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GENETIC DIVERSITY FOR MORPHO-GENETIC TRAITS IN BARLEY GERMPLASM

ZAHEER AHMAD[1], SAIF ULLAH AJMAL[21], MUHAMMAD MUNIR[2], MUHAMMAD ZUBAIR[1] AND MUHAMMAD SHAHID MASOOD[3]

[1]Crop Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan.
[2]Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan.
[3]Plant Genetic Resources Program, National Agricultural Research Centre, Islamabad, Pakistan.

Abstract

Barley (*Hordeum vulgare* L.) germplasm comprising of 133 accessions collected from Pakistan was evaluated for 14 quantitative traits. Significant amount of genetic variation was observed for most of the plant characteristics. All the accessions were grouped into 7 clusters on the basis of morphological similarities. Representative accessions from a cluster of particular group could be chosen for hybridization program. First five principal components having greater than 1 eigenvalue contributed more than 83.40% genetic variation. The PC1 accounted for 33.60% of the total variation. The characters contributing more positively to PC1 were grain yield, 1000 grain weight, heading days, maturity days, plant height, harvest index and biomass. Analysis on the basis of regions revealed that accessions from Northern Areas performed better for early heading, longer grain filling period, broader leaf area, short plant height and number of grains per spike. Long peduncle length was found in accessions from Baluchistan. NWFP material was good for harvest index, 1000 grain weight and tall stature. Scattered diagram based on average regional genetic diversity indicated that material from each region had its own distinctness and grouped them independently. The accessions from Northern Areas were altogether apart from the accessions belonging to NWFP and Baluchistan, while accessions from these provinces were closer to each other. Scattered diagram in relation to altitude showed that barley accessions collected from group 1 (201 to 800 masl), group 4 (2001 to 2600 masl) and group 5 (2601 to 3000 masl) were altogether apart from each other while the group 2 (801 to 1400 masl) and group 3 (1401 to 2000 masl) were closer to each other. The present study provides a comprehensive set of database for the barley accessions from various regions of Pakistan.

Introduction

Barley is the second most important winter cereal in Pakistan. About two third of the area devoted to barley in the country is rain fed and one third is irrigated. It offers great opportunity to bring the marginal areas under cultivation where other crops cannot be grown successfully. It is among the major crops used for food, feed and malt in the world. Nowadays it is one of the most widespread and widely adapted crop grown under contrasting edaphic conditions. Adaptations to new environments, different agricultural practices and selection for different uses have further added to the complex diversity pattern.

Knowledge regarding the amount of genetic variation in germplasm arrays and genetic relationships between genotypes are important considerations for efficient conservation and utilization of germplasm resources (Russel *et al.*, 1997; Davila *et al.*, 1998; and Manjunatha *et al*., 2006). In the context of plant improvement, this information provides a basis for making decisions regarding selection of parental combinations that will maximize gain from selection and maintain genetic diversity. Information on the

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[1] Corresponding author: Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan

amount of genetic variation present, and the location of the genetic determinants of diversity may be useful for germplasm conservation and targeting gene discovery efforts (Sorrels & Wilson, 1997; Jana, 1999; Hou *et al*., 2005).

Assessment of the extent of genetic variability within barley, is fundamental for barley breeding programs and the conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids. The present study was undertaken with the objectives i) to assess and evaluate genetic diversity of barley germplasm based on agro-morphological traits, ii) to establish the geographical distribution pattern of genes contributing the agro-morphological traits and iii) to explore significant variation for future use in selection and breeding programs.

Materials and Methods

One hundred thirty three barley accessions were taken from the gene bank of Institute of Agri. Biotechnology and Genetic Resources, National Agricultural Research Centre Islamabad. This collection comprised of 52 accessions from Baluchistan, 28 accessions from North West Frontier Province (NWFP) and 53 accessions from Northern Areas of Pakistan. Two commercial barley cultivars were also included as check varieties. The accessions were sown at the Experimental Farm of NARC, Islamabad (33^o 43' N and 73^o 06' E) during 2002-03 season following augmented design. Observations were recorded from ten plants of each accession. The data were recorded on days to heading, flag leaf area (cm), grain filling period, days to maturity, number of tillers per plant, peduncle length (cm), plant height (cm), spike length (cm), number of spikelets per spike, number of grains per spike, biomass per plant (g), grain yield per plant (g), 1000 grain weight (g) and harvest index (%). The data were analyzed for basic statistics (mean, range, variance and standard error) following the methods of Steel & Torrie (1980).

Principal Component Analysis was done following the methods of Sneath & Sokal (1973) as adopted by Shafaeddin, (2002) and Liu *et al.*, 2002. Estimates of euclidean distances were made for all pairs of accessions. The resulting Euclidean dissimilarity coefficient matrices were used to establish the relationships between the accessions with cluster analysis using Ward’s method. Scatter diagram based on average genetic diversity was also plotted to provide the variation pattern of regional representation. Germplasm was categorized on the basis of regions and altitude.

Results and Discussion

High level of genetic variation was observed for days taken to heading and maturity, grain filling period, flag leaf area, plant height, spikelets per spike, grains per spike, biomass, 1000 grain weight and harvest index (Table 1). These traits could be utilized efficiently for tailoring a new plant variety according to the need of different regions of the country. The frequency distribution for quantitative traits allows further classification of the variance into different classes, which assist in conservation of desirable gene pool, and its utilization in breeding program for specific plant traits (Yahyaoui *et al.,* 1997; Babu & Hanchinal, 1998).

Cluster analysis: Dissimilarity ranged from a minimum of 1.0 between accessions PAK5109 and PAK5116 to maximum of 11.8 between PAK5168 and Sanober-96. The cluster diagram proposed two major groups. Critical examination of the phenogram revealed seven clusters (Fig. 1). Although cluster analysis grouped the barley accessions with greater morphological similarity, the cluster did not necessarily include all the accessions from the same or adjacent sites. For example in the present study accessions from Baluchistan fell into different clusters. Rabbani *et al*., (1998) also reported the absence of association between morphological characters and geographic origin. The accessions have been grouped in a particular cluster on the basis of morphological trait similarities, thus representative accessions from a cluster of particular group could be chosen for hybridization program. Some potentially important traits have been identified and these can be exploited for specific trait improvement and assemblage of core collection from a bulk genetic stock. Cross (1994) evaluated diverse collection of 1118 spring barley cultivars and found that gremplasm from Algeria, Afghanistan, Argentina, Ethiopia, India, Peru and Turkey were diverse and genetically distinct. On the basis of cluster analysis, he further suggested that a collection strategy based solely on country of origin is inappropriate. Shafaeddin (2002) evaluated a part of gene bank barley collections from northern regions of Iran. By using cluster analysis, he observed good relationship between the genetic and geographical classifications among origins of samples. But no relationship was found between the genetic diversity and geographical classification when all quantitative characters were considered.

The variation studied through Principal Component Analysis revealed that five principal components having greater than 1 eigenvalues contributed 83.40% of the total variation (Table 2). The traits, which contributed more positively to PC1 were yield per plant, 1000 grain weight, heading days, maturity days, plant height, harvest index and biomass. Number of tillers contributed less than 0.5, which showed non-significant genetic variance. Grain filling duration and number of spikelets contributed negatively to the first component.

Further analysis of diversity pattern, among accessions from different regions for quantitative traits revealed that accessions from Baluchistan were good for long peduncle length (Table 3). Accessions from Northern Areas possessed early heading, longer grain filling period, more leaf area, short plant height and more number of grains per spike. NWFP material showed better performance for harvest index, 1000 grain weight and tall plant height. Gilani & Witcombe (1981) studied the accessions of primitive barley collected from Northern Pakistan. Some regions were found homogeneous and the degree of homogeneity appeared to be related to the ease of communication.

Table 1. Basic statistics for quantitative traits in accessions of barley.

S. No.	Traits	Mean ± S.E*	Minimum value	Maximum value	Variance
1.	Days to heading	127.47 ± 1.20	94.00	145.00	191.94
2.	Flag leaf area (cm^2)	29.45 ± 0.66	8.96	58.03	57.93
3.	Grain filling period	35.35 ± 0.90	22.50	63.00	107.52
4.	Days to maturity	162.80 ± 0.45	155.00	171.50	27.28
5.	No. of tillers per plant	12.75 ± 0.34	4.80	30.20	15.00
6.	Peduncle length (cm)	27.20 ± 0.44	15.90	39.10	25.23
7.	Plant height (cm)	98.56 ± 0.84	75.70	116.80	93.34
8.	Spike length (cm)	8.93 ± 0.08	5.70	11.05	0.89
9.	No. of spikelets per spike	65.06 ± 0.82	23.40	85.80	89.94
10.	No. of grains per spike	51.48 ± 0.78	23.30	68.50	81.80
11.	Biomass per plant (g)	42.22 ± 0.96	22.02	80.41	123.20
12.	Grain yield per plant (g)	6.92 ± 0.35	1.03	22.90	16.06
13.	1000 grain weight (g)	22.08 ± 0.46	13.75	40.75	28.06
14.	Harvest index (%)	16.00 ± 0.6	3.82	39.00	40.44

* S.E: Standard error

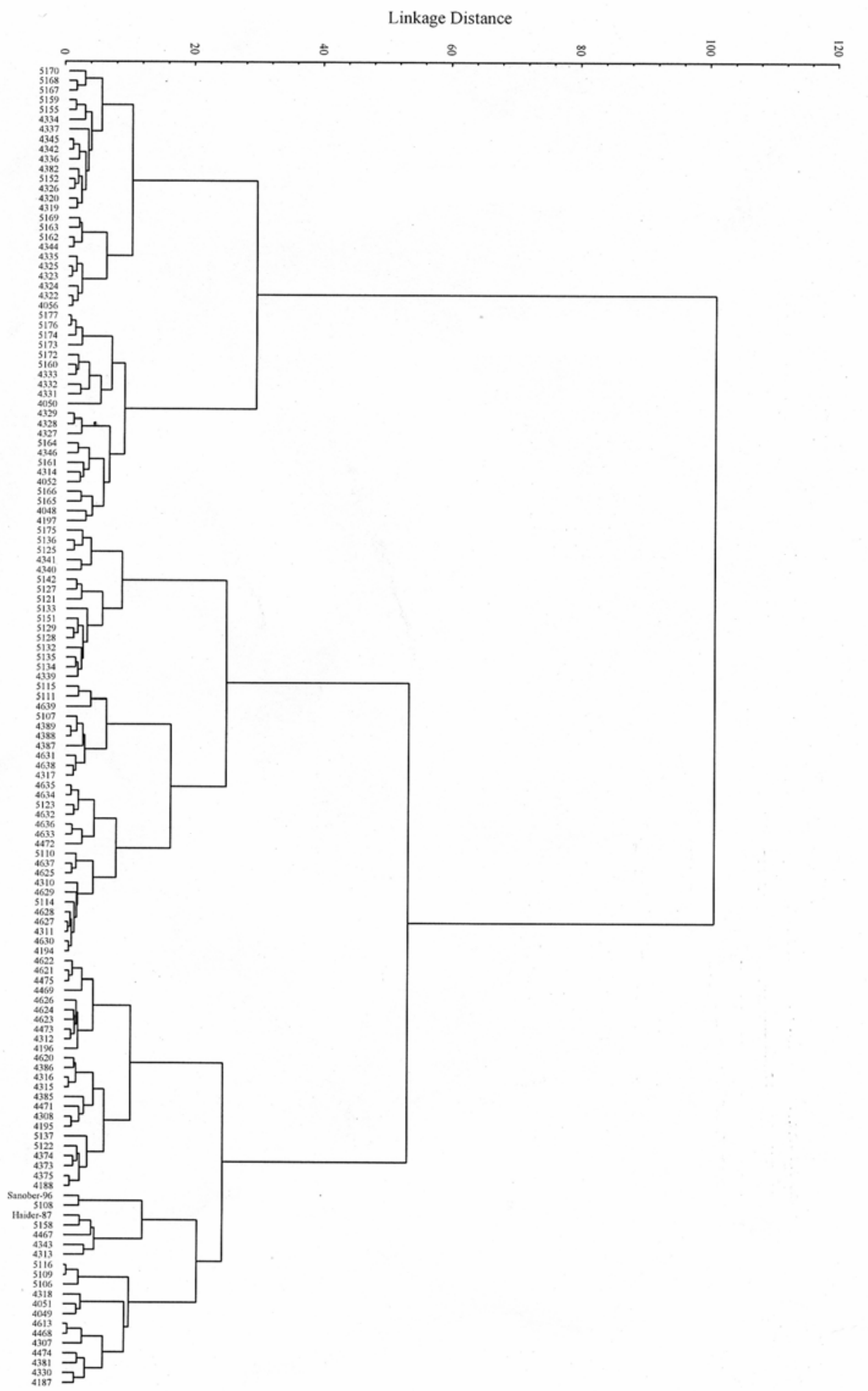
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Table 2. Principal Component analysis of barley accessions.

		PC_1	PC_2	PC_3
Eigen value		4.910	4.003	1.542
Proportion of variance		32.731	26.687	10.281
Cumulative variance		32.731	59.418	69.699
	Communalities	**Eigenvector**		
Days to heading	0.806	-0.883	-0.136	0.091
Flag leaf area	0.626	0.768	-0.089	-0.169
Grain filling period	0.477	0.623	0.229	-0.191
Days to maturity	0.632	-0.792	0.029	-0.063
Number of tillers per plant	0.716	0.209	0.714	-0.402
Peduncle length	0.780	0.087	0.698	0.534
Plant height	0.759	-0.335	0.568	0.569
Spike length	0.688	0.784	0.262	0.074
Number of spikelets per spike	0.780	0.804	0.140	0.338
Number of grains per spike	0.533	0.584	0.182	-0.399
Biomass per plant	0.687	0.256	0.647	-0.451
Grain yield per plant	0.929	-0.009	0.923	-0.277
1000 Grain weight	0.682	-0.610	0.471	-0.297
Harvest index (%)	0.672	-0 .207	0.790	0.073

Scattered diagram regarding average genetic diversity based on various regions showed that material from each region had its own distinctness and was grouped independently (Fig. 2). The barley accessions from Northern Areas were altogether apart from the accessions belonging to NWFP, Baluchistan and the Checks, whereas the accessions from Baluchistan and NWFP were closer to each other. The independent grouping of all the accessions belonging to each region is entirely due to genetic variation both in quantitative and qualitative traits in addition to edaphic variation. This indicated that within the region, there seemed to be high degree of association between morphological characters and geographical origin. This suggests that accessions from a particular region should be used to develop barley cultivars having better adaptability by exploiting the regional germplasm. Similarly Gilani & Witcombe (1980) described the distribution of morphological variability of primitive barley from Northern Pakistan and reported that naked barley showed distinct regional variation.

Germplasm were categorized from 200 masl to 3000 masl altitude with an interval of 600 masl (Table 3). The accessions collected from 200-800 masl showed long peduncle, less leaf area, tall height, high yield, heavier grain weight and high harvest index. High yielding germplasm with other good economic characters was observed in collections from 200 to 800 masl. The accessions collected from 2001-2600 masl were early in heading and maturity, took longer grain filling period and possessed high tillers, short height, long spikes and more number of spikelets and grains per spike. It was observed that the material under investigation gave high variation for days to heading, grain filling duration, leaf area, spikelets per spike, grains per spike and biomass for most of the collection sites on the basis of altitude. Crossing among selected parents from these identified groups may produce desirable recombinants for further selection. A scattered diagram based on average genetic diversity in relation to altitude is presented in Fig. 3. The barley accessions, which were collected from group 1 (201 to 800 masl), group 4 (2001 to 2600 masl) and group 5 (2601 to 3000 masl) were altogether apart from each other while the group 2 (801 to 1400 masl) and group 3 (1401 to 2000 masl) were closer. Jaradat *et al.,* (2004) characterized barley accessions for qualitative and quantitative traits and explored significant variation in morphological traits for selection and breeding.

Table 3. Basic statistics based on regions and altitude of barley accessions.

Traits	Baluchistan (52)	N.A (53)	NWFP (28)	Checks (2)	201-800 (9)	801-1400 (20)	1401-2000 (48)	2001-2600 (41)	2601-3000 (15)
Days to heading	*97 ~145	94 ~139	129 ~145	122 ~135	*97 ~136	101 ~141	97 ~145	94 ~144	106 ~137
	**135 ± 7.6	114 ± 12.1	137 ± 3.7	128 ± 8.8	**129 ±12.3	135 ± 9.6	134 ± 10.0	116 ± 13.8	125 ± 8.98
Flag leaf area (cm2)	13 ~54	8.9 ~46	15 ~58	13 ~14	15 ~28	18 ~39	13 ~54	8 ~39	24~58
	28 ± 6.3	30 ± 6.5	30 ± 10.9	14 ± 0.6	23 ± 3.9	27 ± 5.8	29 ± 7.79	29 ± 5.9	36 ± 9.7
Grain filling period	22 ~ 62	23 ~ 63	26 ~ 36	31 ~ 44	30 ~ 62	25 ~ 53	22 ~ 63	23 ~ 62	26 ~ 53
	29 ± 6.1	43 ± 11	30 ± 2.9	37 ± 9.5	35 ± 10.3	30 ± 6.4	30 ± 7.9	42 ± 11.4	36 ± 8.48
Days to maturity	156 ~170	155 ~ 171	156 ~ 171	166 ~ 167	159 ~168	155 ~ 168	156 ~ 171	156 ~ 171	156 ~ 171
	165 ± 3.3	158 ± 3.9	167 ± 3.0	166 ± 0.7	164 ± 2.50	165 ± 3.7	165 ± 4.0	158 ± 4.5	161 ± 5.6
No. of tillers per plant	6 ~ 30	7 ~ 24	4 ~ 19	12 ~ 25	10~ 19	6 ~ 16	5 ~ 25	4 ~ 30	4 ~ 16
	13 ± 4.5	13 ± 3.3	11 ± 3.5	19 ± 8.9	13 ± 3.0	11 ± 2.3	12 ± 3.9	14 ± 4.4	10 ± 2.7
Peduncle length (cm)	15 ~ 39	17 ~ 36	18 ~ 36	23 ~ 24	26 ~ 39	22 ~ 38	15 ~ 38	17 ~ 36	19 ~ 32
	29 ± 5.1	24 ± 3.5	27 ± 4.8	24 ± 0.6	33 ± 3.7	30 ± 4.1	27 ± 4.9	24 ± 3.7	23 ± 3.7
Plant height(cm)	89 ~116	75 ~106	92 ~112	89 ~93	98 ~111	89 ~115	76 ~116	75 ~115	82 ~112
	104 ± 7.3	89 ± 5.9	103 ± 6.0	91 ± 3.5	107 ± 4.4	105 ± 8.0	102 ± 8.2	90 ± 6.8	93 ± 7.1
Spike length (cm)	6 ~11	6~10	5 ~10	7.6 ~7.9	7 ~10	5~10	6 ~10	6.1 ~11	7.1 ~10.5
	8 ± 0.7	9 ± 1.1	8 ± 0.9	7 ± 0.2	9 ± 0.8	8 ± 0.9	8 ± 0.6	9 ± 1.1	8.± 0.9
No. of spikelets per spike	23 ~85	54 ~84	46 ~71	19 ~57	54 ~76	46 ~82	23 ~77	54 ~85	55 ~75
	60 ± 8.9	71 ± 8.6	61 ± 5.2	38 ± 27	63 ± 6.2	61 ± 8.6	61 ± 8.6	71 ± 9.1	65 ± 6.9
No. of grains per spike	23 ~64	29 ~68	33 ~64	23 ~40	33 ~61	31 ~68	23 ~64	29 ~67	40 ~64
	46 ± 9.1	56 ± 7.7	51 ± 6.4	32 ± 12.4	50 ± 9.16	48 ± 7.7	48 ± 9.6	55 ± 7.57	53 ± 8.49
Biomass per plant (g)	22 ~80	25 ~ 65	26 ~ 62	37 ~ 56	29 ~58	22 ~ 62	25 ~ 80	25 ~ 71	31 ~ 60
	42 ± 13.4	40 ± 9.6	43 ± 8.9	46 ± 13	46 ± 9.26	38 ± 8.7	43 ± 12.5	42 ± 11	41 ± 9.0
Grain yield per plant (g)	1 ~ 22	2 ~ 19	2 ~ 20	16 ~ 18	4 ~ 18	2 ~ 20	1 ~ 22	2 ~ 12	2 ~ 19
	6± 4.5	5.9± 3.0	8 ± 4.1	17 ± 1.1	11± 3.6	6.1± 4.1	7 ± 4.5	5.9± 2.5	6.9± 4.2
1000 grain weight (g)	13 ~34	13 ~35	16 ~40	35 ~37	16 ~29	13 ~40	14 ~34	14 ~27	13 ~35
	21 ± 4.9	20 ± 4.5	26± 4.7	36 ± 1.6	26 ± 3.8	21 ± 5.9	23± 5.4	20 ± 3.9	22 ± 6.3
Harvest index (%)	3 ~33	6 ~38	8 ~32	32 ~43	16~31	6 ~32	3 ~33	6 ~28	8 ~38
	15 ± 6.7	14 ± 5.5	19 ± 6.1	38 ± 8.3	23 ± 4.6	15 ± 6.2	15 ± 6.8	14 ± 4.5	16 ± 7.1

Ranges ** Mean ± Standard deviation

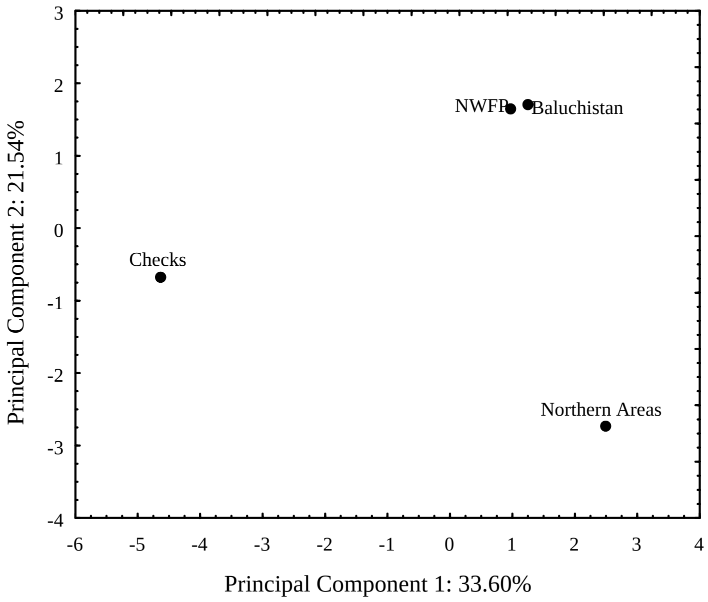

Fig. 2. Scatter diagram based on average regional diversity for two PCs in barley accessions.

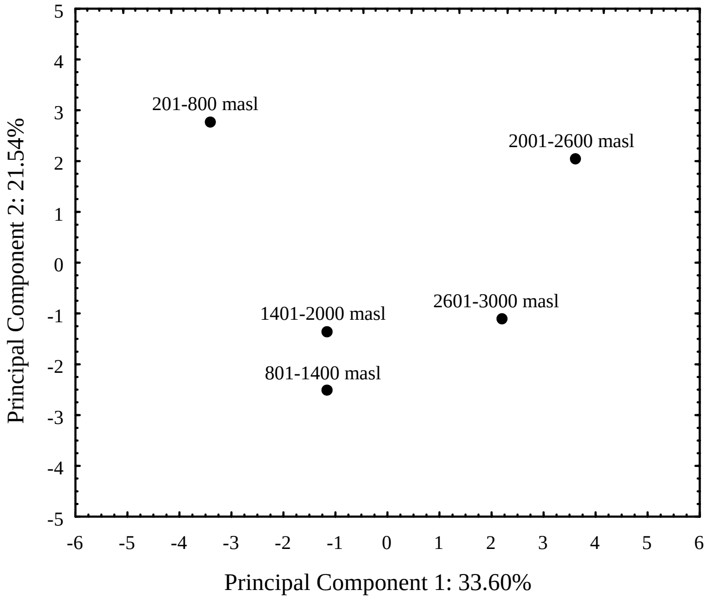

Fig. 3. Scatter diagram of based on average diversity for two PCs in relation to altitude.

Present study provides comprehensive information on barley germplasm collected from different regions of Pakistan and hence could be effectively utilized in breeding program to evolve improved varieties for various agro-climatic zones of the country.

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