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Distinctness, uniformity and stability (DUS) characterization on phenological traits and assessing the diversity of inbreds in maize (*Zea mays* L.)

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Maize is one of the economic crops of global importance. Protection of Plant varieties and Farmers Right Act (2001) insists on Distinctness, Uniformity and Stability (DUS) characterization of extant, farmers and new varieties and recommends the registration of varieties for any one specific novel character. Studies initiated to develop morphological characters in seventeen inbreds including 16 domestic inbreds (UMI 1200, UMI 285, UMI 61, UMI 395, UMI 936(O), UMI 936(W), UMI 176, UMI 80, UMI 112, UMI 551, UMI 190, UMI 136, UMI 101, UMI 69, UMI 1230, UMI 178) of Tamil Nadu Agricultural University (TNAU) and one exotic inbred (CIM Entry 10527) of International Maize and Wheat Improvement Centre (CIMMYT). As per DUS guidelines revealed that, inbreds vary widely in their characters. The results revealed that, among the 17 inbreds, UMI 1200 and UMI 1230 were distinct from other inbreds and UMI 551 had the distinguishable character of tassel anthocyanin colouration at glume base. Dendrograms were constructed based on the morphological characters that established differences among the individuals indicating reportable variation among the 17 maize inbreds which would aid in selection of inbreds with desirable characters for further breeding programme.

Key words: Plant variety protection, morphological markers, selection of inbreds.

INTRODUCTION

Maize (*Zea mays* L.) is the world's third most widely grown cereal (Ayisi and Poswall, 1997) commercially valued economic crop of global importance widely used in poultry and cereal food industries. It provides raw materials for starch, gluten, corn oil, corn syrup, sugar, corn meal and com flour and occupies an important place in Indian agriculture. Anon (2007) highlighted that about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (e.g., starch and oil production) and 1% as seed. This emphasizes the demands of maize in India and there is an urging need to develop high yielding single cross hybrids. Therefore, knowledge on genetic diversity of inbred lines would help the breeder in planning crosses for superior hybrid development.

Researchers used morphological characters of plant,

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Table 1. Characterization based on Plant characters in maize inbreds.

Genotypes	Leaf angle	Leaf attitude	Stem brace root colour	Days to 50% anthesis (DAS)	Tassel anthocyanin colour	Glume colour excluding base
UMI 1200	Small	Straight	Present	60	Absent	present
UMI 285	Wide	Drooping	Present	58	Absent	Absent
UMI 61	Small	Drooping	Present	60	Absent	Present
UMI 395	Small	Drooping	Absent	56	Absent	Present
UMI 936(O)	Wide	Drooping	Absent	58	Absent	Absent
UMI 936(W)	Small	Drooping	Absent	58	Absent	Present
UMI 176	Small	Drooping	Present	50	Absent	Absent
UMI 80	Small	Drooping	Present	52	Absent	Absent
UMI 112	Wide	Drooping	Absent	55	Absent	Present
UMI 551	Wide	Drooping	Absent	51	Present	Present
UMI 190	Small	Drooping	Absent	53	Absent	Present
UMI 136	Wide	Drooping	Absent	53	Absent	Present
UMI 101	Small	Drooping	Absent	48	Absent	Absent
UMI 69	Small	Drooping	Absent	55	Absent	Present
CIM Entry 10527	Wide	Drooping	Absent	56	Absent	Absent
UMI 1230	Wide	Straight	Absent	58	Absent	Absent
UMI 178	Wide	Drooping	Absent	53	Absent	Absent

physical, physiological, biochemical, and molecular characterization of seed in crops like *Vicia faba* (Bond and Crofton, 2001), sorghum (Thangavel, 2003), lucerne (Senthilkumar, 2003) and pearl millet (Arunkumar et al., 2004), oat (Sumathi, 2007), rice (Eevera, 2003), cotton (Ameena, 2009) and rice (Maheshwaran, 2010) for identification of genotypes. Genotypic variation in maize was based on morphological, biochemical and molecular characters for different traits were observed by Grzesiak (2001), Ihsan et al. (2005) and Rakshit et al. (2011).

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) 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 and FRA for the domestic inbreds of Tamil Nadu Agricultural University (TNAU) gene pool and the exotic inbred of International Maize and Wheat Improvement Centre (CIMMYT) gene pool which will help in selection of inbreds for specific breeding program.

MATERIALS AND METHODS

The domestic inbred lines viz., UMI 1200, UMI 285, UMI 61, UMI 395, UMI 936(O), UMI936 (W), UMI 176, UMI 80, UMI 112, UMI 551, UMI 190, UMI 136, UMI 101, UMI 69, UMI 1230, UMI 178 from TNAU gene pool and exotic inbred CIM Entry 10527 from CIMMYT were collected with similar maturity group. Each of the individual inbreds were raised in a Randomized Block Design (RBD) in 4 lines of 6 m row length with the spacing of 75 × 20 cm of inter and intra row spacing with three replications as per the guidelines of PPV and FRA (Anon, 2007). The crop was maintained with recommended package of practices (Anon, 2012). During crop growth, the morphological characters were observed for plant height (cm), stem anthocyanin colour, leaf angle, leaf attitude, days to 50 percent anthesis, tassel anthocyanin coloration, tassel glume colouration, anther colouration, tassel angle, tassel attitude, days to 50% silk emergence and silk colouration. Harvesting was done when the plants had completely senesced with the expression of physiological maturation, the dunken layer formation (Baker, 1973). The harvested cobs were observed for ear (cob) length (cm), ear diameter (cm), ear shape, number of grain rows, grain type, grain colour, kernel row arrangement, and 100 grain weight (g). Both the qualitative and quantitative traits were evaluated, scored and analysed by NTSYS-pc version 2.2 software (Rohlf, 2005), using SIMINT module for cluster analysis and dendrograms were constructed.

RESULTS AND DISCUSSION

The morphological traits were evaluated as per Distinctiveness, Uniformity and Stability (DUS) guidelines, expressed higher variability within the inbreds (Table 1). Abu-Alrub et al. (2006) 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 (Table 1 and Figure 1), 9 inbreds were observed with small leaf angle while 8 inbreds with wider leaf angle. The leaf attitude was drooping in all inbreds except in UMI 1200 and UMI 1230 which were straight. The stem brace root colour was observed as presence of anthocyanin colouration at the

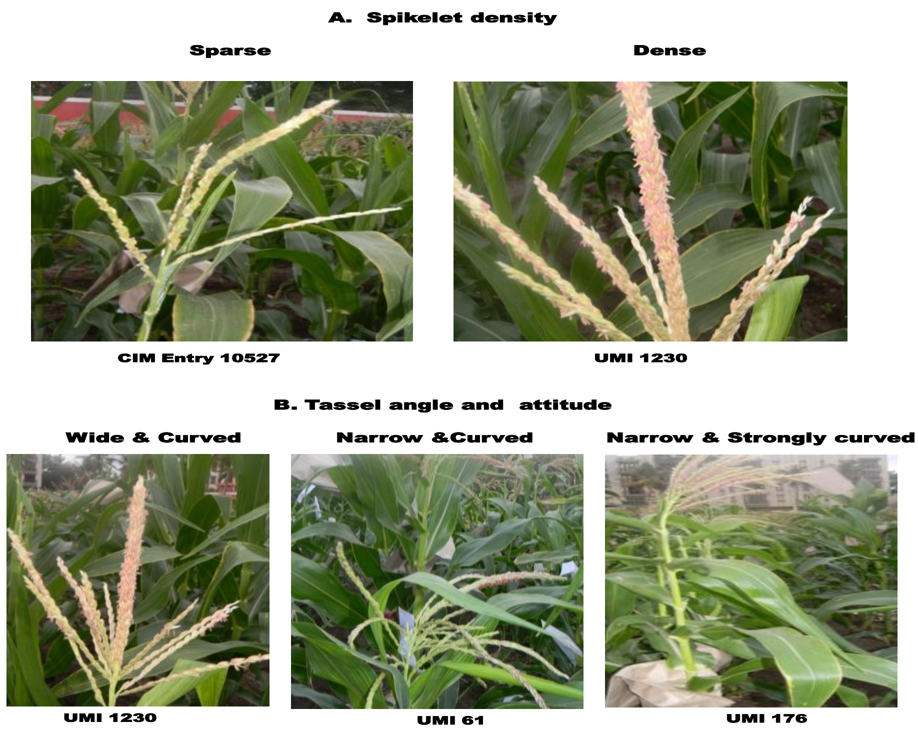

Figure 1. Tassel characterization of maize inbreds.

Table 2. Characterization based on Tassel and silk characters in maize inbreds.

Genotypes	Anther colour	Spikelet density	Tassel angle	Tassel attitude	Days to 50% silking (DAS)	Silk colour	Plant height (cm)
UMI 1200	Present	Dense	Narrow	Straight	62	Present	127
UMI 285	Absent	Dense	Narrow	Straight	61	Absent	120
UMI 61	Present	Dense	Narrow	Strongly curved	63	Present	112
UMI 395	Present	Dense	Narrow	Straight	59	Absent	110
UMI 936(O)	Absent	Dense	Narrow	Curved	60	Absent	128
UMI936(W)	Present	Dense	Narrow	Curved	60	Present	111
UMI 176	Present	Dense	Wide	Strongly curved	53	Absent	127
UMI 80	Present	Dense	Narrow	Curved	54	Absent	116
UMI 112	Present	Dense	Narrow	Curved	57	Present	111
UMI 551	Present	Dense	Narrow	Straight	54	Present	124
UMI 190	Absent	Sparse	Wide	Straight	55	Absent	120
UMI 136	Present	Dense	Wide	Curved	55	Absent	117
UMI 101	Absent	Sparse	Narrow	Straight	51	Absent	97
UMI 69	Present	Dense	Narrow	Straight	57	Absent	130
CIM Entry 10527	Absent	Sparse	Narrow	Curved	59	Absent	110
UMI 1230	Present	Dense	Wide	curved	60	Absent	135
UMI 178	Present	Dense	Wide	Curved	55	Present	128

stem brace root was observed only in 5 inbreds (UMI 1200, UMI 285, UMI 61, UMI 176, and UMI 80). The tassel anthocyanin colouration at glume base which is an easily identifiable character was absent in all the inbreds except UMI 551 (Table 2).

Based on the anthocyanin pigmentation of anthers, the anthers were grouped for presence of anther colour in 12 inbreds and the absence in seven inbreds. Similarly, the spikelet density was dense in almost all inbreds except 3 inbreds viz., UMI 190, UMI 101, and CIM entry 10527

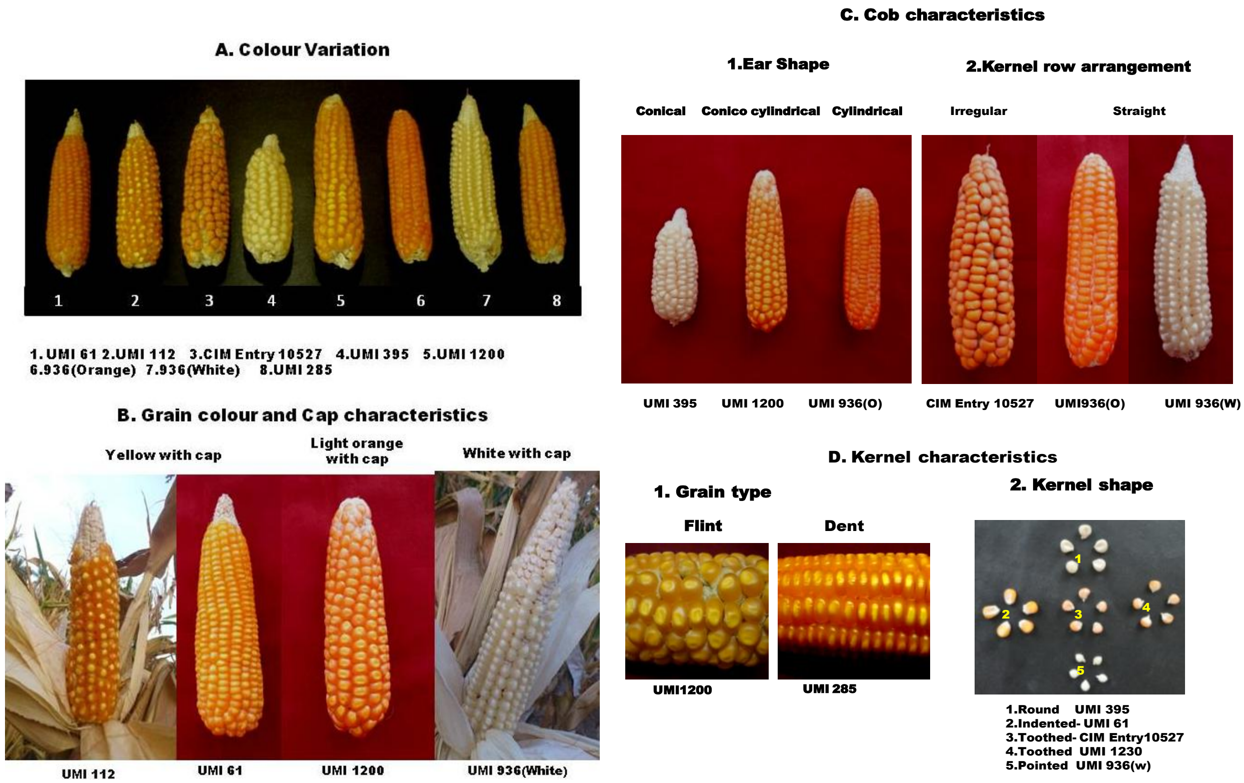

Figure 2. Cob and kernel characterization of maize inbreds.

which were sparse. The tassel angle was either wide or narrow and was shared by 5 and 12 inbreds respectively. The tassel attitude was straight in 7 inbreds, curved in 8 inbreds while it was observed as strongly curved in UMI 61 and UMI 176.

The anthocyanin pigmentation in silk was observed as silk colour and it was present in 6 inbreds while it was absent in 11 inbreds. For easy identification of individual inbreds with specific morphological characters, the flow diagram was constructed (Figure 3). The kernel row arrangement was straight in 15 inbreds while it was irregular in UMI 395 and CIM entry 10527 (Figure 2C). Based on the type of grain, the inbreds were classified into flint, semi flint or semi dent and dent. Among the inbreds, UMI 1200 had the dent type of grain and the remaining were observed to be flint type (Table 4 and Figure 2D)

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, and 100 grain weight. The days taken for 50% silking ranged from 51 days (UMI 101) to 62 days (UMI 1200), while the minimum (97 cm) and maximum (135 cm) plant height were observed in UMI 101 and UMI 1230, respectively. The 100 seed weight was maximum (26 g) in UMI 395 and minimum in UMI 69 (12 g). Based on the phenotypic traits studied, Wietholter et al. (2008) 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. 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.

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) grouped the 41 maize populations through cluster analysis into three main clusters and observed a wide range of overall genetic diversity among these populations. Ranatunga et al. (2009) indicated that, cluster analysis using 8 different qualitative traits across 43 maize genotypes resulted in grouping of genotypes into two major clusters of 19 and 24 genotypes.

In the present study cluster analysis resulted in grouping of inbreds into 6 major clusters of 1, 4, 1, 4,4, 2, and 1 is presented in Table 5 and Figure 4, where the dissimilarity coefficient ranged between 1.09 and 3.24. The maximum numbers of inbreds were included in cluster II, IV, and V having 4 inbreds and the minimum number in cluster I, III and VII having one inbred each (Figure 4). The dendrogram of 17 test inbreds constructed using 15 qualitative and seven quantitative

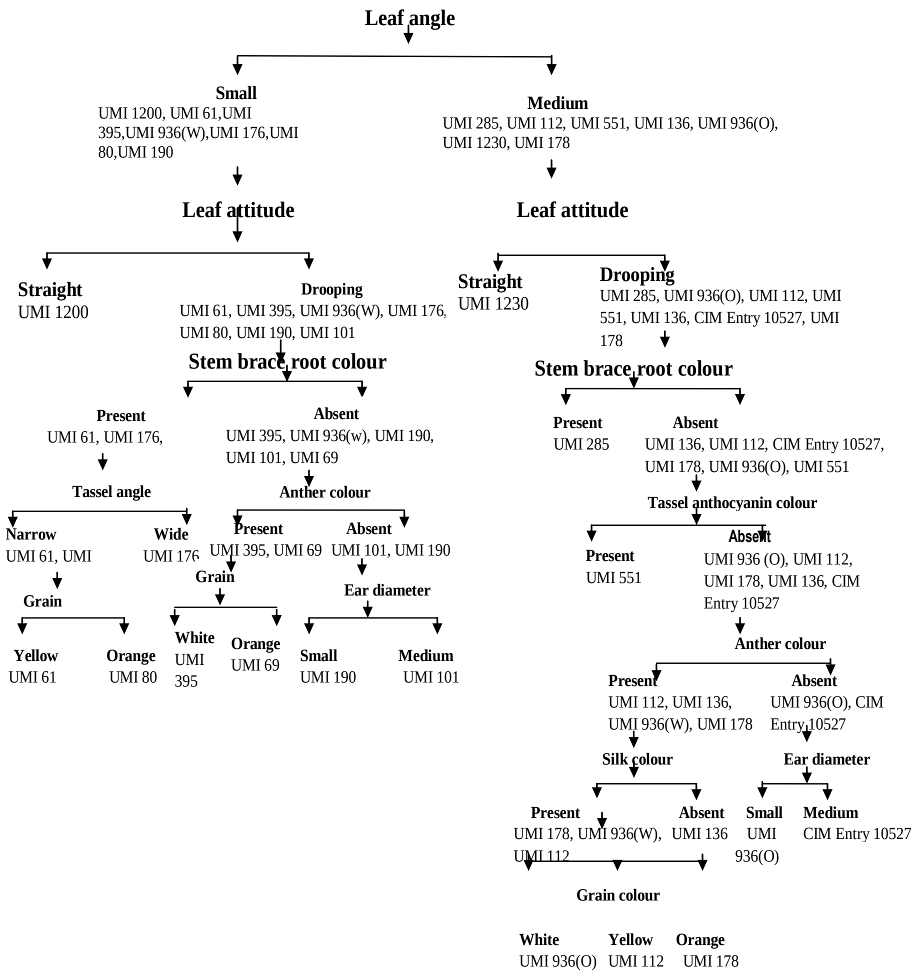

Figure 3. Flow diagram for morphological parameters in maize inbreds.

Table 3. Characterization based on cob characters in maize inbreds.

Genotypes	Cob length (cm)	Diameter	Ear shape	Type of grain	Grain colour
UMI 1200	13.7	Medium	Conico cylinder	Dent	Light orange with yellow cap
UMI 285	13.0	Small	Conico cylinder	Flint	Orange with cap
UMI 61	15.0	Medium	Conical	Flint	Yellow with cap
UMI 395	12.0	Medium	Conical	Flint	White with cap
UMI 936(O)	12.5	Small	Cylindrical	Flint	Orange
UMI 936(W)	14.0	Medium	Conico cylinder	Flint	White with cap
UMI 176	13.0	Medium	Conico cylinder	Flint	Yellow
UMI 80	14.2	Medium	Conico cylinder	Flint	Orange
UMI 112	11.8	Small	Conico cylinder	Flint	Yellow with cap
UMI 551	12.4	Medium	Conico cylinder	Flint	Orange
UMI 190	11.0	Small	Conico cylinder	Flint	Orange
UMI 69	12.8	Medium	Conico cylinder	Flint	Orange
UMI 136	13.4	Medium	Conico cylinder	Flint	Orange
UMI 101	15.0	Medium	Conico cylinder	Flint	Orange
CIM Entry10527	13.0	Medium	Conico cylinder	Flint	Yellow
UMI 1230	12.5	Medium	Conico cylinder	Flint	Light yellow
UMI 178	12.2	Medium	Conico cylinder	Flint	Orange

Table 4. Characterization based on kernel characters in maize inbreds.

Genotypes	Kernel row arrangement	Kernel shape	100 kernel weight (g)	Number of kernel rows
UMI 1200	Straight	Toothed	25.0	14
UMI 285	Straight	Round	24.0	10
UMI 61	Straight	Indented	24.0	12
UMI 395	Irregular	Round	26.0	10
UMI 936(O)	Straight	Round	22.0	12
UMI 936(W)	Straight	Pointed	14.0	12
UMI 176	Straight	Round	22.0	14
UMI 80	Straight	Round	18.8	12
UMI 112	Straight	Toothed	24.0	10
UMI 551	Straight	Toothed	24.5	12
UMI 190	Straight	Toothed	23.0	10
UMI 69	Straight	Round	23.6	12
UMI 136	Straight	Round	16.0	12
UMI 101	Straight	Round	12.0	10
CIM Entry10527	Irregular	Toothed	23.0	8
UMI 1230	Straight	Toothed	15.5	12
UMI 178	Straight	Toothed	24.0	12

Table 5. Cluster analysis in maize inbreds based on their morphological characters.

Cluster	Inbreds
Cluster I	UMI 1200
Cluster II	UMI 61, UMI936 (W), UMI 112 and UMI 178
Cluster III	UMI 551
Cluster IV	UMI 395, UMI 69, UMI 136 and UMI 190
Cluster V	UMI 285, UMI 936(O), CIM Entry 10527 and UMI 101
Cluster VI	UMI 176 and UMI 80
Cluster VII	UMI 1230

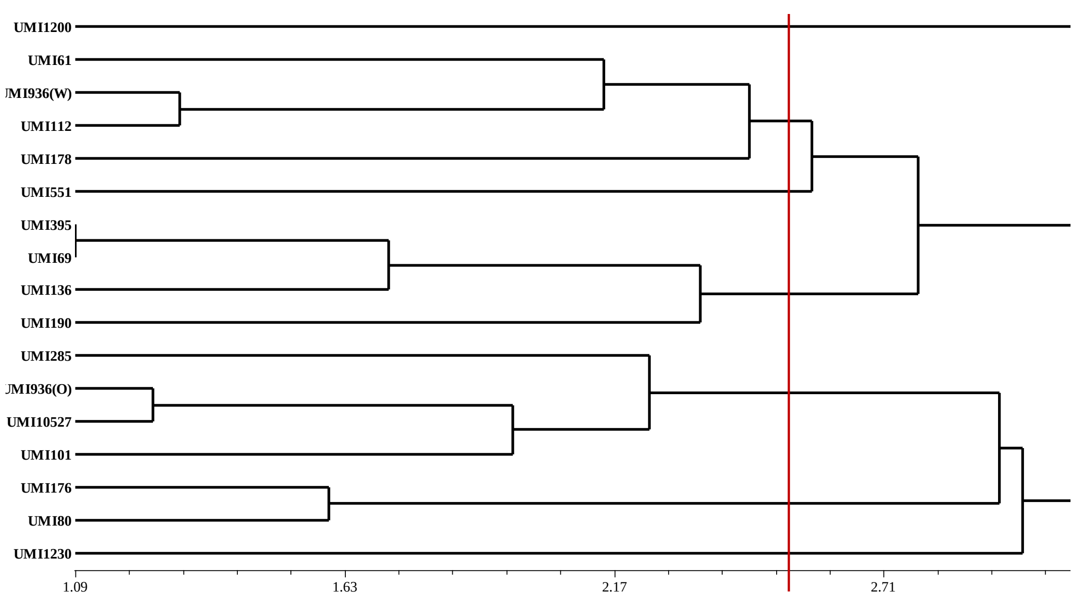

Figure 4. UPGMA dendrogram of 17 maize inbreds on qualitative traits (15) and quantitative traits (7).

traits based on genetic dissimilarity exposed a dissimilarity index of 2.49 between the individuals making up the pair which was distinct from each other.

Among the 17 inbreds, the two inbreds UMI 395 and UMI 69 were the genotypes with maximum similarity based on overall morphological and maturity characteristics with a dissimilarity index of 3.86. The dissimilarity index 4.99 was observed between UMI 936(O) and CIM Entry 10527 and 4.62 observed between UMI 936(W) and UMI 112. The results revealed that, among the 17 inbreds, UMI 1200, UMI 1230, and UMI 551 were distinct from other inbreds.

Conclusion

In summary, our analysis of clustering revealed the distinct characteristics of inbreds for desired selection and indicated that, morphological variations exist with inbreds due to variation in genetic makeup and could be better utilized by breeders in the selection of inbreds based on their specific requirement for breeding programme.

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