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'MH-21,' A NOVEL HIGH-YIELDING AND RUSTS RESISTANT BREAD WHEAT VARIETY FOR IRRIGATED AREAS OF PUNJAB, PAKISTAN

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SUMMARY

Developing high-yielding wheat varieties tolerant to abiotic and biotic stresses is a challenge wheat breeder currently face. Wheat Research Institute (WRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, has released 65 wheat varieties, including MH-21, for several zones since its establishment in 1906. MH-21, with the varietal code V-12304, gave 11.2% and 10.28% more grain yield than the check variety (Faisalabad-08), respectively, in preliminary (2011–2012) and regular yield trials (2012–2013). Then, testing it in outstation yield trials proceeded over 21 locations in Punjab province (2013–2014) and 18 locations all over Pakistan (2014–2015, 2017–2018, and 2019–2020) for yield and disease constancy, simultaneous with agronomic field trials and quality lab testing during 2014–2018 and 2014–2016, respectively. It exhibited an amber seed color, semi-erect growth habit at booting, erect flag leaf attitude, hairiness auricle, awned yellowish ear, and elevated shoulder and beak shape of glumes traits. DNA fingerprinting of MH-21 ensued to compare its genetic background with previously registered 21 wheat varieties. Given the promising stable performance in multi-locations over the years for better grain yield, nutritional quality, and resistance against rusts, the Punjab Seed Council approved V12304 in 2021 with the name 'MH-21' for general cultivation in irrigated areas of Punjab, Pakistan.

Keywords: MH-21, high yielding, rusts resistant, bread wheat irrigated areas, Punjab

Key findings: The approval of MH-21 gives fresh courage to redesign food security for the growers of irrigated areas overall Punjab, particularly in-explicit zones, by wheat yield maximization and tolerating the unpredicted disease threat.

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Abbreviations used: AARI: Ayub Agricultural Research Institute; CDRI: National Wheat Disease Screening Nursery; CSISA: Cereal Systems Initiative for South Asia; CTAB: cetyltrimethyl ammonium bromide; LDSN: Local Disease Screening Nursery; NARC: National Agriculture Research Centre; NUWYT: National Uniform Wheat Yield Trial; NWDSN: National Wheat Disease Screening Nursery; PUWYT: Provincial Uniform Wheat Yield Trial; WRI: Wheat Research Institute; UPGMA: Unweighted Pair Group Method of Arithmetic Means.

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INTRODUCTION

Wheat need is escalating with the steady upsurge of national populations. The supply of wheat requires sustainability to address an overwhelming rising demand. Presently, commercial wheat varieties are trailing their resistance due to anxieties. Replacing current wheat varieties with novel high-yielding and disease-lenient ones is an urgent contest for breeders. Therefore, with an estimated population of 227 million by 2025 in Pakistan and resources becoming increasingly scarce (Anonymous, 2016), a dire need to accelerate wheat breeding strategies requires increasing new variety supply persistently for wheat growers and investment in agriculture research and development immediately.

The WRI, AARI, Faisalabad, Pakistan, has a very glorious history since its establishment in 1906 as a Cereal Section and now as a Directorate. It has developed more than 65 high-yielding varieties of bread wheat, three varieties of durum wheat, and seven for barley, in addition to its production technology and quality considerations. For the last five years, the institute has released six wheat varieties, including MH-21, which demonstrated upright yield potential against different biotic and abiotic stresses under several zones. The intention of the Government of Pakistan to attain self-reliance in wheat production, ascertained more achievable with the release of new wheat varieties.

The recently released varieties play a vital part for the nation's general productivity, and Punjab, specifically, for maximizing wheat yield, disease resistance, and quality traits. The Institute has strong research partnerships with key national and international organizations, indicating its endurance to advance from the green revolution to the gene revolution. Breeders require appraising the anticipated variety in comparison with existing ones for different agronomic, morphological, pathological, physicochemical, and genomics traits in multi-locations to release a new type. This paper explains the execution of necessary breeding steps for the evolution of a high-yielding and disease-resistant wheat line, 'V-12304,' under nationwide multi-locational trials, considering its different traits. Based on the recommendations by an expert subcommittee and with the approval of the Punjab Seed Council in 2021 with the name as 'MH-21' for general cultivation in irrigated areas of Punjab, Pakistan solidified the national food security system in the country.

MATERIALS AND METHODS

Evolving history

The current study took less than a decade to accomplish (2011–2020) by the WRI, AARI, Faisalabad, Pakistan, at 21 locations in Punjab province and 18 in other regions of Pakistan. Selecting MH-21 from CIMMYT material (CSISA-EM E-10223) during 2011–2012 was based on yield performance and rust resistance. The line selection, with parentage WAXWING /4/ SNI/ TRAP#1 /3/ KAUZ*2TRAP // KAUZ /5/TECUE#1 and pedigree CMSS06B00468S-0Y-099Y-099M-1WGY-0B,

Table 1. Development history of "V-12304."

No.	Years	Generations/ trials
1	2011–2012	Preliminary wheat yield trial (A-trial) under CSISA (E-10223)
2	2012–2013	Regular wheat yield trial (B-trial) code No. 12304
3	2013–2014	PUWYT (21 locations in Punjab)
4	2014–2015	NUWYT (18 locations in Pakistan)
		CDRI, NARC, Islamabad
		Agronomic Trials at WRI, Faisalabad
5	2015–2016	CDRI, NARC, Islamabad
		Agronomic Trials at WRI, Faisalabad
6	2016–2017	Agronomic Trials at WRI, Faisalabad
7	2017–2018	NUWYT (18 Locations)
		CDRI, NARC, Islamabad, Pakistan
		Agronomic Trials at WRI, Faisalabad, Pakistan
8	2019-20	NUWYT (18 Locations)

continued later verification in station yield trials A and B-trials under the code name V-12304 during 2012–2013. Showing better performance in these trials than the check variety, it moved to PUWYT for multi-location testing of yield stability and adaptability during 2013–2014. Afterward, V-12304's further promotion in NUWYT ensued in 2014–2015, 2017–2018, and 2019–2020, for broader malleability and yield constancy testing all over Pakistan. Establishing disease screening of V-12304 against leaf and yellow rusts used the modified Cobb's scale (Peterson *et al.*, 1948) during 2014–2015, 2015–2016, and 2017–2018 by CDRI, NARC, Islamabad. Agronomic field trials (sowing date trials) took place during 2014–2018, with seed rate and fertilizer dose trials executed in three consecutive years (2014–2017) at WRI, Faisalabad. Grain samples collected from PUWYT trials were for quality testing of the V-12304 during 2014–2016 at the Cereal Technology Laboratory (ISO certified-17025) of WRI, Faisalabad. For quality testing, the standard procedure adopted was the method from the American Association of Cereal Chemists (Anon., 2000). A detailed summary of V-12304's history appears in Table 1.

DNA fingerprinting

The conduct of DNA fingerprinting of MH-21 compared its genetic background with previously registered 21 wheat varieties. Formerly registered wheat varieties and MH-21 were sown in pots (two for each type) for seedling establishment in the DNA fingerprinting lab of the Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan. The pots kept in a greenhouse at 28 °C followed the standard agricultural practices. Sample harvesting was at the four-five leaf stage, then stored in a -20 °C freezer before further processing for DNA extraction. Genomic DNA extraction employed the reformed CTAB method explained by Iqbal *et al. (*2019*,* 2021b). The Nanodrop Spectrophotometer (ND-2000) measured DNA quality and quantity, running on 0.8% agarose gel electrophoresis. After a quality check, DNA dilutions' preparations consisted of 20 *ng/µl* concentrations, essential for polymerase chain reaction (PCR) (Rehman *et al*., 2022). Fifty most polymorphic simple sequence repeat (SSR) markers attained underwent DNA fingerprinting, which were obtained from different databases, i.e., Grain Genes (https://wheat.pw.usda.gov/GG3/), Gramene (https://www.gramene.org/), and MAS wheat (https://maswheat.ucdavis.edu/) (Table 2). Initially, 233 SSR markers equally spread on A, B, and D, the genomes had all chromosomes selected, with 50 shortlisted based on their polymorphic evidence content described in diverse studies (Iqbal *et al.,* 2021a).

The PCR was accumulated for each of the 50 SSR markers using a 2X DreamTaq Green PCR master mix (https://www.thermofisher.com, K1081) following the standardized procedure (Jamil *et*

Table 2. List of 50 most polymorphic wheat SSR markers used for DNA fingerprinting of newly developed MH-21 variety.

Marker name	Forward primer	Reverse primer
BARC168	GCGATGCATATGAGATAAGGAACAAATG	GCGGCTCTAAGGCGGTTTCAAAT
WMS295	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG
BARC77	GCGTATTCTCCCTCGTTTCCAAGTCTG	GTGGGAATTTCTTGGGAGTCTGTA
CFA2153	TTGTGCATGATGGCTTCAAT	CCAATCCTAATGATCCGCTG
XWMC633	ACACCAGCGGGGATATTTGTTAC	GTGCACAAGACATGAGGTGGATT
CWEM17	GCGATGACTTCGGACAGG	AAGAGCACCGTCTTGGTCTG
CFA2019	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT
CWEM32	ATGCTCAAGCCGAGGAAGTA	TAGACGCCAACAAAGCCACT
GWM169	ACC ACT GCA GAG AAC ACA TAC G	GTG CTC TGC TCT AAG TGT GGG
GWM126	CAC ACG CTC CAC CAT GAC	GTT GAG TTG ATG CGG GAG G
GWM636	CGG TAG TTT TTA GCA AAG AG	CCT TAC AGT TCT TGG CAG AA
XGWM146	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG
BARC70	GCG AAA AAC GAT GCG ACT CAA AG	GCG CCA TAT AAT TCAGAC CCA CAA AA
CWEM22	TCTGGATCCCTTGTCGAATC	GAGGCGAGGATCTCATGGTA
GWM60	TGT CCT ACA CGG ACC ACG T	GCA TTG ACA GAT GCA CAC G
GWM339	AAT TTT CTT CCT CAC TTA TT	AAA CGA ACA ACC ACT CAA TC
GWM410	GCT TGA GAC CGG CAC AGT	CGA GAC CTT GAG GGT CTA GA
WMC617	GAT CTT GGC GCT GAG AGA GA	CTC CGA TGG ATT ACT CGC AC
WMS261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG
BARC23	GCGTGAAATAGTGCAAGCCAGAGAT	GCGCTAACACCTCGGCAAGACAA
BARC210	TGA AGC AAA ACC GCA ATG GGA TAG G	GGA GCC GAA GAG CAG GAA GGT G
GWM497	GTA GTG AAG ACA AGG GCA TT	CCG AAA GTT GGG TGA TAT AC
WMS-190	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG
CWEM06	CCTGCTCTGCCATTACTTGG	TGCACCTCCATCTCCTTCTT
GWEM260	GCC CCC TTG CAC AA TC	CGC AGC TAC AGG AGG CC
GWM473	TCA TAC GGG TAT GGT TGG AC	CAC CCC CTT GTT GGT CAC
GWM515	AAC ACA ATG GCA AAT GCA GA	CCT TCC TAG TAA GTG TGC CTC A
GWM637	AAA GAG GTC TGC CGC TAA CA	TAT ACG GTT TTG TGA GGG GG
CFD15	CTCCCGTATTGAGCAGGAAG	GGCAGGTGTGGTGATGATCT
GWM296	AAT TCA ACC TAC CAA TCT CTG	GCC TAA TAA ACT GAA AAC GAG
GWM311	TCA CGT GGA AGA CGC TCC	CTA CGT GCA CCA CCA TTT TG
GWM369	CTG CAG GCC ATG ATG ATG	ACC GTG GGT GTT GTG AGC
GWM47.2	TTG CTA CCA TGC ATG ACC AT	TTC ACC TCG ATT GAG GTC CT
GWM573	AAG AGA TAA CAT GCA AGA AA	TTC AAA TAT GTG GGA ACT AC
GWM614	GAT CAC ATG CAT GCG TCA TG	TTT TAC CGT TCC GGC CTT
WMS44	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG
CFD2141	GAATGGAAGGCGGACATAGA	GCCTCCACAACAGCCATAAT
CFD49	TGAGTTCTTCTGGTGAGGCA	GAATCGGTTCACAAGGGAAA
GWM156	CCA ACC GTG CTA TTA GTC ATT C	CAA TGC AGG CCC TCC TAA C
GWM205	CGA CCC GGT TCA CTT CAG	AGT CGC CGT TGT ATA GTG CC
GWM334	AAT TTC AAA AAG GAG AGA GA	AAC ATG TGT TTT TAG CTA TC
GWM372	AAT AGA GCC CTG GGA CTG GG	GAA GGA CGA CAT TCC ACC TG
GWM425	GAG CCC ACA AGC TGG CA	TCG TTC TCC CAA GGC TTG
GWM5	GCC AGC TAC CTC GAT ACA ACT C	AGA AAG GGC CAG GCT AGT AGT
WMS639	CTCTCTCCATTCGGTTTTCC	CATGCCCCCCTTTTCTG
WGWM273	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGATC
BARC49	GTC CCA CCA AATTAA CAG CTC CTA	AGG CGC AGT GCT CGA AGA ATA TTA T
GWM165	TGC AGT GGT CAG ATG TTT CC	CTT TTC TTT CAG ATT GCG CC
GWM111	TCT GTA GGC TCT CTC CGA CTG	ACC TGA TCA GAT CCC ACT CG
GWM312	ATC GCA TGA TGC ACG TAG AG	ACA TGC ATG CCT ACC TAA TGG

al., 2020b). Unlike cyclic conditions used in the PCR thermal cycler were as follows: 94 °C for 5 min (initial denaturation), trailed by 35 cycles of 94 °C for 1 min (denaturation), 50 °C – 60 °C for 1 min, depending upon primers (annealing temperature), 72 °C for 1 min (extension), 72 °C for 7 min (final extension), followed by a hold at 4 °C. Determining PCR products progressed on Polyacrylamide Gel Electrophoresis, with alleles counted in a binary matrix, as explained by Jamil *et al.* (2021a). The statistical analysis of the binary data followed with the help of NTSYSpc program version 2.0. Jaccard similarity coefficients assessment used UPGMA. Lastly, using SAHN clustering helped build the dendrogram, with an approximation of the genetic diversity of MH-21 with other wheat varieties carried out (Sneath and Sokal, 2014).

RESULTS AND DISCUSSION

Station yield trials

Preliminary yield trial (2011–2012)

In a preliminary wheat yield trial (A-Trial), CSISA line E-10223 performed superior to the check variety "Lansani-06" among 30 advanced lines during 2011–2012, producing 11.2% more grain yield. Its good performance promoted it further to regular wheat yield trials for varied adaptability testing.

Regular wheat yield trial (2012–2013)

In the regular wheat yield trial (B-Trial), the E-10223 line received a new code as V-12304, which performed better than the check variety "Faisalabad-06" during 2012**–**2013, making 10.28% more grain yield. Its good performance led it to the Punjab uniform wheat yield trial (PUWYT) for wider adaptability testing.

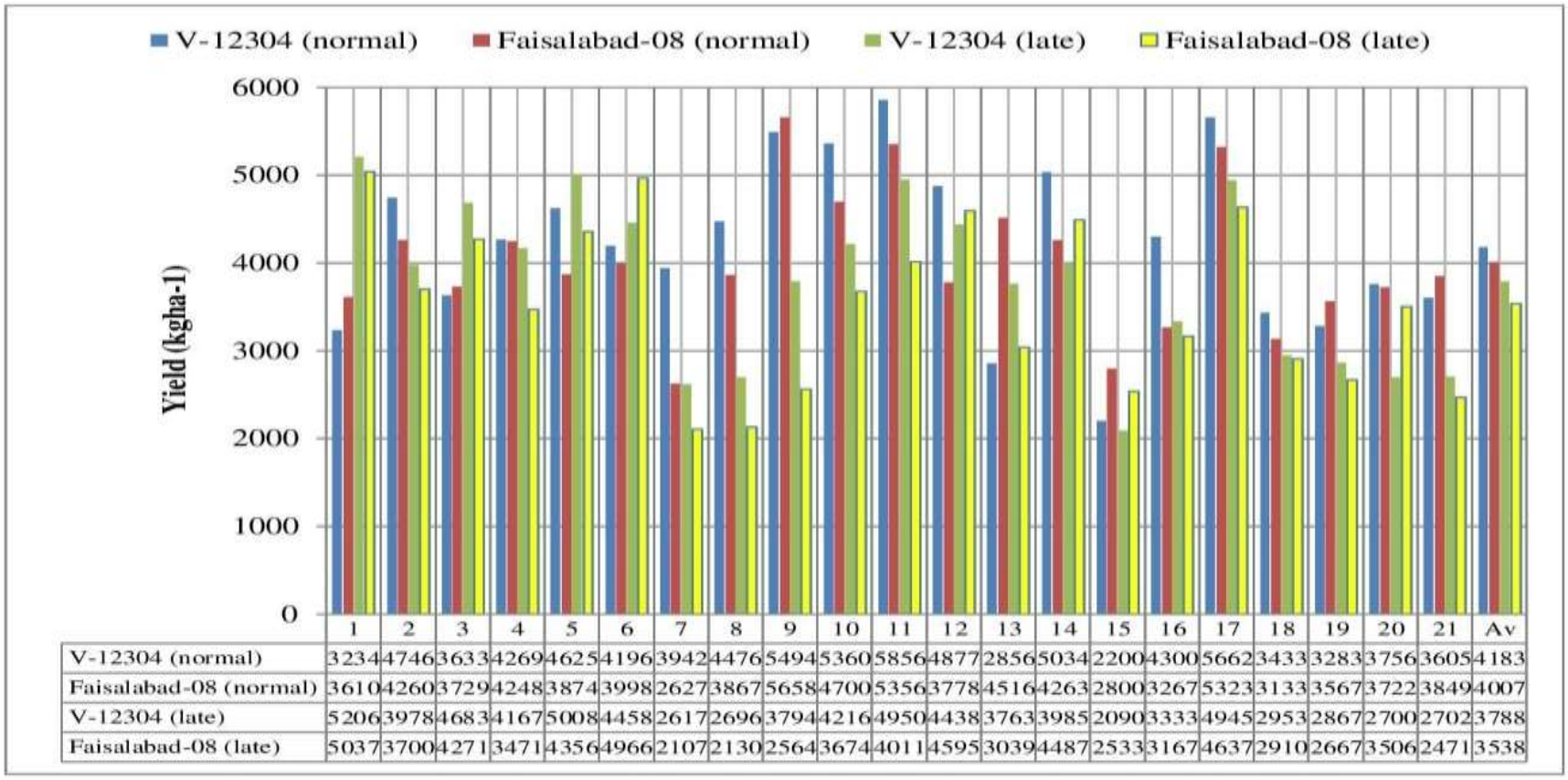
Out-station yield trials

Punjab uniform wheat yield trial (PUWYT, 2013–2014)

Wheat is the foremost crop rotation in Pakistan, after rice and cotton. Stumpy wheat yield potential ensued owing to terminal heat stress, where the planting date is late; it reveals the wheat crop to high temperature at the post-anthesis stage (Zulkiffal *et al.*, 2022). Hence, the vital objective was addressing heat stress in contrast with normal planting for yield and disease resistance by late sowing at multi-locations. V-12304 also delivered superior performance at PUWYT (regular and late) during 2013–2014. Tested at 21 locations, it produced 4.4% more yield than the check "Faisalabad-08" in normal planting and 7.1% more in late planting (Figure 1). This added yield production than the check advanced it further for additional testing in the NUWYT for extensive adaptability and yield steadiness throughout Pakistan.

National uniform wheat yield trial (2014–2015, 2017–2018, and 2019–2020)

It is very significant for a genotype to execute consistency, preferably under diverse agroecological surroundings over the years, where it displays intense turnover and suitable finest while tolerating various biotic and abiotic stresses. In multiple settings, multiple traits are conventionally segregated because they are affected by environmental factors. Among 18 multi-locations throughout Pakistan, V-12304 displayed excellent behavior under NUWYT trials during 2014**–**2015, 2017**–**2018, and 2019**–**2020. In 2014**–**2015, the advanced line V-12304 produced 8.80% more yield than the promising variety 'Faisalabad-08'; in 2017**–**2018, it gave 1.16% added harvest than the same check, while in 2019**–**2020, it delivered 0.60%, 8.94%, and 9.91% more than check varieties Ghazi-19, Pak-13, and Faisalabad-08, respectively (Figure 2).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Av
V-12304 (normal)	3234	4746	3633	4269	4625	4196	3942	4476	5494	5360	5856	4877	2856	5034	2200	4300	5662	3433	3283	3756	3605	4183
Faisalabad-08 (normal)	3610	4260	3729	4248	3874	3998	2627	3867	5658	4700	5356	3778	4516	4263	2800	3267	5323	3133	3567	3722	3849	4007
V-12304 (late)	5206	3978	4683	4167	5008	4458	2617	2696	3794	4216	4950	4438	3763	3985	2090	3333	4945	2953	2867	2700	2702	3788
Faisalabad-08 (late)	5037	3700	4271	3471	4356	4966	2107	2130	2564	3674	4011	4595	3039	4487	2533	3167	4637	2910	2667	3506	2471	3538

1.WRI, Faisalabad, 2. Okara, 3. Sahiwal, 4. Dhakkar, 5. Bahwalanagar, 6.Kala Shah Kaku, 7. Gujranwala, 8. Kot Nana, 9. Khanewal, 10. Multan, 11.Vehari, 12. Karor, 13. Sargodha, 14. Piplan, 15. Rahim Yar Khan, 16. Muzafar Ghar 17. Jalla Arian, 18. Bahawalpur, 19. Bhakkar, 20. ABRI, Faisalabad 21. UA, Faisalabad

Figure 1. Yield performance of V-12304 in PUWYT under normal and late sowing 2013–2014.

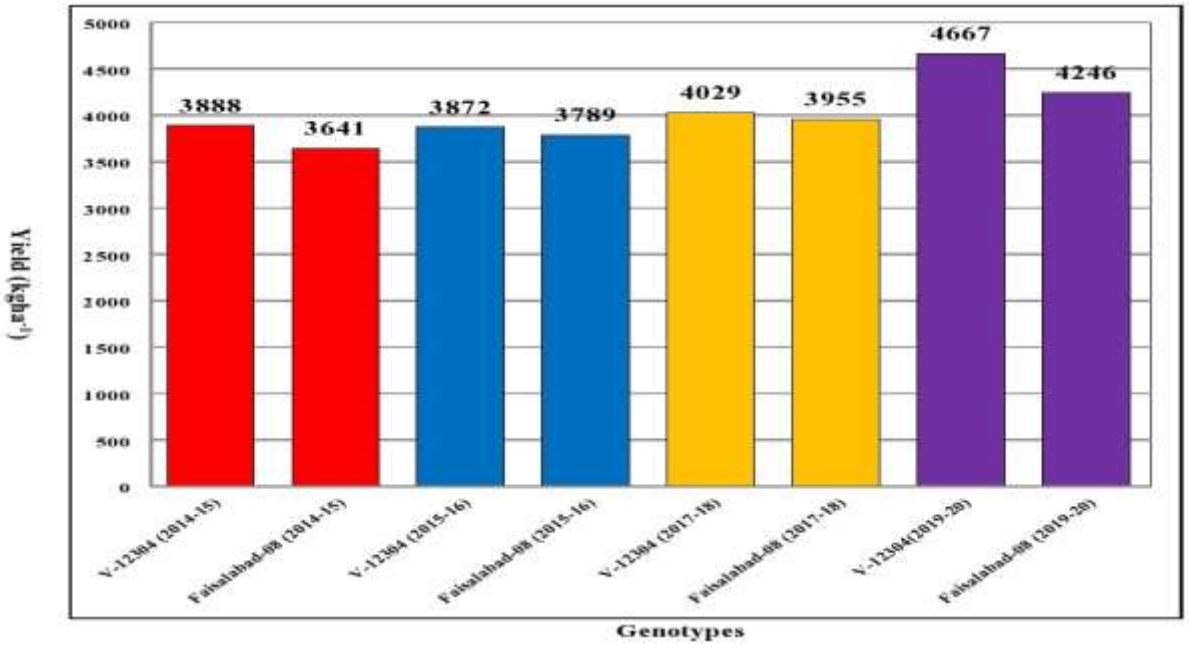


Figure 2. Years average yield performance of V-12304 over 18 locations in NUWYT trials.

Agronomic trials

For yield maximization, conducting the agronomic trials of V-12304 at WRI, Faisalabad, included sowing date, seed rate, and fertilizer levels. In Pakistan, summers are expanding, and winters are shrinking, thus lessening the range of wheat planting, reducing yield by around 1.3% per day for each sowing postponement (Shaukat *et al.,* 2021). Yield and grain weight are complex, quantitative traits, and their performances are significantly impacted by shifting sowing dates. Owing to this need, the sowing date trial took place to know the best sowing time for newly developed wheat lines under changing climate scenarios. The planting trials on seven different sowing dates with 10 days interval started from 1 November 2014 to 30 December 2018. On average, advanced line V-12304 in sowing date trials produced 6.57% more grain yield during 2014–2015, 11.32% in 2015–2016, 2.86% in 2016–2017, and 3.55% in 2017–2018, than the check variety (Faisalabad-08). In all sowing date trials, the 1 November planting date revealed more appropriate for V-12304 to get maximum grain yield. Zulkiffal *et al*. (2022) identified high-yielding and highly stable wheat candidate lines over eight different sowing dates by using methods on parametric stability models. Regarding seed rate trials, V-12304 delivered 4.62% and 5.82% more grain yield than the check during 2014–2015 and 2107–2018, respectively. The 100 kg ha^{-1} seed rate showed best for V-12304 among four tested seed rates (75, 100, 125, and 150 kg ha^{-1}). In fertilizers-application studies, advanced line V-12304 exhibited 11.35% and 8.92% more grain yield than the check during 2014–2015 and 2017–2018, respectively. Among four tested fertilizer doses (0-0-0, 120-90-60, 120-114-60, and 160-171-60 N-P-K kg ha^{-1}), the third dose proved more suitable in acquiring maximum yield from the V-12304.

Disease-screening studies

Present abiotic and biotic stress attributed to the unusual yield loss in Australia and Pakistan, trailed by India and China, while reducing effects for Canada, Russia, USA, and Turkey (Zulkiffal *et al.,* 2021). 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 because of yellow rust in the previous epidemic incident of the disease (Ahmad *et al.,* 2010). Regarding the environment, the genetic diversity for the valuation of resistance could have estimation by exploring disease severity and incidence parameters (Wilcoxson *et al.,* 1975) and the infection rate (Broers *et al*., 1996). Both strategies were adopted to screen V-12304 for yellow and leaf rusts at multiple locations, including the National Wheat Disease Screening Nursery during 2014–2016 and 2017–2018 by CDRI, NARC, Islamabad. The average rust resistance index in V-12304 for yellow and leaf rust was 8.53 and 7.60, respectively, during 2014–2016 and 2017–2018. In LDSN at multiple locations for two years (2014–2016), there was no prevalence of yellow rust, while during 2014–2015, only Faisalabad showed 20S incidents of leaf rust. On the other hand, during 2015–2016, leaf rust occurred in three areas only, i.e., Bahawalpur (30M), Khanewal (30M), and Faisalabad (20M). These results showed the success of V-12304 in the field under multi-locations; hence, it is valuable for further application in wheat breeding programs.

Characteristics

Quality characteristics

The advanced line V-12304 was also impressive for its quality-indicating traits. On average, during 2014–2015 and 2015–2016, the ranges of 1000 grain weight (35.5–39.3 g), test weight (75.95–80.7 kg hl^{-1}), protein content (15.2% – 16.0%), starch content (53.85% – 54.0%), and gluten content (31.0% – 35.0%), considerably fulfilled the quality standards of wheat grains as compared with the check variety (Faisalabad-08).

Botanical characteristics

MH-21 is a medium-tall (95–105cm) variety. It possesses a better tillering capacity at 400 tillers m^{-2}. The detailed botanical description is given in Figure 3.

DNA finger printing

Fifty SSR markers amplified 364 alleles. All were polymorphic for 21 genotypes. On average, 7.28 alleles per SSR marker were amplified, higher than the former study results (Al-Ashkar *et al.,* 2020). Efficacy of the SSR markers attained verification by calculating polymorphic information content using a power marker software which extended from 0.1 to 0.80. Hence, verifying that SSR markers used in this study are more discriminative than previous studies (Al-Tamimi and Al-Janabi., 2019). The resemblance/difference coefficients among varieties were used for cluster analysis using the UPGMA algorithm. The fallouts marked that genetic similarity coefficient between 21 wheat varieties varied from 0.65 to 0.85. Classification of 20 wheat varieties broadly to two discrete groups is evident from Figure 4. Cluster-I comprised of Chakwal-50, Galaxy 13, Anaj-17, AARI-11, Johar-16, F-Bhakkar, FSD-8, Ujala-16, Barani-17, Ihsan-16, Dharabi-11, Millat-11, Punjab-2011, and Ghazi-19. Likewise, Cluster-II consisted of Arooj-22, Akbar-19, MH-21, Dilkash-21, AAS-11, Bhakkar-2020, AAS 11, and Gold-16. The candidate variety MH-21 showed similarities with Chakwal-50 (66.5%), Galaxy-13 (69%), Anaj-17 (66.5%), Arooj-22 (79.1%), Akabr-19 (83%), Dikash-21 (81%), AAS-11 (72.8%), Bhakkar-2020 (73.4%), AAR-11 (63.1%), FSD-08 (61.4%), Millat-11 (61.8%), Punjab-11 (63.7%), Ujala-16 (70.3%), Barani 17 (67.3%), Dharabi-11 (68.1%), Ihsan-16 (66.8%), Johar-16 (68.4%), F-Bhakkar (68.1%), Gold-16 (69.2%), and Ghazi-19 (64.4%). These similarity percentages showed that MH-21 is genetically diverse from the previous 20 developed wheat varieties and may be registered as a new wheat variety.

Figure 3. Botanical description of MH-21.

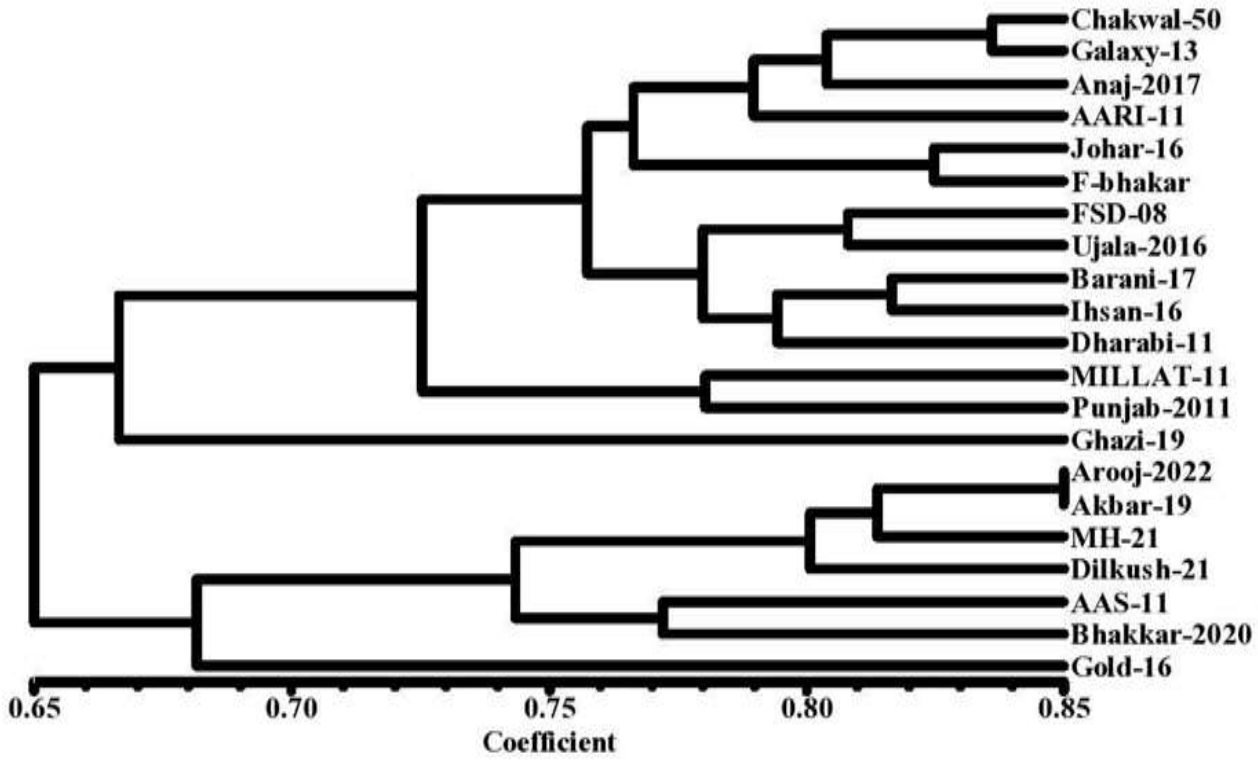


Figure 4. Dendrogram of 21 wheat genotypes constructed based on Jaccard's similarities coefficients following UPGMA Sahn clustering.

CONCLUSIONS

With the passage of stay in the field, old and obsolete wheat varieties are prone to biotic and abiotic stresses. Replacing wheat varieties with new ones increases cost efficiency as it permits manipulating additional genetic gains from wheat breeding. The V-12304 evaluation, in contrast with current ones, transpired from all aspects in multi-locations nationwide. On this basis, it resulted in a recommendation by an expert subcommittee and approved by the Punjab Seed Council with the name "MH-21" for general cultivation in irrigated areas in Punjab, Pakistan, for national food security.

AVAILABILITY

Wheat cultivar MH-21 has been deposited in the Plant Genetic Resource Institute, NARC, Islamabad, Pakistan, for future research retrieval. Seed for commercial use is available from the Punjab Seed Corporation, Lahore, Pakistan.

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REFERENCES

Ahmad S, Afzal M, Noorka IR, Iqbal Z, Akhtar N, Iftkhar Y, Kamran M (2010). Prediction of yield losses in wheat (*Triticum aestivum* L.) caused by yellow rust in relation to epidemiological factors in Faisalabad. *Pak. J. Bot.* 42: 401-407.

Al-Ashkar I, Alderfasi A, Romdhane BW, Seleiman MF, El-Said RA, Al-Doss A (2020). Morphological and genetic diversity within salt tolerance detection in eighteen wheat genotypes. *Plants* 9(3): 287. https://doi.org/10.3390/plants9030287.

Al-Tamimi AJT, Al-Janabi AS (2019). Genetic diversity among bread wheat genotypes using RAPD and SSR markers. *SABRAO J. Breed. Genet.* 51: 325-339.

Anonymous (2016). Ministry of National Health Services, Regulations & Coordination, Government of Pakistan LG&RD Complex, G/5-2, Islamabad. Retrieved from https:// pbit.punjab.gov.pk/system/files/ National%20Vision%20RMNCAH%202016-2020.pdf. December, 2022.

Broers LHM, Subias C, Lopez ARM (1996). Field assessment of quantitative resistance to yellow rust in ten spring bread wheat cultivars. *Euphytica* 90: 9-16.

Iqbal M, Jamil S, Shahzad R, Bilal K, Qaisar R, Nisar A, Kanwal S, Bhatti M (2021a). DNA fingerprinting of crops and its applications in the field of plant breeding. *J. Agric. Res.* 59: 13-28.

Iqbal MZ, Jamil S, Mehmood A, Shahzad R (2019). Identification of seven olive varieties using RAPD molecular markers. *J. Agric. Res.* 57 (1): 07-14.

Iqbal MZ, Jamil S, Shahzad R, Rahman SU (2021b). DNA finger printing and cultivar identification of olive using SSR markers. *Adv. Life Sci*. 8: 143-148.

Jamil S, Shahzad R, Iqbal MZ, Yasmeen E, Rahman SU (2021a). DNA fingerprinting and genetic diversity assessment of GM cotton genotypes for protection of plant breeder's rights. *Int. J. Agric. Biol.* l25: 768-776.

Jamil S, Shahzad R, Kanwal S, Yasmeen E, Rahman SU, Iqbal MZ, (2020a). DNA fingerprinting and population structure of date palm varieties grown in Punjab Pakistan using simple sequence repeat markers. *Int. J. Agric. Biol.* 23: 943-950.

Jamil S, Shahzad R, Yasmeen E, Rahman SU, Younas M, Iqbal MZ (2020b). DNA fingerprinting of Pakistani maize hybrids and parental lines using simple sequence repeat markers. *Pak. J. Bot.* 52: 2133-2145.

Peterson RF, Campbell AB, Hannah AE (1948). A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Can. J. Res.* C 26: 496-500.

Rehman S, Jamil S, Shahzad R, Yasmeen E, Sattar S, Iqbal M (2022). Genetic diversity and DNA fingerprinting of potato varieties using simple sequence repeat (SSR) markers. *J. Anim. Plant Sci.* 32: 775-783.

Shaukat S, Kousar I, Fatima S, Shukat R, Ali A, Ahmad J, Akhtar N, Nadeem M, Farooq J, Ramzan M (2021). Evaluation of spring wheat genotypes for terminal heat stress. *SABRAO J. Breed. Genet.* 53(2): 239-247.

Sneath PHA, Sokal RR (2014). Numerical Taxonomy: The Principles and Practice of Numerical Classification. Taylor and Francis Publishing Ltd. England, pp. 263-268.

Wilcoxson RD, Skovmand B, Atif AS (1975). Evaluation of wheat cultivars for ability to retard development of stem rust. *Ann. Appl. Biol*. 80: 275-281.

Zulkiffal M, Ahmed J, Owais M, Ahmed N, Ahsan A, Rehman A, Anwar J, Riaz M, Ramzan Y, Musa M, Nadeem M (2022). Disclosure of stress driver's traits related to reliability in normal, drought and heat prone settings in bread wheat advanced lines. *Bulg. J. Agric. Sci.* 28 (3): 488-493.

Zulkiffal M, Ahmed J, Riaz M, Ramzan Y, Ahsan A, Kanwal A, Ghafoor I, Nadeem M, Abdullah M (2022). Response of heat-stress tolerant and susceptible wheat lines in diverse planting environments by using parametric stability models. *SABRAO J. Breed. Genet*. 54(1): 127-140.

Zulkiffal M, Ahsan A, Ahmed J, Musa M, Kanwal A, Saleem M, Anwar J, Rehman A, Ajmal S, Gulnaz S, Javaid MM (2021). Heat and drought stresses in wheat (*Triticum aestivum* L.) substantial yield losses, practical achievements, improvement approaches, and adaptive mechanisms. *Plant Stress Physiology Book.* INTECH OPEN, UK. pp 1-24.