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Physiological and Qualitative Responses of Wheat Store under Local Condition

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Abstract

Twenty-two genotypes were kept in local condition for three years to find the storage impact on quality and germination traits. Significant variation was observed on Zeleny sedimentation, wet gluten, dry gluten, gluten index, falling number value, germination, and germination energy, whereas, protein and moisture content showed non-significant difference among genotypes. Mean variance analysis revealed that genotype "Miraj-08" exhibited the highest germination (95.67%) and germination energy (79.73%) while Zeleny value (82.67) was recorded maximum in "Punjab-11" and gluten index (76.67) in "Shahkar-13". The findings indicate that moisture, germination, wet gluten, gluten index, Zeleny value, and falling number are important indicators for evaluating long-term stored wheat. These traits can guide seed storage, wheat trade, utilization of carryover wheat stocks, helping maintain seed quality, reduce post-harvest losses, and ensure the availability of high-quality seed for farmers.

Keywords: Falling number, Gluten index, Germination energy, Zeleny
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Introduction

Food security refers to reliable access to sufficient, safe, and nutritious food for all individuals. It is commonly assessed using economic and non-economic indicators at both household and individual levels to evaluate dietary adequacy. Multiple socio-economic factors, including income, family size, education, health status, inflation, and dietary habits, influence food security and vulnerability to hunger (Marina et al., 2019).

Pakistan is largely self-sufficient in wheat production and ranks among the top global producers. According to the National Nutrition Survey (2018), 63.1% of households were food secure, 36.9% were food insecure, and 18.3% experienced severe food insecurity. Food security varies across provinces, with Punjab, Khyber Pakhtunkhwa, and Gilgit-Baltistan relatively well positioned compared with Sindh and Balochistan (Baah, 2023). Households consumed about 50.8% income for food annually which is sensitive to price inflation (National Nutrition Survey, 2018). It is quite worth mentioning that wheat has 9.0% and 2.2% share in agriculture sector and GDP, respectively. In Pakistan, wheat area (9 to 10 million ha) and production (26–30 million tons) have gradually increased whereas largest share was contributed by Punjab (Economic Advisor's Wing, 2023–24).

Although, wheat production was increased but grain quality was effected by climate change. Biotic and abiotic stresses badly affect grain weight, protein, starch, minerals, anti-nutritional factors and gluten composition (Zahra et al., 2023; Daloz et al., 2021).

Grain quality is important for seeking food security and nutritional needs. Optimum storage condition is conducive to maintain seed viability, germination potential, and consumer acceptance. Seed moisture, temperature, and storage duration strongly influence deterioration in storage systems, including hermetic and conventional methods (Jafri et al., 2022). In Pakistan, farmers store nearly one-third of wheat for household and next-season use, resulting in significant post-harvest losses estimated at Rs. 4,000/ton. In contrast, national storage losses reach Rs. 6–7 billion annually (Prikhodko & Zrilyi, 2013).

Prolonged storage reduces germination and seed vigor due to deterioration under high temperature and humidity (Malaker et al., 2008). Hermetic and low-temperature storage conditions better preserve grain quality by slowing metabolic degradation of proteins, fats, and carbohydrates (Scariot et al., 2017; Zhao et al., 2024). In contrast, high temperatures accelerate gluten degradation and lipid oxidation, reducing nutritional and baking quality.

Given widespread conventional storage practices in Pakistan, significant post-harvest losses persist across households, farmers, and storage facilities. Therefore, this study evaluates the effects of conventional storage duration on wheat seed germination and quality traits to assess implications for producers, handlers, and end users.

Materials and Methods

Twenty-two (n=22) wheat genotypes were sown during the 2nd week of November 2020-21 at research area of Wheat Research Institute (WRI), Faisalabad, Punjab-Pakistan. The seeds of these wheat varieties were stored in the jute bags (113 cm x 71 cm, weight 1100 g, and 50 kg capacity) at local storage conditions with a range of temperature (°C), humidity (%) and rainfall (mm), viz., 5.5-39.1°C, 37.3-89.7% & 0-134.6 mm; 5.9-41.5°C, 24.7-89.4% & 0-241.6 mm; 4.8-44.8°C, 42.6-87.2% & 0-91mm during 2020-21, 2021-22, and 2022-23, respectively, and kept under the conventional/local storerooms for three consecutive years (2020-23). The experiment was carried out in a complete randomized design (CRD) with three replications. The randomly selected five stored samples of each wheat varieties were tested for M, P, Z, WG, DG, GI, FN, G, and GE in triplicate in Cereal Technology Laboratory working under WRI, Faisalabad.

Compositional analysis of wheat genotypes

Determination of moisture % (M), protein % (P), and zeleny (Z) value

M, P and Z value were analysed (Nigro et al., 2017) through NIR based instrument i.e. SpectraAlyzer, Zeutec, Germany (Model No. 2100119). At first, the sample was cleaned and made free from foreign matter such as dirt, dust, stone, glass particles, metallic pieces, straw and other food grains. Then randomly one Kg representative sample of wheat was taken for carrying out the determination of quality characteristics. The said equipment determined M, P, starch, GI, and Z content. Wavelength of the apparatus was set according to the common wheat genotypes because wheat samples other than the common wheat like durum wheat has different wavelength settings during the analysis otherwise the instrument will not work properly. About 600g grain sample was dispensed into the hopper of the instrument and analysis started. Then after the analysis time of 3-4 minutes, results were noted from digital screen. Five random grain samples of each wheat genotypes for the above parameters were run in triplicate and results were recorded.

Determination of wet gluten% (WG), dry gluten% (DG) and gluten index (GI)

WG, DG, and GI were determined using equipment such as the Glutomatic (Perten, Sweden; Model No. GM 2200) in accordance with AACC Method 38-12A (2010). The sample was cleaned and free of foreign matter, including dirt, dust, stone, glass particles, metallic pieces, straw, and other food grains. Then, a 1-kg representative sample of wheat was randomly selected to determine the gluten content. One hundred grams of pre-cleaned wheat grains were milled in the laboratory mill (LM120), Perten, Sweden (Model No. 080138) to form wheat flour. After weighing, 10 grams of flour was loaded into the washing chamber of a glutomatic instrument, with a polyester screen at the bottom of the sample. Two different mesh-size screens were used during the analysis. The fine screen of mesh size 88 micron and a coarse screen of mesh size 840 microns (if the sample is refined flour, then fine sieve/screen is used; otherwise, both fine and coarse sieves are employed during the analysis). Then, a quantity of sodium chloride solution was added to the flour sample to prevent it from dropping through the sieve, which would affect the accuracy of the results, and the machine's start button was pressed. Washing continued for 5 minutes with a 2% sodium chloride solution, during which the starch content, other soluble fractions, and bran were separated. WG was collected from the chamber, placed in the sieve cassette assembly, and centrifuged. WG passed and retained from the sieve were both weighed. DG was obtained by drying WG in a Glutork apparatus at 150 °C for 4 minutes. WG and GI were calculated by using the given formulae.

$$\text{Wet Gluten (\%)} = \frac{\text{Total gluten (g)}}{\text{10 g}} \times 100$$

$$\text{Gluten Index} = \frac{\text{Total gluten} - \text{gluten passed on sieve (g)}}{\text{Total gluten}} \times 100$$

Estimation of Falling Number Value (FNV)

FNV is an indicator of enzymatic activity (alpha-amylase) that was assessed using a Perten Falling Number apparatus (Model No. FN 1310) in accordance with AACC Method 56-81B (2010). Sample weight was determined from the moisture content using the Falling Number table. The moisture content of each analyzed sample was measured using a Kern moisture tester (MLS 50-3 HA 250), Balingen, Germany (Model No. WL 073580). The sample was cleaned and free of foreign matter, including dirt, dust, stones, glass particles, metal fragments, straw, and other food grains. A representative wheat sample was then randomly selected for the Falling Number test. One hundred grams of pre-cleaned wheat grains were milled in the laboratory mill (LM120), Perten, Huddinge, Sweden (Model No. 080106) to produce whole wheat flour. After accurate weighing, about 07g of ground kernels or milled flour was mixed with 25ml of distilled H2O and shaken in a Shakematic (part of the Falling Number apparatus) to obtain a uniform slurry. The slurry was then placed in a Falling Number glass tube with a plunger that fell into the mixture in the boiling water bath (100 °C) of the main Falling Number apparatus. Continuous heating in the tube immersed in the boiling water bath gelatinized the starch, converting it into a dense paste. Digital reading (seconds) from the apparatus was noted after the complete fall of the plunger through sticky stuff in the Falling Number tube. When a sample is ground or milled to perform this test, the sample must weigh at least 300g to ensure the sample is representative. Optimum falling number value (FNV) ranged from 250- 450 sec, which was inverse of alpha-amylase activity (Posner & Hibbs, 2005). A falling number value above 300 sec indicated low enzymatic activity and good quality of wheat grains or their end products. However, a falling number value below 250 seconds indicated higher enzymatic activity, which was associated with sprouted wheat grains (Posner & Hibbs, 2005).

Determination of Germination % (G)

One hundred grains of each wheat variety, stored under local storage conditions for three consecutive years, were randomly selected and placed in autoclaved Petri dishes in three replicates, following the procedure of the International Seed Testing Association (ISTA, 1996). Each petri dish had a diameter of 14 cm, with 100 grains on the top of filter paper, which was placed on the inner side of every dish. All standard requirements were met in accordance with the recommended rules for seed germination. Normal seedlings were counted after eight days from the planting day. Germinated seeds with normal root and shoot development were counted, and results were recorded as germination percentage after the stipulated period in accordance with the guidelines of ISTA (1996) by the subsequent reckoning.

$$\text{Germination \%} = seedlings/ \times 100$$

Calculation of Germination energy % (GE)

GE was calculated as the percentage of germinated grains at the first count (exactly 4 days after seed planting) relative to the total number of seeds planted in the glassware, as described by Ruan et al. (2002).

$$\text{Germination energy (\%)} = days/Total \times 100$$

Data analysis

Wheat grain quality data were analyzed for mean variance of different traits using LSD (Williams & Abdi, 2010). For classification of genotypes and quality traits, scree plot and principal component analysis were practiced using “RStudio” (Team R. C., 2020)

Results and Discussions

The analysis of variance (ANOVA) for various grain quality traits in the present study is shown in Table 1, indicating that M and P were non-significant. In contrast, WG, DG, Z, FN, G, and GE were significant. Mean values for the different quality traits are also presented in Table 2. Among 22 genotypes, “Punjab-11” exhibited the highest Z value

(82.67), whereas the lowest (68.67) was recorded in "Pirsabak-08". Faisalabad-08 exhibited the highest mean values (39.61 and 13.73) and the lowest values (29.47 and 9.067) for WG and DG, respectively (Table 3). Maximum GI was noted (76.67) in "Shahkar-13", while the lowest (39.0) was recorded in the genotype "Barsat 2010". FN was highest (421.33 sec) in "Nifa Lalma-12", whereas the lowest (319.33 sec) was recorded in "Shahkar-13". Genotype "Miraj-08" exhibited the maximum values for both G (95.67%) and GE (79.730%), whereas the lowest was 69.0% in "Nia Saarang-12" and 69.483% in "Pirsabak-13", respectively.

Analysis of variance revealed that moisture % increased over time. The maximum moisture % was observed after the 2nd year due to the highest rainfall (0- 241.6 mm), and the minimum moisture % was observed in fresh material (1st year) due to less rainfall (0- 91 mm). Absorption of atmospheric moisture increased moisture levels over time. This slight increase in moisture levels was due to the absorption of atmospheric humidity. The study revealed that protein levels decreased slightly over time. Zeleny and wet gluten values were highest in the fresh sample and decreased over time, reaching their lowest after three years. Gluten index, germination, and germination energy were highest in the fresh sample, and these parameters decreased with storage period, reaching minimum values after three years of storage.

Table 1. Analysis of variance for various wheat grain quality attributes

Trait	Storage years	Genotypes	Storage years × Genotypes	Error
DF	2	21	42	66
M	3.29**	0.32^{NS}	0.07^{NS}	0.34
P	4.61*	1.11^{NS}	0.15^{NS}	1.02
Z	3584.12**	95.17**	72.44**	2.73
WG	267.46**	51.05**	8.63**	0.83
DG	27.62**	7.27**	1.88NS	2.07
GI	5551.93**	805.80**	125.86**	3.44
FN	77644.50**	5545.10**	397.90**	22.30
G	2788.45**	217.38**	82.90**	2.88
GE	716.71**	47.62**	18.75**	0.91

**= Highly significant, * = Significant, NS = Non-significant, SOV= Source of variation, DF= Degree of freedom

Moisture % (M), Protein % (P), Zeleny (Z), Wet Gluten % (WG), Dry Gluten % (DG), Gluten Index (GI), Falling Number value sec (FN), Germination % (G), Germination Energy % (GE)

Table 2. Mean variance for wheat grain quality traits during 2020-2023

Seed Quality Trait	1st Year (Fresh, 2022–23)	2nd Year (2021–22)	3rd Year (2020–21)	CV (%)
Moisture (%) (M)	9[b]	10a	9b	0.25
Protein (%) (P)	16[a]	16ab	16b	0.43
Zeleny sedimentation value (Z)	83[a]	76b	65c	0.70
Wet gluten (%) (WG)	36[a]	33b	31c	0.39
Dry gluten (%) (DG)	11[a]	11a	10b	0.61
Gluten index (GI)	68[a]	54b	46c	0.79
Falling number (s) (FN)	338[a]	371b	422c	2.01
Germination (%) (G)	93[a]	88b	77c	0.72
Germination energy (%) (GE)	78a	74b	70c	0.41

The moisture % increased significantly during the second year of storage but declined in the third year to a level similar to that of the fresh samples. The maximum moisture % was observed in the second year of storage, whereas the lowest moisture content was recorded in the fresh and third-year samples (Table 2). The temporary increase in moisture may be attributed to the absorption of atmospheric humidity during storage. Bezerra et al. (2015) reported that grain moisture depends on the storage environment, while Athanassiou et al. (2011) observed that condensation during storage increased grain moisture. Sawant (2012) also reported increased moisture content and reduced germination during storage. Mhiko (2012) found that the falling number increased during storage due to reduced α-amylase activity. Mirani and Lee (2015) observed higher germination and falling number in wheat stored in ferrocement bins than in conventionally stored wheat. Chattha et al. (2018) observed germination decline while moisture percentage increased during wheat storage. These results coincided with our study where germination and germination energy decreased with passage of storage time but the response varied in different genotypes.

This study showed that protein values also decreased with storage interval Zeleny sedimentation and wet gluten were observed maximum in fresh wheat grains and gradually declined during storage and the minimum values noted after three years. Gluten index, germination, and germination energy were recorded highest in fresh sample and minimum after three years. Storage conditions (temperature, humidity, CO_2 and O_2 level) affected seed viability and quality (Abdullah et al., 2019). Previous research showed that germination capacity of seed reduced with extended storage period even kept in ideal environment (Siddiqui & Chaudhary, 2008). Similar findings were noted in our work indicating that germination decreased from 93% to 77% after three years (Table 2).

Gluten index is vital parameter for viscoelastic properties of dough which significantly influences kneading performance and gas-holding capacity (Subaşi & Ercan, 2024). About 5% wet gluten reduction was recorded under masonry storage conditions (Sisman & Ergin, 2011). Storage period also declined wet gluten and gluten index at room temperature (Sujitha et al., 2018). Seed moisture content also reduced the gluten index (Abdullah et al., 2019). Gluten index reduced 14 units after one year and 22 units after three year's storage in the present study, however, various genotypes behaved differently.

Subaşi and Ercan (2024) reported falling number values relate with α-amylase activity in bread wheat. Minimum α-amylase activity produced insufficient sugars for yeast, whereas maximum enzyme activity negatively affected dough quality. Koksel et al. (2000) investigated that falling number (<150s) enhanced α-amylase activity, whereas enzyme activity reduced at >300s. In our study, falling number ranged from 338 to 422s during storage that showed gradual decrease in enzyme activity. According to Srivastava and Rao (1994), Zeleny sedimentation test is an important parameter because it is related with gluten quality and water absorption capacity. Storage conditions, therefore, influence Zeleny sedimentation similarly to wet and dry gluten. Previous studies have shown that higher storage temperatures and prolonged storage reduce Zeleny sedimentation values (Sur et al., 1993; Karaoglu et al., 2010). Subaşi and Ercan (2024) also reported that Zeleny sedimentation is an important indicator of protein quality in wheat. Consistent with these findings, the present study observed an 18-unit reduction in the Zeleny sedimentation value after three years of storage, although the response varied among genotypes.

Principal component analysis (PCA)

PCA is also called canonical vector analysis, a type of multivariate analysis in which canonical vectors, or roots, representing axes of differentiation and the proportion of variation accounted for by each axis are produced (Rao, 1952). It also describes the significance of the largest contributor to total variation on each differentiation axis (Nadarajan et al., 2020). Principal component analysis was applied to 08 wheat seed quality traits of twenty-two genotypes to assess the independent impact of each trait, the total variability, and the association of each trait with genotypes. The scree plot showed the percentage of variance explained by each principal component (PC). Further, it was revealed that PC1 to PC4 accounted for 80.3% of the variation, while the remaining PCs accounted for only 19.7%. Maximum variability 33.7%) was contributed by PC1, followed by 16.8% (PC2), 14.98% (PC3), and 14.78% by PC4. Based on the scree plot, PC1 and PC2 explained more variation, which could help select diverse genotypes.

PCA - biplot

A biplot based on PC1 and PC2 explained 50.5% of the total PCA variation and described the relationships among various seed quality traits, providing the best comparison among 22 wheat genotypes based on quality traits (Christina et al., 2021).

Seed quality traits and genotypes were scattered in all four quadrants as shown in Figure 1. This distribution of genotypes across all four quadrants indicated that genetic diversity existed in the material and suggested that certain traits may be more prevalent in specific genotypes, which could be useful for breeding programs. Four genotypes were present in the 1st quadrant, along with two quality traits (FNV and GE). In contrast, three genotypes with three quality traits (WG, DG, and P) were present in the 2nd quadrant, five genotypes with two traits (GI and Z) belonged to the 3rd quadrant, and nine genotypes with one trait (G) were noted in the 4th quadrant.

When selection was based on FN, the genotype coded 15 (Nifa Lalma-12) performed best among the genotypes due to its high PC values. Genotype coded 25 (Faisalabad-08) had a strong association with WG and DG compared with the other genotypes. For M, GI, and Z, the genotype coded 10 (Shahkar-13) was found to be the best among the genotypes. For

G trait, genotype coded 27 (Miraj-08) was noted the best as compared to other genotypes. Based on PC values, the attributes and genotypes with higher values present in the 1st, 2nd, 3^{rd,} and 4th quadrants of the biplot may be considered better. They can be utilized for genotype evaluation when kept under prolonged storage, as well as for wheat trading and for improving quality and germination characteristics in breeding programs, trading, and food processing industries.

Table 3: Variation among wheat genotypes in seed quality parameters

Genotype	Z	WG	DG	GI	FN	G	GE
Ujala-16	74^{fghi}	29^{k}	9^{fg}	64^{def}	353^{k}	82^{jk}	72^{k}
Fateh Jang-16	78^{c}	36^{bc}	11^{bcde}	55^{h}	351^{kl}	93^{b}	78^{b}
Jouhar-16	76^{de}	32^{hi}	10^{defg}	62^{f}	346^{l}	87^{efg}	75^{def}
Borlaug-16	75^{def}	34^{de}	11^{cdefg}	71^{b}	325^{mn}	86^{fg}	74^{fg}
Gandum-4	71^{jk}	34^{d}	11^{cdef}	64^{cdef}	325^{m}	85^{gh}	72^{ijk}
Shahkar-13	81^{b}	29^{k}	9^{g}	77^{a}	319^{n}	83^{ij}	73^{hij}
Pirsabak-13	82^{ab}	35^{cd}	10^{cdefg}	52^{i}	362^{j}	80^{k}	69^{l}
Hamal-13	77^{cd}	32^{gh}	10^{cdefg}	50^{i}	383^{h}	94^{b}	79^{a}
Benazir-13	78^{c}	33^{fgh}	10^{cdefg}	44^{k}	398^{def}	90^{c}	76^{cd}
Nia Saarang-12	78^{c}	31^{jk}	10^{efg}	27^{m}	400^{d}	69^{m}	73^{ghi}
NIFA Lalma-12	74^{efgh}	33^{fg}	10^{cdefg}	58^{g}	421^{a}	83^{ij}	74^{gh}
Punjab-11	83^{a}	37^{b}	11^{bcd}	55^{h}	401^{cd}	89^{cd}	76^{cde}
Millat-11	73^{ghij}	37^{b}	12^{bc}	39^{l}	376^{i}	89^{cd}	75^{ef}
NARC-11	73^{ghi}	36^{b}	11^{bcd}	52^{i}	351^{kl}	88^{def}	76^{cde}
Nia Sunder-11	72^{ij}	34^{d}	11^{bcdef}	59^{g}	407^{bc}	84^{hi}	73^{hij}
Barsat-10	70^{kl}	39^{a}	13^{ab}	39^{l}	393^{fg}	81^{k}	72^{k}
Faisalabad-08	69^{kl}	40^{a}	14^{a}	47^{j}	384^{h}	78^{l}	70^{l}
Lasani-08	78^{c}	30^{k}	10^{efg}	66^{c}	388^{gh}	84^{hi}	72^{k}
Miraj-08	72^{hij}	30^{jk}	9^{fg}	58^{g}	398^{de}	96^{a}	80^{a}
Hashim-08	72^{hij}	31^{ij}	10^{defg}	62^{ef}	406^{bc}	88^{de}	77^{c}
Pirsabak-08	69^{l}	33^{fgh}	10^{cdefg}	65^{cd}	393^{efg}	93^{b}	77^{c}
Fareed-06	74^{efg}	33^{ef}	10^{defg}	64^{cde}	411^{b}	84^{hi}	72^{jk}

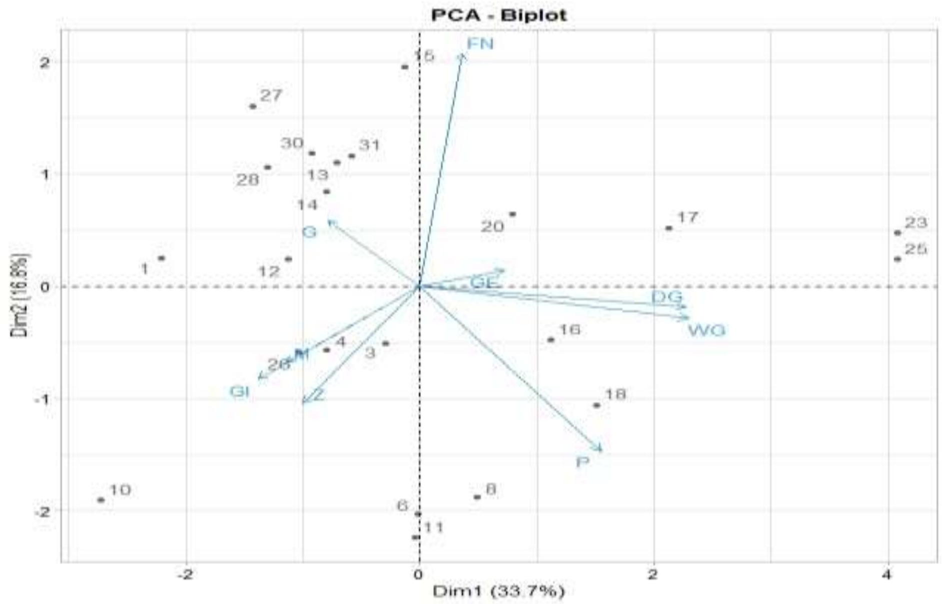

Figure 1: Impact of principal component analysis on wheat quality traits and genotypes.

Conclusions

Z, GI, G, GE, M and FN are more effected than other traits under storage. Based on mean variance, G (95.67%) and GE (79.730%) values were found to be the highest in genotype (Miraj-08), whereas Z (82.67) in Punjab-11 and GI (76.67) were reported best in Shahkar-13. PCA also revealed that M, GI and Z have strong association, whereas WG and DG were found in other quads. This study concluded that M, G, GE, WG, GI, Z, and FN are key quality traits for evaluating any genotype stored for prolonged periods, for trading and food processing purposes.

Conflict of Interests

All authors have not shown any conflict of interest regarding the manuscript publication.

Author Contributions

Muhammad Abdullah conducted the experiment, compiled the data, and helped with proofreading the manuscript; Muhammad Ilyas Khokhar examined the data quality, designed the study's analysis, and wrote up the manuscript; Muhammad Zulkiffal assisted with proofreading; Javed Ahmad supervised the research; and Amir Hameed helped the statistical analysis.

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