The Pharma Innovation Journal 2020; 9(5): 381-389

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(5): 381-389

www.thepharmajournal.com
Received: 25-03-2020
Accepted: 27-04-2020

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Characterization of maize germplasm (*Zea mays* L.) based on morphological descriptors and molecular markers under mid hill conditions of North-Western Himalayas

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DOI: https://doi.org/10.22271/tpi.2020.v9.i5g.4728

Abstract
A major challenge facing those involved in the testing of new plant varieties for Distinctness, Uniformity and Stability (DUS) is the need to compare them against all those of 'common knowledge'. A set of thirty three exotic germplasm lines was used to compare how morphological characterization and SSR molecular marker described variety relationships. All the exotic germplasm were confirmed distinct on the basis of morphological and molecular marker. The results revealed that, among the 33 germplasm lines, non-hierarchical Euclidean cluster analysis germplasm lines were grouped into nine clusters. Among them cluster IV, V, VI, VIII and IX were monogenotypic whereas rests were polygenotypic based on genetic divergence and EC444416 had the distinguishable character of absence of anthocyanin colouration of brace roots. Based on polymorphism exhibited by SSR markers, dendrogram was constructed using Jaccard's similarity coefficient the germplasm lines were grouped into two major clusters. In the present study, the molecular markers also exposed useful genetic diversity and the visual displays appeared to disperse the lines somewhat more evenly over the plot than the morphological methods, suggesting that the maize germplasm collection is a rich source of material with adequate variation for future use in breeding programs.

Keywords: DUS, plant variety protection, germplasm, morphological markers, SSR markers

Introduction
Maize (*Zea mays* L.) is the world's third most important cereal commercially valued economic crop of global importance widely used in poultry and cereal food industries next to wheat and rice. As it has higher yield potential than any other cereals, hence, it is referred to as "miracle crop" or the "queen" of cereals. Maize is grown both as food for human beings and fodder for animals. It is a model system for the study of genetics, evolution, and domestication. From the centre of origin in Mexico, maize populations were introduced to an array of growing conditions in tropical, subtropical, and temperate regions (Rebourg CM, 2003; Dubreuil PM, 2006) [27, 6], leading to the existence of diverse landraces in several countries worldwide. The maize was cultivated in an area of 191.89 million ha with a production of 1099.19 million tonnes in the world and in India it is cultivated in an area of 9.50 million ha with a production of 29.00 million tonnes with the productivity of 30.50 quintals per ha. In Himachal Pradesh it is cultivated in an area of 0.29 million ha with production of 0.78 million tonnes and productivity of 26.72 quintals per ha (Anonymous, 2018) [4]. It provides raw materials for starch, gluten, corn oil, corn syrup, sugar, corn meal and corn flour and occupies an important place in Indian agriculture.

It is predominantly a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographical adaptability. This genetic diversity offers incredible opportunities for genetic enhancement. Many primitive maize landraces cultivated in hilly areas possesses useful characteristics like resistance to stalk rot, stem borer and can withstand water logging and are sweet in taste. Despite the advent of hybrid varieties, about 70% area is still under local landraces. These local cultivars are adapted to the agricultural system characterized by limited use of chemical fertilizer and also to consumption preference by people. However, these desirable alleles are often scattered over a wide array of landraces or populations.

There is an important role of morphological data in the management of genetic resources that are conserved in ex-situ gene-banks. Many tools are now available to study the relationships among the cultivars, including various types of molecular markers; however, morphological characterization is the first step in the description and classification of germplasm. The characterization of morphological variability is useful tool to identify accessions with desirable characteristics such as earliness, disease resistance, or improved ear trait. The characterization and grouping of lines helps the breeders to avoid duplication in sampling populations and aid in the identification of varieties and hybrids. Breeders used morphological characters of plant, physical, physiological, biochemical, and molecular characterization of seed in crops like *Vicia faba* (Bond and Crofton, 2001) [5], sorghum (Thangavel, 2003) [36], lucerne (Senthilkumar, 2003) [32] and pearl millet (Kumar ABM, 2004) [15], oat (Sumathi, 2007) [35], rice (Eevera, 2003) [7], and rice (Maheshwaran, 2010) [16] for identification of genotypes. Genotypic variation in maize was based on morphological, biochemical and molecular characters for different traits were observed by Messmer MM, 1992 [18] and Ihsan *et al.* (2005) [13].

Protection of Plant varieties and Farmers Right authority insists on characterization and registration of extant, farmers and new varieties as a part of national and botanical asset. Pinnisch *et al.* (2012) [25] also indicated that, inbred lines serve as the seed parent to estimate the profitability of commercial maize genotypes. Hence studies were initiated to develop varietal characteristics as per the guidelines of PPV&FRA for the germplasm of CSK HPKV gene pool and the exotic germplasm of International Maize and Wheat Improvement Centre (CIMMYT) gene pool which will help in selection of exotic germplasm for specific breeding program.

2. Materials and Methods

The experimental material for the present study consisted of 33 maize genotypes which comprised 3 checks namely Bajaura Makka, Girija, and Jaisinghpur L. These checks were used to compare different genotypes. The genotypes were evaluated for different morphological and quality trait in α-RBD design during *Kharif* 2015 with plot size of 3.0×2.4 m^2 with row to row and plant to plant distance of 60 cm and 20 cm, respectively with 2 replications, 11 blocks per replication and 3 entries per block in the experimental farm of Department of Crop Improvement, CSK HPKV Palampur (32°6' N latitude, 76°3' E longitude and 1290.8 m altitude) as per the guidelines of PPV and FRA (Anonymous, 2007) [3]. The crop was raised by following standard agronomic practices.

DUS Testing is one of the important criteria to test germplasm lines for distinctness, uniformity and stability and one of the requirements for granting Plant Breeders Rights (PBR) and it is conducted according to national guidelines prepared on the basis of UPOV guidelines. During crop growth, the morphological characters were observed for plant height (cm), stem anthocyanin colour, days to 50% anthesis, tassel anthocyanin coloration, tassel glume colouration, anther colouration, days to 50% silk emergence and silk colouration. Anthocyanin colouration of glume excluding base, density of spikelet (Sparse, Dense), Attitude of blade (Erect, Drooping), Anthocyanin colouration of brace roots.

2.1 Molecular Analysis

2.1.1 DNA Extraction

Young leaves of each accession were used for DNA extraction following CTAB method (Murray and Thompson, 1980) [20] with some modifications. DNA stocks were prepared in TE buffer and quantification of DNA was done on 0.8% agarose gel by comparing with lambda DNA (Fermentas, Lithuania). Working stock of each sample was prepared by diluting it to make a final concentration of 13ng/µl. These dilutions were further checked on 0.8% agarose before being used in PCR reactions.

2.1.2. SSR Genotyping

A total of 60 SSR primers were initially screened for polymorphism, of which only 28 were selected on the basis of polymorphism and reproducible amplification products. PCR reactions were carried out using these primers developed in maize. For amplification of genomic DNA, the PCR reactions were carried out in 10.0 µl final volumes containing 4.65 µl sterilized distilled water, 1.0 µl template DNA (13 ng/µl), 1.0 µl of dNTP mix (0.2 mM each of dATP, dCTP, dGTP, dTTP), 1.25 µl 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.0 µl of $MgCl_2$ (25 mM), 0.5 µl of each primer (5 µM) and 0.1 µl Taq polymerase (5U/ µl). PCR amplification was carried out in S1000TM Thermal Cycler (BIO-RAD) and PCR reactions were performed at 1 cycle of 4 min at 94^0C as initial denaturation, followed by 35 cycles with a denaturation step at 94^0C for 1 min, an annealing step for 1 min at respective annealing temperature of each primer in a range of 49-57^0C and an extension step at 72^0C for 1 min, followed by last cycle of extension at 72^0C for 7 min. The amplified products were electrophoresed in 3% agarose gel and stained with ethidium bromide (0.5µg/ml). The PCR products were visualized and photographed using the Gel-Documentation Unit (BIORAD). Sizing of alleles was done with the help of 50-bp DNA ladder (Fermentas, Lithuania).

Molecular Data Analysis

All fragments were scored manually and converted into binary data, i.e. 1 for presence of the band and 0 for absence of the band. Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of NTSYS pc2.0 (Rohlf, 1993) [29]. Neighborjoining (N-J) tree was constructed with the help of DARwin software (Perrier, 2003) [24]. Bootstrapping with 1000 replicates was also performed with DARwin software.

3. Results and Discussion

Plant variety protection can only be granted in respect of a new plant variety after examination for criteria *viz.* distinctness (D), uniformity (U) and stability (S) 'DUS' in short. It involves comparison of new (candidate) variety against existing varieties for recording a number of morphological/ physiological characters, by growing new and existing varieties side-by-side. The morphological traits were evaluated as per Distinctiveness, Uniformity and Stability (DUS) guidelines, expressed higher variability within the inbreds (Table 2). Nayak *et al.* (2015) [22] studied 55 early maturity maize inbred lines based on 27 morphological characters as per the DUS guidelines and significant differences were observed for different traits viz., anthocyanin coloration of brace roots, glumes in tassel and silks; leaf angle

and leaf width; tassel characters such as density of spikelets, and angle between the main axis and lateral branches; ear characters such as ear length, number of rows of grain, kernel arrangement and thousand kernel weight. Aboyi *et al.* (2004) [1] used kernel traits as the best descriptors for classifying Peruvian highland maize germplasm, followed by ear traits and also expressed that, tassel traits were found to be less reliable descriptors for classifying the germplasm. On evaluation of morphological characters Anthocyanin colouration at glume base is an easily identifiable character and was present in all the germplasm lines (Table 2). Anthocyanin pigmentation of glumes excluding base, the germplasm lines were grouped for presence of anther colour in twenty eight germplasm lines and the absence in five lines *viz.*, EC444416, EC232174, EC232213, EC287341 and EC5444362 (Table 2). Based on the anthocyanin pigmentation of anthers, the genotypes were grouped for presence of anther colour in thirty germplasm lines and the absence in three germplasm lines *viz.*, EC232200, EC444371 and Jaisinghpur L.

The anthocyanin pigmentation in silk was observed as silk colour and it was present in twenty four germplasm lines while it was absent in nine germplasm lines *viz.*, EC287286, EC287302, EC5444341, EC232174, EC444453, EC232200, EC444435, EC287283 and Girija (Table 3). Two contrasting traits *viz.*, Sparse and Dense were observed in the case of density of spikelet. Most of the line expressed sparse type of spikelet and only few of them expressed dense type of spikelet. Ten type of germplasm lines *viz.*, EC287286, EC232216, EC444375, EC232174, EC287341, EC5444334, EC444434, EC444429, EC5444332 and Girija dense type density and twenty three exhibit sparse spikelet. Two alternative forms of blade attitude *viz.*, Drooping and straight were observed in the germplasm lines. The leaf attitude was drooping for sixteen germplasm lines *viz.*, EC444416, EC287286, EC287334, EC287299, EC343292, EC444441, EC232174, EC44445, EC232213, EC232200, EC444434, EC5444362, EC5444363, EC232161, EC5444332 and EC34328 and was straight for rest of 17 germplasm lines. The stem brace root colour was observed for the presence of anthocyanin. Colouration at the stem brace root was present in all the germplasm lines except EC444416.

Among the germplasm, EC5444332 and EC5444334 have the dent type of grain and the remaining were observed to be flint type. For grain colour among the germplasm EC5444332, EC232161, EC444386, EC232213 and EC232216 have yellow grain colour whereas remaining were of orange colour (Table 4). The observed quantitative characters also expressed a considerable amount of variation among the seven quantitative traits *viz.*, days to 50% anthesis, days to 50% silking, plant height, cob length, cob girth and 100-grain weight. The days taken for 50% anthesis ranged from 59 days (Bajaura Makka) to 73 days (EC287279), while a day to 50% silking varies from 61 days (Bajaura Makka) to 74.50 days (EC287279 and EC5444334). Among the germplasm lines plant height maximum plant height was observed for Jaisinghpur L. (280.20 cm) and minimum for EC444416 (158.20 cm). Cob length varies from 10.74 cm (EC232161) to 18.38 cm (Jaisinghpur L.), whereas, cob diameter (cm) was highest for EC444435 (15.13) and was minimum for EC232200 (10.51) (Table 4). Kernel rows/ear was highest for EC5444332 (15.20) and minimum for EC5444363 (10.40), while minimum (18.80) and maximum (33.50) grains/row was observed for EC232161 and EC287299, respectively. The 100-seed weight was maximum (33.41 g) in EC444375 and minimum in EC287341 (15.27 g) (Table 5). Based on the phenotypic traits studied, Wietholter *et al.* (2008) [39] concluded that, the traits contributed majorly to the classification of Brazilian corn landraces were plant height, ear insertion, female flowering, male flowering and kernel row number per ear. Nayak *et al.* (2015) [22] studied 55 early maturity maize inbred lines based on 27 morphological characters as per the DUS guidelines and significant differences were observed for different traits viz., anthocyanin coloration of brace roots, glumes in tassel and silks; leaf angle and leaf width; tassel characters such as density of spikelets, and angle between the main axis and lateral branches; ear characters such as ear length, number of rows of grain, kernel arrangement and thousand kernel weight. Though both qualitative and quantitative characters could be a better descriptive for grouping the maize genotypes, but high heritable traits are much useful in selection of inbreds for further breeding programme. Though both qualitative and quantitative characters could be a better descriptive for grouping the maize genotypes, but high heritable traits are much useful in selection of germplasm lines for further breeding programme.

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. Genetic divergence is a useful tool in quantifying the degree of divergence between biological populations at genotypic level and also to assess the relative contribution of different components to the total divergence both at inter and intra cluster level (Nair and Mukherjee, 1960) [21]. The importance of intra-specific divergence in plant breeding has also been emphasized by Hawkes (1981) [11].

D^2-statistics is a powerful tool for estimating genetic diversity among different germplasm lines and to identify the parents for hybridization to obtain desirable recombinants. The assessment of genetic diversity helps in reducing the number of breeding lines from the large germplasm and the progenies derived from diverse parents were expected to show a broad spectrum of genetic variability and provide better scope to isolate superior recombinants. Cluster analysis of any tested populations is based on morphological characters to group them into different clusters is suggested by several scientist. Ali *et al.* (2008) [2] grouped the 41 maize populations through cluster analysis into three main clusters and observed a wide range of overall genetic diversity among these populations. Wang *et al.* (2009) [37] observed seven groups in cluster analysis based on phenotypic data and most of germplasm lines were clustered into group I. Reddy *et al.* (2013) [28] observed eight groups in cluster analysis and most of the lines were clustered in cluster III. Salazar *et al.* (2016) [30] clustered 48 germplasm lines into 5 groups.

In the present investigation with non-hierarchical Euclidean cluster analysis, 33 germplasm lines of maize were grouped into nine clusters. Among them cluster IV, V, VI, VIII and IX were monogenotypic whereas rests were polygenotypic based on genetic divergence (Table 6).

3.1 Molecular characterization

The question of Plant Variety Protection (PVP) has been brought into worldwide focus by the agreement on Trade Related Aspects of Intellectual Property Right (TRIPS), which is a part of GATT (General Agreement on Tariffs and Trade) Agreement establishing the World Trade Organization (WTO) in 1995. The PBR concept is based on the realization

that if commercial plant breeding is to be encouraged for the benefit of agriculture and society, measures have to be taken to allow breeders to profit from their product (Mishra, 1999) [19]. Further, it makes possible to define a plant grouping with sufficient specificity to allow the unambiguous assignment and enforcement of property rights. Analysis of genetic diversity and of relationship among the elite breeding materials can significantly aid in crop improvement (Hallauer *et al.*, 1988) [10]. In maize, this information is useful in planning for hybrid and line development, assigning lines to heterotic groups and in plant variety protection (Yuan *et al.*, 2002) [40], molecular markers are more powerful in assessing genetic diversity in comparison with the morphological data, pedigree data and biochemical data, because these markers reveal differences at the level of DNA (Melchinger, 1999) [17]. The lines used in this study were a small but representative sample of existing commercial hybrids, and so typified the kind of diversity encountered by the testing authorities conducting registration tests. They were all morphologically distinct, as would be expected.

Based on polymorphism exhibited by SSR markers, dendrogram was constructed using Jaccard's similarity coefficient using UPGMA method of NTSYS-PC package (version 2.02); the germplasm lines were grouped into two major clusters with a genetic similarity of 35%. Two clusters separated local germplasm into two groups. Clustering of populations into two distinct groups, represent the diversity between populations and indicates a significant influence of environment on genetic diversity. Cluster A comprised of 18 germplasm lines, whereas cluster B comprised of 15 germplasm lines. Cluster A and cluster B was further divided into various sub-clusters as shown in Table 7. Within two sub-clusters of group A, maximum germplasm lines were found in A_2. Sub-cluster A_1 comprised of seven germplasm lines *viz.*, EC444416, EC287334, EC287286, EC287302, EC285604, EC5444341 and EC232216. Sub-cluster A_2 comprising of eleven germplasm lines *viz.*, EC444386, EC5444334, EC5444363, EC343287, EC287283, EC444371, EC232161, EC444429, Jaisinghpur L., Bajaura Makka and EC5444332. Cluster B was also divided into two sub clusters. Cluster B_1 comprised of ten germplasm lines *viz.*, EC444375, EC444453, EC232174, EC232213, EC287341, EC444418, EC444434, EC5444362, EC232200 and EC444435 (Table 7). Cluster B_2 consist of five germplasm lines *viz.*, EC287299, Girija, EC343292, EC444441 and EC287279. Clustering of population represents population density between two distinct groups, which indicates a significant influence of environment on genetic diversity. In sub-clusters several germplasm lines from different regional origin were classified into same cluster. It seems there was slight genetic difference between these populations grouped in the same sub-cluster. It mainly happens for population evolved in environments which differs slightly from each other in terms of climatic conditions. Such a classification may reflect gene flow among different maize population in different regions or environments. The discrepancy between the known pedigree of some germplasm lines and the dendogram placement could be due to relatively low number of SSRs used. Another explanation could be that they would be diverged from the original one due to natural selection in maintaining environment, genetic drift, unintentional outcrossing and mutations. This was in confirmation with the results of Enoki *et al.* (2002) [8] and Hoxha *et al.* (2004) [12]. Effectiveness of SSR markers in the genetic diversity analysis of maize germplasm has been well documented by different workers (Pabendon *et al.*, 2010; Guo *et al.*, 2011) [23, 9]. Yuan-Li *et al.* (2000) [41] studied genetic diversity and heterotic groups in maize and compared different types of DNA markers and suggested their use for analysis of genetic diversity. Prasanna and Hoisington (2003) [26] also studied the genetic diversity in the Indian maize germplasm using microsatellite markers. Group I includes germplasm lines, with similarity coefficients of between 0.62 and 0.89. Group II includes fifteen germplasm lines, with similarity coefficients of between 0.64 and 0.89. Guo *et al.* (2011) [9] studied genetic diversity of 77 maize relatives by simple sequence repeats (SSR) markers and divided them into seven groups by UPGMA method based on SSR fingerprinting. Kanagarasu *et al.* (2013) [14] reported that dendrogram generated with UPGMA cluster analysis revealed five major clusters with 0.62 similarity coefficients. Sivaranjani *et al.* (2014) [33] analysed 24 diverse maize germplasm lines using 36 simple sequence repeat markers.

In the present study, the molecular markers also exposed useful genetic diversity, and the visual displays appeared to disperse the line somewhat more evenly over the plot than the morphological method. However, there was little agreement on variety relationships between the morphology and the molecular methods. Lines that display high phenotypic dissimilarity need not be genetically dissimilar. The purpose of pre-screening would be to subdivide candidate varieties into groups, so reducing the number of controls and pair-wise comparisons that have to be examined in the morphology test. However, this process assumes that the pre-screening characters guarantee that varieties placed in different groups are distinct in the morphological characters used for registration. Clearly, this would not be the case as the present study showed that molecular and morphological differences were not correlated. Therefore, using molecular markers as grouping characters would by default, require acceptance of their use as a distinguishing characters, at least for the most divergent inbred lines. An alternative way to deal with the poor correlation between genetic and morphological distances could be to select only molecular markers linked to phenotypic traits in DUS testing. The diversity patterns of the inbred lines revealed a large amount of diversity that did not allow a clear-cut distinction between groups. This case is similar to that of the CIMMYT populations, which served as germplasm sources for many of the Asian lines (Warburton *et al.*, 2002) [38], where a large amount a diversity within, relative to between, source populations was observed. On the other hand, the heterotic groups in the US and European temperate maize were clearly differentiated in previous studies using SSRs (Senior *et al.*, 1998 [31]; Smith *et al.*, 1997 [34]).

Table 1: List of germplasm lines of maize (*Zea mays*. L) evaluated under the study

Sr. No	Genotypes	Source/ Pedigree	Sr. No	Genotypes	Source/Pedigree
1.	EC444416	Mexico	18.	EC287341	Mexico
2.	EC287286	Mexico	19.	EC5444334	Mexico
3.	EC287302	Mexico	20.	EC444418	Mexico
4.	EC5444341	Mexico	21.	EC444435	Mexico
5.	EC285604	Mexico	22.	EC444434	Mexico
6.	EC232216	Mexico	23.	EC5444362	Mexico
7.	EC287334	Mexico	24.	EC5444363	Mexico
8.	EC444375	Mexico	25.	EC444371	Mexico
9.	EC287299	Mexico	26.	EC232161	Mexico
10.	EC343292	Indonesia	27.	EC444429	Mexico
11.	EC444441	Mexico	28.	EC287283	Mexico
12.	EC232174	Mexico	29.	EC5444332	Mexico
13.	EC444453	Mexico	30.	EC343287	Indonesia
14.	EC232213	Mexico	31.	Jaisinghpur L.	Kangra
15.	EC287279	Mexico	32.	Bajaura Makka	PS 62/FH 3209/ FH3198/ FH 3202/EC
16.	EC444386	Mexico			
17.	EC232200	Mexico	33.	Girija	NAVJOT/PARVATI/ KH9405/ZC 2810/ MMH 81/MMH 60/PRO 306/ICI 736/ L 110/ ZC 2733/ JH 1136 /JH 1146

L =Local

Table 2: Characterization based on Plant characters in maize germplasm

Genotypes	Brace root	Days to 50% anthesis	Tassel anthocyanin colour at base of glume	Tassel anthocyanin colour excluding base	Leaf attitude
EC444416	A	60.50**	P	A	Dr
EC287286	P	69.50	P	P	Dr
EC287302	P	71.00	P	P	Sr
EC5444341	P	72.00	P	P	Sr
EC285604	P	67.50	P	P	Sr
EC232216	P	70.00	P	P	Sr
EC287334	P	68.50	P	P	Dr
EC444375	P	68.00	P	P	Sr
EC287299	P	61.50**	P	P	Dr
EC343292	P	63.00	P	P	Dr
EC444441	P	69.00	P	P	Dr
EC232174	P	63.50	P	A	Dr
EC444453	P	69.00	P	P	Dr
EC232213	P	68.00	P	A	Dr
EC287279	P	73.00	P	P	Sr
EC444386	P	70.00	P	P	Sr
EC232200	P	64.00	P	P	Dr
EC287341	P	63.50	P	A	Sr
EC5444334	P	72.00	P	P	Sr
EC444418	P	64.50	P	P	Sr
EC444435	P	65.00	P	P	Sr
EC444434	P	68.00	P	P	Dr
EC5444362	P	61.00**	P	A	Dr
EC5444363	P	61.50**	P	P	Dr
EC444371	P	68.50	P	P	Sr
EC232161	P	63.00	P	P	Dr
EC444429	P	64.00	P	P	Sr
EC287283	P	69.50	P	P	Sr
EC5444332	P	71.00	P	P	Dr
EC343287	P	70.00	P	P	Dr
Jaisinghpur L.	P	61.00	P	P	Sr
Bajaura Makka	P	59.00	P	P	Sr
Girija	P	63.50	P	P	Sr

**- statistically at par with check; P-Present; A-Absent; Sr-Straight; Dr-Drooping

Table 3: Characterization based on Tassel and silk characters in maize germplasm

Genotypes	Anther Colour	Silk Colour	Days to 50% silking	Plant Height	Density of spikelet
EC444416	P	P	62.50*	158.20	S
EC287286	P	A	72.50	202.20*	D
EC287302	P	A	73.50	220.10*	S
EC5444341	P	A	73.00	234.40	S
EC285604	P	P	71.50	214.80*	S
EC232216	P	P	72.50	265.20	D
EC287334	P	P	73.00	263.10	S
EC444375	P	P	70.00	235.90	D
EC287299	P	P	63.00*	191.70	S
EC343292	P	P	65.50	227.90	S
EC444441	P	P	73.00	211.10*	S
EC232174	P	A	67.00	225.20*	D
EC444453	P	A	71.00	223.80*	S
EC232213	P	P	71.00	275.75	S
EC287279	P	P	74.50	239.10	S
EC444386	P	P	72.50	222.60*	S
EC232200	A	A	67.50	222.70*	S
EC287341	P	P	65.00	168.80	D
EC5444334	P	P	74.50	222.90*	D
EC444418	P	P	66.00	214.40*	S
EC444435	P	A	69.00	236.20	S
EC444434	P	P	70.50	212.60*	D
EC5444362	P	P	65.00	170.40	S
EC5444363	P	P	63.50*	198.20	S
EC444371	A	P	71.50	229.00	S
EC232161	P	P	67.50	213.50*	S
EC444429	P	P	67.00	242.00	D
EC287283	P	A	73.50	279.05	S
EC5444332	P	P	72.00	258.00	D
EC343287	P	P	71.50	180.50	S
Jaisinghpur L.	A	P	63.50	280.20	S
Bajaura Makka	P	P	61.00	210.70	S
Girija	P	A	66.00	243.80	D

*-significantly superior over best check; S-Sparse; D-Dense

Table 4: Characterization based on cob characters in maize germplasm

Genotypes	Cob length (cm)	Cob Diameter (cm)	Type of grain	Grain Colour
EC444416	16.13**	13.08**	Flint	Orange
EC287286	13.13	11.62	Flint	Orange
EC287302	12.86	12.58	Flint	Orange
EC5444341	12.23	12.76	Flint	Orange
EC285604	13.40	12.37	Flint	Orange
EC232216	13.91	13.70**	Flint	Yellow
EC287334	15.69**	14.87**	Flint	Orange
EC444375	13.87	13.81**	Flint	Orange
EC287299	15.98**	10.66	Flint	Orange
EC343292	15.74**	13.19**	Flint	Orange
EC444441	13.37	13.37**	Flint	Orange
EC232174	11.19	11.27	Flint	Orange
EC444453	14.87	12.91	Flint	Orange
EC232213	15.63**	12.33	Flint	Yellow
EC287279	12.38	12.31	Flint	Orange
EC444386	11.70	13.17**	Flint	Yellow
EC232200	12.12	10.51	Flint	Orange
EC287341	12.35	11.29	Flint	Orange
EC5444334	12.81	14.03**	Dent	Orange
EC444418	14.38	13.81**	Flint	Orange
EC444435	12.59	15.13**	Flint	Orange
EC444434	13.17	12.36	Flint	Orange
EC5444362	11.98	12.75	Flint	Orange
EC5444363	11.07	11.44	Flint	Orange
EC444371	11.99	13.00	Flint	Orange
EC232161	10.74	10.55	Flint	Yellow
EC444429	16.12**	14.90**	Flint	Orange
EC287283	15.17	13.64**	Flint	Orange

EC5444332	11.94	13.86**	Dent	Yellow
EC343287	12.41	12.07	Flint	Orange
Jaisinghpur L.	18.38	14.07	Flint	Orange
Bajaura Makka	16.62	13.62	Flint	Orange
Girija	16.55	13.28	Flint	Orange

**- statistically at par with check

Table 5: Characterization based on kernel characters in maize germplasm

Genotype	Kernel rows/ear	Grains/row	100-kernel weight (gm)
EC444416	12.20	28.60	29.36**
EC287286	14.60	28.60	16.40
EC287302	12.60	23.90	24.10
EC5444341	12.20	22.60	30.57**
EC285604	12.40	27.20	22.90
EC232216	12.00	30.00	24.66
EC287334	14.80	32.10**	23.02
EC444375	11.40	25.50	33.41**
EC287299	12.80	33.50**	20.21
EC343292	14.80	31.70**	22.68
EC444441	11.40	26.10	29.97**
EC232174	12.20	26.30	19.01
EC444453	12.00	28.70	25.05
EC232213	12.60	29.60	20.72
EC287279	12.20	23.80	25.62**
EC444386	11.20	27.90	27.16**
EC232200	11.60	21.75	19.68
EC287341	13.40	27.10	15.27
EC5444334	14.60	25.50	19.99
EC444418	13.70	28.90	21.72
EC444435	15.00*	26.00	17.78
EC444434	13.00	24.50	28.50**
EC5444362	14.60	23.10	18.96
EC5444363	10.40	22.40	21.23
EC444371	14.40	25.53	25.52**
EC232161	12.30	18.80	24.88
EC444429	14.00	33.30**	30.33**
EC287283	12.80	30.60	30.97**
EC5444332	15.20*	22.60	24.14
EC343287	12.20	23.70	25.49**
Jaisinghpur L.	11.60	38.30	34.04
Bajaura Makka	13.60	34.70	30.42
Girija	13.40	31.30	26.49

**- statistically at par with check; *- significantly superior over best check

Table 6: Distribution of maize germplasm lines among different clusters on the basis of Mahalanobis D^2-analysis

Clusters	Number of germplasm lines	Germplasm lines
I	10	EC544341, EC285604, EC444453, EC287302, EC444434, EC287279, EC5444363, EC444441, EC5444334, EC343287
II	8	EC444418, EC444429, Girija, EC444375, EC444386, EC287283, EC287334, EC232213
III	8	EC232174, EC5444363, EC232200, EC287341, EC287299, EC343292, EC5444362, EC287286
IV	1	EC444435
V	1	EC232216
VI	1	EC5444332
VII	2	EC444416 and Bajaura Makka.
VIII	1	Jaisinghpur L.
IX	1	EC232161

Table 7: Grouping of maize germplasm lines into different clusters on the basis of SSR data

Clusters	Sub Cluster	Number of germplasm lines	Germplasm lines
A	A_1	7	EC444416, EC287334, EC287286, EC287302, EC285604, EC544434, EC232216
	A_2	11	EC444386, EC5444334, EC5444363, EC343287, EC287283, EC444371, EC232161, EC444429, Jaisinghpur L., Bajaura Makka, EC5444332
B	B_1	10	EC444375, EC444453, EC232174, EC232213, EC287341, EC444418, EC444434, EC5444362, EC232200, EC444435
	B_2	5	EC287299, Girija, EC343292, EC444441, EC287279

4. Conclusion

This study is an initial attempt to characterize the breadth of

germplasm diversity, from which we concluded that breeding activity at Palampur has not caused a decline in the overall amount of diversity in germplasm. In sum and substance, it can be stated that although the work had concentrated on DUS testing, it is myth and less a reality. There are only small numbers of descriptors available in released and notified cultivars in India and their parental lines. If an attempt is made by considering a large number of descriptors, establishment of clear distinguishability for each material may not be difficult. Morphological markers and molecular markers with insufficient primers do not generate sufficient diversity in the population. So sufficient primers which cover whole genome should be used in the further studies on DUS testing to make the success.

5. Acknowledgement

Authors are thankful to NBPGR, New Delhi for providing the seed material and help

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