasing.

Rust diseases are among the most important biotic stress factors limiting wheat production in Türkiye, which has an important place in the world as a wheat producer. In the country, the yield loss caused by different types of rust in wheat plants has been recorded as ranging from 5% to 90% (Aktaş, 2001; Başer, 2020; Bilgen et al., 2023). The development of resistant varieties is the most preferred method in combating the disease.

According to the research results, it was revealed that wheat varieties (Torres and Sunco) carrying the brown rust tolerance gene (*Lr24*) can be used as parents in future breeding programs to develop brown rust tolerant varieties. In addition, it was determined that the SCAR marker can be used for the pre-selection of the material to be used as parents in classical breeding programs. Thanks to this finding, it is possible to contribute to the purposeful selection of parents for the breeding program and to prevent time and economic loss in the breeding process.

CONCLUSIONS

Leaf rust is an important fungal disease in wheat and causes significant yield losses worldwide. The best solution for the disease is to grow resistant genotypes. The presence of the *Lr24* gene, which shows resistance to brown rust disease, has been registered in Torres and Sunco varieties, but the relevant gene has not been detected in other wheat varieties subject to this research. It is planned to introduce the *Lr24* gene to these varieties with the breeding programs to be created.

ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies. (Grant Number: TAGEM/TBAD/T1/23/A10/P1/6470). The authors wish to thank for their support. The authors also would like to thank Charles E. Fiero for editing this article.

REFERENCES

Aktaş, H., 2001. *Önemli Hububat Hastalıkları ve Sürvey Yöntemleri Kitapçığı*. Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research, Department of Plant Health Research, Ankara.

Anonymous, 2014. Agric.wa.gov.au/news/media-releases/testing-confirms-increased-leaf-rust-risk-popular-wheat-varieties-wa/Testing confirms increased leaf rust risk in popular wheat varieties in WA (Access Date: 03.09.2024).

FAO, 2023. *Crops and livestock products*. https://www.fao.org/faostat/en/#data/QCL (Access Date: 21.03.2025).

Başer, İ., 2020. *Comparison of Bread Wheat Genotypes for Leaf Rust Resistance Genes*. Journal of Agricultural Sciences, 26(1): 22-31. https://doi.org/10.15832/ankutbd.447752

Bilgen, B.B., Yürük, B., Nasirian, H., 2023. *Brown rust resistance screening and molecular characterization of wheat cultivars by molecular markers*, Turkish Journal of Agriculture and Forestry, 47(5), Article 16. https://doi.org/10.55730/1300-011X.3127

Davis, P.H. (eds.), 1965-1985. *Flora of Turkey and the East Aegean Islands*. Vol.1-9, Edinburgh Univ. Press, Edinburgh.

Salam, K.P., Thomas, G.J., Beard, C., Loughman, R., MacLeod, W.J., Salam, M.U., 2013. *Application of meta-analysis in plant pathology: a case study examining the impact of fungicides on wheat yield loss from the yellow spot - septoria nodorum blotch disease complex in Western Australia*. Food Sec., 5: 319-325. https://doi.org/10.1007/s12571-013-0255-y

Gupta, S.K., Charpe, A., Koul, S., Haque, Q., Prabhu, K., 2006. *Development and Validation of SCAR Markers Cosegregating with an Agropyron elongatum Derived Leaf Rust Resistance Gene Lr24 in Wheat*. Euphytica, 150: 233-240. https://doi.org/10.1007/s10681-006-9113-8

Kumla, S., Doolgindachbaporn, S., Sudmoon, R., Sattayasai, N., 2012. *Genetic Variation, Population Structure and Identification of Yellow Catfish, Mystus nemurus (C&V) in Thailand Using RAPD, ISSR and SCAR Marker*. Molecular Biology Reports, 39(5): 5201-5210. https://doi.org/10.1007/s11033-011-1317-x

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. *Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture*. Frontiers in Public Health, 4, 148. https://doi.org/10.3389/fpubh.2016.00148

Örs, E., 2018. *Morphological and molecular characteristics of natural conditions of leaf rust resistance in bread wheat (Triticum aestivum L.) F2 populations*. Namık Kemal University, Institute of Science (Master's Thesis).

Ren, X., Wang, C., Ren, Z., Wang, J., Zhang, P., Zhao, S., Li, M., Yuan, M., Yu, X., Li, Z., Chen, S., Wang, X., 2023. *Genetics of Resistance to Leaf Rust in Wheat: An Overview in a Genome-Wide Level*. Sustainability, 15(4), 3247. https://doi.org/10.3390/su15043247

Singh, R., Datta, D., Singh, S., Tiwari, R., 2004. *Marker-Assisted Selection for Leaf Rust Resistance Genes Lr19 and Lr24 in Wheat (Triticum aestivum L.)*. Journal of Applied Genetics, 45(4): 399-404. https://pubmed.ncbi.nlm.nih.gov/15586436/

Yang, L., Fu, S., Khan, M.A., Zeng, W., Fu, J., 2013. *Molecular Cloning and Development of RAPD-SCAR Markers for Dimocarpus Longan Variety Authentication*. SpringerPlus, 2, 501. https://doi.org/10.1186/2193-1801-2-501

Zuev, E.V., Lebedeva T.V., Yakovleva O.V., Kolesova M.A., Brykova A.N., Lysenko N.S., Tyryshkin L.G., 2024. *Genetic Diversity for Effective Resistance in Wheat Landraces from Ethiopia and Eritrea to Fungal Diseases and Toxic Aluminum Ions*. Plants (Basel), 13(8): 1166. doi:10.3390/plants13081166