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RESEARCH ARTICLE

Phenotypic Diversity of Maize Landraces from North-Western India and Exploring their Potentiality

Jyoti Kumari*, Shivani Sharma, Sherry R Jacob, Ram Kumar Sharma, Preeti Jakhar, Sapna Langyan, Ishwar Singh, KS Hooda and Ashok Kumar

Abstract

Collection of local landraces is essential for preserving the genetic variability and their evaluation and utilization through plant breeding provide economic benefits to the farmers and mankind. The present study aimed to evaluate the diversity present in the maize collections of North-Western (NW) regions of India on the basis of agro-morphological traits. About 294 diverse accessions collected from remote areas of the North-Western region (Rajasthan and Gujarat) were evaluated for 26 agro-morphological descriptor traits for two consecutive years, 2015 and 2016. Based on qualitative traits, the predominance of pink tassel anther, glume color and silk color green, droopy and leathery leaves and yellow-colored kernels were found in the collected accessions. These accessions had wide variability for green cob yield/plant, tassel branching, leaf width, number of ears per plant and ear height. Boxplot analysis revealed that accessions of Rajasthan had relatively higher mean values for leaf length, leaf width, ear width and 100-seed weight, whereas accessions from Gujarat were early maturing. The cluster analysis based on the phenotyping data divided 294 accessions into six classes and the spatial diversity of accessions in biplot clearly depicted that the accessions of Rajasthan had more diversity in comparison to those from Gujarat. Multi-trait specific accessions (IC331107 with high ear length and width, kernel rows and number; IC280465, early maturing with less plant and ear height; IC331144 and IC470443 with earliness and bold seededness) could be selected for further research and utilization in breeding and genetics studies.

Keywords: Maize landraces, Characterization, Genetic resources, Diversity analysis, North-Western India

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Introduction

Maize (*Zea mays* L.) is the third most important food crop after rice and wheat, having diverse uses as human food, animal feed, fodder and industrial products. Globally, maize is known as a queen of cereals owing to its highest genetic yield potential and is one of the cereal crops that can be grown in different seasons, ecologies and altitudes. Maize has high variation for kernel types like normal yellow/white grain corn, sweet corn, baby corn, popcorn, waxy corn, high amylase corn, high oil corn, quality protein maize, etc. It is considered a *Kharif* crop since 80% of its sown area is under cultivation in the *Kharif* season. In terms of area and production, the USA is the largest producer of maize in the world. The cultivation of maize in India is practiced in 9.86 m ha with the production of 32.42 m tonnes and 3.29 tonnes ha^{-1} productivity (https://agriwelfare.gov.in/en/StatHortEst). Among Indian states, Karnataka has the highest area and production under maize (1.59 m ha and 5.22 m tonnes) followed by Madhya Pradesh (1.40 m ha and 4.57 m tonnes). Among other states, Maharashtra (1.29 m ha and 3.53 m tonnes), Rajasthan (0.95 m ha and 2.04 m tonnes) and Bihar (0.66 m ha and 2.52 m tonnes) has the major share in area and production (Agricultural Statistics at a Glance, 2022).

The maize crop is considered to be domesticated in Southern Mexico from teosinte (*Z. mays* L. subsp. *parviglumis*) between 6000 to 10,000 years ago (Matsuoka *et al*., 2002). It further spread to other parts of the world and got adapted to diverse climates and soil types such as the deserts and the high elevations of the mountains. Grant and Wellhausen (1955) studied the racial diversity of maize in India and observed extensive variability in plant, tassel and ear characteristics in the North-Eastern (NEH) region and North-western highlands of India. Mukherjee *et al.* (1971) reported that the varieties of Northern Plains and Peninsular India resembled Mexican and Columbian germplasm.

Morphological characterization is a pre-requisite for the assessment, description and classification of germplasm collections to enhance their use in maize breeding (Prasanna and Sharma, 2005; Wasala *et al.,* 2013; Kumari *et al*., 2017). The importance of phenotyping maize landraces from India and other countries has been highlighted in various studies (Salazar *et al*., 2016; Kumari *et al.,* 2017; Islam *et al.,* 2020; Nelimor *et al*., 2020). The National Genebank of India (NGB) housed in ICAR National Bureau of Plant Genetic Resources (NBPGR) conserves around 11,094 maize accessions comprising both indigenous and exotic collections. Of these, 331 accessions have been collected from Rajasthan and 181 accessions from Gujarat as landraces and local collections over the years. These regions fall in arid and semi-arid regions, having fragile ecosystems and receiving very less rainfall. Different forms of diversified uses of maize are found in the west and central plains of India. It is used as corn soup, *daliya*, roasted cob, popped kernel, corn oil, forage/fodder, wafers/*papad, sattu* and *chapatti*/flatbread. Some of the local cultivars/landraces grown in the western plain region of India included the flint types, Doodhmogar and Sameri, known for early maturity and drought tolerance; Gulla and Chandan with drought tolerance and Sathi, with insect-pest resistance trait (Pandey *et al.,* 2015). Dhillon *et al.* (2005) reported some promising landraces from the North-Western (NW) region as Sathi local from Punjab with heat and drought tolerance, very early maturity, excellent yield and high adaptability, Basi local and Dausa local from Rajasthan with multiple traits like excellent yield characters, drought tolerance trait and wider adaptability. This untapped valuable diversity needs to be collected, conserved, systematically evaluated and effectively utilized. Therefore, the present study was undertaken to characterize and evaluate the landraces and local collections from Rajasthan and Gujarat for agro-morphological traits and, to analyze phenotypic diversity among the accessions collected and to identify the trait-specific germplasm for utilization in maize improvement.

Materials and Methods

About 294 accessions conserved in the National Gene Bank (NGB), comprising 190 accessions from Rajasthan and 104 from Gujarat, were used for characterization and evaluation purposes in this study (Supplementary Table 1, Figure 1). The accessions from Gujarat were collected from the districts of Amreli, Banaskantha, Baroda, Bharuch, Dahod, Godhra, Jamnagar, Junagadh, Narmada, Panchmahal, Sabarkantha, Surat, Vadodara and Vijay Nagar. Similarly, from Rajasthan, the collections belonged to remote villages in the districts of Ajmer, Alwar, Banswara, Baran, Bharatpur, Bhilwara, Bundi, Chittorgarh, Dausa, Dungerpur, Jaipur, Jodhpur, Jhalawar, Jhunjhunu, Karauli, Kota, Pali, Pratapgarh, Rajsamand, Sawai Madhopur, Sirohi, Tonk and Udaipur. The altitude of the collection site varied from 13 m above sea level (Surat) to 300 m above sea level (Dahod) in Gujarat.

These accessions were evaluated in Augmented Block Design at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, during *Kharif* 2015 and 2016 with four checks HQPM-1, Bio9637, Jawahar Pop Corn and Pusa Composite 3 randomized in each 15 blocks of size 40, with the last block as an incomplete block. Each experiment comprised a row plot with a row length of 4 m and row-to-row spacing as 75 cm. The experimental farm is situated at a latitude of 28°40′ N and longitude of 77°12′ E and an altitude of 228.6 m above the mean sea level (Arabian Sea). The mean annual rainfall of Delhi during the crop season was 650 mm and annual temperature ranged between 12 and 32°C. The data were recorded for 26 agro-morphological descriptors as per the minimal descriptors and descriptor's states developed by ICAR-NBPGR (Mahajan *et al.,* 2000). The 15 agronomic characters included days to tassel, days to silk, plant height (cm), ear height (cm), ear length (cm), ear diameter (cm), leaf length (cm), leaf width (cm), tassel branching, number of kernel rows/ear, number of kernels/rows, days to maturity, number of ears/plant, grain weight (g) and green cob yield/plant (g). The 11 qualitative characters undertaken in this study were early plant vigor, tassel texture, tassel anther glume color, silk color at emergence, leaf color, leaf orientation, leaf pubescence, leaf texture, grain texture, grain size and kernel color.

Analysis of variance (ANOVA) for the individual year was carried out as per the augmented design and then the adjusted mean of both years was subjected to combined analysis after ascertaining the homogeneity of error variance using Bartlett's test (Arsham, 2010). Adjusted mean values for each trait were used for further analyses. Boxplot was generated for 15 quantitative traits to compare the performance of accessions from both states. Genetic diversity analysis was performed using a hierarchical algorithm with Euclidean distances and Ward's method of minimum variance for clustering the accessions. Principal component analysis (PCA) was carried out based on a phenotypic correlation matrix for the 15 descriptors. Each principal component was calculated by taking a linear combination of an Eigenvector of the correlation matrix with a variable. The biplot based on two principal components

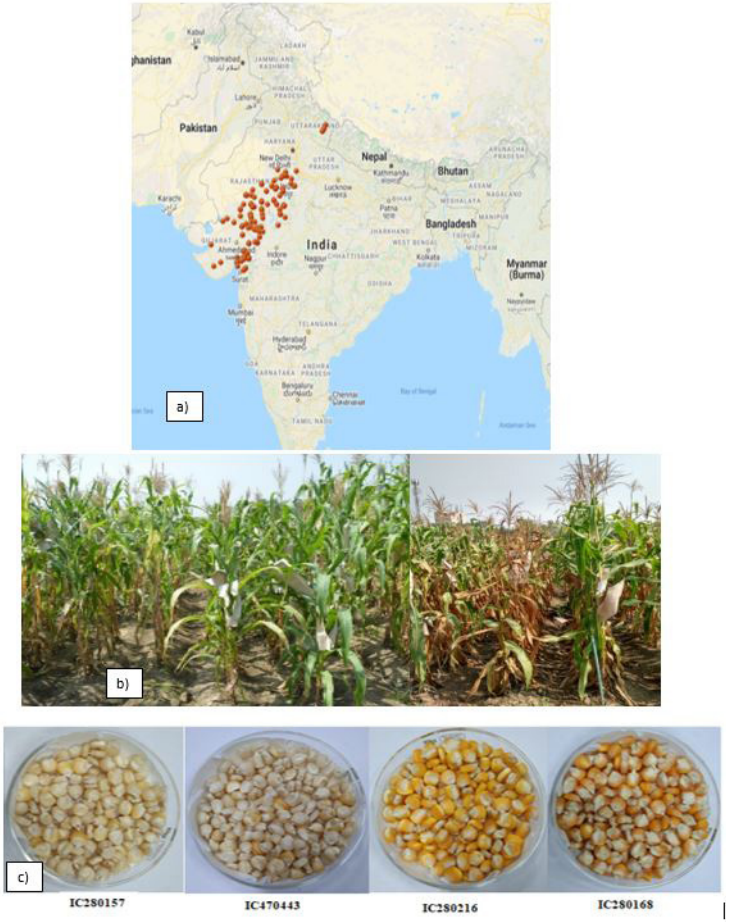

Figure 1: a) GIS map depicting collection area of maize accessions, b) Field view showing early maturing accession; c) Maize accessions with high grain weight

was also generated to depict the two-dimensional view of accession scores as well as the loading of characters. ANOVA was estimated using SAS 9.3, whereas Boxplot, frequency distribution, clustering and PCA were performed using JMP 15 software.

Results

Agro-morphological Characterization

Characterization for qualitative traits describes the accessions about their distinctiveness, uniqueness and specific traits. The frequency distribution of some qualitative traits (early plant vigor, tassel texture, silk color, leaf color, leaf pubescence, leaf texture, grain texture, grain size, kernel color) are depicted in Figure 2. Agro-morphological characterization for silk and tassel-related traits revealed that the majority of the accessions had pink tassel anther glume color (185), followed by light purple (83), green (23) and purple color(3); however, tassel glume base color was absent in 293 accessions. The silk color at emergence was pink in 174, while green in 115 accessions. Tassel texture was either medium or lax except 18 accessions with dense type. For leaf color, the majority of them were green (136) and dark green color (91). Leaf orientation was found to be erect in 53 while drooping in 238 accessions. Another trait, leaf pubescence, was present in 50 accessions. For leaf texture, 121 accessions had leathery texture, followed by 116 as normal and, 213 accessions had medium leaf width and 78 had narrow leaf width. For kernel traits, yellow-colored kernels (160) were predominant, followed by white (60). Most of the accessions had either indented or round type grain shapes. Grain size, the important parameter for yield determinant, was medium in the majority of the accessions. However, bold size was recorded in six accessions from Rajasthan, namely, IC280216, IC280435, IC280169, IC280212, IC437070 and IC470443. The ear shape was cylindrical with an intermediate husk cover and regular kernel row arrangement in most of the accessions.

Phenotypic Variability and Identification of Trait-specific Germplasm

Analysis of genetic variability and diversity in maize collections of specific regions is of utmost importance to know the prevalence of specific traits in the landraces. We observed sufficient variation for all 15 quantitative traits (Table 1 and Supplementary Figure 1). Days to tassel

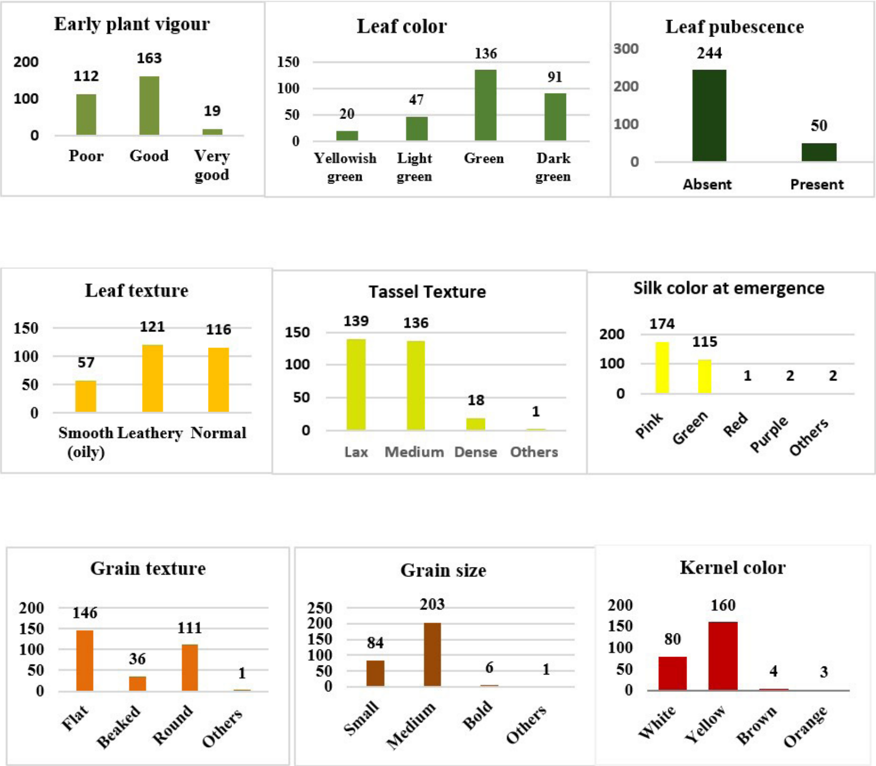

Figure 2: Frequency distribution for nine qualitative traits in maize accessions

varied from 41.5 to 73 days with a mean value of 53.37 days; days to silk varied from 45 to 75 days with mean value of 6.69 days; grain yield/plant showed a range from 50 to 390 g with an average of 175.11 g; tassel branching varied between 12.60 to 38 with a mean value of 20.83; plant height ranged from 141.10 to 239.25 cm with a mean value of 200.44 cm; ear height ranged from 73.60 to 149.5 cm with mean value of 105.28 cm; a number of ears/plants ranged from 1.15 to 2.45 with mean of 1.61. Leaf length varied from 54.80 to 105.30 cm with a mean value of 86.16 cm and leaf width ranged from 6 to 12.90 cm with a mean value of 9.36 cm. These accessions matured between 80.50 to 98.11 days with a mean value of 89.65 days. Among ear traits, ear length varied between 10.25 to 21.80 cm with a mean value of 15.61 cm, ear width ranged from 2.30 to 4.90 cm with a mean value of 3.50 cm, kernel rows/cob ranged between 8.95 to 16, with a mean value of 12.74; a number of kernels/row varied between 19.70 to 41.30 with range of 29.79 and 100-grain weight varied between 14.15 to 31.40 g with average of 20.82 g.

Boxplot analysis revealed that accessions from Rajasthan showed a wider range of variability than that of Gujarat for traits days to tassel, days to silk, tassel branching, ear height, leaf width, ear width and 100 seed weight. Similarly, accessions from Gujarat showed more variation than Rajasthan for green cob yield/plant, numbers of ears/plant, ear length, and number of kernel rows and for traits like plant height, leaf length and number of kernels per row, accessions from both states showed similar range of variability (Supplementary Figure 2).

Promising maize germplasm was identified for different traits (Table 1), such as, for green cob yield per plant, IC331150, IC337022, IC325844, IC280180, IC313646, IC325961, IC331147, IC325856 (>310.00 g). For 100-seed weight, IC280216, IC470443, IC280168, IC280157, IC273359, IC331144 (>27 g) were found promising. Maize accessions IC331141, IC331144 and IC280167 had tasseling and silking in less than 45 days and IC280465, IC449437 and IC344701 came in maturity in about 80 days. High kernel numbers per row, *i.e.*, more than 38, were found in accessions IC331205, IC273356, IC337035, IC331107, IC437070, IC280182, IC337025, IC470459. We also identified accessions with multiple traits, namely IC331107 (Medium plant height, long and wide ear, high number of kernel rows and kernels per row) and IC280465 (earliness and medium plant height), IC331144 and IC470443 (earliness and seed weight). These promising germplasms were validated in replicated trial during *Kharif* 2017 and 2018, consecutively for two years.

Multivariate Analysis

Correlation analysis was carried out among 15 quantitative parameters as shown in Supplementary Table 2. Days to tasseling showed a positive correlation with days to silking

Table 1: Mean and range of traits studied and trait-specific germplasm identified in maize collections

Traits	*Range*	*Mean ± SE*	*Trait specific germplasm*
Days to tasseling	41.50–73.00	53.37 ± 0.45	IC331144(Guj), IC331153(Guj), IC280167(Raj), IC331141(Guj), IC330939(Raj), IC280465(Raj), IC470443(Raj), IC280406 (Raj) (<45.00)
Days to silking	45.00–75.50	56.69 ± 0.23	IC331141(Guj), IC331144(Guj), IC280167(Raj), IC330939(Raj), IC331153(Guj), IC280465(Raj), IC470443(Raj), IC280405(Raj) (<48.50)
Green cob yield/plant	50.00–390.00	175.11 ± 3.27	IC331150(Guj), IC337022(Guj), IC325844(Raj), IC280180(Raj), IC313646(Raj), IC325961(Guj), IC331147(Guj), IC325856(Raj) (>310.00)
Tassel branching	12.60–38.00	20.83 ± 0.19	IC280429(Raj), IC325961(Guj), IC280421(Raj), IC331198(Guj), IC280422(Raj), IC337023(Guj), IC337022(Guj) (>28.00)
Plant height (cm)	141.10–239.25	200.44 ± 1.02	IC344658(Guj), IC280168(Raj), IC280465(Raj), IC331107(Guj), IC280445(Raj), IC77602(Raj), IC337090(Guj), IC344794(Guj) (<166.00)
Ear height (cm)	73.60–139.45	105.28 ± 0.66	IC82002(Guj), IC280426(Raj), IC344658(Guj), IC77602(Raj), IC280444(Raj), IC280465(Raj), IC280461(Raj), IC449432(Raj) (<83.45)
No. of ears per plant	1.15–2.45	1.61 ± 0.01	IC81998(Guj), IC273353(Raj), IC280164(Raj), IC273346(Raj), IC330966(Guj), IC331193(Guj), IC273358(Raj), IC280415(Raj) (>2.00)
Leaf length (cm)	54.80–105.30	86.16 ± 0.52	IC337006(Guj), IC280212(Raj), IC298557(Guj), IC280200(Raj), IC82089(Raj), IC310656(Raj), IC81991(Guj) (>102.60)
Leaf width (cm)	6.00–12.90	9.36 ± 0.07	IC280431(Raj), IC280157(Raj), IC280429(Raj), IC273338(Guj), IC280165(Raj), IC280174(Raj), IC280408(Raj), IC325876(Raj) (>10.80)
Days to maturity	80.50–98.11	89.65 ± 0.18	IC280465(Raj), IC449437(Raj), IC344701(Guj), IC344716(Guj), IC280405(Raj), IC280459(Raj), IC280462(Raj), IC449432(Raj) (<82.00)
Ear length (cm)	10.25–21.80	15.61 ± 0.09	IC333328(Guj), IC331107(Guj), IC337042(Guj), IC313643(Raj), IC331205(Guj), IC337038(Guj), IC330965(Guj), IC331093(Guj) (>19.00)
Ear width (cm)	2.30–4.90	3.50 ± 0.02	IC280450(Raj), IC77611(Guj), IC331107(Guj), IC280429(Raj), IC337439(Guj), IC331205(Guj), IC337024(Guj), IC331118(Guj) (<4.00)
No. of kernel rows	8.95–16.00	12.74 ± 0.07	IC331107(Guj), IC331098(Guj), IC273342(Guj), IC280161(Raj), IC391316(Raj), IC82089(Raj), IC336989(Guj), IC337022(Guj) (>15.00)
No. of kernels/row	19.70–41.30	29.79 ± 0.20	IC331205(Guj), IC273356(Raj), IC337035(Guj), IC331107(Guj), IC437070(Raj), IC280182(Raj), IC337025(Guj), IC470459(Raj) (>37.92)
100 Seed weight (g)	14.15–31.40	20.82 ± 0.15	IC280216(Raj), IC470443(Raj), IC280168(Raj), IC280157(Raj), IC273359(Raj), IC331144(Guj), IC437070(Raj), IC273332(Guj) (>25.40)

(0.97**), days to maturity (0.75 **), green cob yield/plant (0.30**), leaf length (0.20**) and grain weight (0.29**). Days to silking had a positive association with yield per plant (0.29**), leaf length (0.22**), days to maturity (0.77**) and grain weight (0.31**). Yield per plant showed a positive correlation with leaf length (0.16**) and days to maturity (0.32**). Tassel branching showed a positive correlation with leaf width (0.36**) and ear width (0.23**). Plant height was positively associated with ear height (0.79**), leaf length (0.17**) and days to maturity (0.17**), whereas ear height showed a positive correlation with leaf length (0.18**), leaf width (0.17**), days to maturity (0.16**), ear length (0.17**) and ear width (0.17**). Leaf length showed positive correlations with leaf width (0.25**), days to maturity (0.22**), ear length (0.16**), ear width (0.24**) and grain weight (0.26**), while leaf width had a positive correlation with ear length (0.31**), ear width (0.32**), number of kernel rows (0.19**) and grain weight (0.19**). Ear length showed positive correlations with ear width (0.45**), number of kernel rows (0.23**), number of kernels/row (0.61**) and grain weight (0.35**), whereas ear width had a positive correlation with the number of kernel rows (0.54**), number of kernels/row (0.35**) and grain weight (0.42**).

Genetic diversity analysis was performed using hierarchical cluster analysis with Ward's method. All the accessions were grouped into six clusters comprising 46, 80, 20, 73, 38 and 40 accessions, respectively (Supplementary Figure 3). Among all the clusters, cluster 2 was the largest and cluster 3 was the smallest one. Cluster I comprised accessions with high grain weight with high numbers of ears per plant, whereas cluster III comprised early maturing accessions. Cluster IV had accessions with high yield per plant, leaf length, ear length, ear width and number of kernels per row, while cluster V had accessions with high leaf width and number of kernel rows. Cluster VI comprised accessions having tall plant height and ear height (Supplementary Table 3).

The PCA based on correlation was used to study interrelationships among the different parameters and their contribution to the overall variability. The first five

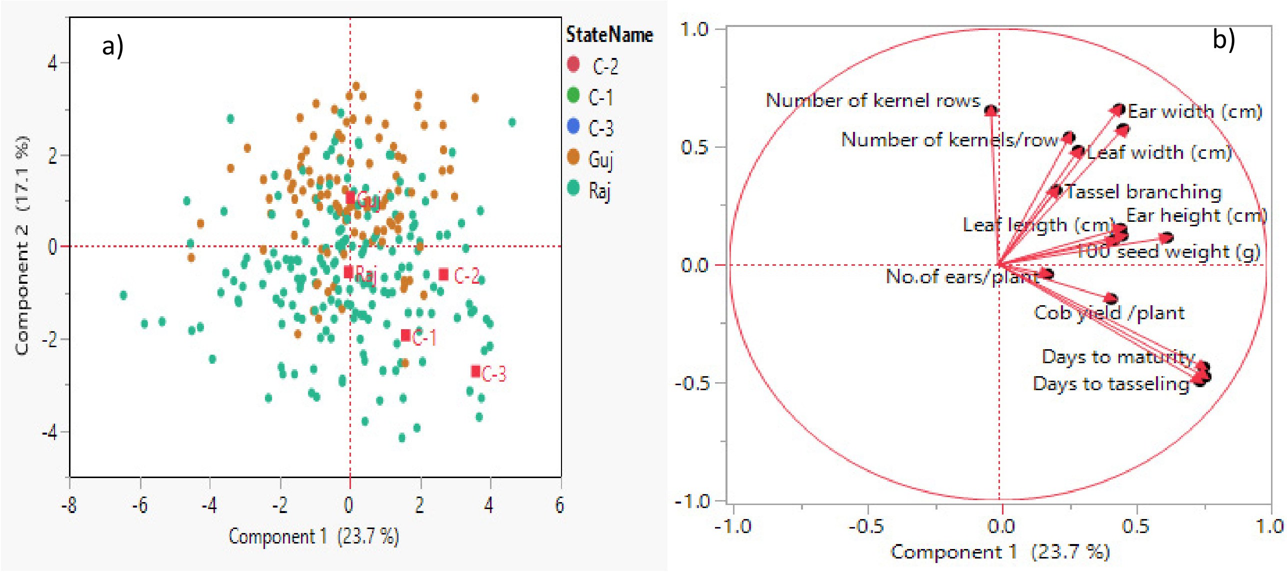

Figure 3: Principal component biplot showing the distribution of maize accessions: a) Genotype biplot; b) Trait biplot

components provided major summary of data and explained 67.49% variance in maize germplasm. The first principal component (PC I) accounted for 23.72% of the variation and was mainly contributed by days to silking, days to tasseling, days to maturity and 100-grain weight. The second principal component accounted for 40.78% of variation and was mainly contributed by ear width, number of kernel rows, number of kernel/rows and ear width (Supplementary Table 4). Two-dimensional principal components plots displayed significant variation in accessions from both states, with visible intra-state diversity (Figure 3). However, some accessions from adjoining districts or from the inter-state boundary area are overlapping, which might be due to the gene flow resulting from cross-pollinating behavior of maize.

Discussion

Characterization and evaluation of any landrace is the primary step to gaining knowledge about the specific traits of the germplasm accession collected for conservation and further utilization in breeding programs. Evaluation of genetic resources for specific traits is essential for finding new sources of donor for target traits and their introgression into elite lines for specific breeding purposes. The maize accessions from this fragile ecosystem of NW India had mainly pink tassel anther glume color, absence of tassel glume color base, pink or green silk color, medium or lax tassel texture, green and dark green leaf color, drooping leaf orientation, absence of leaf pubescence and leathery or normal leaf texture and medium leaf width. With regard to kernel traits, these accessions had majorly yellow colored kernels followed by white, indented or round type grain shape and medium grain size. However, large-size grain was recorded in six accessions from Rajasthan, namely, IC280216, IC280435, IC280169, IC280212, IC437070 and IC470443. Ear shape was cylindrical with intermediate husk cover and regular kernel row arrangement in most of the accessions, which meant that farmers preferred these types during selection on their farmland. The phenotypic evaluation of maize landraces was reported by earlier researchers in various countries, including Canada (Azar *et al.* 1997), Turkey (Ilarslan *et al.* 2002), Mexico (Pressoir and Berthaud 2004), India (Prasanna and Sharma 2005, Kumar *et al.,* 2015 and Kumari *et al.*, 2017) and China (Wei *et al.* 2009).

Positive correlation among days to tasseling and silking with green cob yield/plant, leaf length and grain weight suggested more time is required for biological growth and photosynthate accumulation in plants. Similarly, a positive association of leaf length and leaf width with ear length, ear width and grain weight was observed, which is due to the role of larger leaf area in translocation of photosynthates from source to sink. Ear length showed a positive association with ear width, number of kernel rows, number of kernels/row and grain weight. Thus, for enhanced maize yield, medium-maturity germplasm with large leaf size and ear length should be preferred. Similar kind of association between grain yield, 100-seed weight, ear girth and ear length have been reported previously (Selvaraj & Nagarajan, 2011; Jambagi & Wali, 2016).

Knowledge of the extent and pattern of genetic diversity within germplasm accessions, particularly collections of a specific region, is important for effective future collection, development of conservation strategies and efficient use of these genetic resources (Frankel *et al.* 1995). Ecological characteristics of any region influence the genotypic constitution of landraces during domestication. Genetic diversity provides breeders with basic germplasm material for crossing programs and broadening of genetic base. Here, the cluster analysis revealed a sufficient level of variability in the maize germplasm and accessions from a diverse group, such as groups 3 and 6 for plant and ear height and groups 1 and 3 for tasselling and silking, which can be used in the hybridization program. Cluster analysis used by Gouesnard *et al.,* 1997 Sharma *et al*., 2010 Kumar *et al*., 2015; Kumari *et al*., 2017 and Goyanka *et al*., 2021 also differentiated maize landraces based on morpho-agronomic characters.

In this study, the principal component biplot supported the result obtained by cluster analysis. The first five components provided a major summary of data and explained most of the variation in maize germplasm. The first principal component was mainly contributed by days to silking, days to tasseling, days to maturity and 100-grain weight. The two-dimensional principal components plot showed good variation in accessions from both states, with overlapping in accessions from adjacent locations across the state boundaries due to gene flow caused by cross-pollination. Several researchers used principal component analysis to analyze diversity patterns in maize germplasm (Mohammadi and Prasanna, 2003; Angelo *et al.,* 2008; Lu *et al.,* 2009). Semagn *et al.* (2012) in their study obtained three groups among 450 germplasm based on PCA using 1065 SNPs and principal component analysis supported the clustering pattern.

Being a crop with varied uses, maize has immense potential to be utilized as feed, fodder, food and various industrial uses. Hence, there is a need to search for novel germplasm that can be used directly as a product or indirectly through further improvement. This study led to the identification of unique germplasm for specific traits like green cob yield per plant (IC331150, IC337022, IC325844), early tasseling and silking less than 45 days (IC331144, IC331153, IC280167), early maturity, high kernel number per row (IC331205, IC273356, IC337035), kernel rows (IC331107, IC331098, IC273342), ear length (IC333328, IC331107, IC337042) and grain weight (IC280216, IC470443). The accessions with multiple traits (IC331107 with high ear length and width, kernel rows and number; IC280465, early maturing with less ear height; IC331144 and IC470443 with earliness and bold seededness) were also identified for further use in a breeding program. Therefore, evaluation of the untapped gene pool of maize is necessary to find out the variation present and to promote their conservation and utilization.

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Supplementary Files

https://ispgr.in/public/site/supplementary-files/2600-supplementary-file.pdf