Diversity in Nepalese Wheat Genetic Resources as Revealed by Agro-morphological Markers

Krishna Hari Ghimire[1], Raju Thada Magar[2]

Abstract—Wheat (*Triticum aestivum* L.) is the third most important crop of Nepal after rice and maize. Large numbers of local landraces are still grown in western and far western hills of the country. Most of them are conserved ex-situ but their utilization in breeding is very less. This study was carried out to study level of phenotypic variability among wheat genetic resources conserved National Agriculture Genetic Resource Centre (Genebank) based on twelve quantitative and ten qualitative traits. A total of 237 accessions were planted at Khumaltar (1360masl) with non-replicated rod row design. Both univariate and multivariate statistics were used to determine the extent of variation among germplasm. Wide range of variation has been observed among the accessions for both quantitative and qualitative traits, revealing Standardized Shannon-Weaver diversity (H') ranging from 0.55 to 0.87 for quantitative traits and 0.24 to 0.94 for qualitative traits. The dendrogram constructed to describe the relationship among the landraces, resulted in six clusters at 73.7% of similarity, based on average linkage and Euclidian distance. Correlation analysis between quantitative traits showed significant positive correlation among days to heading and days to maturity (r=0.54), 1000-grain weight and grain width (r=0.43), 1000-grain weight and seed length (0.39). The study of variability among accession for different quantitative and qualitative traits could be used to select advantageous adaptive traits for crosses in the breeding program, direct use by farmers and also can be used as an important tool for the management of crop germplasm collection.

Index Terms—characterization, diversity, genetic resources, landraces, Nepal, quantitative and qualitative traits, wheat

[1] Senior Scientist, Genebank, Nepal Agricultural Research Council, Kathmandu, Nepal; ghimirekh@gmail.com
[2] Research Intern, Genebank, Nepal Agricultural Research Council, Kathmandu, Nepal; rujanthada@gmail.com

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1 INTRODUCTION

WHEAT is one of the oldest cereals and is the first cereal crop known to have been domesticated. It is a nutritious, convenient and economical source of food, provides about 20% of the world's food calories [1], and staple food for 35% of the world population [2]. In Nepal, it has been growing since time immemorial particularly in Far and Mid-Western hills of Nepal. Before the introduction of Mexican semi-dwarf wheat varieties, it was considered as a minor cereal in the country, after the introduction of semi-dwarf varieties from Mexico, the area and production of wheat in Nepal has been increased dramatically and now it has significant contribution to the national food supply. At present national average wheat productivity is 2,454 kg/ha, it is grown in 791,573 ha with total production of 1,942,870 mt. It occupies 23% of total cereal area and contributes 22.5% of the total cereal production in the country [3]. Wheat is widely adapted with its coverage in all the agro-climatic regions of Nepal, ranging from 50 m to 4000 m above sea level.

Genetic variability present in collection and preserved germplasm are important resource in generating new plant ideotypes having desired traits that help to increase crop production and thus improve the level of human nutrition [4]. Nepal circumscribe the existence of many local landraces of wheat, [5] reported many landraces and 10 wild relatives of wheat in Nepal. After the establishment of National Genebank in 2010, collection and conservation of wheat germplasm got high priority. Now, NAGRC holds more than 1,700 wheat genetic resources in medium and long-term conservation [6], [7].

Wheat is third most important crop of Nepal. Although it's great contribution on total national AGDP, food security and, Nepalese economy, the overall level of public-sector support to agricultural research remains relatively low and insufficient. There are lots of germplasm at the wild state in the different part of country, they are neglected or underutilized by farmer due to their undesirable agronomic characters such as low yield, harvesting and threshing problem etc. At different geographical location of the country we have locally adapted wheat landraces with better quality (in terms of disease resistance, nutritional quality, drought resistance, early maturity etc.,) but none or very few have been used in crop improvement program. Such trends lead to genetic erosion of the wheat genetic diversity [8]. Characterization of Nepalese germplasm at genotypic level cannot be accessed at proper level yet.

Morphological characterization is the foundation of genetic diversity research at any taxonomic level [9]. It is still an important tool for management of crop germplasm [10]. Characterization of germplasm therefore is essential to provide information on the traits of germplasm assuring the maximum utilization of the germplasm collection to the final users in breeding program and for any other purposes. Characterization of accessions based on multiple traits can be used as a management tool in regenerations to allow validating the identity of an accession.

2 MATERIALS AND METHODS

A total of 237 wheat accession including landraces from different district, released varieties and breeding line from NWRP Bhairahawa conserved at NAGRC were characterized at research site of NAGRC Khumaltar from December 2015 to June 2016, located in $85^{o}20'$ east longitude and $27^{o}40'$ north latitude at an altitude of 1368 m above sea level. The climate of experimental field was semi temperate with average $17^{o}C$ temperature and a total of 326mm rain fall during entire study. The soil of the experimental site was dominantly silty loam with pH 6.0. A total of 237 wheat accessions were characterized in non-replicated rod row design, each accession were planted in the plot size 2m length and 1m width, five rows of 2m length were maintained with 25cm spacing between rows and continuous sowing within rows. Two times irrigation at the time of germination and at the time of flowering was given. Hand weeding of a noxious weed Ragate jhar (Phalaris minor) and other minor weeds were carried out before the flowering stage. Well decomposed farm yard manure (FYM) at the rate of 5 ton/ha as well as chemical fertilizers at the rate of 50:50:20 kg/ha N:P:K were applied at the time of land preparation.

For the yield component analysis, 3 random spikes of each plot were taken and these sample spikes and other remaining spikes were well dried in sunlight and threshing and cleaning were carried out manually. For the precise data recording and analysis, observation of the field was done regularly. Purposive sample for 12 quantitative and 10 qualitative traits were taken. Observation and data collection were carried out as per as directed in Revised Descriptor List of Wheat (*Triticum* spp.)[11].

Descriptive statistics, standardized Shannon-Weaver diversity index (H'), cluster analysis and frequency diagram were employed to estimate and analyze the diversity via MS Excel. Correlation analysis between the 9 quantitative characters was done by using MINI-TAB-17 software. Shannon-Weaver diversity indices [12] were calculated for each quantitative and qualitative traits recorded with Microsoft Excel using the formula:

$$H' = \left[\sum (n/N) * \{\log 2(n/N) * (-1)\}\right] / \log 2k$$

Where, H' is standardized Shannon Weaver diversity index, k is the number of phenotypic classes for a character, n is the frequency of phenotypic class of that character and N is the total number of observation for characters. For the quantitative traits, accession were divided into 10 phenotypic classes as <x-2sd, x-2sd, x-1.5sd, x-1sd, x-0.5sd, x, x+0.5sd, x+1sd, x=1.5sd, x+2sd, >x+2sd are as the margin of the

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classes, where x is average and sd is standard deviation. The diversity index has been extensively used in estimation of diversity in germplasm collection of barley [13] and used for measurement and comparison pattern of phenotypic diversity in germplasm collection of wheat [14], [15], [16]. The relationship between collected accessions of measured parameters was studied using cluster analysis that results in the grouping of variables into cluster. Distance between clusters were analyzed and reported as a dendrogram of Euclidean distances via MINITAB-17.

3 RESULTS AND DISCUSSION

3.1 Descriptor statistics and Shannon-Weaver diversity

The results obtain from the univariate and multivariate analysis for the investigated characteristics indicated the presence of a large variation for both quantitative traits (table 1) and qualitative traits (table 2). Among quantitative traits flag leaf length, plant height, grain length and number of seeds per spike shows higher Shannon-Weaver diversity index (H') of 0.87, whereas minimum index (H') of 0.55 was shown by flag leaf width. Among qualitative traits, tillering capacity shows higher Shannon-Weaver diversity index (H'=0.93) whereas glume color shows lower diversity index (H'=0.24).

Table 1: Descriptive statistics and Shannon-Weaver diversity indices (H') for 11 quantitative traits Nepalese wheat accessions

SN	Traits	Maximum	Minimum	Mean	Std. Dev.	H'
1	Days to heading	136	89	110	5.95	0.81
2	Days to flowering	143	105	116	4.67	0.84
3	Days to maturity	139	181	156	4.60	0.85
4	Flag leaf length, cm	27	11	17.6	3.17	0.87
5	Flag leaf width, cm	6	0.8	1.5	0.56	0.55
6	Plant height, cm	126	48	86	12.70	0.87
7	1000-Seed weight, g	66.7	18.5	46.8	7.14	0.85
8	Grain length, mm	7.9	5.3	6.5	0.41	0.87
9	Grain width, mm	4.2	1.9	3.0	0.26	0.80
10	Number of seeds/spike	97	28.7	52.4	11.81	0.87
11	Spike length, cm	21	5.9	10.2	1.62	0.84

Higher percentages of the germplasm were emerged between 15 to 18 days after seeding i.e. 39.2%. The studied germplasm were headed between 89 to 139 days after sowing with the majority (28.7%) were headed at 108-111 days. The days to maturity were ranges from 139 to 181 days after the day of sowing. The higher number i.e. 22.8% of the accessions was matured at 154-156 days. The plant height was ranges from 48 to 126 cm with the majority between 86 to 92 cm. A wider range was observed for spike length (5.9 cm to 21 cm). About 49% of the accessions have shorter spike length than the average 10.2 cm. Number of seed per spike was ranges from 29 to 97 with mean 52.42. Grain length was ranges from 5.3 mm to 7.9 mm with the majority in between the range of 6.3 to 6.5 mm. Average 1000-seed weight was observed 46.8 g, ranging from 18.5 to 66.7 g, where 47% accessions showed lower 1000-grain weight than average.

For growth habit, 75% of the accessions were of erect type. Similarly, 89% accessions were awned, 94% accessions had white glume color, 92% accessions were without glume hairiness. More than 94% accessions were found moderately resistant to highly resistant against yellow rust disease, which is a major problem in hilly areas.

Table 2 : Descriptors used for estimating qualitative trait diversity, proportion (%) of phenotypic classes and phenotypic diversity index (H') for each trait

SN	Morphological trait	Observed phenotypic class	Proportion, %	H′
1	Growth class	Prostrate	3	0.58
		Intermediate	22	
		Erect	75	
2	Spike density (SD)	Very lax	0	0.79
		Lax	9	
		Intermediate	23	
		Dense	41	
		Very dense	17	
3	Tillering capacity	Low	23	0.94
		Medium	51	
		High	25	
4	Awnedness	Awnless	5	0.37
		Awnletted	8	
		Awned	89	
5	Glume color (GC)	white	94	0.24
		red to brown	5	
		purple to black	1	
6	Glume hairiness (GH)	Absent	92	0.31
		Hairy (low)	2	
		Hairy (high)	6	
7	Seed size	Small	0	0.61
		Intermediate	16	
		Large	59	
		Very large	15	
8	Seed color	white-1	67	0.66
		Red	31	
		Amber	3	
9	Yellow rust susceptibility	Highly resistance	47	0.56
		Moderately resistance	48	
		Low resistance	4	
		Susceptible	2	
		Highly susceptible	0	

3.2 Cluster analysis

The relationship between collected accessions of measured parameters was studied using cluster analysis that results in the grouping of variables into cluster. The dendrogram constructed to describe the relationship among the landraces. The similarity matrix was constructed using Euclidean distance to access the phenotypic relatedness among 237 Nepalese wheat germplasm shown in Fig 1. Based on the average linkage method in Euclidean distance, 6 cluster are formed, on which cluster I, II, III, IV, V and VI consists of 210, 9, 3, 7, 1 and 7 accessions. The clustering pattern of the wheat landraces revealed that the wheat landraces showed considerable genetic diversity among themselves. Accession with more similarity were grouped on one cluster (accession in each cluster were not clearly visible in the dendrogram). The analysis had demonstrated the ability of agro-morphological traits to detect the variability among the accessions. Wheat landraces under study are grouped depending on specific traits useful for wheat improvement program.

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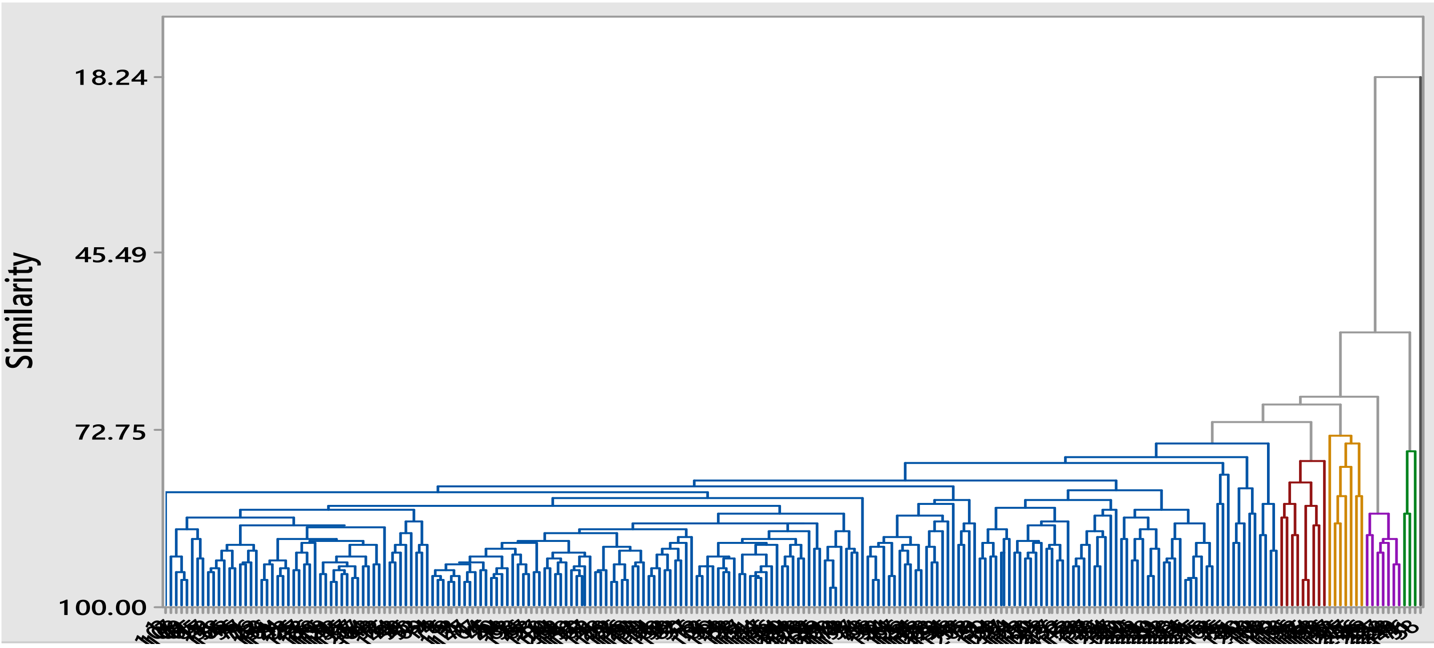

Figure 1: Dendrogram showing the clustering patterns in phenotypic variation of 13 quantitative characters of wheat genetic resource

3.3 Correlation among quantitative traits

Correlation analysis between quantitative traits showed significant positive correlation among days to heading and days to maturity, 1000-grain weight with grain length and grain width with Pearson Correlation value r=0.544, 0.353 and 0.431, respectively. This showed that longer as well as bold grains having higher 1000-grain weight, which is considered as a preferable trait. In contrast, significant negative correlation was observed among days to maturity with spike length, 1000-grain weight, grain width and flag leaf length. Days to heading was also negatively correlated with 1000-grain weight and grain weight, which suggests that early maturing entries could be selected for higher grain yield. The study of variability among accession in different quantitative and qualitative traits could be used to select advantageous adaptive traits for crosses in the breeding program, direct use by farmers and also can be used as an important tool for the management of crop germplasm collection.

Table 3: Correlation among nine quantitative traits observed over 237 wheat accessions

	DTH	DTM	FLL	FLW	PHT	GPS	SL	TGW	GL
DTM	0.544**	-	-	-	-	-	-	-	-
FLL	0.004	-0.159*	-	-	-	-	-	-	-
FLW	0.003	-0.010	0.098	-	-	-	-	-	-
PHT	0.042	0.043	0.077	-0.177**	-	-	-	-	-
GPS	0.060	0.109	0.193**	0.036	-0.142*	-	-	-	-
SL	0.032	-0.343**	0.269**	-0.021	0.004	0.106	-	-	-
TGW	-0.273**	-0.256**	0.153*	0.154*	-0.104	-0.026	-0.020	-	-
GL	-0.036	0.112	0.061	0.093	-0.021	-0.044	-0.007	0.393**	-
GW	-0.196**	-0.185**	-0.049	0.071	-0.152*	-0.034	-0.097	0.431**	0.127

DTH=Days to 50% heading, DTM=Days to 50% maturity, FLL=Flag leaf length (cm), FLW=Flag leaf width (cm), PHT=Plant height (cm), GPS= Number of grains per spike, SL=Spike length (cm), TGW=1000-grains weight (gm), GL=Grain length (mm) and GW=Grain width (mm).

4 CONCLUSION

Variation of traits is a primary need of any plant breeding effort that involves the natural evolution and cause sustainable crop production under different environment. Wide range of variation has been observed in both quantitative and qualitative traits as revealed by Shannon-Weaver diversity indices (H'). The dendrogram constructed to describe the relationship among the landraces, resulted in six clusters at 73.67 % of similarity, based on average linkage and Euclidian distance. This variability among accessions is expressed in differences of earliness, plant height, thousands kernel weight and spike characters and they could be used to select advantageous adaptive traits for crosses in the breeding programs and the result of this study created comprehensive documentation on phenotypic characters of 237 Nepalese wheat accessions.

Landraces CB0448, CB0381, CB0340, CB0148, CB0513, CB0470, CB077, Baitadi-15, CB0492 have early days to heading and maturity. Landraces with early flowering and maturity days should be used to develop early maturing varieties so that farmers of high hills and mountain areas would prefer such varieties. Early maturing landraces are also suitable for drought stress condition since they can escape severe drought during reproductive stages. Landraces CB0440, CB0513, CB0804, CB0384 have shorter plant height. Landraces with lower plant height are tolerant to lodging and these genotypes should be used for high fertility areas. Plant height is also negatively correlated with 1000-seed weight or number of grains/spike in this study; thus we can suggest and recommend that germplasm with lower plant height are also demonstrated by high yielding traits. Landraces CB0333, C06844, CB0130, CB0210, CB0642, CB0169, CB0141, CB0249, CB0503, CB0138 have higher 1000-seed weight and good level of number of grain per spike. Germplasm with bold seeds, higher 1000-seed weight and higher number of grains per spike are the high yielding genotypes because these are the yield attributing traits. Thus such genetic resources could be utilized efficiently in variety improvement program.

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