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Development of a new hexaploid spring wheat (*Triticum aestivum* L.) variety "NIFA NIJAT-23" for commercial cultivation in irrigated areas of Khyber Pakhtunkhwa

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Abstract

Sustainable wheat farming requires an ongoing process of variety development, release, and quality seed production of new high yielding and disease resistant varieties. Farmers in Khyber Pakhtunkhwa are currently cultivating several wheat cultivars, including NIFA AMAN-17, NIFA AWAZ-19, PIRSABAK-21, ZARGHUN-21, PIRSABAK-19, GULZAR-19, KHAISTA-17, PIRSABAK-15, INSAF-15, KP-15 and LALMA-13 under both irrigated and rainfed circumstances. The frequent changes in disease virulence, particularly yellow rust and leaf rust observed frequently over the last three years in KP, have forced breeders to develop new resistant varieties in order to increase productivity. The new hexaploid spring wheat variety NIFA NIJAT-23 (CTHN-162056) developed at NIFA has demonstrated resistance to yellow rust during testing in the Khyber Pakhtunkhwa Wheat Yield Trials (KPWYT) at 13 distinct locations in 2019-20, and this variety with a grain yield of 3337 kg ha⁻ ¹ has been placed at the third position among 24 test entries. Under normal planting conditions in 2020-21, the NUWYT (National Uniform Wheat Yield Trial) pooled analysis revealed that CTHN-162056 secured the 1st position and provided the greatest mean grain yield of 4701 kg ha⁻¹ across all the candidate lines throughout Pakistan at 31 locations. According to the data reported by the Crop Diseases Research Institute (CDRI), Department of Plant and Environmental Protection, National Agricultural Research Centre (NARC), Islamabad, it has demonstrated highly desirable relative resistance index (RRI) for two consecutive years (2020-21 & 2021-22). The Provincial Seed Council (PSC), Khyber Pakhtunkhwa approved CTHN-162056 and registered as a potential variety "NIFA NIJAT-23".

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Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop grown around the globe (Rahmatov et al., 2019). Wheat plays a crucial role in the world economy, ensuring food security due to high caloric and protein values (Rahmatov et al., 2019; Venske et al., 2019). In Pakistan, annual production of wheat was recorded above 26 million tonnes in 2023 (FAO, 2023), which was slightly higher than that of previous years. Although wheat possesses adaptation to dry and warm humid climates due to its diverse genome (Shiferaw et al., 2013; Subhan et al., 2022), different varieties with varying genetic make-up perform

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variably under filed conditions depending on the intensity and prevalence of biotic and abiotic factors therein. Each province of the country has its own research set-ups wherein breeding programs for developing varieties of different crops including wheat are in place. Depending on the specific environmental conditions of each province, wheat varieties well adapted to such conditions are usually developed. In 2023, the Crop Reporting Services of the Department of Agriculture in Khyber Pakhtunkhwa (KP) reported the cultivation of wheat on 1,931,012 acres (781,452 ha) of land with a net production of 1.2 to 1.3 million metric tons per year, while the annual requirements of the province ranges about 5 million metric tons (TNN, 2023). Presently, the KP farmers are growing various wheat cultivars including NIFA AMAN-17, NIFA AWAZ-2019, PIRSABAK-21, ZARGHUN-21, PIRSABAK-19, GULZAR-19, KHAISTA-17, PIRSABAK-2015, INSAF-2015, LALMA-2013, and KP-15 under irrigated and rainfed settings. The recurrent variation in disease virulence, predominantly yellow rust and leaf in these varieties, enforced the breeders to develop new varieties by providing significant nutrition, better processing value, maximum yield and resistance against different stresses (Subhan et al., 2004; Khan et al., 2007; Mondala et al., 2016). The tremendous increase in population by 2050, requires an estimated 37.14 million tonnes of wheat grains, therefore the country needs to have sufficient wheat production in the coming years (AARI, 2023).

The minimum average yield per hectare of wheat is attributed to high diseases and biophysical/socio-economic restraints. Thus, development of new varieties with maximum yield and disease resistance are crucially needed for increasing and stabilizing production of wheat in KP. The main objective of NIFA's wheat breeding program is to create promising wheat varieties for improving wheat yield and nutritional quality. The previously irrigated varieties reported by NIFA, including BAKHTAWAR, FAKHRE SARHAD, BATHOOR and NIFA AMAN-17 are key steps in enhancing wheat production. The current research work highlights the development of a newly developed wheat variety "NIFA NIJAT-23".

Materials and Methods

One genotype "S # 2056" of spring wheat (*Triticum aestivum* L.) was selected from 26th High Rainfall Wheat Screening Nursery (HRWSN) during 2015-16. The selection and evaluation of the genotype obtained from the nursery were carried out with respect to maximum yield and highest resistance to yellow and leaf rust. Primarily, the genotype was subjected to regular yield trial evaluations during 2016-17, and subsequently, multi-locational field evaluation was performed for four succeeding crop seasons (**Table 1**). The genotype was given a code name "CTHN-162056". The experiment was carried out with Randomized Complete Block Design (RCBD) for yield trials with 5 m long plot size having 4 rows, maintaining 30 cm spacing among individual plants and rows, correspondingly. The entire cultural practices were performed with standard procedures as suggested for irrigated cultivation.

Year	Selection source	Remarks
2015-16	26 th High Rainfall Wheat	Based on high grain yield and rust resistance (Yr & Lr); the said
	Screening Nursery (HRWSN)	entry as S # 2056 was initially selected and designated as CTHN-
		162056 (Table 3).
2016-17	Preliminary Yield Trial-II	CTHN-162056 produced 8583 kg/ha grain yield against the check
	(Normal)	cultivar KP-15 produced 7666 kg ha ⁻¹ (Table 3).
2017-18	Advance Selection Yield Trial-II	Ranked 3 rd and it out-yielded both the check varieties NIFA
	(Normal)	AMAN-17 and KP-15 (Table 4).
2018-19	Multi-location Trial	Excelled both check varieties NIFA AMAN-17 and KHAISTA-17
		after producing 6609, 6914 and 5167 kg ha ⁻¹ grain yield on three
		different locations (Table 5).
2019-20	KPWYT	Ranked third at 13 sites in KP with 3337 kg/ha grain yield
2020.24		compared to check cultivars PIRSABAK-13 (2883 kg ha ⁻¹ , Table 6).
2020-21	NUWYT 1 st Year	Based on mean yield performance, ranked 1 st in Punjab and
2024 22	NU NAVYT 200 Veer	country level, and 3 rd at KP level (Table 7).
2021-22	NUWYT 2 nd Year	Produced grain yield of 4396 kg ha ⁻¹ at 35 locations in the country and was at par with two checks (Table 8).
2020-21	Disease Screening	Showed highly desirable relative resistance index (RRI) of 8.79 for
2020-21	Disease screening	<i>Yr</i> and 7.25 for <i>Lr</i> (Table 9, 11).
2021-22	Disease Screening	Showed desirable RRI of ≈ 6 for Yr and 8.23 for Lr (Table 9 and 11).
2021-22	Quality Evaluation	Met the recommended quality standard with 14.6 percent protein
2020-21		during 1 st year (Table 13).
2021-22	Quality Evaluation	Met the recommended quality standard with 14.6 percent protein
2021 22		during 2 nd year (Table 14).

Table 1. Summary	f developmental history	of CTHN-162056
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Results and Discussion

The yield performance of the current wheat variety CTHN-162056 (NIFA NIJIAT-23) is presented in Tables 3 to 8. Mean grain yield of 26th High Rainfall Wheat Screening Nursery (HRWSN) sown during 2015-16, while Preliminary Yield Trial (PYT) performed during 2016-17 at NIFA revealed that CTHN-162056 obtained maximum grain production of 6933 kg ha⁻¹ and 8583 kg ha⁻¹ with respect to that of the check variety BATHOOR-08 as 6666 kg ha⁻¹ and 7666 kg ha⁻¹, correspondingly (**Table 3**). Advanced Yield Trials (AYTs) were carried out during 2017-18 and the data on grain yields of CTHN-162056 showed maximum yield of 4722 kg ha⁻¹ over that of the check varieties NIFA AMAN-17 (4361 kg ha⁻¹) and KP-15 (2722 kg ha⁻¹) (Table 4). Likewise, Micro-Plot Trials (MPT) studies were performed at three locations in the irrigated areas of Khyber Pakhtunkhwa (NIFA Peshawar, CCRI Pirsabak Nowshera, and KP Agri. Univ. Peshawar) during 2018-19. Mean grain yield data revealed that CTHN-162056 exhibited a maximum increase in grain yield of 6230 kg ha⁻¹ as compared to that of the check varieties NIFA AMAN-17 (3433 kg ha⁻¹) and Khaista-2017 (5726 kg ha⁻¹) (Table 5). During the subsequent year (2019-20), the candidate line gave a maximal average grain yield of 3367 kg ha⁻¹ at 13 sites of Khyber Pakhtunkhwa in KPWYT over that of the local checks NIFA AMAN-17 (2781 kg ha⁻¹) and PIRSABAK-2013 (2883 kg ha⁻¹), and stood at 3rd position among all varieties tested simultaneously (Table 7). Further assessment of the variety was carried out at the National Uniform Wheat Yield Trials (NUWYTs) by the National Wheat Coordinator at National Agricultural Research Centre (NARC), Islamabad for two consecutive years 2020-21 and 2021-22. The CTHN-162056 ranked 4th in NUWYT 2020-21, at 5 sites in KP having a net production of 5903 kg ha⁻¹, and it stood 1st of all varieties tested across Pakistan with a total production of 4701 kg ha⁻¹ at 31 locations (Table 7). CTHN-162056 displayed significant outcomes against the check varieties used for comparison in the National Uniform Wheat Yield Trials (NUWYTs) in the second mandatory evaluation in NUWYT (2021-22). The genotype was ranked 18th at 6 sites across KP and stood 26th at 40 sites across Pakistan having mean grain yield of 4396 kg ha⁻¹, compared with that of the local checks (4105 kg ha⁻¹), PAK-2013 (4360 kg ha⁻¹) and AKBAR-19 (4508 kg ha⁻¹) during 2021-22 (Table 8). Globally, research studies for sustaining wheat breeding programs may give rise maximum yield for meeting the food necessities of the growing human population (Borlaug, 2007; Dadrasi et al., 2023).

Table 2. Main charac	steristics of NIFA NIJIAT-23
Parentage	PREMIO//JNRB.5/PIFED/3/KA/NAC//TRCH
Pedigree	CMSA08Y00308T-050M-050Y-040ZTM-050Y-52BMX-012Y-0BMX113-14\M23HRWYT \1003
Species	Triticum aestivum L.
Origin	Exotic
Breeding method	Introduction
Areas of Adaptation	KP irrigated areas
Sowing Time	1 st November to 30 th November
Plant height (cm)	Range (104 - 110)
Seed color	Light red
Seed size	Medium
Protein (%)	14.5
Bread quality	Good
Grain yield	4701 kg ha ⁻¹ NUWYT Average on Pakistan Basis
Stem rust	Resistant
Leaf rust	Resistant
Stripe rust	Resistant

Table 2. Main characteristics of NIFA NIJIAT-23

One of the overwhelming diseases of wheat reported so far is yellow rust that causes 100% loss in grain yield, affecting grain quality and fodder value (Waqar et al., 2018). Rust also causes severe infection in barley, rye and grasses (Subhan et al., 2022). Moreover, newly established crop varieties generally show disease resistance in addition to enhanced yield (Rahmatov et al., 2019). Consequently, the use of genetically tolerant varieties is a more convenient and cost-effective way to avoid infections in developing countries (Khan et al., 2009; Ellis et al., 2014). The genotype was also evaluated for tolerance potential against the rust at the Crop Disease Research Institute (CDRI), Islamabad during 2020-21 and 2021-22, that revealed desired Relative Resistance Index (RRI) of 8.89 and \approx 6 (acceptable: > 6) for yellow rust (*Yr*), 7.86 and 8.23 (acceptable: > 5) for leaf rust (*Lr*) (Tables 9, 10, 11 & 12). Development of new wheat varieties having high yield and maximum tolerance for diseases is vital for filling the gap left by exhausted varieties (Zuniga et al., 2023).

The NIFA NIJIAT-23 grains possess relatively high gluten content and maximum protein content essential for bread quality and baking industry (Tables 13 and 14). One of the key steps is the availability of quality seed to the farmers for evolving heritably better-quality wheat varieties having higher grain

yield (Hashim e al., 2011). Thus, introduction of elite wheat germplasm also overcomes the current deficiency in yield production. The adaptation trial under different environments leading to production of promising genotypes will be further used for utilization in future breeding programs through gene pyramiding of agronomic traits in the current varieties and those of other crops (Zulfiqar et al., 2014; Joshi et al., 2017; Khattak et al., 2019). Furthermore, the current varieties have frequently been conquered due to introduction and rapid evolution of the pathogens (Subhan et al., 2004; Khan et al., 2009). Thus, the newly developed variety NIFA NIJAT-23 possesses maximum yield, considerable adaptability, tolerance against diseases and high baking value.

Year	Trial / Nursery	Grai	Grain Yield (kg/ha)		
		CTHN-162056	Check(s)		
2015 – 16	26 th HRWSN (Entry # 2056)	6933	6666 (BATHOOR-08)	+ 4.00	
2016 – 17	PYT-2 Normal (Entry # 60)	8583	7666 (KP-15)	+ 11.9	
2016 – 17	PYT-2 Late (Entry # 60)	4767	4733 (KP-15)	+ 0.72	
Mean value	s (PYT-2 Normal & Late)	6675	6200	+ 7.7	

Table 3. Yield performance of CTHN-162056 in 26th HRWSN (2015-16) and in Preliminary Yield Trial (PYT-2) at NIFA, 2016-17

Table 4. Yield performance of CTHN-162056 in Advanced Yield Trials (AYT-2) at NIFA, 2017-18

Trial		Grain Yield (kg/ha)	—— % ± over Check
IIIdi	CTHN-162056	Check (s)	% ± over check
AYT-2 Normal (Entry # 24)	4722	4361 (NIFA AMAN-17)	8.28
ATT-2 NOTTIAI (ETILTY # 24)	4722	2722 (KP-15)	73.47
AVT 2 ata (Eatry # 24)	4000	3244 (NIFA AMAN-17),	23.30
AYT-2 Late (Entry # 24)	4000	2967 (KP-15)	34.81
Mean values	4361	3803 (NIFA AMAN-17),	14.67
	4301	2967 (KP-15)	53.28

Table 5. Mean grain yield of CTHN-162056 in Micro-Plot Trial at NIFA, Peshawar, 2018-19

Locations	Grain Y	Grain Yield (kg/ha)				
Locations	CTHN-162056 (Ent. # 05))	Check (s)	—— % ± over Check			
NIFA, Peshawar	6609	4293 NIFA AMAN-17	53.9			
	0009	6481 KHAISTA-17	3.0			
CCRI Pirsabak, Nowshera	6914	2939 NIFA AMAN-17	118.			
	0914	6197 KHAISTA-17	3.5			
KP Agri. Univ. Peshawar	5167	3067 NIFA AMAN-17	68.5			
	5107	4500 KHAISTA-17	14.8			
Mean values	6230	3433 NIFA AMAN-17	81.5			
	0230	5726 KHAISTA-17	8.8			

Table 6. Mean yield (kg/ha) performance of CTHN-162056 in Khyber Pakhtunkhwa Wheat Yield Trial (KPWYTNormal & Late) planted in different irrigated zones (13 locations) of Khyber Pakhtunkhwa (KP) during 2019-20

		Danking on			
Entry	Normal (NIFA)	Normal (NIFA) Late (NIFA)		— Ranking on KP basis	
CTHN-162056	5148	3325	3367		
Local Check (NIFA AMAN-17)	4433	2400	2781	3 rd	
Local Check (PIRSABAK-13)	4630	2350	2883	3.4	
% ± over Checks	11.2 - 16.2	38.5 - 41.5	16.8 - 21.1		

Table 7. Mean yield (kg/ha) performance of CTHN-162056 in NUWYT at 31 locations across Pakistan during 2020-21.

Entry	Punjab	Sindh	KP	Balochistan	Pakistan	Position	
CTHN-162056	4670	4058	5903	3763	4701	Pakistan	1 st
Local Check	4312	3394	5407	2945	4261	Punjab	1 st
GHAZI-19	4155	4011	5339	3995	4279	KP	3 rd
PAKISTAN-13	4038	3199	5013	2892	3992	Sindh	11 th
% ± over high yielding Check	+8.3	+1.2	+9.2	-5.4	+9.9	Balochistan	11 th

Table 8. Mean yield performance of CTHN-162056 in NUWYT planted at different irrigated zones (35 locations) across Pakistan during 2021-22.

S. #	Entry	Punjab (23)	Sind (6)	KP (6)	Pakistan (35)
05	CTHN-162056	4209	4799	4181	4396
23	PAKISTAN-13	4074	4620	4387	4360
46	AKBAR-19 (Check)	4257	5154	4114	4508
70	Local Check	4289	4168	3859	4105
	Grand Mean	4132	4613	3950	4232

Year	C #	Finitian d		Yr			Lr	Sr
	S. #	Entry	TR	ACI	RRI	ACI	RRI	TR
2020-21	07	CTHN-162056	10R	0.67	8.89	7.71	7.86	0
2021-22	05	CTHN-162056	10MR	24.33	≈6	8.52	8.23	1.8

Table 9. Terminal disease reaction, resistance indices and cooperative data of NUWYT irrigated during2020-21 and 2021-22

TR= Terminal resistance, ACI= Average Coefficient Index, RRI= Relative Resistance Index, MR= Moderately Resistant.

Table 10. Response of CTHN-162056 to yellow rust along with their Average Coefficient Infection (ACI), Country Average Relative Percent Attack (CARPA) and Relative Resistance Index (RRI) during 2021-22

S. #	Entry	Islamabad	Swabi	Nowshera	ACIs	CARPA	RRI
5	CTHN-162056	40MRMS	30MRMS	10MR	24.33	33.82	≈6

MRMS= Moderately Resistant Moderately Susceptible, MR= Moderately Resistance CARPA= Combined Adult Plant and Seedling Resistance

Table 11. Response of candidate lines to leaf rust along with their Average Coefficient Infection (ACI), Country Average Relative Percent Attack (CARPA) and Relative Resistance Index (RRI) during 2020-21

Entry	Karachi	T. jam	Sakrand	Kunri	Thatta	BWP	F. bad	ACIs	CARPA	RRI
KT-22	0	0	0	0	0	0	0	0	0	0
CTHN162009	10MSS	10MSS	10MSS	10MSS	10MSS	20MSS	20S	11.86	19.43	7.25
CTHN162056	5MSS	5MSS	5MSS	5MSS	10MSS	20MSS	10MSS	7.71	12.64	7.86
NRL-1664	5MSS	5MSS	5MSS	10MSS	5MSS	10MSS	30MSS	9	14.75	7.67
NRL-1643	10MSS	5MSS	10MSS	5MSS	5MSS	5MSS	10MSS	6.43	10.54	8.05
PR-133	10MSS	5MSS	10MSS	5MSS	5MSS	10MSS	5MSS	6.43	10.54	8.05
	KT-22 CTHN162009 CTHN162056 NRL-1664 NRL-1643	KT-22 0 CTHN162009 10MSS CTHN162056 5MSS NRL-1664 5MSS NRL-1643 10MSS PR-133 10MSS	KT-22 0 0 CTHN162009 10MSS 10MSS CTHN162056 5MSS 5MSS NRL-1664 5MSS 5MSS NRL-1643 10MSS 5MSS PR-133 10MSS 5MSS	KT-22 0 0 0 CTHN162009 10MSS 10MSS 10MSS CTHN162056 5MSS 5MSS 5MSS NRL-1664 5MSS 5MSS 5MSS NRL-1643 10MSS 5MSS 10MSS PR-133 10MSS 5MSS 10MSS	KT-22 0 0 0 0 CTHN162009 10MSS 10MSS 10MSS 10MSS CTHN162056 5MSS 5MSS 5MSS 5MSS NRL-1664 5MSS 5MSS 5MSS 10MSS NRL-1643 10MSS 5MSS 10MSS 5MSS PR-133 10MSS 5MSS 10MSS 5MSS	KT-22 0 0 0 0 0 0 CTHN162009 10MSS 10MSS 10MSS 10MSS 10MSS 10MSS CTHN162056 5MSS 5MSS 5MSS 5MSS 10MSS NRL-1664 5MSS 5MSS 5MSS 10MSS 5MSS NRL-1643 10MSS 5MSS 10MSS 5MSS 5MSS PR-133 10MSS 5MSS 10MSS 5MSS 5MSS	KT-22 0 <td>KT-22 0<td>KT-22 0 0 0 0 0 0 0 0 0 CTHN162009 10MSS 10MSS 10MSS 10MSS 10MSS 20MSS 20S 11.86 CTHN162056 5MSS 5MSS 5MSS 5MSS 10MSS 20MSS 10MSS 7.71 NRL-1664 5MSS 5MSS 5MSS 10MSS 5MSS 30MSS 9 NRL-1643 10MSS 5MSS 10MSS 5MSS 5MSS 5MSS 6.43 PR-133 10MSS 5MSS 10MSS 5MSS 5MSS 6.43</td><td>KT-2200</td></td>	KT-22 0 <td>KT-22 0 0 0 0 0 0 0 0 0 CTHN162009 10MSS 10MSS 10MSS 10MSS 10MSS 20MSS 20S 11.86 CTHN162056 5MSS 5MSS 5MSS 5MSS 10MSS 20MSS 10MSS 7.71 NRL-1664 5MSS 5MSS 5MSS 10MSS 5MSS 30MSS 9 NRL-1643 10MSS 5MSS 10MSS 5MSS 5MSS 5MSS 6.43 PR-133 10MSS 5MSS 10MSS 5MSS 5MSS 6.43</td> <td>KT-2200</td>	KT-22 0 0 0 0 0 0 0 0 0 CTHN162009 10MSS 10MSS 10MSS 10MSS 10MSS 20MSS 20S 11.86 CTHN162056 5MSS 5MSS 5MSS 5MSS 10MSS 20MSS 10MSS 7.71 NRL-1664 5MSS 5MSS 5MSS 10MSS 5MSS 30MSS 9 NRL-1643 10MSS 5MSS 10MSS 5MSS 5MSS 5MSS 6.43 PR-133 10MSS 5MSS 10MSS 5MSS 5MSS 6.43	KT-2200

MSS= Moderately susceptible to susceptibility; MR= Moderately Resistant.

Table 12. Response of candidate lines to leaf rust along with their Average Coefficient of Infection (ACI), and Country Average Relative Percent Attack and Relative Resistance Index (CARPA) during 2021-22

S. #	Entry	Karachi	T J.am	Sakrand	Kunri	Thatta	FSD	BWP	Multan	ACIs	CARPA	RRI
1	CTHN- 162056	0	0	0	0	0	0	MRMS	RMS	6	8.52	8.23
2	V-7	20MSS	5MSS	40MSS	20MSS	40MSS	5MS	10MRMS	40MSS	19.81	28.13	6.47
3	NR-550	10MSS	20MSS	10MSS	20MSS	10MSS	0	10MRMS	20MSS	10.88	15.44	7.61
4	NW-9	5MSS	5MSS	5MSS	5MSS	5MSS	0	20MSS	0	5.06	7.19	8.35
5	AUP- 1413015	TMSS/LS	TMSS	TMSS/LS	TMSS/LS	0/LS	0	10MRMS	10MSS	2.33	3.30	8.70

MSS= Moderate Susceptible to Susceptibility, TMSS/LS= Terminal Moderately Susceptible to Susceptible / Late Susceptibility

Table	13. Quality	evaluation of Natio	nal Uni	form Wheat Yield Trials	(irrigated) fo	r CTHN-162056 durin	1g2020-21
S. #	Entry	НКѠ	ΤW	Starch % MC	GP	GDW	GWW

7	CTHN-162056	41.9	72.9	52.5	9.9	23.0	45.5	19.0
HKW	1000 kernel wt (g); 1	TW: Test wt	. (kg/h); I	MC: Moisture	(%) GP:	: Grain protein (% dry wt basis);	GDW: Gluten

HKW: 1000 kernel wt (g); I W: lest wt. (kg/h); MC: Moisture (%) GP: Grain protein (% dry wt basis); GDW: Gluten dry wt basis (%); GWW: Gluten wet weight basis (%)

Table 14. Quality evaluation of National Uniform Wheat Yield Trials (irrigated) for CTHN-162056 during 2021-22								
S. #	Entry	HKW	TW	Protein (%)	Starch (%)	Gluten (%)		
6	CTHN-162056	38.37	71.8	14.6	50.1	27.5		

HKW: 1000 kernel wt (g); TW: Test wt. (kg/h)

Author(s), Editor(s) and Publisher's declarations

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Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Contribution of authors

Research supervised: STS, MIK. Conduction of experiment: STS, MIK. Data collection, visualization and interpretation: MIK, STS. Preparation of initial draft: STS, MIK. Review of initial draft: STS.

Ethical approval

This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All authors contributed in designing and writing the entire article. All contributors have critically read this manuscript and agreed to publish in IJAaEB.

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