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Original Research

Tarnab Rehbar: A New High-Yielding, Rust-Resistant and Zinc Bio Fortified Bread Wheat Variety for Late and Irrigated Areas of Khyber Pakhtunkhwa, Pakistan

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Abstract

Developing high-yielding wheat varieties that are tolerant to both abiotic and biotic stresses is a significant challenge currently faced by wheat breeders. The Agriculture Research Institute (ARI), Tarnab, Peshawar, Pakistan, has released a new wheat variety, Tarnab Rehbar (varietal code TRB-2-103). Tarnab Rehbar outperformed the check variety Pirsabak-15, yielding 27.3% and 10.8% higher grain yields in preliminary (2017-2018) and regular yield trials (2018-2019), respectively. Subsequent outstation yield trials conducted across 14 locations in Khyber Pakhtunkhwa (2019-2020) and 31 and 40 locations nationwide (2020-2021, 2021-2022) confirmed the variety's stable performance and disease resistance. Agronomic field trials and quality lab testing were conducted simultaneously. Tarnab Rehbar is characterized by medium plant height, dark green color at booting, a strong stem, and a well-developed peduncle. The spikes are well-structured with moderate awns, and the seeds are uniform in size, white, and have a notable 1000-grain weight. Additionally, it boasts a high protein content and impressive yield potential. DNA fingerprinting was performed to compare the genetic background of Tarnab Rehbar with 21 previously registered wheat varieties. Due to its consistent performance across

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multiple locations, improved grain yield, nutritional quality, and rust resistance, the Provincial Seed Council approved the release of Tarnab Rehbar in 2023 for general cultivation in the late and irrigated areas of Khyber Pakhtunkhwa. Introducing this variety into wheat production systems can help reduce fungicide use, promote healthier ecosystems, minimize pesticide residues in wheat products, and address health challenges such as regional malnutrition and zinc deficiencies.

Keywords: Tarnab Rehbar, high yielding, rust resistant, bread wheat, irrigated, late areas, Khyber Pakhtunkhwa, zinc biofortified

Introduction

Pakistan's population is projected to grow at an annual rate of 2%, potentially doubling to 400 million in the near future. This rapid population growth poses a significant threat to food security [1]. Ensuring the country's food security necessitates focusing on wheat production, which is a staple crop in Pakistan. Wheat is currently grown on 9.60 million hectares, yielding an annual production of 31.40 million tons [2]. As the primary food source for millions of people, maintaining and enhancing wheat production is critical for sustaining the country's growing population. However, climate change poses a significant threat to wheat productivity due to increasing temperatures, erratic rainfall patterns, and frequent droughts. These climatic stresses can lead to reduced grain-filling duration, lower yields, and increased vulnerability to pests and diseases. Therefore, developing climate-resilient wheat varieties is essential to ensure stable production under changing environmental conditions [3-5].

Biofortification of staple crops with key micronutrients is an alternative strategy for reaching some of the country's more remote areas, where food fortification coverage is impractical [6, 7]. This involves the enhancement of the nutrient content of the crop through traditional selective breeding techniques, genetic modification, and/or agronomic techniques, including the application of micronutrient fertilizers [8, 9]. Globally, several biofortified crop varieties have been released, including iron-rich pearl millet in India [10], zinc-rich rice in Bangladesh [11], and zinc-rich wheat in India and Pakistan [12]. The daily Zn requirement for the human body ranges from 8 to 13 mg [13]. Hence, zinc-biofortified wheat varieties can fulfill this daily intake without causing nutritional toxicity. However, zinc deficiency is associated with various health issues, such as impaired learning, weakened immunity, higher susceptibility to infections, and stunted physical growth.

Approximately 208 wheat varieties in Pakistan have been released, including five zinc-biofortified varieties. Among these, Nawab-21 and Akbar-19 from Punjab, along with Zincol-2016 from the National Agricultural Research Center (NARC), Islamabad, are recognized as zinc-biofortified wheat varieties. For the first time, the Agriculture Research Institute, Tarnab, Peshawar, has introduced two new high-yielding zinc-biofortified wheat varieties, namely, Tarnab Rehabar and Tarnab Gandum-I, in Khyber Pakhtunkhwa, reinforcing efforts toward wheat zinc biofortification.

Stripe rust (also known as yellow rust, YR) and leaf rust (LR), caused by *Puccinia striiformis* f. sp. *Tritici* (*Pst*) and *P. triticina* (*Pt*), respectively, can cause total crop loss when an early infection strikes in susceptible varieties. Annually, wheat rust pathogens contribute to global yield losses of approximately 15 million tons, equating to around US$2.9 billion, with yellow rust alone responsible for 5.5 million tons in losses [14, 15]. Durable rust resistance, characterized by race-nonspecific and long-lasting effectiveness, is crucial for sustained productivity [16]. YR generally occurs in cool and moist environments, whereas LR is more adapted to warmer environments coupled with ideal moisture conditions, but migrating and evolving YR races have infected wheat crops in previously unaffected areas [17, 18].

Wheat has three common rust resistance mechanisms: race-specific seedling/all-stage resistance, race-specific adult plant resistance (APR), and race-non-specific APR [19]. Race-specific resistance genes (R genes) offer relatively high resistance and are easier to select in breeding programs. However, these R genes are often overcome quickly by evolving rust races, especially when used singly, leading to "boom and bust" cycles. A total of 83 stripe rust (YR) and 80 leaf rust (LR) resistance genes have been cataloged in wheat [20, 21]. Most of these are R-genes, and many have already lost effectiveness due to pathogen virulence. Three pleiotropic APR genes – *Lr34/Yr18/Sr57/Pm38/Ltn1*, *Lr67/Yr46/Sr55/Pm46/Ltn3,* and *Lr46/Yr29/Sr58/Pm39/Ltn2* – offer resistance to LR, YR, stem rust (SR), and powdery mildew (PM). The first two pleiotropic genes have already been cloned and characterized [22]. Combining 4-5 APR genes can result in near-immune rust resistance in CIMMYT wheat germplasm [23]. In light of the above, the present article aims to promote Tarnab Rehbar (TRB-2-103) as a high-yielding, zinc-biofortified, and rust-resistant wheat variety for irrigated and late sowing in Khyber Pakhtunkhwa.

Table 1. Development history of Tarnab Rehbar *TRB-2-103*.

S. No.	Years	Generations/ trials
1.	2017-18	38th ESWYT, Entry No. 103
2.	2018-19	Regular wheat yield trial under code No. TRB-2-103
3.	2019-20	8th KPWYT
4.	2020-21	NUWYT 1st year
5.	2021-22	NUWYT 2nd year

Materials and Methods

Evolving History

The wheat line *TRB-2-103* represents the outcome of a systematic and rigorous selection process conducted by the Cereal Research Program at the Agricultural Research Institute (ARI), Tarnab, Peshawar. Originally introduced by CIMMYT, Mexico, in 2017-18 as Entry No. 103 under the 38th Elite Spring Wheat Yield Trial (ESWYT), the line was designated *TRB-2-103* for local testing and evaluation (Table 1). Its journey began with inclusion in the Preliminary Wheat Yield Trial during the 2017-18 cropping season at ARI, Tarnab. Among the 50 entries, including the check variety Pirsabak-15, *TRB-2-103* exhibited outstanding yield performance by producing 6,692 kg/ha, 27.3% higher than the yield of the check cultivar (5,256 kg/ha). In addition to its superior productivity, the line showed strong resistance to yellow rust, with a terminal reaction of 0-TR. These results justified its advancement to the Regular B-Trial for further evaluation. In the 2018-19 Regular Wheat Yield Trial, a total of 26 promising lines, including *TRB-2-103*, were tested against two check varieties: Pirsabak-15 (PS-15) and Khaista-17. The line continued to demonstrate strong performance by yielding 13.80% and 10.8% more than PS-15 and Khaista-17, respectively. Its consistent superiority secured its progression to the provincial-level multi-location testing phase. During the 2019-20 season, *TRB-2-103* was evaluated in the 8th Khyber Pakhtunkhwa Wheat Yield Trials (KPWYT), conducted across diverse agro-ecological locations under both normal/irrigated and late sowing conditions. Under normal/irrigated conditions at 14 different locations, *TRB-2-103* achieved a mean yield of 3,233 kg/ha, which was 6.3% and 0.09% higher than the general check cultivar Pirsabak-2013 (3,040 kg/ha) and the local check (3,230 kg/ha), respectively (Fig. 1). Similarly, under late sowing conditions at 10 locations, *TRB-2-103* maintained its performance by producing a mean grain yield of 2,586 kg/ha, outperforming Pirsabak-2013 (2,357 kg/ha) and the local check (2,364 kg/ha) by 9.7 and 9.3%, respectively (Fig. 2). This extensive and multi-stage evaluation underscores the genetic potential and adaptability of *TRB-2-103* under diverse wheat-growing environments. Its consistent yield superiority and disease resistance position it as a promising candidate for strengthening regional wheat production.

Results and Discussion

National Uniform Wheat Yield Trials

NUWYT (Normal/Irrigated) 2020-21 and 2021-22 at Khyber Pakhtunkhwa

In NUWYT (normal) trials, the line *TRB-2-103* was tested across 4 and 6 locations in Khyber Pakhtunkhwa during 2020-21 and 2021-22, respectively (Table 2). Averaged over two years, *TRB-2-103* produced a grain yield of 5084 kg/ha, which was 7.40 and 9.70% higher than the general checks (Pakistan-13, Ghazi-19, Rustam-20, and Akbar-19) and local checks, respectively.

NUWYT (Normal/Irrigated) 2020-

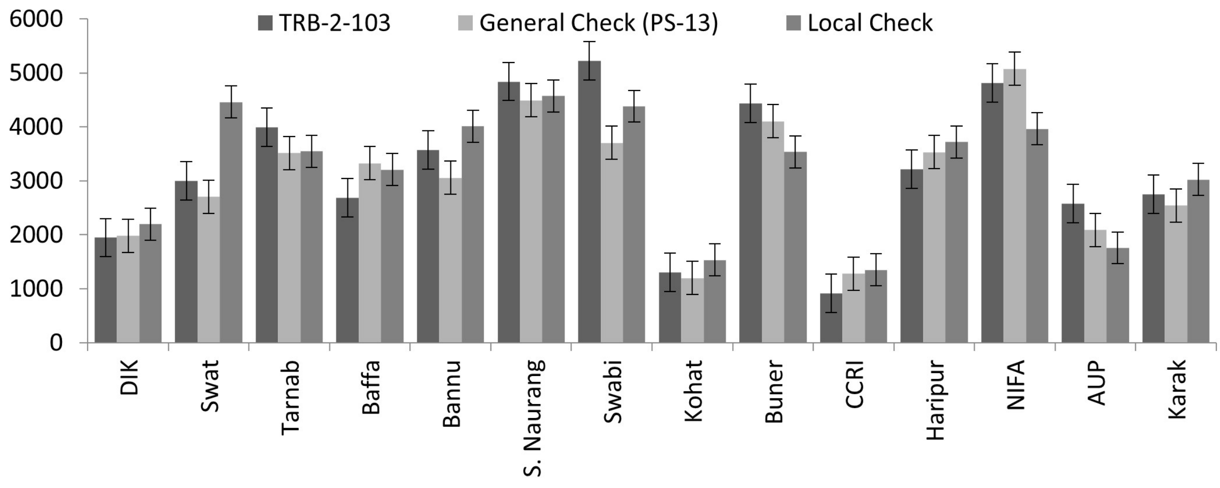

Fig. 1 Yield (Kg ha^{-1}) *TRB-2-103* in KPWYT across 14 locations during 2019-20.

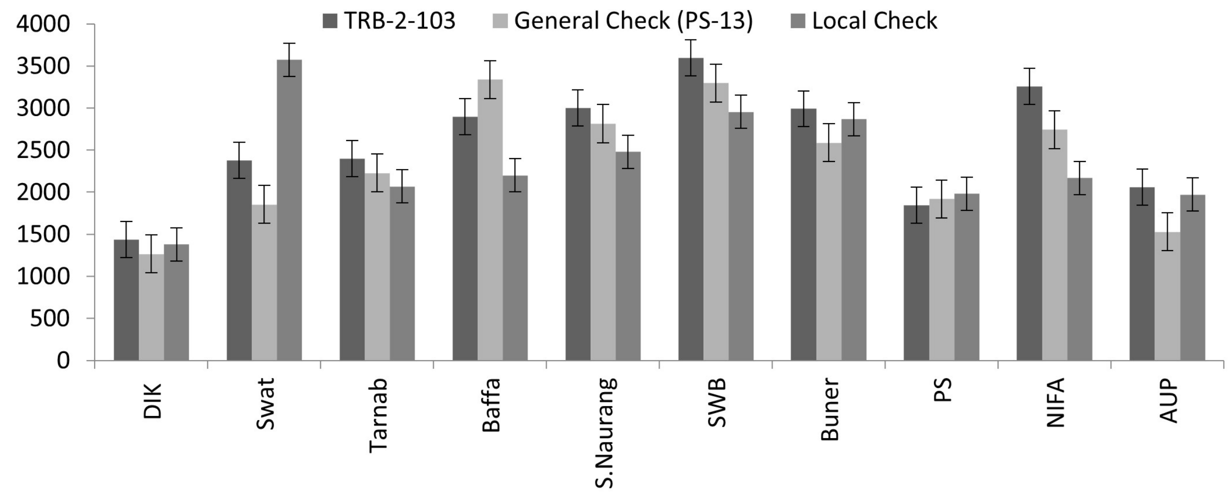

Fig. 2 Yield (Kg ha^{-1}) *TRB-2-103* in KPWYT across 10 locations during 2019-20.

Table 2. Grain Yield Performance of *TRB-2-103* across two years (2020-21 and 2021-22) in NUWYT Irrigated in Khyber Pakhtunkhwa.

S. No	Entry/Line	Year		Average
		2020-21 (04-Loc)	2021-22 (06-Loc)	
1	TRB-2-103	5999	4168	5084
2	General Checks	5176	4281	4729
3	Local Checks	5407	3859	4633
Percent +/- of *TRB-2-103* over G. Checks			7.4	
Percent +/- of *TRB-2-103* over L. Checks			9.7	

Table 3. Grain Yield Performance of *TRB-2-103* across two years (2020-21 and 2021-22) in NUWYT Irrigated in Pakistan.

S. No	Entry/Line	Year		Average
		2020-21 (31-Loc)	2021-22 (40-Loc)	
1	TRB-2-103	4616	4162	4389
2	General Checks	4136	4066	4101
3	Local Checks	4261	4080	4171
Percent +/- of *TRB-2-103* over G. Checks			7.0	
Percent +/- of *TRB-2-103* over L. Checks			5.2	

21 and 2021-22 in Pakistan

To study the yield dynamics of the line *TRB-2-103* and its wider adaptability, it was tested at 31 and 40 locations across Pakistan during 2020-21 and 2021-22, respectively (Table 3). Combined over two years across all locations, the line *TRB-2-103* produced a grain yield of 4389 kg/ha and surpassed the general checks (Pakistan-13, Ghazi-19, Rustam-20, and Akbar-19) and local check varieties by 7.1 and 5.20%, respectively.

Agronomic Trials

Agronomic trials conducted at ARI, Tarnab, Peshawar, focused on optimizing planting time, fertilizer levels, and seed rate to achieve maximum grain yield. Sowing date trials revealed that October 20th was the most suitable time for planting Tarnab-Rehbar (*TRB-2-103*), yielding 5477 kg/ha, while the lowest yield of 2400 kg/ha was recorded for the December 20th sowing. Fertilizer trials indicated that an NPK application of 120:90:60 kg/ha was optimal, resulting in a maximum yield of 6096 kg/ha. The study emphasized the genetic variability of wheat lines in nutrient utilization,

Table 4. The variety specific nucleotide barcode at the specified SNP positions using KASP markers.

SNP	Allele	Chromosome	Position	Tarnab Rehbar (TRB-2-103)
AX-110438364	T/C	chr1A	39871488	CC
AX-94388408	G/C	chr1A	481692078	TT
AX-110999783	C/G	chr1A	30193606	GG
AX-95652854	T/C	chr1B	488211547	CC
AX-110514958	G/C	chr1B	589778206	GG
AX-110961936	C/T	chr1B	687773466	CT
AX-112287874	A/G	chr2A	50206255	GG
AX-111232531	T/C	chr2A	86266686	TT
AX-86185273	G/C	chr2A	598161025	GG
AX-94464095	A/G	chr2B	44424336	CG
AX-111467298	T/C	chr2B	73989699	NN
AX-112286114	T/C	chr2B	763924649	CC
AX-109437310	C/G	chr2D	636190644	GG
AX-111514932	T/C	chr3A	642160277	CT
AX-95659854	A/G	chr3B	764185624	GG
AX-108818546	A/G	chr3B	796459163	GG
AX-111861704	C/T	chr4A	40242560	CC
AX-109397053	C/G	chr4A	626063259	GG
AX-95209376	C/G	chr4B	650599974	GG
AX-111111185	C/T	chr4B	671667802	CC
AX-110934942	T/A	chr4D	455761167	TT
AX-111556373	A/G	chr4D	489187250	GG
AX-109336640	A/G	chr5A	478821075	AA
AX-94504479	A/G	chr5A	610750842	GG
AX-110954664	A/G	chr5A	611558441	GG
AX-95100031	C/G	chr5B	531222411	GG
AX-94667285	T/C	chr5B	671196095	TT
AX-89705852	G/T	chr6A	77084804	TT
AX-108834903	C/G	chr6A	601951605	GG
AX-110498226	C/G	chr6B	167360426	CC
AX-89560976	G/C	chr7A	162521385	GG
AX-94533089	G/A	chrUn	255787724	AA

underscoring the importance of tailored fertilizer strategies in variety development. Seed rate trials demonstrated that a seeding rate of 120 kg/ha produced the highest yield of 5937 kg/ha. These findings highlight the significance of agronomic optimization in enhancing yield potential.

Characteristics

Quality Characteristics

The proposed wheat line *TRB-2-103* demonstrated strong performance in various quality traits. It exhibited a test weight of 71.80 kg/ha, a protein content of 15.4%, a starch content of 54.4%, and a gluten content of 30%.

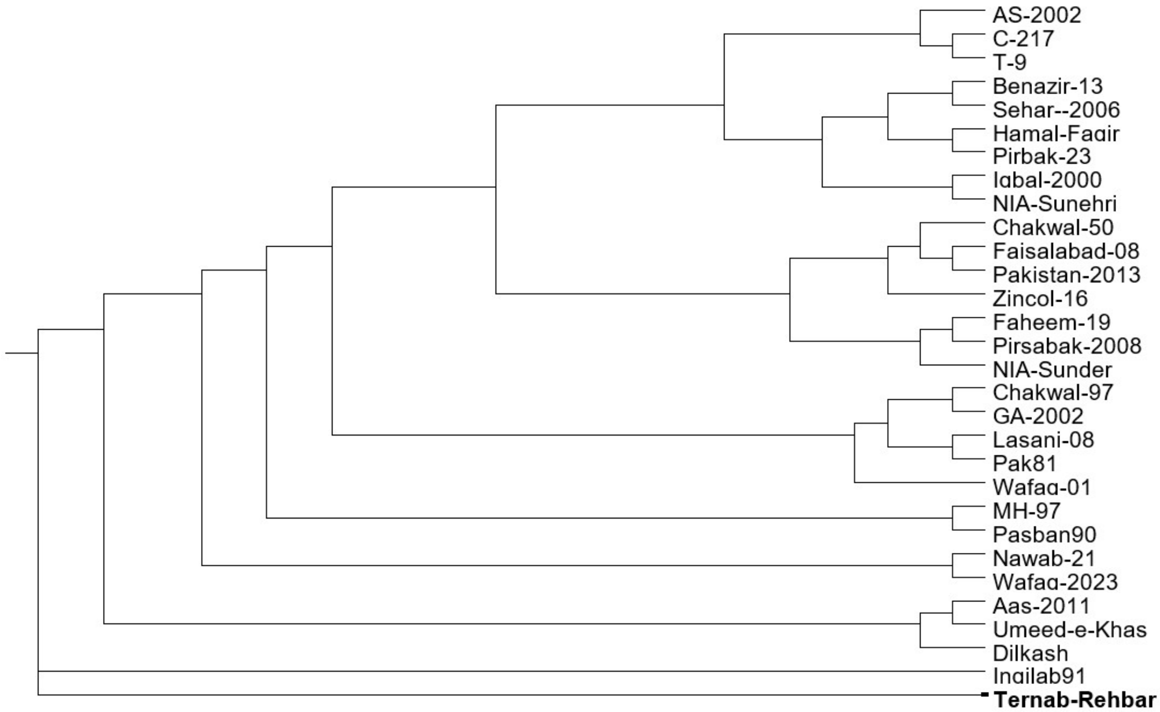

Fig. 3. Phylogenetic relationship between Tarnab wheat line TRB-2-103 (Tarnab Rehbar) vs. other major wheat.

All these quality parameters align with Pakistan's standard requirements.

Botanical Characteristics

Tarnab Rehbar exhibited a plant height of 95-105 cm, a dark green color at booting, and a stem diameter of 3-4 mm. It had a peduncle length of 12 cm and an average of 430 tillers per m^2. The flag leaf measured 26 cm in length and 2.5 cm in width. The crop reached heading at 115 days, with a spike length of 12 cm and an awn length of 4-5 cm. The seeds were white, 6.5-7.5 mm long, with a thousand-grain weight of 35 g. The genotype produced 55-60 seeds per spike, had a protein content of 15.40%, and yielded 6.7 tons per hectare.

Disease-Screening Studies

Present abiotic and biotic stress is attributed to the unusual yield loss in Australia and Pakistan, followed by India and China, while reducing effects for Canada, Russia, the USA, and Turkey [24]. The occurrence and extent of these losses may increase further due to changing climatic situations and hazards of a rust epidemic. Nearly half of the production losses were due to yellow rust in the previous disease epidemic [25]. Regarding the environment, the genetic diversity for the valuation of resistance could have been estimated by exploring disease severity and incidence parameters and the infection rate [26]. Both strategies were adopted to screen *TRB-2-103* for yellow and leaf rusts at multiple locations, including the National Wheat Disease Screening Nursery during 2020-21 and 2021-22 at CDRI, NARC, Islamabad. The average rust resistance index in *TRB-2-103* for yellow and leaf rust was 7.95 and 8.26, respectively, during 2020-21 and 2021-22. The wheat line (*TRB-2-103*) demonstrated promising resistance to leaf rust (*Puccinia triticina*) during the 2021-22 cropping season, as detailed in the CDRI report (page 27, table 5). Evaluated across multiple environments, the line showed a coefficient of infection (CI) ranging from 0 in Faisalabad (immune) to 40 MSS in Bahawalpur, with other locations such as Karachi TMSS, Tandojam (5 MSS), Sakrand TMSS, Kunri TMSS, Thatta TMSS, and Multan (5 MSS) showing mild to moderate disease expression. These findings suggest that *TRB-2-103* carries race-specific or adult plant resistance (APR) genes contributing to durable and broad-spectrum resistance. From a breeding perspective, such lines are vital for incorporating stable rust resistance into new cultivars, especially for rainfed regions like Khyber Pakhtunkhwa, where disease pressure can vary significantly. The overall resistance performance of *TRB-2-103* highlights its potential as a parental line in future wheat improvement programs targeting both yield stability and rust resistance.

DNA Profiling

The Department of Plant Sciences at Quaid-i-Azam University, Islamabad, carried out DNA profiling of Tarnab Rehbar. Seeds were germinated under controlled conditions to facilitate DNA barcoding. Genomic DNA was isolated from 3-5 cm long seedling leaves grown in plastic trays filled with peat moss, following a two-

Department of Plant Sciences Quaid-i-Azam University, Islamabad

Fig. 4. 2-D Digital Barcode of the Nucleotides of Tarnab Rehber.

step CTAB extraction method described by [27-29]. The extracted DNA was assessed on a 1% agarose gel, ensuring a 50 ng μl^{-1} concentration for subsequent SNP analysis.

SNP Genotyping

Single Nucleotide Polymorphism (SNP) genotyping was conducted using 32 carefully selected SNP markers from the 55K SNP array data, chosen for their effectiveness in distinguishing wheat cultivars [30, 31]. The genotyping process employed Kompetitive Allele Specific PCR (KASP) technology, a high-throughput platform widely used for analyzing key wheat traits. DNA samples were distributed into a 384-well clear plate using an automated SNPLine™ dispenser (LGC®, London, UK), air-dried at 55°C for 30 minutes, and mixed with KASP Master-Mix and specific primers. PCR amplification followed standard protocols, and fluorescence signals (FAM, HEX, and ROX) were detected at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), Haidian-Beijing, China. The genotyping results are presented in Table 4.

DNA Barcoding

DNA barcodes were developed using polymorphism data from 32 SNP markers analyzed through KASP technology, following modified methods described by [32, 33]. Fig. 3 presents the dendrogram of Tarnab Rehbar, while Fig. 4 displays the 2-D barcode illustrating variety-specific nucleotides at the designated SNP positions identified by KASP markers. The nucleotide barcode of Tarnab Rehbar is distinct compared to 29 other Pakistani wheat cultivars, showing no similarity to any previously released varieties in the country.

Conclusions

Old and obsolete wheat varieties become vulnerable to biotic and abiotic stresses with prolonged field exposure. Replacing them with improved varieties enhances cost efficiency by utilizing additional genetic gains through wheat breeding. Tarnab Rehbar, a zinc-biofortified variety, was evaluated across multiple locations nationwide and demonstrated superior yield potential and resistance to yellow and leaf rust. Its biofortification helps combat malnutrition, while its reduced reliance on chemical inputs makes it a cost-effective and environmentally friendly option. Based on these attributes, Tarnab Rehbar was approved by the Provincial Seed Council for general cultivation in late and irrigated areas of Khyber Pakhtunkhwa, Pakistan, contributing to sustainable agriculture and national food security.

Availability

Wheat cultivar Tarnab Rehbar (TRB-2-103) has been deposited in the Plant Genetic Resource Institute, NARC, Islamabad, Pakistan, for future research retrieval. Seed for commercial use is available from the Agriculture Extension Department, Khyber Pakhtunkhwa.

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Conflict of Interest

The authors declare no conflict of interest.

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