



Research Article

Evaluation of Genetic Diversity in Barley Landraces through Agro-Morphological and Biochemical Characterization

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Abstract | Genetic diversity can be investigated through different methods such as morphological traits, biochemical (SDS PAGE) and molecular markers. The present work was conducted on the basis of morphological and biochemical characterization in order to estimate the genetic diversity among the barley landraces; to explore significant variation which can be used in breeding programs. For this purpose, 40 barley landraces were collected from Dir Lower and Swat, districts of KP, Pakistan. A total of 18 traits were recorded, 10 qualitative characters including spike density, awn barbs, glume awn, lemma type and awn color, length of the rachilla hairs, lemma color, auricle pigmentation, photoperiod sensitivity and stem pigmentation. Variation was observed in these qualitative traits through frequency distribution. In quantitative traits maximum variation was found for plant height ranging from 36cm to 50cm, days to flowering range from 130 to 133 days), while 100 seed weight ranged from 36 to 86 g and plant biomass range from 50 to 199.5 g per plant. SDS-PAGE analyses of total seed storage protein resulted in a total of 18 polymorphic bands. Total genetic diversity on the basis of total seed storage protein analysis was 17.5%. In Band, the 14 total genetic diversity was (0.60%) followed by Band 16 (0.58%) and Band 17 (0.55%). Similarly, Band 3 showed diversity, while Band 8, 4 and 5 indicated 0.45, 0.38 and 0.35%, respectively. A cluster dendrogram tree was constructed which was divided into two linkage and further divided into seven clusters. The variation was found among the clusters. The information obtained from the study is useful in planning further crop improvement.

Received | February 26, 2021; **Accepted** | May 18, 2021; **Published** | July 11, 2021

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Citation | Khan, A., M. Ihsan, M. Nisar, A. Hazrat, M. Ali, R.U. Haq, K. Khan, K. Gul and S. Faisal. 2021. Evaluation of genetic diversity in barley landraces through agro-morphological and biochemical characterization. *Sarhad Journal of Agriculture*, 37(3): 984-992.

DOI | <https://dx.doi.org/10.17582/journal.sja/2021/37.3.984.992>

Keywords | Barley, Morphology, Cluster analysis, PCA, SDS-PAGE

Intorduction

Barley is an important cereal crop and ranks around fourth in the world cereal production statistics following wheat, maize and rice (USDA-2008). It is estimated that annually over 133.85 million tones barley is produced worldwide. Barley is used as feed, food and for the brewing and distilling industries

(Morris and Bryce, 2000). Barley is containing 80% carbohydrates which is an important source of energy. In the roman times, barley was the most popular food of providing a high energy, before going to the circus. The gladiators were fed on barley diet, so they were called "hordeari" from hordeium. Researchers also found that the amount of cholesterol in humans is decreased in blood serum due to the consistency of

non-starch polysaccharides in barley, also known as glucans (Zeeshan *et al.*, 2012).

As well as being more important to humans, barley is a major nutritional part of the human diet, contributing to a significant portion of the global human diet. Barley is also used as beer and fodder form of animal feed (Finnie and Svensson, 2009). Due to barley tolerant, various environmental factor it adopt itself. Barley is distributed all over the globe in different areas such as the sub-Arctic, cooler tropical highlands and sub-tropics, temperate climates, high altitudes and saline soils of the maritime zone. Indeed, one of the few areas where barley is not found on Earth is the tropical lowlands because it is more hot and humid regions on earth (Ali *et al.*, 2020). As early as 13,000 years ago in Eurasia (Ukraine) the first cultivated plant was barley. In various cultures barley used in soups and stews and barley bread. Barley grains are commonly made into malt in a traditional and ancient method of preparation (Zohary and Hopf, 2020).

In order to effectively conserve, evaluate, and use germplasm, researchers must look into the estimate of genetic variation available. Genetic diversity is an integral aspect of any agricultural production system, in breeding, modification in germplasm effectively, the investigation of genetic diversity important, in the first part of the last century, recognized the importance of genetic variation in the germplasm of crop organisms (Smykal *et al.*, 2018). Morphological characterization is considered an important step in description and classification of crop germplasm because a breeding program depends on the magnitude of genetic variability, among biochemical techniques, Sodium Dodecyl Sulphate Poly acryl amide Gel Electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm (Raza *et al.*, 2020). The development of cultivars with enhanced agronomic characteristics is most easily accomplished in genetically diverse plants. Consequently, the presence and estimation of genetic variability within such organisms is important for plant breeding programs (Ghafoor *et al.*, 2005). Genetic variation within and between crop populations is determined by techniques such as morphological characterization, Protein profiling and DNA (or molecular) marker analysis (Nisar *et al.*, 2008; Ghafoor and Arshad, 2008).

One important step forward is the assessment of

diversity among varieties and section of best variety. To analyze diversity, molecular techniques such as protein electrophoresis due to high polymorphism and morphological characterization are acceptable methods. Via morphological characterization, total seed storage proteins and selection of elite (high yielding) genotypes, the purpose of this study is to estimate genetic diversity among 40 barley landraces.

Materials and Methods

The present work was carried out under field condition in the Department of Botany, Botanical Garden University of Malakand, KP, Pakistan. Total of 40 landraces were evaluated for both morphological and biochemical characterizations. The seeds were collected from various locations of District Dir and Swat that represent a wide range of ecological zones from dry mountains to irrigated plains. The Landraces were planted on October 28, 2019. Block field design was used for the experiment (following Randomized Completer Blocked Design with three replicates), the row length was 2 m with 20 cm space between rows. Each genotype was planted in adjacent rows, where intra variety distance (between the seed) was 12 cm.

Morphological/ agronomic characterization

Both quantitative and qualitative traits were measured. 10 were qualitative which are Aluerone color, Lemma color, Grain color, Awn color, Awn barbs, Kernel covering, Seed color (SC), Glumes color, Stem pigmentation, Auricle pigmentation, Length of rechilla hairs, Lemma type, Lemma with awn, Glume and Glume Awns, and 8 quantitative traits which are Days to germination (DG), Days to Flowering (DF), Days to Maturity (DM), Plant height, Spike length, Seed per spike, 100 Seed Weight, biomass/plant.

Biochemical characterization

All the collected genotypes used in agro morphological characterizations, subject to the total seed storage proteins based on SDS-PAGE analyses. Five healthy and mature seeds with mortar and pestle were ground manually. A sample of 0.02 g grinded was weighed and transferred to 1.5 ml centrifuge tubes, where the tube was added with a 400µl protein extraction buffer. The powder was homogenized at room temperature for 1 minute before centrifugation at 12,000 rpm for 10 minutes (RT). The 12. 25% polyacrylamide gels were used for total seed protein, following Laemmli's 1970 protocol (He, 2011).

Table 1: Descriptive statistics for eight quantitative character of 40 barley genotype.

Parameters	Mean	Standard error	Standard deviation	Sample variance	Minimum	Maximum	CV%
Days to maturity	178.4	1.05	6.65	44.25	167	185	3.73
Days to flowering	140.6	0.74	4.7	22.09	133	146	3.34
Plant height	43.78	0.6	3.77	14.23	36	50	8.62
Spike length	10.4	0.22	1.41	1.99	7	13	13.56
Seed per spike	72.25	2.38	15.07	227.12	36	108	20.86
100seed weight	56.59	2.09	13.21	174.45	37.9	86.5	23.34
Biomass/plant	119.3	7.95	50.27	2526.77	50	199.6	42.13

Table 2: Correlation among the eight quantitative traits of 40 germplasm.

Parameters	DM	DF	PH	SPKL	S/SPK	100 SWT	BM	HI
DM	1							
DF	.387*	1						
PH	0.125	.689**	1					
SPKL	-0.05	-0.169	-0.118	1				
S/SPK	0.007	-0.017	0.027	-0.051	1			
100SWT	-0.208	-.557**	-.524**	0.083	-0.11	1		
BM	0.198	0.239	.340*	0.022	-0.142	-0.139	1	
HI	0.193	0.208	0.02	0.04	0.001	-0.204	.553**	1

Five plants were randomly selected to measure genetic diversity, and then the mean value of each genotype was used for data analysis. Using Microsoft Excel, Basic statistics were computed (mean, maximum, minimum, and coefficient of variance). Using cluster and main component analysis, a total of 14 morphological characteristics were identified and analyzed. Correlation analysis was estimated using Statistica 7 and version 2.1 of NTSys PC (Applied Biostatistics Inc, USA). Binary matrix data for total seeds storage proteins were measured as band presence and absence. Dendrogram was computed using the unweighted pair group approach with the arithmetic average (UPGMA) algorithm for cluster analysis. For possible pairs of electrophograms, the electrophoresis band spectra and similarity index were computed.

Results and Discussion

Genetic diversity based on agro morphological characterization. During the present study both quantitative (8) and qualitative (10) traits were studied in the collected genotypes of Barley from Dir Lower and Swat districts. The purpose of the study to identify with the aid of morphological character the genetic diversity among the barley genotypes and to find out high yielding genotype using protein

profiling. During the present analysis (correlation analysis Principal Component Analysis and Cluster analysis) of both qualitative and quantitative traits of 40 barley genotypes were examined. Morphological and agronomic characteristics of the germplasm collected promote the identification and selection of suitable characteristics (Ghafoor *et al.*, 2002; Khan *et al.*, 2019).

Quantitative parameters

During the present study eight quantitative characters were studied as presented in Table 3, such as, days to maturity, days to flowering, plant height, spike length, seed per spike, 100 seed weight, biomass per plant, harvest index. Mean value, standard error, standard deviation, sample variance, minimum, maximum and CV% as presented in Table 3. During the present study a significant diversity was found in days to flowering, early (130 to 133days), moderate (137 to 138 days) and high (144 to 146). With the mean value of 140.60 and sample variance 22.09. A wide range of variation were noted for days to maturity with the range of 167 to 185 days, with the mean value of 178.40 and sample variance 44.25. During the present a number of variation was found for spike length, low (range from 7-9 cm), medium (range 10-11 cm) and high (range from 12-13 cm) with mean

value of 10.40 and sample variance 1.99. Maximum no. of landraces had medium spike length (55%) followed by low spike length (25%) and high spike length (20%). In case of plant height, there was a wide range of variability found among the landraces. The range of plant height between 36cm to 50cm, with mean value of 43.78, and sample variance of 14.23. Low (36 to 40cm), medium (41 to 44cm) and high (45 to 50cm). The number of seed per spike in 40 landraces are divided into three categories, low (36 to 72) medium (72 to 80) and high (82 to 108), with the mean value of 72.25 and sample variance 227.12. A wide range of variation was found among the landraces on the base of 100 seed weight. Range from 37g to 86.5g with mean value of 56.59 and sample variance 174.45. Significant variation was observed during the study on the base of biomass per plant range from (50 to 199.5). In present study, high variation was found in spike length (from 7 to 11cm show 80%) in barley germplasm. [Lasa et al. \(2001\)](#) investigated the morphological characterization, both qualitative and quantitative traits, which shown similarities and variation like present study, especially in kernel covering. [Shegaw et al. \(2013\)](#) also reported a wide range of variation in plant height, days to maturity and grain yield. Similarly, ([Alemayehu and Parlevliet, 1997](#)), reported days to maturity, plant height, during this present study the germplasm had broad range of days to maturity (167 to 185 days) and plant height (36cm to 50cm). The results of this study are very much in accordance with those of [Gerano et al. \(2014\)](#) assessed 22 morphological variability accessions for sorghum. Nine qualitative and 20 quantitative parameters were studied and found to be highly diversified in agro-morphological characters, including panicle compactness and shape, glume color, flowering days to 50%, plant height, panicle width, grain yield, seed color and leaf midrib color.

Qualitative traits

During the present study, a total of 10 qualitative traits were studied. On the base of spike density, there are two types of spike density (lax and dense) were observed. In 40 landraces of barley, the frequency of lax (15%) 6, while frequency of intermediate was (85%) 34, which show divergence among the landraces. Among the 40 landraces the awn barbs found are smooth which have (87.5%) frequency 35, intermediate (12.5%) frequency 5. The glume with awn length compare kernel length glumes and awn shorter than kernel, which present in (40%). In 16 landraces

(out of 40), length of the glumes and awn longer than kernel with frequency (27.5%). In 11 and glume plus awn longer than kernel with a frequency (12.5%), and in 5 showed variation among these landraces. A wide range of variability found in lemma type, on lemma have no teeth was found with frequency (22.5%) 9 and lemma with teeth with frequency (77.5%). The lemma color, normal (green color) with frequency (95%) 38, red color frequency had (2.5%) and purple color with frequency (2.5%) ([Table 1](#)). In case of awn color, the white color was found with frequency of (17.5%) 7, the yellow color with frequency of (77.5%) 31, reddish color had (5%) 2. The length of rachilla hairs was recorded which are of two types, long hair with frequency (52.5%) 21 and short with frequency (47.5%) 19. There were three type color on the base of auricle pigmentation, which were observed in these landraces, the dark purple color were not found, the green color was found with frequency (90%) 36, pale purple with frequency (7.5%) 3 and the purple color had 1 with 2.5%. There were three type of stem pigmentation was found, 5 with green color (12.5%), 32 with purple color (80%) and 3 of them was with purple color (half or more) 7.5% ([Table 1](#)). [Zeeshan et al. \(2012\)](#) studied 151 accessions of barley for agro morphological variation. A total of 4 traits were quantitative and 10 were qualitative with a significant variation. It includes spike density, glume color, awn roughness, awn color, number of spikelet per spike, rows of per spike, glume and glume awns, kernel covering, lemma color, rachilla hairs, grain color, awn length, 100 seed weight, spike length.

Correlation analysis

Correlation analysis of 40 landraces was conducted for eight agro-morphological characters by SPSS 16.1. During the present study days to flowering was found significantly correlated with days to maturity with the value of .387*, followed by plant height found strongly significant with days to flowering. 100 seed weight 0 was found negatively correlated with days to flowering -.557** and plant height with the value of -.524**. Similarly, plant biomass was found significantly correlated with plant height with the value of .340* and harvest index was found strongly negatively with plant biomass with the value of -.553** respectively ([Table 2](#)). The same result was obtained by [Eshaghi \(2009\)](#) also reported the same result for the positive and negative correlation of the barley germplasm for this study he used 63 genotype for agro morphological and biochemical variation

among barley germplasm. Ebrahim *et al.* (2015) also investigate the same result of correlation for quantitative traits of 20 genotype of barley germplasm. Similar results for grain yield and plant height in barley were reported by other studies (Kisana *et al.*, 1999; Wisal *et al.*, 2109; Arshad *et al.*, 2019).

Table 3: Principal component analysis of 9 quantitative traits of 40 Barely landraces.

AXIS	PC1	PC2	PC3	PC4
% of Variance	38.25	18.12	14.18	10.69
Cum. % of Var.	38.25	56.37	70.55	81.24
Eigenvalue	2.829	1.829	1.329	0.996
DM	-0.34	0.608	0.009	0.015
DG	-0.34	0.608	0.009	0.015
DF	-0.43	-0.08	-0.25	-0.13
PH	-0.38	-0.33	-0.2	-0.04
SL	0.077	0.025	0.428	0.695
Sp/s	0.006	0.013	-0.51	0.679
100 W	0.352	0.206	0.313	-0.15
Bm/p	-0.37	-0.17	0.511	0.043
Hi	0.424	0.265	-0.3	-0.11

Principal component analysis

Principle component analysis was conducted based on morphological traits of different Barely genotypes. It was observed that the total variation was 81.24% with the Eigen value of 0.99% (Table 3; Figure 1). In PC1 the total variation was 38.25% and it was found that days to maturity-0.34, days to germination-0.34, days to flowering-0.43, plant height -0.38 and biomass -0.37 were found negative weight while seed length -0.07, seed per spike 0.006 were found positive weight. In PC2 the total variation was 56.37% and it was observed that days to maturity 0.60, days to germination 0.60, seed length 0.02, 100 seed weight 0.20 and harvest index 0.26 were found positive while days to flowering -0.08, plant height -0.33and plant biomass -0.17 were found negative weight. In addition, the total variation in PC3 was 70.55% and it was found that days to maturity 0.09, days to germination 0.08, seed length 0.42, 100seed weight 0.31 and biomass 0.51 were found positive while the remaining traits days to flowering -0.25, plant height -0.20, and seed per spike -0.51 were contributed negative weight. Similarly, in PC4 the total variation was 681.24% and the contribution of days to maturity 0.01, days to germination 0.01, seed length 0.69, seed

per spike 0.67 and biomass 0.04 were found positive while days to flowering -0.13, plant height -0.04 and harvest index -0.11 were found negative weight in PC4. (Table 3). Similarly, the scattering plot is also closely related for morphological analyzes which show genetic heterogeneity in the 40 genotypes in quantitative traits and similarities between closely associated genotype, which demonstrates similarity. The scatter plot was divided into two groups in term of direction. Mzid *et al.* (2016) who reported different quantitative traits in his study.

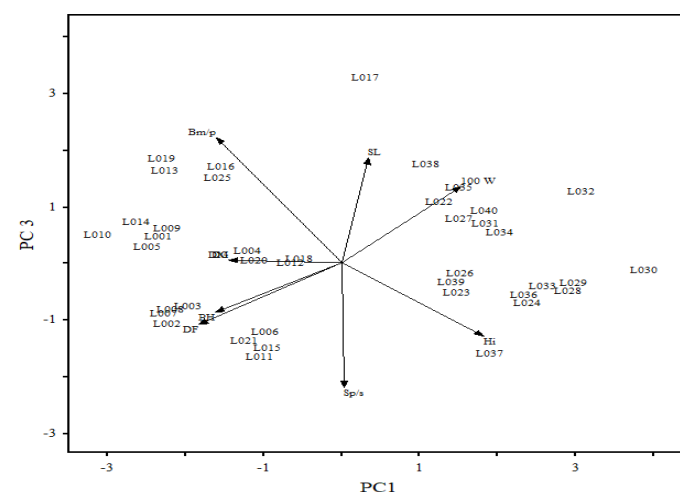


Figure 1: Scatter diagram of PC1 and PC3 of agro morphological traits of Barely genotypes.

Total seed storage proteins

To find out the genetic divergence in the landraces, SDS PAGE was used. It was found that genotypes showed substantial levels of genetic divergence on the basis of protein studies. Fifteen percent polyacrylamide gel was used during the current work. A total of 18 polymorphic subunit were observed. The total genetic diversity found in Barley was f 17.5 percent. In Band 14 the genetic variation was (0.60%), followed by Band 16 (0.58%), Band 17and Band10 was (0.55%) and Band 2, Band 11, Band 12, Band 15 (0.53%) polymorphism respectively. Band 1, Band 6, Band 18 (0.50%), Band 7 and Band 13 (0.48%), Band 8 (0.45), Band 4, Band 9 (0.38%) and Band 5 (0.35%). Similarly, Band 3 revealed low level of genetic variation (0.33%). Here the binary data matrix of total seed storage protein a cluster dendrogram was constructed and all the 40 genotypes were divided into 2 main lineages at a distance of 12.5% and further divided into 7 sub cluster. Lineage 1 divided into 5 sub cluster while lineage 2 divided into 2 sub cluster. Each cluster members shows less variation to one another but shows high number of genetic variation to the members of the other groups.

Figure 2 cluster 1 of lineage 1 consists of 8 landraces, such as (L001, L008, L011, L002, L005, L007, L010, L040), cluster 2 contain 3 landraces (L003, L022, L033), cluster 3 contain 4 landraces (L012, L016, L014, L017) cluster 4 also contain 7 landraces (L013, L015, L026, L021, L024, L035, L027), cluster 5 contain 7 landraces (L018, L029, L032, L020, L038, L028, L031), cluster 6 contain 4 landraces (L004, L006, L009, L039) and cluster 7 had contain consist of 7 landraces (L016, L025, L030, L037, L023, L034, L036). Total seed protein analysis based on SDS-PAGE play an important role in estimation of genetic diversity (Zahoor *et al.*, 2015). It was estimated since several years that the morphological character of the different crop species (Nisar *et al.*, 2016), but the biochemical methods of genetic diversity are now more beneficial than classical morphology (Akdeniz *et al.*, 2004). PAGE for SDS Different plant grains have already been produced by electrophoresis of seed protein (Ghafoor *et al.*, 2002; Nisar *et al.*, 2008, 2009; Hameed *et al.*, 2009). SDS-PAGE is a biochemical technology used to identify the genetic structure of different plants because it is simple, reliable and free of fluctuations. Protein profiling (Ghafoor *et al.*, 2005). Researchers approved the valid evidence for detection of intra-specific variation and evaluation of inter-specific relationships in the SDS PAGE biochemical protein analysis (Hameed *et al.*, 2009; Irfan *et al.*, 2010). SDS PAGE data was not suited for analysis, according to (Zeeshan *et al.*, 2012) says that it worked on the 200 barley accession, but bands were not clear.

Similarly, in other crops (Abdul and Tahir, 2014) who study 23 genotype pea total 21 bands was found which show variation. In present study of Barely a significant variation was observe in bands 14 (60%) and Band 16 (58%). The variation in protein bands express the genetic diversity present in the seed storage protein, the selection of elite variety or improvement in crops are depend on genetic variation (Figure 1). Daniele *et al.* (2015), according to this when the genetic diversity high in a species then higher the chance of improvement in that species. Dendrogram of the population, Different landraces that are genetically different from each other are discriminated against by a separate cluster in a tree in different classes or genetically identical genotypes (Govindaraj *et al.*, 2015). PCA is a multivariate method that is employed to complement the cluster analysis information and produce a dimensional germplasm scatter plot more

descriptive of genotype distance (Hassan *et al.*, 2016). For the current analysis, PCA (scatter plot) also showed variations between lands that were divided into different classes based on their similarities and differences (Figure 2).

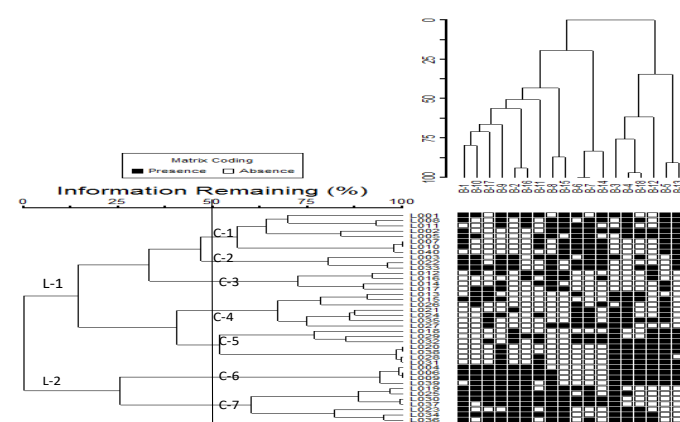


Figure 2: Dendrogram tree for the protein data profiling of 40 landraces of barley.

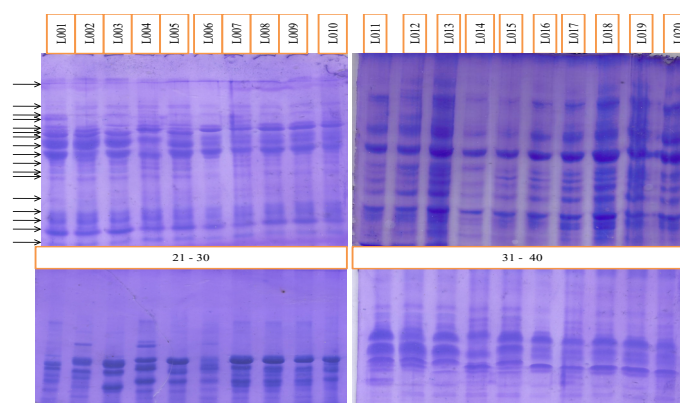


Figure 3: The gel show the protein bands pattern and their existing, of 10 landraces out of 40 germplasm of barley.

The landraces were collected from the districts of Dir Lower and Swat, KP- Pakistan. Passport information of the collected genotypes was presented in Table 4.

Conclusions and Recommendations

It was concluded for investigation of genetic divergence, ten qualitative traits utmost level of variation was found. In case of quantitative characters, maximum variation was found for plant height and days to flowering, while minimum variation was observed for 100 seed weight. A considerable variation was observed in SDS-PAGE analyses of seed storage protein given significant result. In 18 Bands, variation was observed among the clusters. It was suggested that representative landraces from different clusters could be chosen for the future breeding program.

Table 4: Passport information of the present genotypes.

Genotype no.	Location name	District
L001	Serai	Dir lower
L002	Tarnao	Dir lower
L003	Kityari	Dir lower
L004	Birand	Dir lower
L005	Bagh derai	Dir lower
L006	Danda	Dir lower
L007	Asbanr	Dir lower
L008	Khawas	Dir lower
L009	Khadagzai	Dir lower
L010	Khadang talash	Dir lower
L011	Osakai	Dir lower
L012	Taknai	Dir lower
L013	Kabaloo	Dir lower
L014	Bnada talash	Dir lower
L015	Dando shah	Dir lower
L016	Fazal mamond	Dir lower
L017	Hashm monda	Dir lower
L018	Ughaz	Dir lower
L019	Nawagi	Dir lower
L020	Seyyah	Dir lower
L021	Batkhela	Malakand
L022	Kamala	Malakand
L023	Sogyar	Malakand
L024	Pirana	Malakand
L025	Aladand	Malakand
L026	Nagram	Dir upper
L027	U-1	Dir upper
L028	U-2	Dir upper
L029	Bahrain	Swat
L030	Madayn	Swat
L031	Kalkot	Swat
L032	Beshagram	Swat
L033	Shegal	Swat
L034	Durshkhela	Swat
L035	Matta	Swat
L036	Sakhra	Swat
L037	Bartana	Swat
L038	Sinpora	Swat
L039	Nalkot	Swat
L040	Chupral	Swat

Novelty Statement

This study aimed to identify diversity in barley landraces in the hilly areas. This activity was novel and was not carried out in this area.

Author's Contribution

Arshad Khan: Result collection and field work, objective and title configuration.

Mohammad Ihsan and Mohammad Nisar: Result calibration with softwares.

Ali Hazrat and Murad Ali: Discussion calibration with result.

Rashid Ul-Haq: Helped in collection of data from the field.

Khalid Khan: References designing according to the journal standard.

Karishma Gul: Review of literature.

Shah Faisal: Overall compilation of the paper

Conflict of interest

The authors have declared no conflict of interest.

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