

# Diversity among maize landraces in North West Himalayan region of India assessed by agro-morphological and quality traits

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## Abstract

Fifty-one accessions collected from remote areas of NWH region (Himachal Pradesh and Jammu and Kashmir) were evaluated for genetic diversity on the basis of phenotypic and grain quality data. Significant differences were found among accessions for agro-morphological as well as quality traits. These accessions had relatively greater variability for yield per plant (g), grain weight (g), plant height (cm), ear height (cm), kernel rows, number of kernels per row, leaf width (cm) and tryptophan content. The cluster analysis based on the phenotyping and biochemical data divided 51 accessions into four clusters; all accessions from Jammu and Kashmir region grouped into cluster 1. Principal component analysis revealed that plant height, ear height, protein, oil, sugar, starch contents and leaf length (cm) were major contributor towards diversity. The grouping pattern obtained in the cluster analysis and PC biplot was congruent with geographical relationship among the accessions. Accessions such as IC556421 with high protein (13.27%) and sugar (4.53%) content, IC568267 with high oil content (4.94%) and IC568265 with high tryptophan content (0.56%) could be utilized in future research programme.

**Key words:** Maize, cluster analysis, genetic resources, grain yield, protein, sugar

## Introduction

Maize (*Zea mays* L.) is grown under diverse environmental conditions ranging from the tropical to the temperate regions (Singh 1977; Prasanna and Sharma 2005; Prasanna 2010). The adaptability of landraces to any specific local environments has created variation for agronomic and quality traits including resistance to biotic and abiotic stresses. The genetic diversity that has evolved under the influence of natural and artificial selections is being cultivated

and maintained in the traditional agricultural production systems worldwide including North-Western Himalayan (NWH) region of India. Farmers characterize these landraces based on ear characteristics and maintain them through conservative selection, though there is a considerable gene flow (Louette and Smale 2000; Mahar et al. 2009). Social and cultural values and rituals of different tribes and ethnic communities who are cultivating maize for centuries have played an important role in the conservation of landraces. Owing to tremendous diversity in the Indian Himalayan regions, Anderson (1941) considered maize to have an Asiatic origin while Dhawan (1964) defined several landraces from NE Himalayan regions as Sikkim primitives, which have primitive characters of popcorns and high prolificacy. The primitive group comprised of several races of popcorn, which had differentiated at various altitudes and under diverse conditions was distributed throughout the eastern Himalayan region. This group included Poorvi Botapa, Murli sub-race of Poorvi Botapa, Tirap Nag-Sahypung, Arun Tepi and Alok Sapa. Further, based on evolutionary history and cultivation practices, Singh (1977) grouped Indian maize races into four categories viz., (i) primitive (ii) advanced or derived (iii) recent introductions, and (iv) hybrids.

The National Bureau of Plant Genetic Resources (NBPGR) conserves around 9,000 maize germplasm accessions of both indigenous and exotic origin, and about 60% of these collections are landraces and populations. Although majority of the accessions have been characterized for various agronomic descriptors,

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still many are needed to be evaluated for advanced agronomic and quality traits (Goodman, 1968; Chandel and Bhat, 1989; Prasanna and Sharma, 2005; Wasala et al. 2013). Many studies have emphasized that phenotypic and phenological information based on descriptors continues to be the first step for the assessment, description and classification of germplasm collections to enhance their use in maize breeding (Prasanna and Sharma 2005; Wasala et al. 2013; Nass et al. 1993 and Brandolini and Brandolini 2001). Also the identification of local landraces through germplasm collection will be useful to preserve the genetic variability and their value assessment will promote their use and provide economic profits to the farmers. The present study was, therefore, undertaken to generate agronomic, biochemical and genetic knowledge on 51 maize landrace accessions (acc.) collected from NWH region of India.

## Materials and methods

### Collection sites

The states of Jammu and Kashmir (J&K) and Himachal Pradesh (HP) are situated at 31.83 N to 32.72 N and 74.85 E to 77.69 E in the NWH region of India. The germplasm of maize landraces were collected from J&K (11 acc.) and HP (40 acc.) (Table 1) during 2007 and 2008 respectively.

### Agronomic and biochemical evaluation

These germplasm accessions were grown at NBPGR New Area Farm, New Delhi during *kharif* of 2009 and 2010 for characterization and evaluation in Augmented Block Design with three checks *viz.*, Madhuri, Navjot and Jawahar Pop Corn. The data were recorded on five randomly selected plants for 16 agronomic and six biochemical characters as per the minimal descriptors developed by NBPGR (Mahajan et al. 2013). The agronomic characters included days to tassel, days to silk, plant height (cm), ear height (cm), leaf length (cm), leaf width (cm), number of leaves, tassel length (cm), number of tassel branches, ear length (cm), ear diameter (cm), kernel rows, kernels per row, days to maturity, number of ears per plant, grain weight (g) and grain yield per plant (g). Biochemical characters were protein content, oil content, sugar content, starch content, specific gravity and tryptophan content in protein measured on dry weight basis using triplicate samples from each accession. All the accessions were maintained by bulk sibbing for seed increase and biochemical evaluation by collecting pollens from tassel of each plants in the

particular plot and putting the bulked pollen on the emerging silk of all plants following proper bagging techniques.

The kernels were oven-dried and ground to powder and de-fatted by using petroleum ether and subsequently stored in desiccators for analysis. Protein content was determined by Micro Kjeldahl method of AOAC (AOAC, 1970) while tryptophan content was estimated by papain hydrolysis method (Villegas E. and Mertz E.T. 1971). The optical density was recorded against blank at 545 nm and tryptophan content was calculated by using a standard curve. Starch content was determined by the method of Clegg (1956) using anthrone reagent. After extraction of starch with perchloric acid, it is further hydrolyzed (in an acidic medium) into glucose, which forms a green colour compound on reaction with anthrone reagent. The optical density was recorded against blank at 620 nm. Oil content was estimated following AOAC (1970) using solvent extractor system in which extraction of oil was done by using non-polar solvent petroleum ether (Chaudhary et al. 2012).

### Statistical analysis

Analysis of variance for individual year was carried out as per the augmented design and then adjusted mean of both years were subjected to combined analysis after ascertaining the homogeneity of error variance using the Bartlett's test. Mean values were used for statistical analysis using SAS JMP software. Cluster analysis of the accessions was performed using all continuous data pertaining to agronomic and biochemical traits recorded in the study. Euclidean distances were estimated and the matrix was further analyzed using Ward's method of minimum variance for clustering the accessions. Principal component biplot was generated using principal component option with correlation method.

## Results and discussion

### Genetic variability

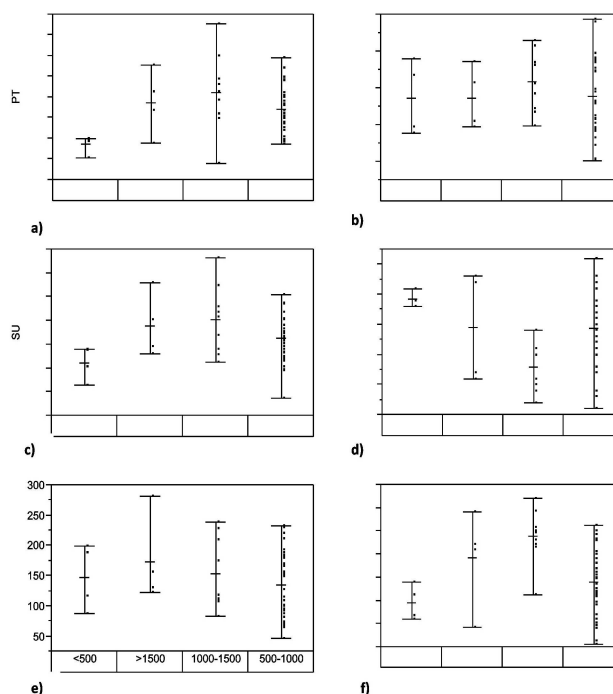
The statistical analysis of data for quantitative traits showed wide range of variability among the accessions. These accessions had relatively greater variability for yield per plant, grain weight, plant height, ear height, kernel rows, number of kernels per row, leaf width and tryptophan content based on coefficient of variance. Environmental effect was significant for all the agronomic traits except ear width and grain weight. The mean value of days to tassel was 53.41

**Table 1.** Details of selected maize landraces from North Western Himalayan region

States	Districts	Latitude, Longitude	Altitude (m)	Accessions			
Jammu & Kashmir	Doda	33.14N, 75.54 E	1107	IC556400 <sup>YD</sup> , IC556401 <sup>DRF</sup> , IC556409 <sup>YD</sup> , IC556410 <sup>YD</sup> (1-4)			
	Kishtwar	33.31N, 75.76E	1685	IC556411 <sup>YF</sup> , IC556413 <sup>OF</sup> (5-6)			
	Ramban	33.24N, 75.23E	1156	IC556414 <sup>YF</sup> , IC556415 <sup>YF</sup> , IC556416 <sup>WF</sup> , IC556419 <sup>DRF</sup> , IC556421 <sup>DRF</sup> (7-11)			
Himachal Pradesh	Chamba	32.55N, 76.12E	996	IC556424 <sup>YD</sup> , IC556425 <sup>YD</sup> , IC556429 <sup>YF</sup> (12-14), IC568265 <sup>YD</sup> , IC568267 <sup>WF</sup> , IC568269 <sup>LYD</sup> (31-33), IC568304 <sup>YD</sup> , IC568306 <sup>BD</sup> , IC568307 <sup>DRD</sup> , IC568310 <sup>YF</sup> , IC568312 <sup>YF</sup> (47-51)			
				Kangra	32.10N, 76.27E	733	IC556430 <sup>YD</sup> , IC556431 <sup>YD</sup> , IC556432 <sup>WF</sup> , IC556433 <sup>WF</sup> (15-18), IC568272 <sup>YF</sup> , IC568274 <sup>YD</sup> , IC568279 <sup>LYD</sup> , IC568282 <sup>DOF</sup> , IC568283 <sup>DRF</sup> , IC568286 <sup>DO/RF</sup> , IC568290 <sup>DRD</sup> , IC568292 <sup>DRF</sup> , IC568293 <sup>LRF</sup> , IC568295 <sup>YD</sup> , IC568296 <sup>DRD</sup> , IC568298 <sup>YD</sup> , IC568299 <sup>OD</sup> (34-46)
				Hamirpur	31.68N, 76.52E	785	IC556435 <sup>WF</sup> , IC556436 <sup>YF</sup> (19-20), IC568243 <sup>YF</sup> , IC568244 <sup>WF</sup> , IC568245 <sup>YF</sup> , IC568247 <sup>YD</sup> (23-26)
	Mandi	31.70N, 76.93E	1524	IC568235 <sup>YD</sup> , IC568238 <sup>YD</sup> (21,22)			
	Kullu	31.95N, 77.10E	120	IC568248 <sup>WD</sup> , IC568251 <sup>YD</sup> , IC568254 <sup>LY</sup> , IC568256 <sup>YD</sup> (27-30)			

Value in parentheses indicates serial number of accessions used in the study; Detail of the abbreviations for kernel colour and grain type (superscripts) are as follows: YD=Yellow, Dent; DRF=Dark Red, Flint; YF=Yellow, Flint; OF=Orange, Flint; WF=White, Flint; LYD=Light Yellow, Dent; BD=Black, Dent; DRD: Dark Red, Dent; DO/RF=Dark Orange/Red Flint; LRF= Light Red, Flint; OD=Orange, Dent; WD=White, Dent

ranging from 41 for accession IC556432 and IC556433 to 63 days for accession IC568279. Leaf length and width, both were highest for accession IC568304 with an average of 84.42 and 9.05 cm respectively. Plant height was highest for IC556414 (288 cm) and shortest for IC568267 (162.60 cm) with mean value of 223 cm. Ear length was highest for IC568269 (19.6 cm) and shortest for IC568267 (12 cm) with an average of 16.63 cm. For 100 seed weight, accession IC568307 had maximum value 38.3, whereas IC556401 had minimum value of 19.65 g. The protein content ranged from 9.89% in IC556415 to 13.27% in IC556421 with the mean value of 11.24%, whereas the sugar content ranged from 3.35% to 4.53% in IC556421. Tryptophan content in protein was minimum in the accession IC556425 and maximum in IC568265. The collected maize accessions belonged to varied altitudes from 120 m i.e. valley of North Himalayan region like Kullu to Kishtwar in Jammu Kashmir at 1685 m level. Altitude wise variability was shown graphically for six traits for the altitude ranges <500, 500-1000, 1000-1500 and >1500 meter above sea level (Fig. 1). It was observed that accessions belonging to 500-1000 m altitude had greater variability for the traits oil content, tryptophan and plant height and accessions belonging to 1000-1500 m altitude were more variable for protein content. Accessions collected from lower altitude were relatively



**Fig 1.** Altitude wise variability in maize accessions for a) Protein (PT), b) Oil, c) Sugar (SU), and d) Tryptophan (TR) content along with e) Yield per plant (g) (YP) and f) Plant height (cm) (PH) for altitude ranges <500, 500-1000, 1000-1500 and > 1500 meter above sea level

less variable, which might be due to less number of collections from that altitude.

The evaluation of maize landraces from NWH region revealed that although there was variation in kernel colour ranging from white, light yellow, yellow, dark orange to dark red but most of them were either white or yellow colour with flint or dent type. The maize accessions also showed considerable range for crude protein content, total grain yield, test weight, ear height and tryptophan content, etc. Chandel and Bhat (1989) reported existence of high genetic variability in landraces of NWH region for most of the cobs and seed characters, plant height etc. and mentioned this region as the potential centre of landrace diversity in maize. The results obtained in the present study indicated that landraces of J&K had lower anthesis to silk interval; this may be indicative of source of drought tolerance genes, as the trait is negatively associated with high drought tolerance (Ngugi et al. 2013). The high morphological variability observed in the NWH accessions could be due to open pollinated nature of local accessions and can result from their specific

adaption to local conditions and the continuous use of seeds maintained by the farmers. Long back Collins (1930) reported that isolations of maize into locations having different environments was a source of diversification of crop resources. The nutritive value of maize landraces is very important to study and generate the knowledge and idea of variability of nutritional contents. We found that accessions of Jammu and Kashmir had higher average values for protein, oil, sugar and starch content whereas accessions of Himachal Pradesh had more tryptophan content and specific gravity. In the present study, the accession, IC556421 collected from Ramban area had highest protein (13.27 %) and sugar content (4.53%) and moderate amount of oil content (4.06%). Similarly, with respect to agronomic traits, accessions from Jammu and Kashmir were taller with high ear placement and early maturity. In fact, maize is the second most important crop after rice and is a staple food of tribal people such as Gujjar and Bakarwal (nomadic race) of JK (Najeeb et al. 2012). In Kashmir valley maize is grown as a sole crop at an altitude range of 1850-2300 m above mean sea level. However,

**Table 2.** Promising accessions identified based on their performance at Delhi location for different traits

Trait	Mean $\pm$ SE	Range	CV(%)	Promising accessions
Protein content (%)	11.3 $\pm$ 0.1	9.9-13.3	6.7	IC556421, IC556400, IC556425, IC556413, IC568307, IC556432 ( $\geq$ 12.0)
Oil content (%)	3.9 $\pm$ 0.1	3.0-4.9	12.8	IC568267, IC568244, IC556409, IC556419, IC556432 ( $\geq$ 4.48)
Sugar content (%)	3.9 $\pm$ 0.1	3.4-4.5	6.5	IC556421, IC556413, IC556400, IC556425, IC556430, IC556433, IC568279 ( $\geq$ 4.14)
Starch content (%)	70.2 $\pm$ 0.1	68.7-71.3	1.0	IC556400, IC556416, IC556401, IC556425, IC568307 ( $\geq$ 71.03)
Tryptophan content (%)	0.4 $\pm$ 0.0	0.3-0.6	15.3	IC568265, IC568290, IC568245, IC568238, IC568269, IC568235 ( $\geq$ 0.52)
Ear length (cm)	16.6 $\pm$ 0.2	12.0-19.6	10.1	IC568269, IC568293, IC568421, IC568251 ( $\geq$ 19.0)
Ear diameter (cm)	3.9 $\pm$ 0.1	3.2-4.6	8.9	IC556424, IC556425, IC568290, IC556429 ( $\geq$ 4.40)
Number of kernel rows	11.5 $\pm$ 0.3	4.4-15.6	18.4	IC556411, IC556409, IC556429 ( $\geq$ 14.0)
Number of kernels per row	33.3 $\pm$ 0.8	21.0-45.4	16.3	IC568235, IC568293, IC568269, IC568238, IC568272 ( $\geq$ 39.80)
Yield per plant (g)	142.8 $\pm$ 8.2	47.0-281.6	41.7	IC568238, IC556419, IC556435, IC556429, IC556421 ( $\geq$ 228.31)
100 seed weight (g)	29.3 $\pm$ 0.7	19.7-38.3	17.3	IC568286, IC568298, IC568235, IC568269, IC556430, IC568248 ( $\geq$ 33.43)
Fodder type* (1-9 scale)	-	-	-	IC568235, IC568254, IC568269, IC568272, IC568279, IC568286, IC568292

\*Ordinal data, hence mean, range and CV not presented; SE= Standard Error

it also occupies plain belts of the valley in few pockets with limited moisture availability. Accessions IC568267 from Himachal region had highest oil content (4.94%) and IC5468265 had the largest seed weight (36.59g), hence, may be used for base population or inbred development. Several landraces of Himachal Pradesh like IC568235, IC568254, IC568269, IC568272, IC568279, IC568286, IC568292, IC568293, IC568295, IC568299, IC568307 (Table 2) were of fodder type with stay green habit and late maturity which could be good source of drought tolerance. Accessions belonging to Kangra had mostly dark red, dark orange and yellow coloured kernel hence may be a good source of carotenoid content.

### **Association among studied traits**

Measurement of correlation coefficient is important in plant breeding because it measures the degree of association (genetic and non-genetic) between two or more traits (Dewey and Lu, 1959). In the presence of high correlations or association between two traits, selection in one trait will cause a change in its mean through additive gene effects of selected individuals and simultaneously cause an indirect change in the mean of the other trait. Association study was carried out among the quality parameters and agromorphological traits including yield components to understand their inter-relationships that may help in chalking out effective breeding strategies. In the present study, protein content was positively correlated with sugar (0.718\*\*), starch (0.659\*\*), plant height (0.524\*\*) and ear height (0.493\*\*). Similar results of association between protein and sugar were also found by Kumari et al. (2007). All the biochemical parameters were associated with plant height and ear height in positive direction, whereas with grain weight in negative direction, except for tryptophan content. Phenological parameters had positive influence on grain weight of maize kernel, tryptophan content, node length, tassel length and ear length which meant that late maturing accessions will have higher values of these traits. Grain yield was positively associated with sugar content, plant height, node length, leaf length, tassel length, number of tassel branches, ear length, ear diameter, kernel rows and kernels per row. It is also important to notice that ear height is not positively related to yield, which might be due to the reason that high ear placement causes lodging of the plant hence evolutionary not selected by the nature with high yield. Further with regards to the biochemical traits, protein and sugar can be improved together due to their positive association. An important finding of the present study

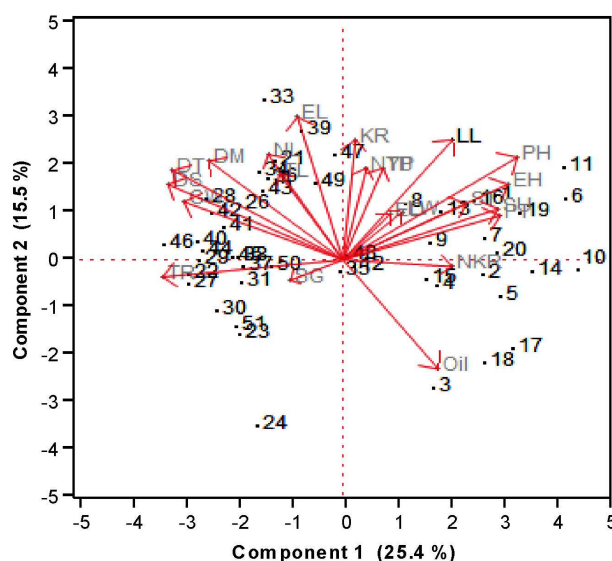
was the significant positive correlation observed between protein with sugar and starch content which implied that breeding for sugar, starch and protein could be strategized simultaneously, however this finding differs from the results obtained by Dudley et al. (2007) and Zhang et al. (2008) who reported significant negative correlation between protein and starch concentration in maize grains. Tryptophan and lysine are two limiting amino acids in maize. The nutritional quality of maize protein is poor because of the deficiencies of these two main essential amino acids. Protein content exhibited a significant negative correlation between tryptophan showing that protein quality may also improve without increase in protein quantity. Protein content and sugar content showed a non-significant negative correlation with 100-kernel weight. This indicates that protein and sugar content can be increased through reciprocal recurrent selection without sacrificing the kernel weight or size at least to the degree that reduction in kernel size affects yield. This observation is in agreement with the studies conducted previously. It has been reported that protein and kernel weight were not correlated in random mated population (Chaudhary et al. 2012). Also tryptophan content can be increased without compromising for the grain yield. The correlation between seed protein and oil content was found positive but non-significant. Panthee et al. (2005) in their study found an inverse relationship between seed protein and oil concentration making it difficult to improve both traits simultaneously. However, Sentayehu (2008) has observed increased concentrations of protein, lysine and other limited amino acids in the high oil maize. The starch content was not related to sugar content in this study although it was found negatively correlated by Kumari et al. (2007). Based on this study inference can be drawn that higher gain in yield improvement may be achieved by indirect selection of high kernel rows, kernels per row, ear diameter, ear length, leaf length and moderate plant height. Further, with regards to the biochemical traits protein and sugar can be improved together due to their positive association.

### **Cluster and principal component analysis**

Understanding the extent and patterns of genetic diversity within germplasm accessions, particularly landraces of a particular region, is important for effective future collection, development of conservation strategies and efficient use of these genetic resources (Frankel et al. 1995). Agronomic and ecological characteristics influence the genotypic constitution of landraces during domestication, and hence a relation

exists between the agro-ecology of the exploration sites and the morpho-physiological make-up of the landraces. Also the analysis of genetic variability and diversity in specific regions is important mainly because to know the prevalence of specific traits in the landraces. The significance of phenotypic evaluation of maize landraces was highlighted by studies undertaken in various countries, including Canada (Azar et al. 1997), Turkey (Ilarslan et al. 2002), Mexico (Pressoir and Berthaud 2004), India (Prasanna and sharma 2005) and China (Wei et al. 2009).

Cluster analysis was performed using Ward linkage method and Euclidean distance measure. Four major clusters comprising 19, 5, 18 and 9 accessions, respectively were formed. Cluster 1 had all 11 accessions from Jammu and Kashmir and 8 accessions from Himachal Pradesh. Cluster III was further divided into subclusters III A (14 acc.) and III B (4 acc.). Cluster IIIA comprised all 3 accessions from Kullu districts and cluster 4 had all accessions from Kangra district belonging to altitude level 500-1000 meter above sea level. As the characters were interrelated, principal component analysis (PCA) was undertaken to have an idea about their independent impact on variability. The first six components in the PCA analysis with Eigen values >1 contributed 72.59% of the variability among accessions evaluated for different agro-morphological traits however major share of variance (40.92%) in maize landraces was explained by first two components. Principal component one (PC1) contributed 25.41 % of the total variation that was loaded on protein with loading of 0.66, sugar, with loading of 0.65, plant height (0.73), ear height (0.69), leaf length (0.46), number of kernel row (0.46), and yield per plant (0.17), while PC2 accounted for 15.5% of total variation with loading on protein (0.20), sugar (0.65), starch (0.52), leaf length (0.46), plant height (0.48) and ear height (0.35). Two dimensional plot based on the PC1 and PC2 revealed similar type of grouping pattern as revealed by clustering. Accessions from Jammu and Kashmir clustered together in the biplot based on two principal components (Fig. 2). Grouping large number of germplasm accessions into few numbers of homogenous clusters facilitates the selection of diverse parents for crossing programme. Cluster analysis as well as principal component analysis categorized the 51 accessions in similar manner. PCA was performed to classify populations on the basis of the most discriminatory traits. Among the traits analyzed plant height, ear height, protein, oil, sugar, starch, leaf length, kernel row played an



**Fig. 2. Principal component biplot showing loading scores of traits and grouping pattern of maize accessions based on two principal components (PCs); 1-11 refer accessions from J&K and 12-51 from HP as mentioned in Table 1**

important role in differentiating maize accessions. Cluster analysis and PCA were also used by the Gouesnard et al. (1997) and Sharma et al. (2010) to differentiate maize landraces based on morpho-agronomic characters. However, in the present study both cluster analysis and PCA could effectively discriminate the accessions from different geographical regions of NWH region. Thus, it can be inferred that phenotypic attributes and biochemical characteristics of landraces do have an important role in genetics and breeding studies and form useful methods of preliminary evaluation of diversity.

The phenotypic and biochemical evaluation of 51 maize accessions under study allowed distinguishing landraces from different geographical regions and identifying accessions with specific traits. Plant breeders need genetically diverse germplasm to improve yield and its contributing traits, morphological attributes and grain quality traits. This collection of maize accessions from North Western Himalayan regions represents a valuable genetic patrimony with great potential for the utilization by breeders and farmers. The promising accessions for the important traits with high sugar and, protein contents alongwith high yield may be exploited in breeding programme. The genetic relationship revealed by cluster analysis as well as PCA was largely in

congruent with the geographical origin and provided a more comprehensive insight into the genetic relationship. This set of maize accessions were diverse and had relevant traits important for climate change and nutritional security. However, further study in this direction is warranted to analyze diversity based on molecular markers and more detailed study on specific characteristics such as biotic and abiotic stresses and nutritional aspects of these landraces.

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