J. Plantarum., 7(2): 107-119

ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

APPRAISAL OF YIELD AND QUALITY TRAITS IN TWO AND SIX ROWED BARLEY

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Received on: 20-12-24; Reviewed on: 12-05-25; Accepted on: 24-08-2025; Published on: 15-12-2025

Abstract

Ten barley genotypes were evaluated for yield and quality traits and found significant variance among these traits. Mean variance study revealed that protein (12-13%), starch (59-60%), wet gluten (8-9%) and dry gluten (3-3%) values were at par in both six and two-rowed barley, while 1000-grain weight (47-43g), test weight (61-58kg/hl) and gluten index (96-91) were greater in two rowed, whereas falling number values (263-261sec) were slightly higher in six rowed. Significant positive associations were found among starch, protein, and falling number values (FNV), whereas 1000-grain weight (GW), test weight (TW), gluten index (GI), wet gluten (WG), and dry gluten (DG) revealed positive links in 2-rowed and 6-rowed barley. Based on a principal component analysis (PCA), barley genotype "B-21034" was found in the first quadrant group with the highest values and positively correlated with GI, WG, DG, and GW. B-21008 and B-21022 were noted in the second quadrant with positive correlation with TW. PCA also showed that FNV and TW are far apart, whereas protein and starch reported in the fourth quadrant are too close. PCA revealed that genotype B-21034 showed highest index of WG, DG, GI and GW in first quadrant and can be utilized in breeding programs. For industrial purposes, Sultan-17 may be the best option due to highest values of starch and protein.

Keywords: Yield attributes and genetic variability

INTRODUCTION

Barley (*Hordeum vulgare* L.), belong to the grass family, ranked fourth among important cereal crops after wheat, rice, and corn, prominent for its manifold usages as human food, animal forage and malting substrate (Loskutov and Khlestkina, 2021). Globally, barley is cultivated at about 47 million hectares with approximately total production of 151.62 million metric tons during the year 2022-2023 with Europe being the largest producer, contributing over 60% of the world's barley output (Mittal, 2022; Bouchetat, 2023).

In Pakistan, barley holds particular importance, especially in the arid and semi-arid regions where other cereal crops like wheat may not thrive as well (Tariq *et al*., 2021). From the last twelve years 2010-2022, data regarding barley area (77-41 thousand hectare) and production (71-44 thousand tons) showed declining trend. Barley is grown on an area of 40.7 thousand hectares, with a total production of around 43.6 thousand tons in 2022-23 (Pakistan Bureau of Statistics, 2022-23). Area under cultivation shared by different provinces during 2022–23, as mentioned in Figure 1, (Pakistan Bureau of Statistics, 2022-23).

Barley is categorized into two and six rowed with the difference of their grain's arrangement on barley head. The difference between these types lies in the arrangement of the grains on the barley head. Two-rowed barley typically has larger grains with higher starch content and lower protein levels, making it preferable for malting, especially in brewing industries (Knudsen *et al*., 2020). On the other hand, six-rowed barley has a higher protein content, which is more suitable for animal feed and value-added food products (Lang *et al*., 2013; Zwirek *et al*., 2019).

The major objective of this study was to evaluate two and six rowed barley genotypes in relation to yield and malt quality for industries that will contribute to enhance profitability. This study can also be helpful for developing desirable nutritional quality and high yielding cultivars through breeding programs resultantly increased cultivated area.

MATERIAL AND METHODS

The trial was conducted in a randomized complete block design (RCBD) with three replications having plot size 6m x 4 rows with 30cm inter-row spacing, using 10 barley genotypes, consisting of six 2-rowed (Sultan-17, B-21025, B-21034, B-21015, B-21008 and B-21022) and four 6-rowed (Pearl-21, B-21045, B-21046 and B-21044) at Wheat Research Institute (WRI), during the 2nd week of November, 2023-24. At maturity, crops were harvested and data were noted for test weight (kg/hl), starch (%), protein (%), wet gluten (%), dry gluten (%), gluten index (GI), falling number value (Sec) and 1000-grain weight (g) in ISO-17025 accredited, Cereal Technology Laboratory of WRI, Faisalabad. 1000-Grain weight (GW) was determined using Numigral II seed counter (Chopin, France) and electronic weighing balance (AND Company, Japan), whereas test weight (TW) was assessed with the help of bushel weight apparatus (Seedburo Company, USA). Falling number value (FNV) is an indicator of alpha amylase enzyme activity that was assessed with Falling Number apparatus, Parten, Sweden (Model No. FN 1310) in accordance with AACC Method 56-81B (2010). Starch and protein were measured by using Kernelyzer/Omeg Analyzer (Bruins instruments, Germany) while wet gluten (WG), dry gluten (DG) and gluten index (GI) were determined with glutomatic apparatus (Perten Instruments Company, Sweden) according to the AACC Method 38-12A (2010). DG was attained by drying of WG in Glutork apparatus at 150 ºC for 4 minutes. WG and GI were calculated by using the given formulae.

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

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

DATA ANALYSIS

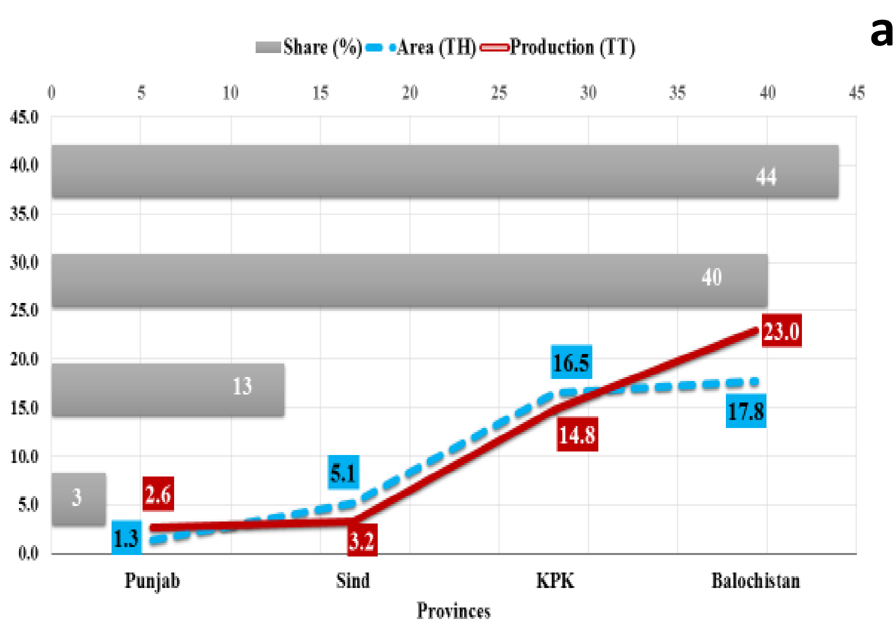
The data were subjected to analysis for mean variance of different traits using Tukey's test at 5% significance level (Tukey, 1991). For studying classification and screening of genotypes and traits, correlation plot and principal component analysis were practiced using "R Studio" (Team, 2020).

RESULTS AND DISCUSSION

Figure 1b described that mean values of P, WG, DG, and S were slightly greater in 2 rowed; however, GW, TW, and GI were found to be higher in 2-rowed as compared to 6-rowed barley genotypes. Mean variance of different quality attributes was given in Table 1. Among 2-rowed and 6-rowed barley, 2-rowed barley genotypes "B-21022" exhibited the lowest 11.4% as well as the highest 14.2% protein in cultivar "Sultan-17". Highest mean variance 49.9g for

GW was noted in 2-rowed "B-21008" and lowest 38.6g in 6-rowed "Pearl-21".

Two rowed "B-21034" had the highest 49.3Kg/hl TW whereas, 6-rowed "Pearl-21" had the lowest 62.4Kg/hl. Starch mean variance was observed maximum (62.6%) in 2-rowed barley variety "Pearl", while the lowest 57.6% was found in 6-rowed genotype "B-21044". Six rowed "Pearl-21" had the maximum FNV (277sec), while the lowest (256.3sec) was in 2-rowed "B-21022.".



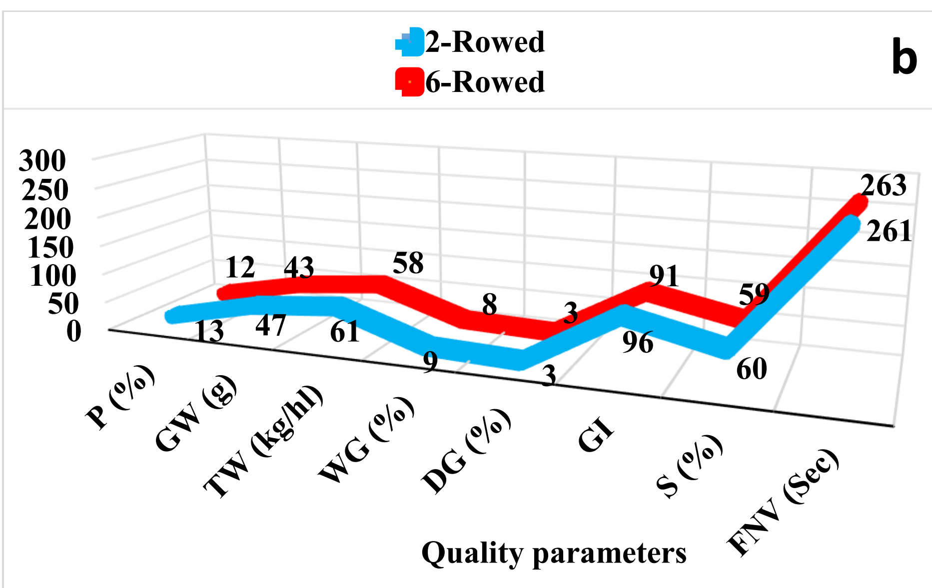


Figure 1: a) Barley area (thousand hectare), production (thousand tones) and percent area share in different Pakistan provinces (2022-23) b) Mean values of various quality traits of 2 and 6-rowed barley genotypes where P: Protein, GW: 1000-Grain weight, TW: Test weight, WG: Wet gluten, DG: Dry gluten, GI: gluten index, S: Starch, FNV: Falling number value

Table 1. Mean variance of various quality traits of barley

Sr. No.	**Genotypes**	**Rowed type**	**P** (%)	**GW (g)**	**TW** (kg/hl^{-1})	**WG** (%)	**DG** (%)	**S** (%)	**FNV (Sec)**
1.	Sultan-17	2	14.2a	44.7d	60.2f	10.0c	3.6a	62.4b	257.7g
2.	Pearl-21	6	14.1a	38.5h	49.3j	7.5	2.9a	62.6a	277.0a
3.	B-21025	2	12.8b	45.2c	61.3d	7.3f	2.4a	60.6c	266.3b
4.	B-21034	2	12.6b	51.2a	62.4a	11.2a	4.1a	59.0d	258.3g
5.	B-21015	2	12.1c	43.6d	58.5i	8.2e	3.2a	59.0d	265.3c
6.	B-21008	2	11.8c	49.9b	62.3b	8.0e	2.6a	58.0g	259.3f
7.	B-21045	6	11.8c	41.7g	60.8e	9.1d	3.3a	58.0g	262.7d
8.	B-21046	6	11.7cd	42.6e	59.7g	10.6b	3.7a	58.2f	261.3e
9.	B-21044	6	11.6cd	41.1g	59.6h	7.1f	2.5a	57.6h	256.4h
10.	B-21022	2	11.4de	49.9b	61.5c	8.1e	3.7a	58.4e	256.3h
C.V.			0.2	0.34	0.08	0.33	3.48	0.20	0.45

P: Protein, GW: 1000-Grain weight, TW: Test weight, WG: Wet gluten, DG: Dry gluten, S: Starch, FNV: Falling number value

Fregeau-Reid *et al*. (2001) reported protein content of 2-rowed barley higher than 6-rowed barley. Singh *et al.* (2005) found a range of protein (7.58 to 11.66%) for 6-rowed and (8.16 to 12.25%) for 2-rowed barley; the lowest value of 7.58% was noted in “K445-180” (6-rowed) and 8.16% in “K144-56” (2-rowed) genotypes. Lahouar *et al.* (2017) noted protein was higher in 6-rowed than 2-rowed genotypes. Elia *et al*. (2010) conducted a study on European 2-rowed barley with American 6-rowed barley and found more protein alleles in 2-rowed barley. Kandic *et al.* (2019) described that environment had strongest influence on protein and test weight (TW) in 2 and 6-rowed barley. Singh *et al.* (2005) observed 6-rowed barley had lower protein but highest starch values, maximum starch (73.08%) in 6-rowed and 71.86% in 2-rowed barley. Kong *et al.* (1995) stated 2-rowed barley had more starch than 6-rowed, while Eastern 2-rowed had more protein than Western 2-rowed barley. Hullless barley had more protein and starch than hulled barley, however, the amount of protein changed from year to year and with location. Bhatty (1993) reported that barley having 15% protein and used for feed. Moisture stress enhanced protein contents in barley (Grant *et al.* 1991). Kong *et al.* (1995) found that 2-rowed barley contained higher starch than 6-rowed; however, barley protein was affected by numerous factors including weather, soil pH, moisture level, sowing time and nitrogen rate. Ozturk (2019) noted 2-rowed barley had more 1000-GW and protein content than 6-rowed. Kolodinska-Brantestam *et al.* (2008); Ozturk *et al.* (2023) reported 1000-GW in 2-rowed than 6-rowed barley. Bensemane *et al.* (2011) noted 1000-GW higher in 2-rowed (49.9g) than 6–rowed (45.9g). Our findings regarding GW in 2-rowed barley are quite comparable to earlier researchers.

Protein levels in both types of barley in the current study were found to be comparable as reported by prior scientists. Seeds undergo metabolic changes under stress scenario in the field, whether natural or artificial, that result in shrinkage of the seeds, which raises protein levels and decreases the yield. Six-rowed barley "Sultan-17" exhibited maximum protein contents and can be utilized as animal feed, manufacturing malt as well as in crossing program with 6-rowed barley to enhance protein contents in filial generations. Further, the current study revealed that minimum protein identified in " Sultan-17" which is a nice gesture for the malting industry.

According to Kandic *et al.* (2019) test weight (TW) is a grain density or also called weight per unit volume of a grain and observed high TW in 2-rowed as compared to 6-rowed barley. Fregeau-Reid *et al.* (2001); Dekic *et al*. (2017) noted that 2-rowed barley produced larger seeds with high TW. Yadav *et al.* (2001) reported that TW of 2-rowed barley was higher than 6-rowed. Deivasigamani and Swaminathan (2018) stated TW is a key predictor for determining grain density, grain quality and milling yield. Robert Kratochvil (2016) reported that maximum TW was attained when crop was harvested at optimum moisture level. According to Conley and John (2013), TW is a crucial trait that was significantly affected by environment. Our results in the current study depicted that TW in 2-rowed barley was found to be higher than 6-rowed, as per prior scientist's findings. "B-21034" might be a suitable option for higher quality grain in the present study because of its highest TW and GW.

Gebregewergis (2024) described that enzyme activity was measured by FNV that affect product quality, excessive enzyme activity suggested high sugar content and low starch value that caused sticky dough and poor texture of the final product. High FNV (> 300 sec) showed less enzyme activity and good quality flour, whereas low FNV (< 250 sec) described substantial enzyme activity that made the flour unfit for consumer end products. In present study, FNV was observed higher than 250 seconds in all genotypes which depicted that flour of these genotypes may be quite suitable for most types of bakery products.

According to Mauro, (1996) starch is composed of polymers, amylose and amylopectin. Barley starch contained 70 to 80% amylose and 20 to 30% amylopectin which were controlled by genetic factor (Xue *et al*., 1997). Washington *et al*. (2000) described that a gene (amo 1) at 7th chromosome regulated the formation of amylose, whereas another gene (wx) at the 7th chromosome controlled the production of amylopectin. High alpha amylase activity in barley causes quality issues such as sticky crumb, low viscosity, low falling number, collapsed loaves as well as lower grain yield and financial loss (Fu *et al.* 2014). Alpha amylase activity also enhanced when humidity of the storage facilities was higher than the recommended relative humidity < 60%. At high alpha amylase activity, amylose and amylopectin broke down into simple sugar which caused grain germination and sometime affected grains may turn black (Zhao, 2018).

Albumin, globulin, prolamin, gliadin, and glutenin are five major proteins found in cereals. First three are minor while the last two are major proteins, which form gluten. Gluten forms more than 80% of total cereal protein. In gluten, gliadin provides extensibility while glutenin gives elasticity. Therefore, gluten has viscoelastic property, which is desirable trait for chapatti, bread and cakes production. Gluten index (GI) is the gluten quality and its range from 0-100. Barley has less wet and dry gluten than wheat and can be added to a variety of food products for value addition.

Oikonomou *et al.* (2015) categorized GI into four grades based on concentration; poor (less than 50), moderate (51-70), strong (70–85) and very high (above 86). In this study, B-21022, B-21034, B-21045, B-21015, B-21044, and Sultan-17 have 98 GI, B-21008 has 90, Pearl-21 and B-21025 have 85 and B-21046 has 80 which shows that all genotypes are best for bakery products and good quality flour as well as their addition in the nutritive edibles. Environmental factors including sowing date, fertilizes, irrigations and soil status had great impact on yield and quality characteristics of different barley genotypes in different regions. Late sowing and scarce irrigation increase the protein contents while reduce GW and starch content. However, judicious use of fertilizers in deficient soil increase the protein and gluten content of various genotypes as similar findings noted by Kong *et al.* (1995).

CORRELATION AMONG QUALITY ATTRIBUTES

1000-Grain weight (GW) showed positive significant correlation with TW, WG, and DG while negative association with protein, starch and FNV as depicted in Figure 2. Grain weight depicted highest positive significant correlation with TW, while highest negative relation with FNV. Protein exhibited positive correlation with starch, FNV and WG while negative association with GW, TW and GI. Protein revealed maximum positive association with starch and the highest negative association with TW.

GI positively correlated with GW, TW, DG, and WG while negative associated with protein, starch and FNV. GI showed maximum positive correlation with TW and most negative correlation with FNV whereas, FNV revealed highest positive association with starch and lowest correlation with TW. WG and DG showed positive relations with GW, TW, protein, DG, and GI while negatively correlated with starch and FNV. WG showed the highest correlation with DG and the highly negative association with FNV. Higher WG and DG values revealed that barley cultivars had good quantity of gluten which are important type of protein.

Nielsen (2014) stated that a correlation is found between test TW and grain yield. Deivasigamani and Swaminathan (2018) studied that 1000-GW significantly impacted TW but had no appreciable impact on germination. The finding of earlier researchers strongly supports our results that GW positively correlates with TW. A strong positive correlation between GW and TW is encouraging because genotypes selected on the basis of these traits always produced high yield and good quality grain. Moreover, higher GW may enhance malt quality of barley cultivars considerably.

Singh *et al.* (2005) observed that protein had inverse correlation with starch in 6 and 2-rowed barley. Kandic *et al.* (2019) reported that protein contents were negatively correlated with yield in both barley types. Reinhardt *et al.* (2013) reported that high temperature at grain filling stage increased the protein contents of barley. Kandic *et al.* (2019) reported that yield and protein contents had strong associations in 2-rowed barley while negative correlation was noted for these traits in 6-rowed under both normal and drought conditions.

Fregeau-Reid *et al.* (2001) noted that protein and β-glucan were not correlated in 2 and 6-rowed barley. Our study strongly supports to some earlier scientist's findings that protein has negative association with GW for both types of barley under normal condition. Khande *et al*. (2023) found that protein had positive and highly significant correlation with GI while negative and non-significant correlation with TW in wheat under different environments. Dogan (2009) reported that agronomic traits inversely correlate with quality parameters; however, 1000-GW had a positive correlation with TW. Desheva and Deshev (2022) found that 1000-GW had a negative and non-significant correlation with crude protein (-0.205), but WG and DG had a substantial and positive correlation (0.952) and the highest R^2 (0.9064) value between WG and DG.

Gulia and Khatkar (2015) and Desheva (2016) reported significant association between WG and DG. Ozturk (2019) noted 1000-GW and TW had slightly significant effect on protein contents in 6-rowed barley. Warechowska *et al.* (2013) and Upadhyay (2020) studied that TW increased with 1000-GW and stronger correlation were found between these traits. In present study, protein and starch have inverse linked with GW and GI, however, both TW and GI positively correlate with GW. The quality traits including GW, starch and protein contents significantly contribute for improving barley malt quality.

PRINCIPAL COMPONENT ANALYSIS (PCA)

Principal component analysis (PCA) is mostly utilized in many breeding programs to know contribution of each trait and total variability. PCA was applied on eight barley quality traits of 10 genotypes in order to assess independent effects of each trait and genotype. Figure 3a showed the genotype scores in the scoring plot while the loading plot illustrated the trait scores.

INDIVIDUALS - PCA

Genotypes located on the basis of PC1 and PC2 are depicted by the score plot in Figure 3b. First principal component (PC1) contributed 47.2%, second principal component (PC2) added 24.2% and total variability comprised about 71.4%. The first quadrant showed positive correlation with PC1 and PC2 having genotypes B-21034 and B-21046. The second quadrant positively correlated with PC1 and negatively correlated with PC2 that depicted five genotypes, viz. B-21022, B-21008, B-21034, B-21044, and B-21045. Third quadrant contained B-21025 and B-21015 which were negatively linked with PC1 and PC2. Fourth quadrant positively correlated with PC2 but negatively with PC1, consisting barley cultivars “Pearl-21 and Sultan-17”.

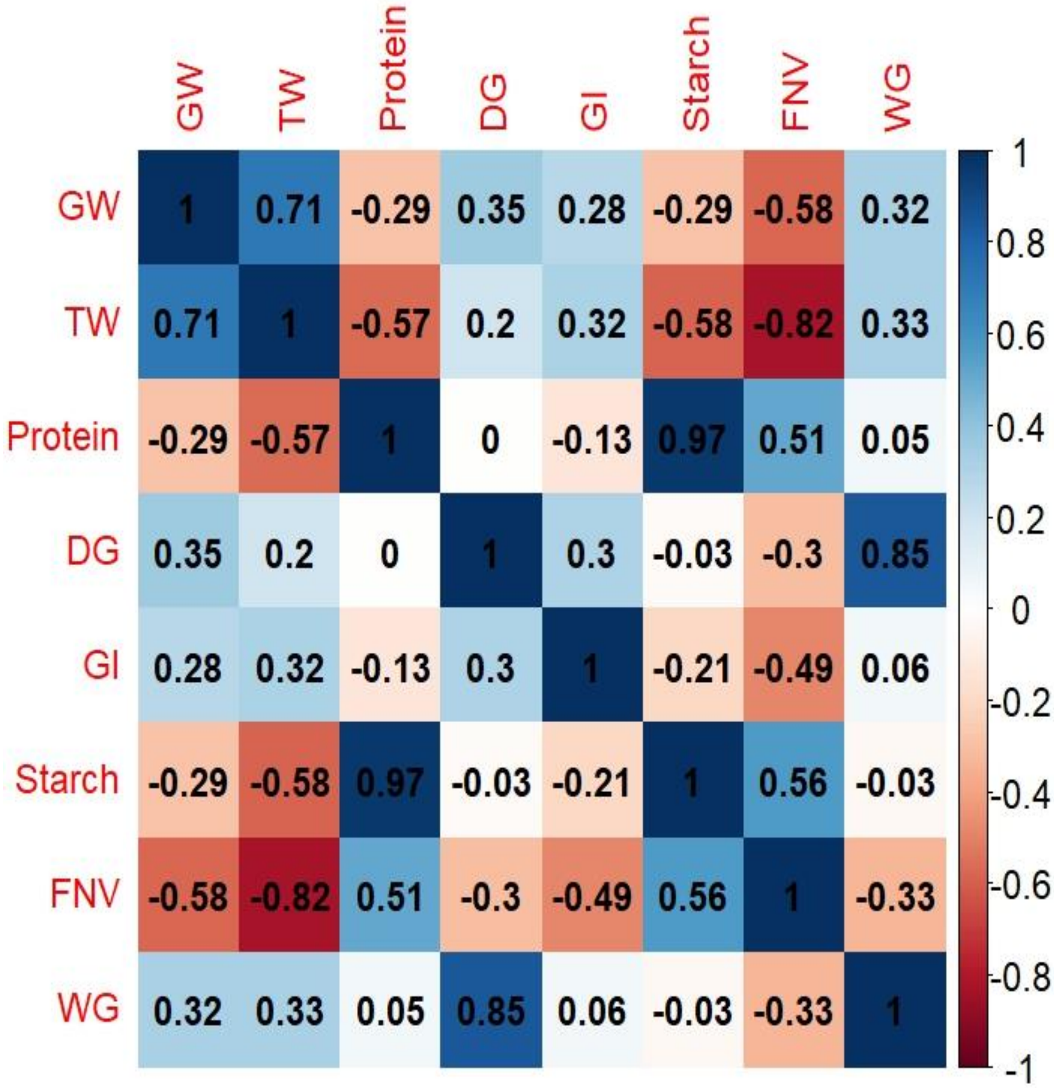


Figure 2: Correlation plot study between quality parameters of barley

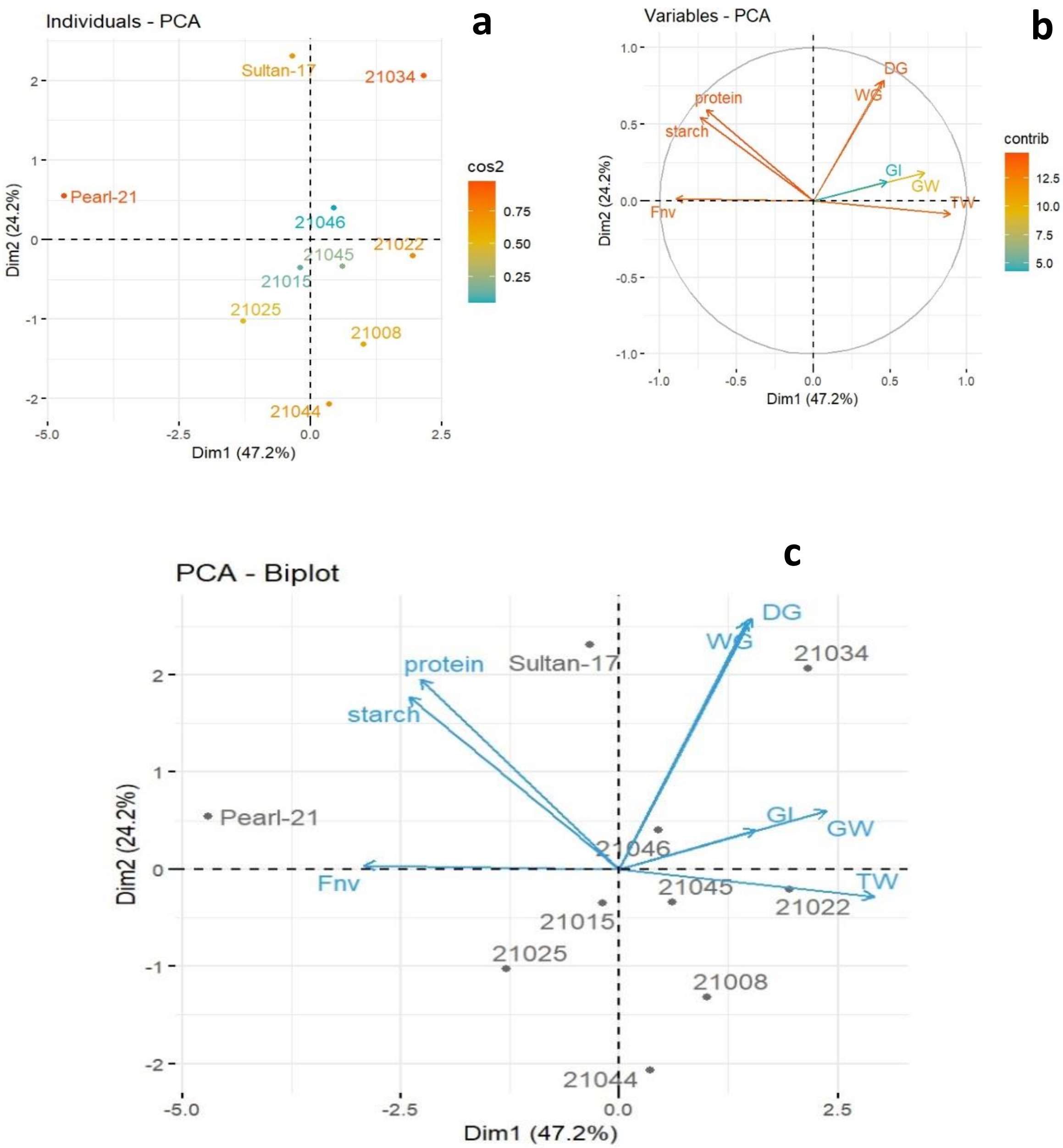


Figure 3: Effect of principal component analysis on 2 and 6-rowed barley a) genotypes b) quality parameters c) genotypes and quality traits

Variables - PCA

Figure 3c showed that loading plot was created between PC1 and PC2 using the variability of both components to find interaction in various quality traits. First quadrant comprised of DG, WG, GI and GW, however DG and WG are highly associated whereas GI and GW also showed the same trend. TW is only the trait present in second quadrant. There were no quality traits in the third quadrant but the fourth quadrant exhibited protein and starch which are highly linked. According to Bensemane *et al*. (2011), PCA for two and six-rowed lines revealed that row type had a significant impact on GW. Christina *et al.* (2021) stated that traits had acute angle (< 90°) between them in each group showed variations that were comparable, so each trait in a specific group can be noted rather than the other traits within the same group. Based on the loading plot, a very small angle was observed between GI and GW as well as for WG and DG. Both GI and GW had acute angle (< 90°) with WG & DG which suggested that they had interrelated traits and showed positive correlations. Conversely, GI and GW exhibited obtuse angles (> 90°) with respect to starch, protein and FNV, indicating a negative association between these variables.

PCA - BIPLOT

A biplot was created between PC1 and PC2 using variability of various traits and genotypes for interaction between the components. According to Christina *et al.* (2021), biplot provides a better image for comparison between different genotypes, based on different traits observed at the same time. Quality traits were found in the first, second, and fourth quarters, whereas the genotypes were scattered throughout all four quadrants in Figure 3c. Genotypes B-21034" had strong association with the quality characters viz., DG, WG, GI, and GW in the 1^{st} quadrant. Genotype "B-21008" had high PC values and strongly associated with TW as compared to other genotypes in 2^{nd} quadrant. Sultan-17 existed in 4^{th} quadrant with strong association for protein and starch, Genotypes present in a similar direction of trait vectors showed greater values for that trait. The information obtained from this study can be utilized in crossing programs for maximum genetic diversity and transferring high quality features in next generations.

CONCLUSION

This study concludes that GW, TW and GI were higher in 2-rowed barley, while FNV were greater in 6-rowed barley. Positive associations were found among starch, protein, and FNV while GW, TW, GI, WG and DG revealed positive link. Grain weight helps for increasing grain yield in two and six rowed barley genotypes. Grain weight, starch and protein content play significant role to improve malt quality and thus useful for industrial products. Breeders should choose best genotypes based on protein, starch, WG and GW for evolving cultivars of high yield and better nutritive quality. Based on PCA and mean variance, B-21034 was found to be the best line with high WG, DG, GI and GW values for yield while Sultan-17 can be utilized in industries due to highest values of starch and protein.

ACKNOWLEDGEMENTS

The authors acknowledged the efforts of Statistical Section, Ayub Agricultural Research Institute, Faisalabad, for providing such useful data visualization using R-Software.

CONFLICT OF INTERESTS

All author has not any conflicts of interest.

AUTHOR CONTRIBUTIONS

Muhammad Ilyas Khokhar collected the relevant material and wrote up the manuscript; Muhammad Awais Ashraf conducted the experiment; Muhammad Abdullah examined the quality data and assisted in proofreading; Majid Nadeem helped to collect references; Javed Ahmad supervised the research, Muhammad Zulkiffal helped in proofreading and Amir Hameed analyzed the data.

FUNDING:

There is no funding to show

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