P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i02.05

Indian Journal of
Plant Genetic Resources

RESEARCH ARTICLE

# Eco-geographic Survey, *In-situ* Characterization and Spatial Analysis of Phenotypic Traits Diversity among the Maize Landraces Collected from Chhattisgarh

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## Abstract

Exploration and germplasm collection expedition undertaken in the three districts of Chhattisgarh state in the present study resulted in the collection of 43 diverse local maize genotypes. The collected accessions were characterized *in-situ* on a farmer's field for six ear and seed-specific quantitative characters and the recorded data was analyzed for quantification and analysis of the genetic variability among the accessions. Significant variability was recorded in all the studied traits *viz*. ear length (7.33–26.67 cm), ear girth (8.00–16.33 cm), number of kernel rows per ear (8-18), number of kernels per ear row (12.67–45.33), ear weight (25.45–204.88 g) and 100 kernel weight (9.74–38.66 g). The first three components explained 99.61% of the total variation; the first principal component represented 97.33%. Cluster analysis based on Euclidean distances classified 43 maize genotypes into five clusters with clustering together of 9, 18, 13, 2 and 1 accessions. Geo-referencing analysis of recorded data was done using DIVA-GIS software. The diversity pattern of maize germplasm accessions revealed by the grid maps confirmed the existence of considerable genetic diversity for all the six quantitative traits in the surveyed area also discussed in this study.

**Keywords**: *Zea mays*, Germplasm, *In-situ* characterization, Diversity analysis, DIVA-GIS.

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**Received:** 28/04/2022 **Revised:** 26/04/2023

**Accepted:** 26/04/2023

**How to cite this article:** Bishnoi, S.K., Malav, P.K., Singh, K., Phuke, R.M., houdhary, S.B., Sharma, H.K. and Ahlawat, S.P. (2023). Eco-geographic Survey, In-situ Characterization and Spatial Analysis of Phenotypic Traits Diversity among the Maize Landraces Collected from Chhattisgarh. *Indian J. Plant Genetic Resources*. 36(2), 233-242. **DOI:** 10.61949/0976-1926.2023.v36i02.05

## Introduction

The breeding progress in maize is dependent on the extent of genetic diversity in its gene pool, most importantly the landraces (Delucchi *et al.,* 2012; Azeez *et al.,* 2018). Therefore, the collection and *ex-situ* conservation of the landraces is a pre-requisite for appreciating their importance as genetic resources and for the development of complementary conservation methodologies (Vilaro *et al.,* 2020). There is a lacking of agronomic and genetic data for the collected landraces limiting their use and management (Azeez *et al.,* 2018). Modern commercial agricultural practices involving homogeneous high-yielding cultivars have resulted in the decline of landrace diversity fueled by global climate change (Mir *et al.,* 2020). The landrace diversity should not only be conserved from a gene source point of view but also from the point of view of sustainable agriculture because of their resiliency/adaptation towards abiotic and biotic stresses prevalent in a particular geography and that they have a preferred taste and sensory properties (Tokatlidis and Vlachostergios, 2016; Dwivedi *et al.,* 2016; Casanas *et al.,* 2017).

The eco-geographic data associated with the collected germplasm accessions has proved extremely useful for trait identification in different crops, including maize (Vilaro *et al.,* 2020). A germplasm accession accompanied with complete passport information and preliminary statistics about key ear and kernel traits is of immense value to the breeders for starting of a pre-breeding programme.

The maize germplasm from India represents a very important component in the world's maize collections. The role of farmer conservators has been well recognized in India in the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPVF&RA, 2001) and the Biological Diversity Act, 2002 (BDA, 2002).

Indian maize landraces are a very important source of single cross maize hybrids (SCHs). The maize germplasm diversity having primitive characters of popcorns and prolificacy from North-Eastern and North-Western Hill regions of the country have been substantially collected and conserved, but less emphasis has been laid on the maize germplasm from other parts (Prasanna 2010, 2012; Yadav *et al.,* 2015 and Dar *et al.,* 2018).

A total of 11699 maize accessions are conserved in the Indian National Gene Bank til now. Out of these only 275 accessions are conserved from Chhattisgarh state and 43 accessions are targeted for the present study. Important gaps were reported in the collections from Chhattisgarh (Pandey *et al.,* 2015); *http://www.nbpgr.ernet.in/PGR_Databases.aspx*), which needed to be filled for building of a comprehensive national collection of maize germplasm. Further, the state of Chhattisgarh is the target for the popularization of SCHs. However, a low hybrid adoption rate of less than 30% was reported (Yadav *et al.,* 2015) which presented a unique opportunity to collect the indigenous maize diversity from the previously unexplored areas of the state.

Therefore, the present study was undertaken with an objective of filling the maize germplasm gaps by undertaking an eco-geographic survey, collection and *in-situ* characterization mission from the three ethnically and eco-geographically diverse districts of Chhattisgarh *viz*. Jaspur, Koriya and Korba.

## Material and Methods

### *Area Prioritization*

In the present study, three districts *viz*. Jaspur, Koriya and Korba located in the North-Eastern part of the Chhattisgarh state were targeted for execution of the eco-geographic survey and germplasm collection mission. The geographic area explored was prioritized based on the gaps in the maize collections of the National Gene Bank. The exploration and germplasm collection mission was included in the National Exploration Plan of the ICAR-National Bureau of Plant Genetic Resources, New Delhi for 2017. The prioritized area represents previously unexplored, predominantly tribal with primitive agriculture and highly inclined for hybrid maize adoption with high probability of the erosion of maize genetic diversity.

### *The Eco-geographic Survey*

As suggested by Brown and Marshall (1995), the modalities of the eco-geographic survey were worked out beforehand, including the itinerary to be undertaken and the establishment of prior contacts with the local Krishi Vigyan Kendras which are the governmental grassroots level extension institutions, to help locating the potential sites of maize germplasm diversity in the targeted areas. The total time of survey was divided based on the prior information about the presence of diversity received from the local collaborators. The exploration and germplasm collection mission was executed from 21–30th September 2017. The time of the survey and collection mission was so adjusted to coincide with the physiological maturity of the maize crop in most of the targeted areas. The starting point and the direction of the transacts were randomized at the time of preparation of the itinerary and minimum distance among the sampling points were employed as per Brown and Marshall (1995).

### *Germplasm Sampling*

The targeted area, with particular focus on deep-seated interior germplasm diversity pockets, was transacted as per the pre-planned itinerary with 100 to 150 km (Singh and Srivastava, 2004) per day and germplasm collection was attempted as and when sighted. Large samples were particularly attempted in case of highly heterogeneous populations (Brown and Briggs, 1991). Random sampling consisting of a magnitude of different genotypes as per Brown and Marshall (1995) to ensure maximum "allelic richness" which is the direct measure of the value of an accession. The ripe cobs (ears) were collected from the farmer's field, threshing yard and/or from the farm stores employing the bulk random sampling and the frequency of sampling (number of samples per site) was adjusted based on "on-the-spot" observations on the variability available. Each collected maize germplasm accession was assigned a specific collector number and status of the population as a landrace/non-improved cultivar was confirmed by interpersonal interactions with the concerned farmers and the household elderlies.

### *In-situ Data Recording*

The germplasm collection and subsequent characterization are often not in a continuum under real conditions and the beginning of characterization may take an unexpectedly long time due to many logistic and other constraints. *In-situ* germplasm characterization on reproductive/commercial plant parts (ear and kernels in case of maize) makes the preliminary identification of trait-specific germplasm easy as it gives a broad insight about the genetic worth of the collected material based on the observations recorded during collection. The *in-situ* data recording involved on the spot recording of two different kinds of data, the passport data and the quantitative data on ear/seed-specific traits.

The information under the passport data was recorded in the "standard passport data book" of the ICAR-NBPGR and involved assigning of a specific collector number to the collected accession, vernacular name of the species, name of the landrace, biological status (landrace/variety/population), type of material (kernels/ear), date of collection, collecting site/acquisition source (farmer's field/farm store/threshing yard), frequency of occurrence, sample type (population/individual), sampling method (random/specific), habitat (cultivates/wild), site of the collection (Basti (locality)/Village/Developmental block/District/State) and the latitude, longitude and altitude of the collection site using a hand-held Garmin GPS device. All the essential fields of the passport data book were duly filled on the spot. The ears were shelled and seeds were mixed after recording the data. The data on six ear and kernel-specific traits *viz.* ear length, ear girth, number of kernel rows per ear, number of kernels per ear-row, ear weight and 100 kernel weight were recorded on three randomly selected ears. The kernel color, kernel-row arrangement and kernel type (flint/dent/pop) was recorded on a visual basis.

### *Data Analysis*

The public domain software DIVA-GIS version 7.5 (Hijmans *et al.*, 2012) freely available on *www.diva-gis.org* was used for spatial analysis and geo-referencing of the collected maize germplasm diversity by generation of grid maps displaying presence points of the trait specific maize germplasm in a specific geographic area along with the map showing vegetation index i.e. intensity of vegetation in the collected area representative of cropping intensity. The coordinates of the collection were recorded in decimals and used for geo-mapping of maize germplasm accessions. Trait-wise geo-referencing (raster cell size=20 minute) was done using statistics option of points to grid tab of the analysis option of DIVA GIS software. The data recorded on six quantitative traits was analyzed for descriptive statistics, principle component analysis using PAST software (Hammer *et al.*, 2001). Cluster analysis based on quantitative traits was done using NTSYSpc v. 2.20 (Rohlf, 2005). For cluster analysis first estimates of Euclidean distance coefficients were carried out for all pairs of genotypes using SIMINT module of NTSYS software. The resulted matrices of Euclidean dissimilarity coefficient were applied to the SAHN programme of NTSYS software to construct UPGMA dendrogram. Simple correlation coefficients between all pairs of quantitative traits were obtained using mean values (Steel and Torrie, 1980).

## Results

### *The Eco-geographic Survey, Germplasm Collection and Passport Data*

During the present eco-geographic survey and collection expedition, a total of 43 maize accessions were collected from a geographic area spanning three districts *viz.* Jaspur, Koriya and Korba of the Chhattisgarh state. The collected germplasm accessions varied primarily in morpho-phenology, spatial origin, eco-geography of the collection site and anthropo-ethnic backdrop. During the survey, the maize germplasm was collected from the farmer's field (32), threshing yard (8) and farm store (3) with the number of accessions acquired from each source given in the parenthesis. The number of accessions acquired from each source signifies the relative maturity duration as late maturing accessions were still in the farmer's field, medium maturity accessions were those collected from the threshing yard and early to very early maturing accessions already harvested, threshed and stored in the farm store, therefore collected from there. It is evident that most of the germplasm accessions collected during the present study fall into the category of late maturing types. The altitudinal range of the collected material ranged from 312 meters from mean sea level to 1089 meters from the mean sea level (Table 1). This range was classified into different categories of altitude of 100 MSL. By this categorization, a total of 16 accessions were collected between the altitudinal ranges of 451–550 MSL. The altitudinal range of 851–1050 MSL was found to be devoid of maize cultivation in the area explored under the present study. The passport details of all the collected accessions along with the geographic coordinates of the collection, are presented in Table 1.

### *In-situ Phenotypic Characterization*

Significant variation in kernel color, ear shape, ear size, kernel type, row arrangement, ear length, number of kernel rows per ear, number of kernels per ear, number of kernels per row and 100 kernel weight was observed and collected. The collected accessions in the present study were classified into categories based on the differences in the kernel colors *viz.* yellow, yellow and white, yellow with white tinge, pale, white, etc. (Table 1). However, the base color of most of the collected accessions was yellow or the variants thereof, signifying the local farmer's preference for yellow or related colors. The accessions collected in the present study (Figure 1) exhibited a particular pattern regarding their ear shape. The ears were

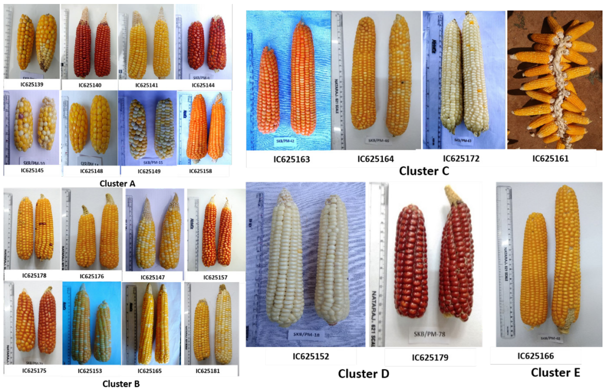

**Figure 1:** Phenotypic diversity in ears of maize germplasm accessions collected from Chhattisgarh, India

**Table 1:** The passport details of maize accessions collected from Chhattisgarh

| Sr. No. | Collector No. | IC No. | Landrace name | Kernel color |
|---|---|---|---|---|
| 1 | SKB/PM-1 | IC625139 | Makai | Yellow |
| 2 | SKB/PM-5 | IC625140 | Makai | Purple |
| 3 | SKB/PM-6 | IC625141 | Makai | Yellow and few white kernels |
| 4 | SKB/PM-7 | IC625142 | Dehati makka | Yellow and few white kernels |
| 5 | SKB/PM-8 | IC625143 | Jowari makka | Yellow with many white kernels |
| 6 | SKB/PM-9 | IC625144 | Lal makka | Purple |
| 7 | SKB/PM-10 | IC625145 | Makai | Pale, white and few purple kernels |
| 8 | SKB/PM-11 | IC625146 | Makai | Yellow |
| 9 | SKB/PM-12 | IC625147 | Makai | Yellow with many white kernels |
| 10 | SKB/PM-14 | IC625148 | Makai | Yellow |
| 11 | SKB/PM-15 | IC625149 | Dehati Makai | Pale and white |
| 12 | SKB/PM-16 | IC625150 | Dehati Makka | Pale |
| 13 | SKB/PM-17 | IC625151 | Dehati Makka | Yellow and white kernels |
| 14 | SKB/PM-18 | IC625152 | Desi Makka | White |
| 15 | SKB/PM-19 | IC625153 | Pili Makka | Yellow with many white kernels |
| 16 | SKB/PM-21 | IC625154 | Dehati Makai | White with many pale kernels |
| 17 | SKB/PM-22 | IC625155 | Makai | Yellow |
| 18 | SKB/PM-24 | IC625156 | Makai | Pale |
| 19 | SKB/PM-28 | IC625157 | Lal makai | Orange with a purple tinge |
| 20 | SKB/PM-31 | IC625158 | Makka | Orange |
| 21 | SKB/PM-35 | IC625159 | Bhutta Makka | Yellow |
| 22 | SKB/PM-36 | IC625160 | Makka | Yellow and white |
| 23 | SKB/PM-39 | IC625161 | Makka | Yellow |
| 24 | SKB/PM-40 | IC625162 | Makka | Yellow with white tinge |
| 25 | SKB/PM-42 | IC625163 | Makka | Orange |
| 26 | SKB/PM-46 | IC625164 | Makka | Yellow |
| 27 | SKB/PM-47 | IC625165 | Makka | Yellow with few white kernels |
| 28 | SKB/PM-48 | IC625166 | Makai | Yellow |
| 29 | SKB/PM-50 | IC625167 | Makai | Orange |
| 30 | SKB/PM-54 | IC625168 | Makai | Yellow |
| 31 | SKB/PM-56 | IC625169 | Makai | Orange |
| 32 | SKB/PM-58 | IC625170 | Makai | yellow |
| 33 | SKB/PM-61 | IC625171 | Makai | Separate orange and yellow ears |
| 34 | SKB/PM-63 | IC625172 | Safed Makka | White with few yellow kernels |
| 35 | SKB/PM-66 | IC625173 | Makka | Orange with few purple kernels |
| 36 | SKB/PM-71 | IC625174 | Pila makka | Yellow with few white kernels |
| 37 | SKB/PM-73 | IC625175 | Makka | Light purple |
| 38 | SKB/PM-75 | IC625176 | Makai | Yellow |
| 39 | SKB/PM-76 | IC625177 | Bhusri makka (Pila) | Purple |
| 40 | SKB/PM-77 | IC625178 | Bhusri makka (Safed) | Yellow with few purple kernels |
| 41 | SKB/PM-78 | IC625179 | Makai | Purple |
| 42 | SKB/PM-79 | IC625180 | Makai | Variegated |
| 43 | SKB/PM-83 | IC625181 | Makai | Separate yellow and white with many yellow kernels |

observed to be either mostly long and thin with a broad base and having yellow kernels or short but compact with yellow or orange-colored kernels. The numbers of kernel rows per ear were found to be of even numbers for all the collected accessions and ranged from 8–18 rows per ear.

The descriptive statistics (mean, range, standard error of mean, standard deviation and coefficient of variation) were computed for all the quantitative variables based on the data recorded on six quantitative traits for 43 accessions collected from Chhattisgarh and it is presented in Table 2.

Based on the quantitative range of the studied trait *viz.* ear length (7.33–26.67 cm), ear girth (8–16.33 cm), number of kernel rows per ear (8.00–18.00), number of kernels per ear row (12.67–45.33), ear weight (25.45–204.88 g) and 100 kernel weight (9.74–38.66 g), with the range of each trait given in the parenthesis, it was concluded that significant intra-trait variability was present in the collected accessions. The coefficient of variation, which is the ratio of standard deviation to the mean and represents the extent of variability in a trait relative to the population mean was maximum for ear weight (43%) followed by 100 kernel weight (26.34%), ear length (26.26), number of kernels per ear row (25.92%), number of kernel rows per ear (17.71%) and ear girth (17.10%). Based on the computed CV, the traits can be classified into highly variable (ear weight), moderately variable (100 kernel weight, ear length, number of kernels per ear row) and relatively less variable or stable (number of kernel rows per ear and ear girth). The statistical inferences drawn on *in-situ* germplasm characterization were utilized to identify trait-specific superior accessions even before the actual comprehensive evaluation exercise of the collected accessions (Table 3). The maximum ear length (26.67 cm), ear girth (16.33 cm), number of kernel rows per ear (18), number of kernels per ear row (45), ear weight (204.88 g) and 100 kernel weight (38.66 g) were recorded for the accessions IC625166, IC625161, IC625169 and IC625161, IC625173, IC625167 and IC625152 respectively. However, maize being a highly cross-pollinated crop the multi-location and multi-year characterization of superior accessions is required for validation of the findings of the present study.

**Table 2:** Descriptive statistics for six quantitative traits in 43 maize accessions collected from Chhattisgarh, India

| *Statistics* | *Ear length (cm)* | *Ear diameter (cm)* | *Rows/ ear* | *Kernels/ row* | *Ear weight (g)* | *100 Kernel weight (g)* |
|---|---|---|---|---|---|---|
| N | 43.00 | 43.00 | 43.00 | 43.00 | 43.00 | 43.00 |
| Min. | 7.33 | 8.00 | 8.00 | 12.67 | 25.45 | 9.74 |
| Max. | 26.67 | 16.33 | 18.00 | 45.33 | 204.88 | 38.66 |
| Mean | 15.05 | 11.64 | 13.40 | 29.22 | 112.88 | 20.45 |
| SE | 0.60 | 0.30 | 0.36 | 1.16 | 7.40 | 0.82 |
| Variance | 15.61 | 3.97 | 5.63 | 57.40 | 2356.23 | 29.01 |
| SD | 3.95 | 1.99 | 2.37 | 7.58 | 48.54 | 5.39 |
| CV% | 26.26 | 17.10 | 17.71 | 25.92 | 43.00 | 26.34 |

**Table 3:** Trait-wise superior accessions were identified among 43 maize accessions collected from Chhattisgarh, India.

| *S. No.* | *Character* | *Superior accessions* |
|---|---|---|
| 1 | Ear length (cm) | IC625166 (26.67), IC625171(20.67), IC625173 (20.67) |
| 2 | Ear girth (cm) | IC625161 (16.33), IC625164 (16.00), IC625169 (15.33) |
| 3 | Rows/ear | IC625169 (18), IC625161 (18), IC625162 (16) |
| 4 | Kernels/row | IC625173 (45), IC625163, (43), IC625166 (43) |
| 5 | Ear weight (g) | IC625167 (204.88), IC625166 (197.19), IC625163 (196.44) |
| 6 | 100 kernels weight (g) | IC625152 (38.66), IC625166 (30.82), IC625179 (31.44) |

### *Principle Component Analysis*

Principle component analysis (PCA) is routinely used in germplasm characterization trials for quantification of the genetic variability based on the phenotypic traits, and thus it helps to identify the breeding value of a genotype. PCA analysis was carried out to classify the collected maize genotypes on the basis of the most discriminatory traits.

The Eigen values, percent variance and cumulative variance were estimated for six quantitative traits using PAST software. The first three components explained 99.61% of the total variation in the set of maize landraces collected from Chhattisgarh (Table 4).

The first component explained 97.33% of the total variation followed by PC II, PC III, PC IV, PC V and PC VI which explained 1.67, 0.61, 0.19, 0.13 and 0.06% of the total variation, respectively. The major contributing trait for PC I was ear weight with 0.99 loading factor and the remaining of the traits contributed positively but with very small loadings. Ear weight loaded negatively for remaining of the five principal

**Table 4:** The Eigenvalues, the proportion of the total variance (%) represented by first six principle components, cumulative variance (%) and component loading of six quantitative traits in 43 maize landraces collected from Chhattisgarh, India.

| Trait | PC I | PC II | PC III | PC IV | PC V | PC VI |
|---|---|---|---|---|---|---|
| Ear length | 0.06 | 0.33 | 0.16 | -0.01 | 0.84 | 0.40 |
| Ear girth | 0.02 | 0.08 | -0.04 | 0.58 | 0.33 | -0.74 |
| Rows/ear | 0.02 | 0.01 | -0.35 | 0.75 | -0.18 | 0.53 |
| Kernels/row | 0.10 | 0.90 | 0.19 | 0.03 | -0.38 | -0.05 |
| Ear weight | 0.99 | -0.09 | -0.09 | -0.05 | -0.01 | -0.02 |
| 100 kernel weight | 0.08 | -0.26 | 0.90 | 0.31 | -0.13 | 0.11 |
| Eigen value | 2401.99 | 41.26 | 15.04 | 4.78 | 3.18 | 1.59 |
| Variance (%) | 97.33 | 1.67 | 0.61 | 0.19 | 0.13 | 0.06 |
| Cumulative variance (%) | 97.33 | 99.00 | 99.61 | 99.81 | 99.94 | 100.00 |

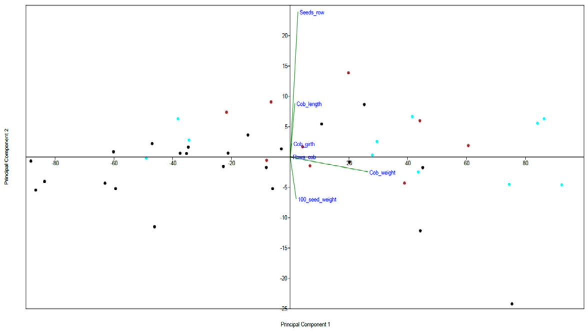

**Figure 2:** Scatter plot derived from the PCA analysis of 6 quantitative traits of maize

components. The trait number of kernels per ear row (0.90) contributed maximally towards the variability present in PC II and loaded negatively for PC V and PC VI. The 100-kernel weight (0.90) was the major loading trait for PC III and it loaded negatively for PC II and PC V. The number of kernel rows per ear (0.75), loaded maximum for PC IV and negative for PC III and V. The ear length (0.84) loaded positively and maximum for PC V and negatively for PC IV. For PC VI number of kernel rows per ear loaded maximum (0.53) while ear girth loaded negatively for PC III and PC VI. During the generation advancements, the positively loading traits are expected to have high propensity of being inherited together and vice versa. The PCA plotted using first two principal components (Figure 2) showed that the germplasm collected from three different districts are diverse and even within a single district, the genotypes were distributed away from each other. Among the collected accessions, ear weight was the most significant contributing trait to the total variability. The character association was performed by estimating the correlation coefficients for all combinations of six quantitative traits (Table 5). A highly significant positive correlation was found between most of the traits except a negative non-significant correlation between number of kernel rows/ear and 100 kernel weight, indicative of a strong inherent association between the studied traits.

**Table 5:** Correlation among different traits in maize accessions collected from Chhattisgarh, India

| *Trait* | *Ear length* | *Ear girth* | *Kernel rows/ear* | *Kernels/ ear-row* | *Ear weight* | *100 kernel weight* |
|---|---|---|---|---|---|---|
| Ear length | - | | | | | |
| Ear girth | 0.52** | - | | | | |
| Kernel rows/ ear | 0.13* | 0.46** | - | | | |
| Kernels/ear-row | 0.85** | 0.47** | 0.17** | - | | |
| Ear weight | 0.71** | 0.45** | 0.32** | 0.62** | - | |
| 100 kernel weight | 0.42** | 0.24** | -0.06 | 0.27** | 0.68** | - |

## *Genetic Diversity Analysis (Cluster analysis)*

The cluster analysis based on the Euclidean distances estimated on the quantitative traits was conducted in the present study. The Euclidean distance is only appropriate for data measured on the same scale which holds good for the present study. A dendrogram was constructed based on the estimated Euclidian distances among all the collected maize germplasm accessions (Figure 3). The cluster analysis grouped the 43 maize accessions into five distinctive groups *viz*. A, B, C, D, and E with each cluster having 9, 18, 13, 2 and 1 accessions. Cluster A had eight accessions originating from the district Jaspur while only one accession originating from Korba while cluster B had 11 accessions from Jaspur, four accessions from Koriya and three accessions from the Korba districts, making it geographically most divergent cluster as far as the collection sites are concerned. The accessions clustered together in cluster C originated mostly from Korba (6 accessions) and Koriya (5 accessions) districts with only two accessions originating from the Jaspur district. The cluster D had only two accessions, both from the Jaspur district, while cluster E comprised only one accession originating from the Korba district. The clusters B and C had genotypes originating from all three districts. However, there was observed a linearity of the spread among accessions clustering together i.e., the accessions collected consecutively on a geographic trail clustered together irrespective of the administrative district boundaries and geographical attributes of the collection sites, which can be understood in terms of germplasm diffusion along narrow geographic tracks. The number and percent of genotypes in each cluster and their associated phenotypic characteristics pertaining to the six quantitative traits recorded in the present study is presented in Table 6. Genotypes located in different clusters should be considered for genetic improvement of maize, particularly as components of heterotic pools in the hybrid breeding programs. The cluster means and standard deviation for six quantitative traits in maize germplasm accessions collected from Chhattisgarh, India is given in Table 7. The genotypes

**Table 6:** Clustering together and phenotypic characteristics of 43 maize accessions collected from Chhattisgarh

| *Cluster* | *No. of genotypes* | *Percentage* | *Phenotypic characteristics* |
|---|---|---|---|
| A | 9 | 20.93 | Short ears, low ear girth, medium number of kernel rows per ear, low number of kernels per row, lowest ear weight, lowest seed weight, collected from medium elevation. |
| B | 18 | 41.86 | Medium ear length, lowest ear girth, lowest number of kernel rows per ear, medium number of kernels per row, medium ear weight, medium 100 kernel weight, collected from medium elevation. |
| C | 13 | 30.23 | High ear length, medium to high ear girth, highest number of kernel rows per ear, medium to high number of kernels per ear row, medium ear weight, low to medium 100 kernel weight, collected from medium elevation. |
| D | 2 | 4.65 | Low ear length, low ear girth, low kernel rows per ear, low number of kernels per ear row, high ear weight, highest 100 kernel weight, collected from high elevation. |
| E | 1 | 2.33 | Highest ear length, highest ear girth, high number of kernel rows per ear, highest number of kernels per ear row, highest ear weight, high hundred kernel weight, collected from low elevation. |

clustered together in cluster C had maximum average ear length (18.26 ± 2.09), ear girth (13.41 ± 1.45), kernel rows per ear (15.08 ± 2.10) and number of kernels per row (36.59 ± 4.23) while cluster D had highest average ear weight (173.28 ± 22.47) and highest average 100 kernel weight (35.05 ± 5.11) with the trait mean ± standard deviation given in the parenthesis. The cluster E had only one genotype, so average and standard deviation could not be computed. However, singly it ranked first in all the traits except 100 kernel weight. The clustering together of genotypes resulted due to the distinct phenotypic traits utilized for cluster analysis.

### *Geo-spatial Analysis of Genetic Diversity*

The grid maps (20 minute raster cell) were generated for diversity analysis of the six quantitative traits *viz.* ear girth, ear weight, ear length, number of kernel rows per ear, number of kernels per ear row and 100 kernel weight in the collected maize germplasm accessions (Figure 4). The grid colors are indicative of extent of trait-specific diversity in a particular geographic location in the explored areas. The diversity pattern of maize germplasm accessions revealed by the grid maps confirmed the existence of considerable genetic diversity for all six quantitative traits in the surveyed area.

The maize germplasm accession IC624139 with the smallest ears (7.33 cm) and eventually minimum ear weight (25.45) was collected from Jaspur district and that with the longest ear IC625166 (26.67 cm) was collected from Korba district of the Chhattisgarh state. The ear girth was found to be minimum (8 cm) in IC625180 collected from Jaspur and a maximum (16.33 cm) in IC625161 collected from Koriya. The number of kernel rows per ear were a minimum (8) in the accession number IC625142 collected from Jaspur and a maximum (18) in two accessions (IC625161 and IC625168) both collected from Korba district. The number of kernels per ear row was minimum (12.67) in IC625149 collected from Jaspur and a maximum (45.33) in IC625173 collected from Koriya. The ear weight was recorded to be maximum (204.88 g) in accession number IC625167 collected from Korba. The accessions with minimum (9.74) and maximum (38.66 g) 100 kernel weights, i.e., IC625144 and IC625152, were collected from Jaspur district.

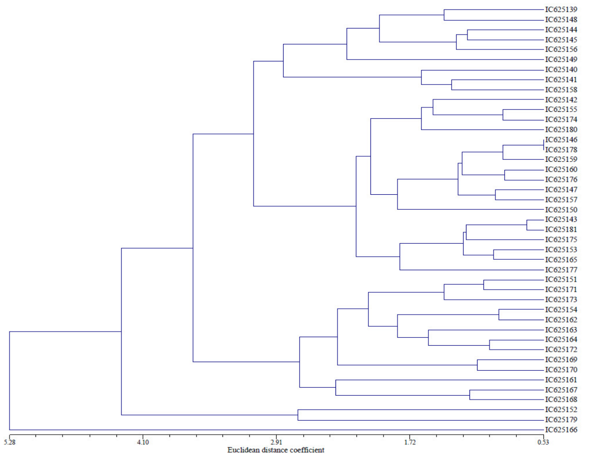

**Figure 3:** Dendrogram generated based on quantitative traits of 43 maize genotypes based on Euclidean distances

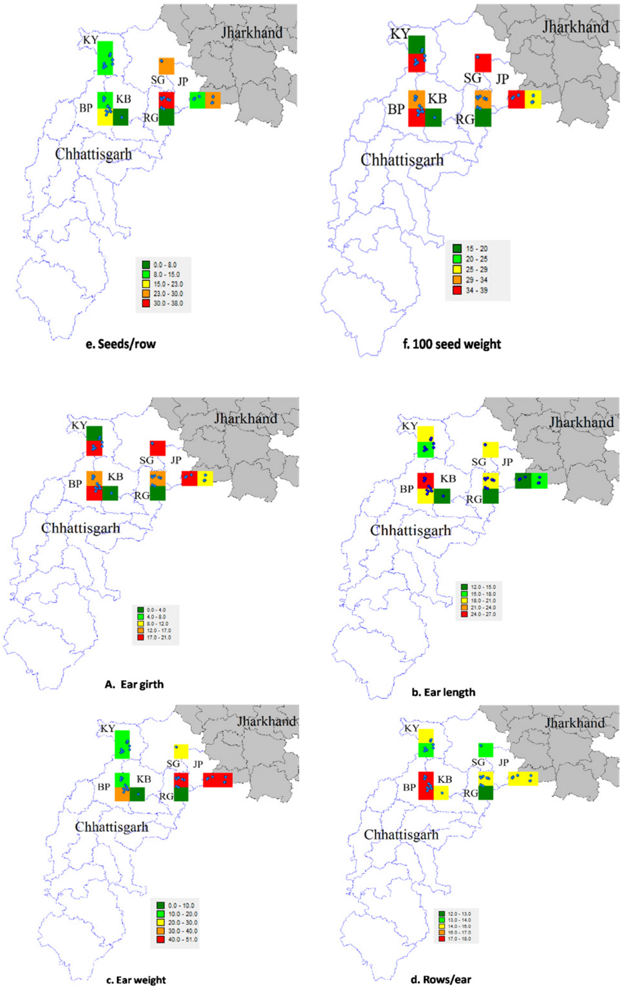

**Figure 4:** Trait-wise geo-referenced source sites for maize germplasm from Chhattisgarh, India

**Table 7:** Cluster means and standard deviation for six quantitative traits in maize germplasm accessions collected from Chhattisgarh, India

| | *Ear length (cm)* | *Ear girth (cm)* | *Rows/ear* | *Kernels/row* | *Ear weight* | *100 kernel weight* | *Average elevation* |
|---|---|---|---|---|---|---|---|
| Cluster A | 10.52 ± 2.64 | 11.33 ± 1.76 | 14.22 ± 2.11 | 19.56 ± 4.30 | 57.60 ± 28.08 | 15.12 ± 3.05 | 556.88 ± 141.47 |
| Cluster B | 14.61 ± 2.11 | 10.28 ± 1.10 | 11.89 ± 1.75 | 28.98 ± 2.68 | 101.93 ± 30.29 | 20.51 ± 3.29 | 576.83 ± 221.83 |
| Cluster C | 18.26 ± 2.09 | 13.41 ± 1.45 | 15.08 ± 2.10 | 36.59 ± 4.23 | 150.54 ± 34.21 | 21.02 ± 3.42 | 540.85 ± 161.16 |
| Cluster D | 12.67 ± 1.89 | 11.67 ± 1.41 | 12.00 ± 2.83 | 20.17 ± 4.95 | 173.28 ± 22.47 | 35.05 ± 5.11 | 1086.00 ± 4.24 |
| Cluster E | 26.67 | 16.00 | 14.00 | 43.00 | 197.19 | 30.82 | 488 |

The grid maps generated by DIVA-GIS successfully explained the existence and distribution of trait specific diversity in the maize germplasm in the explored regions of the Chhattisgarh state. Overall, the district of Jaspur has germplasm diversity with small ear size, smaller ear girth, a smaller number of kernel rows per ear and a smaller number of kernels per ear. However, the diversity for both small seed size and bold seeds was observed and collected from this district. The maize germplasm diversity for long ear length, a greater number of kernel rows per ear and high ear weight was found to be present in the Korba district while the district of Koriya exhibited diversity for high ear girth and number of kernels per ear row.

It could be concluded that the diversity of maize germplasm in the explored area is spatially dispersed and different regions have evolved different traits may be due to adaptation to differential eco-geographies or farmer's preferences. The similar eco-geographies may be targeted elsewhere in the country for collection, conservation and utilization of specific traits in the maize improvement programmes, particularly for introgression of various adaptive traits in the hybrid development for targeted areas. Most of the germplasm accessions were acquired from the difficult terrain regions where the cropping intensity is very low and they are comprised of subsistence small scale agriculture which possesses the non-improved local indigenous cultivars. Therefore, the primary objective of the study to target the interior diversity pockets with primitive agriculture for collection of local unimproved germplasm/landraces/farmer's varieties and their subsequent conservation, thus, stood fulfilled.

## Discussion

The eco-geographic attributes and socioeconomic variables are the main determining factors influencing the distribution pattern of crop genetic diversity in a particular area through the process of local adaptation (Vilaro *et al.,* 2020; Vidal *et al.,* 2020). The agricultural significance of the locally adapted crop landraces can only be realized through their collection and their subsequent use as a source of genes or their refinement through exercising selection on this variability (Casanas *et al.*, 2017). In this context, the sampling technique employed assumes great significance because effective sampling is the one that takes into account all these above-mentioned parameters. Recently, Vidal *et al.* (2020) have described a sampling methodology named the maximization method that successfully captured the maximum variability evaluated in the diversity census from the smallest sample size in maize.

The present study, which was aimed at the exploration and collection of maize landraces diversity from the Eastern Indian state of Chhattisgarh, resulted into a collection of 43 highly diverse accessions as reflected in their unique morphological attributes. Kumar *et al.* (2015a) have attributed the existence of high morphological variation in the maize landraces collected from the North-Western Himalayan region to the open-pollinated nature of local accessions, their adaption to local conditions and the continuous use of seeds by the farmers.

Most of the collections pertaining to the present study fell into late maturing flint type maize genotypes, indicating that this trait has had an adaptive advantage in the localized germplasm acclimatization. The evolution and perpetuation of late-maturing genotypes in North-Eastern India has been previously described by Singh and Singh (1967), and further validated by Singh (1977). The late maturing genotypes have been endowed with inherent drought tolerance ability. As such, it is considered to be the reason behind the evolution and perpetuation of late-maturing genotypes. Moreover, the late maturing accessions are at an advantage in having higher values of grain weight, tryptophan content, node length, tassel length and ear length, translating into higher yield as well as quality (Kumar *et al.,* 2015a). It was found in the present study that the altitudinal elevation of the geographic sites of cultivation systems have a definite bearing on the cultivation/non-cultivation of specific maize landraces in a particular area. This finding is in agreement with those of Singh (1977) who concluded that altitude, being a complex geographic barrier, is the main factor influencing the distribution of Indian races of maize. Mercer *et al.* (2008) found that the adjusted fitness of the highland landraces of Mexico was greater than that of the midland landraces under highland conditions. It was also reported that the landraces being cultivated under lower elevations are more prone to replacement by improved varieties/hybrids than the highland landraces. However, climate change makes the highland landraces more prone to temperature rise. Therefore, the prioritization of the collected landraces in the present study for utilization in the breeding programmes and further micro-level diversity collection should be undertaken while taking into consideration the elevation of their collecting sites.

The results of the principal component analysis are in agreement with those of Sharma *et al.* (2010) who found that 100-kernel weight, ear length, ear girth, number of kernels per ear, grain yield and days to flowering were the major differentiating traits in PCA conducted to differentiate the 'Sikkim Primitives' from the rest of the accessions in maize genotypes from North-Eastern India. Kumar *et al.* (2015a) have also reported the important role of kernel rows per ear as a major differentiating trait, among others. Similarly, Kumari *et al.* (2017) in their study comprising of characterization of maize landraces originating from North-Eastern India have also reported the high discriminatory power of the traits, days to tasseling, days to silking, ear length, ear weight, days to maturity and grain yield, kernels per ear row, kernel rows per ear and kernel weight which are in agreement with the present study. The results obtained by Dar *et al.* (2018), i.e. ear weight being the most important trait contributing to the total genetic variability present in the germplasm set and as the most loading trait in the first principle component, also substantiate the present study's findings.

### *Character Association*

The results of the inter-trait correlation-based character association are in agreement with Kumar *et al.,* (2015b), who reported that the traits plant height, ear height, ear length, ear girth, number of kernel rows per ear, number of kernels per row and 100 kernels weight registered positive and significant correlation with each other. Similar findings for significant positive correlation among different yield-attributing traits have also been reported by Shukla (2017). The correlation analysis conducted by Kumar *et al.* (2015a), revealed that the phenological parameters had a positive influence on maize kernel weight, tryptophan content, node length, tassel length and ear length. The study provided insight for the reason for the perpetual of the late maturity genotypes as the late maturing accessions will have higher values of these traits, meaning enhanced yield and quality.

### *Cluster Analysis*

The cluster analysis findings in the present study do not agree with those of Kumari *et al.* (2017), who reported the clustering of maize accessions from North-Eastern India according to their geographical sites of origin. Similarly, Kumar *et al.* (2015a) also obtained clustering together of accessions depending on the site of origin and the linearity of the spread of diversity as observed in the present study, was absent. The finding in the present study indicates the natural gene flow as well as diffusion of germplasm in the shared terrain and linear eco-geography. Therefore, it may be implied that the genotypes clustered together should ideally have more genetic relatedness than those clustered in different clusters. In the cluster analysis studies conducted by Prasanna *et al.* (2009), the 'Sikkim Primitives' clustered separately from the accessions from elsewhere in India and it was attributed to the alleles associated with the unique features of the 'Sikkim Primitives'. Similar results were reported by Sharma *et al.* (2010), where accessions from Sikkim formed a discrete cluster, indicating their uniqueness among maize landraces in India based on the similarity of phenotypic attributes among accessions that clustered together. Dar *et al.* (2018) have also reported similar cluster analyses based on morphological characterization of maize accessions collected from the North-Western Himalayan region.

The present study is the maiden attempt on cluster analysis on the germplasm originating from elsewhere in India's North-Eastern or North-Western Himalayan regions. Dar *et al.* (2018) evaluated a diverse set of maize inbred lines sourced from all over India, using DIVA-GIS and through generation of grid maps, successfully locating the sites from which trait-specific germplasm for eleven quantitative traits can be sourced. Kumari *et al.* (2017) generated grid maps for the diversity index of 100 seed weight in maize accessions collected from western, central and southern parts of Nagaland state of India using DIVA-GIS software and identified geographic locations exhibiting diversity for this important yield-attributing trait. Various workers already use the DIVA-GIS tool for geo-referencing the diversity of different important crops. Jatropha (Sunil *et al.,* 2009), linseed (Sivaraj *et al.,* 2009, 2012), black gram (Babu *et al.,* 2010), onion (Kamala *et al.,* 2011) and common bean (Sultan *et al.,* 2014), have not been utilized to its full potential for plant genetic resources management in India to fully understand the distribution of diversity of major crops on the geographical scale (Dar *et al.,* 2018). As such, the present study revealed many new information about the adaptation and evolution of maize germplasm from a previously little-explored area of India.

## Conclusion

The present study resulted in the collection and conservation of genetically diverse maize landraces besides the identification of several trait-specific superior accessions and their geo-referenced collection sites for further prioritization and acquisition of the landraces that might possess unique traits. These accessions can be utilized as potential sources of novel allelic combinations using novel cross-disciplinary research tools such as genoplasmics, genome wide association studies, genomic selection, and allele mining.

The collected accessions represent the sub-tropical maize germplasm and can be utilized for sub-tropical x temperate crosses for introgression of adaptiveness in the exotic temperate cultivars, which perform poorly in the sub-tropical Indian conditions. The study should also be helpful to future maize germplasm explorers and collectors for understanding the geographic patterns of genetic diversity within germplasm accessions for effective and efficient germplasm acquisition, conservation and utilization.

## Acknowledgements

The authors sincerely thank the farmer conservators of the Chhattisgarh state for their custodianship of the precious diversity of maize, the Krishi Vigyan Kendra of Jaspur, Koriya and Korba for their help in locating and collecting the diversity and Dr. Kuldeep Singh, Director, ICAR-NBPGR, Pusa Campus, New Delhi for the research facilities.

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