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Comparative evaluation of maize (*Zea mays* L.) genotypes based on distinctness, uniformity and stability (DUS) testing using physiological and morphological characters

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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 maize inbred lines was used to compare how morphological and physio- logical characterization described variety relationships. An experiment was carried out to evaluate test of Distinctiveness, Uniformity and Stability using 26 physiological and 12 morphological characters. Minimum days for 50 % tasseling (50.66 and 50.66 days), minimum days for 50 % silking (53.66 and 53.66 days), minimum days for anthesis silking interval (3.0 and 2.6 days), maximum tassel branching (22.66 and 21.66), maximum cob height (89.70 and 89.16 cm) and maximum cob length (16.96 and 17.75 cm) were recorded in genotype AAIMS-1 in both experiments (2011 and 2012 respectively) and maximum cob width (12.51 and 13.11 cm) and maximum number of grain rows per cob (12.66 and 12.66) were recorded in genotype AAIMS-2 in both experiments (2011 and 2012 respectively). But maximum plant height (155.13 and 153.71cm), minimum days for maturity (86.00 and 88.00 days), maximum grain yield per plant (72.80 and 72.00 g) and maximum 100 seed weight (21.51 and 20.96 g) were recorded in genotype AAIMS-2 and AAIMS-1 respectively in both experiments conducted at experimental farm of Department of Genetics and Plant Breeding, Sam Higginbottom Institute of Agriculture, Technology & Sciences during the year 2011 and 2012 respectively.

Keywords: DUS, Genotypes, Maize, Morphological characters, Physiological characters

INTRODUCTION

Maize is a cereal crop which is cultivated widely throughout the world and has the highest production among all the cereals. USA is the largest producer of maize in the world, followed by China and Brazil. The worldwide production of maize was more than 960 MnMT in 2013-14. It is an important food staple in many countries and is also used in animal feed and many industrial applications. The crop has tremendous genetic variability, which enables it to thrive in tropical, subtropical, and temperate climates (Anonymous, 2014).

The area and production of maize in India is 9.58 mha and 24.35 mt respectively with productivity ranging about 2707 kg/ha (2014-2015). In Uttar Pradesh, the area and production during 2014-15 was 0.74 mha and 1236.6 tonnes respectively with productivity of 1671 kg/ha. Maize is grown worldwide on an approximately 161 million ha annually with a production of 685 million metric tons (Agriculture Statistics at a Glance, 2015).

DUS Testing is one of the important criteria to test inbred lines for distinctness, uniformity and stability. DUS Testing of cultivars is 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. The system accepted and in operation in a large number of countries is as provided by UPOV. Information is, thus, generated on the basis of internationally accepted and followed norms, thereby providing a basis for appropriate comparison of materials identified under the national agricultural research system (NARS) alongside materials from other sources (Yadav and Singh, 2010).

Morpho-physiological characters have been used to study the genetic diversity in maize (Beyene *et al.,* 2005). In addition, morphological characters have been recognized to constitute universally undisputed descriptors for varietal characterization of crop species and establishing the distinctness, uniformity and stability (DUS) of crop species in Plant Variety Protection (PVP) systems (Begum and Kumar, 2011). The traits used in assessing crop variety for DUS have been carefully selected taking into account the plasticity of morphological characteristics and thus the efficient for comparing varieties (Law *et al.*, 2011). However the measurement of morphological and physiological traits is expensive, requiring more space, time consuming (Smykal *et al.,* 2008) and traits

and stability (DUS) testing using physiological and morphological characters.

MATERIALS AND METHODS

Morpho-agronomic studies of maize genotypes on UPOV harmonized characteristics; generally as per DUS test guidelines were undertaken.

Seed materials used and test conditions: Four maize genotypes which were taken from Department of Genetics and Plant Breeding, Sam Higgin bottom Institute of Agriculture, Technology & Sciences (Deemed-to-be -University) Allahabad (U.P.), were grown at Central Research Field, SHIATS, Allahabad. Two evaluation trails were conducted during *kharif* season of 2011 and 2012.

Characteristics used for morphological and physiological evaluation: UPOV's DUS (International Union for the Protection of New Varieties of Plants- Distinctiveness, Uniformity and Stability) test guidelines were generally followed beginning from the trial layout to recording of the last field related observation. In UPOV many morphological and physiological characteristics to be recorded in maize at different stages of plant growth are given. Keeping this in view, a total number of 38 characteristics were selected for observations. Characters considered for testing of inbred lines (Sujay and Singh, 2011) are given in Table 1.

Data analysis: Analysis of variance of different morphological characteristics, visually assessed characteristics, analysis of variance of measurable characteristics was done for distinctiveness in maize, analysis for uniformity in maize, and analysis for stability in maize.

RESULTS AND DISCUSSION

Morphological characters: The mean performance of different morphological characteristics is depicted in table 2 which revealed that minimum days required for 50 % tasseling and for 50 % silking wear 50.66 and 53.66 days in genotype AAIMS-1 in both the years of study. Further maximum tassel branching, maximum cob height and maximum cob length 22.66, 21.66, 89.70, 89.16, and 16.96, 17.75 respectively were also recorded in genotype AIMS-1 in both years, these results were in accordance with Olakojo and Olaoye, 2005, Salami *et al.*, 2007; and Nazir *et al.*, 2010. The maximum cob width and maximum number of grain rows per cob 12.51, 13.11 and 12.66 respectively were recorded in genotype AAIMS-2 in both years. But maximum plant height, minimum days for maturity, maximum grain yield per plant and maximum 100 seed weight 155.13, 153.71 cm, 86.00, 88.00, 72.80, 78.00g and 21.51, 20.96g days were recorded in genotype AAIMS-2 in 2011 maximum grain yield were reported by Beyene *et al*,. 2005 and in AAIMS-1 in 2012 and earliest anthesis silking interval (ASI) was recorded in genotype AAIMS-1 in 2012 and late ASI was recorded in genotype MRM-3777 in 2011. The results of present studies also got support from the findings of Jha and Ghosh (1998).

Physiological characters: Twenty two visually assessed characteristics of maize was also evaluated which has given in table 3 and no variation was found among these characters. States of different measurable physiological characteristics with over two years mean performance of four measurable physiological characteristics have been presented (Table 4).

Analysis of distinctiveness: Significant differences were observed among the genotypes for all the characteristics. Analysis of distinctiveness was also evaluated which revealed that all the genotypes were showing distinctiveness with respect to each other (Table 5).

Analysis of uniformity: For uniformity analysis twenty six characteristics were studied. Out of which, twenty two visually assessed characteristics did not exhibit any variation during the two years of study. All four measured characteristics were uniformity for tassel length in these genotypes is at the lowest level of number of occasions at which the within years standard deviation exceeds the UPOV criterion (Table 6).

Analysis of stability: After the analysis for stability it was recorded that twenty two visually assessed characteristics exhibited stable performance over both the years of experiment as there was no variation in expression of these characteristics over the years. Four measurable characteristics were subjected to statistical analysis for stability by comparison of respective values of PCV and GCV based of pooled morphological data (Table 7). Hence the characters leaf: width of blade and ear: diameter without husk characters were considered relatively less stable as compared to other measurable characters *viz*. Tassel: length of main axis above lowest side branch and Plant: ear placement. Akande and Lamidi 2006; Olaoye, 2009 reported that the two years could be due to differences in environmental conditions which vary from year to year.

In this study, distinctiveness, uniformity and stability tests were evaluated with 13 morphological and 26 physiological characters and all morphological characters did not show any variation in their states of expression over two years study. But in case of physiological characters, four measurable physiological characters were present in which two characters *viz.* leaf: width of blade and ear: diameter without husk characters showed comparatively greater magnitude of differences between GCV and PCV showed which indicated the larger role of environmental factors in bringing variation for these characters in stability test.

Conclusion

In present study, significant differences were observed among the genotypes for all the characteristics for distinctiveness. In case of uniformity, twenty six characters had observed, out of which twenty two characters did not exhibit any variation but four measured characteristics were uniformity for tassel length in

Table 1. Characters used in DUS testing of maize.

S.N.	Characteristics	States	Note	Stage of observation	Type of assess-ment
A. Physiological characteristics					
1	Leaf: Angle between blade and stem	Narrow Wide	3 7	Beginning of anthesis	VG
2	Leaf: Attitude of blade	Erect Drooping	1 9	Beginning of anthesis	VG
3	Leaf: Anthocyanin colouration of blade & sheath	Absent Present	1 9	Anthesis halfway- medium milk	VG
4	Leaf: Hairs on the blade	Absent Present	1 9	Beginning of anthesis	VG
5	Leaf: Anthocyanin colouration of auricle	Absent Present	1 9	Anthesis halfway- medium milk	VG
6	Leaf: Hairs on the auricle	Absent Present	1 9	Anthesis halfway	VG
7	Leaf: Width of blade	Narrow (<8 cm) Medium (8-9 cm) Broad (>9 cm)	3 5 7	Medium milk	MS
8	Stem: Anthocyanin colouration of stem	Absent Present	1 9	Anthesis halfway- medium milk	VG
9	Stem: Anthocyanin colouration of brace roots	Absent Present	1 9	Anthesis halfway	VG
10	Tassel: time of anthesis	Very early (<45 days) Early (45-50 days) Medium (50-55 days) Late (>55 days)	1 3 5 7	Anthesis halfway	VG
11	Tassel: Anthocyanin colouration at base of glume	Absent Present	1 9	Anthesis halfway	VS
12	Tassel: Anthocyanin colouration of glumes excluding base	Absent Present	1 9	Anthesis halfway	VS
13	Tassel: Anthocyanin colouration of anthers	Absent Present	1 9	Anthesis halfway	VG
14	Tassel: Density of spikelets	Sparse Dense	1 9	Anthesis halfway	VG
15	Tassel: Attitude of lateral branches	Straight Curved Strongly curved	1 5 9	Anthesis halfway	VG
16	Tassel: length of main axis above lower side branch	Short (<120 cm) Medium (120-130 cm) Long (>130 cm)	3 5 7	Caryopsis watery ripe	MS
17	Ear: Anthocyanin colouration of silks	Absent Present	1 9	Anthesis halfway	VG
18	Plant: Ear placement	Low Medium High	3 5 7	Medium milk	MS
19	Ear: Time of silk emergence (50% plants)	Very early (<48 days) Early (48-53 days) Medium (53-58 days) Late (>58 days)	1 3 5 7	Anthesis halfway	VG
20	Ear: Anthocyanin colouration of glumes of cob	White Light purple Dark purple	1 2 3	Caryopsis loosening day-time	VG
21	Ear: Type of grain	Flint Semi flint Dent	1 2 3	Caryopsis hard (can no longer be dented by thumb-nails)	VG
22	Ear: Colour of top of grain	White White with cap Yellow Yellow with cap Orange Brown	2 3 4 5 6 7	Caryopsis hard (can no longer be dented by thumb-nails)	VG

Contd.

23	Ear: Diameter without husk	Small (<4 cm) Medium (4-5 cm) Large (>5 cm)	3 5 7	Caryopsis hard (can no longer be dented by thumb-nails)	MS
24	Ear: Shape	Conical Conico-cylindrical Cylindrical	1 2 3	Caryopsis hard (can no longer be dented by thumb-nails)	VG
25	Kernel: Shape	Shrunken Round Toothed Flattened	2 3 4 5	Caryopsis loosening day-time	VG
26	Kernel: Row arrangement	Straight Spiral Irregular	1 2 3	Caryopsis loosening day-time	VG
B. Morphological/Quantitative characters					
27	Days to tasseling, DAY_TASS	To be recorded as number of days from sowing to when 50% of the plants have shed pollen. Pollen shading on the central axis is recorded as tassel emergence			
28	Days to silking, DAS_SILK	Number of days from sowing to when silks have emerged on 50% of the plants. Silk emergence in plants is recorded as days to silk			
29	Tassel branching, TASS_BRN	To be recorded after tasseling			
30	Plant height (cm), PLT_HGT	To be measured from ground level to the base of the tassel (after milk stage)			
31	Ear height (cm), EAR_HGT	To be measured from base of the plant to the point bearing the first ear			
32	Ear length (cm), EAR_LT	To be measured as distance from the base of the tip of the ear			
33	Ear width (cm), EAR_WD	To be measured at the central part of the upper most ear as maximum girth of the ear			
34	Number of kernel rows, KER_ROW	To be recorded as number of kernel – rows in the central part of the upper-most ear			
35	Number of kernels/row, KER_PROW	To be recorded as average number of ker- nels/five – rows of five respective ears			
36	100 seed weight (g), SED_WGT	To be recorded after harvesting			
37	Grain yield / plant (g), YLD_PLT	Average yield of five random plants are scored			

Table 2. Mean performance of three replications for various morphological characteristics in maize (*kharif* 2011 and 2012).

S. N.	Observations	Genotypes			
		AAIMS-1	**AAIMS-2**	**MRM-3777**	**SUPER-36**
1	Days to 50% tasseling	50.66* (50.66)**	51.33* 52.00**	52.33* 53.66**	52.66* 54.66**
2	Days to 50% silking	53.66* 53.66**	54.33* 54.66**	55.33* 56.66**	56.00* 57.33**
3	Anthesissilking interval	3.000* 2.666**	3.000* 3.000**	3.333* 3.000**	3.000* 3.000**
4	Tassel branching	22.66* 21.66**	22.33* 20.66**	20.33* 19.66**	22.33* 20.00**
5	Plant height	151.86* 153.71**	155.13* 149.06**	141.53* 142.91**	142.63* 143.68**
6	Cob height	89.70* 89.16**	88.30* 88.80**	85.35* 85.48**	86.05* 85.50**
7	Days to maturity	87.33* 88.00**	86.00* 87.00**	89.00* 90.00**	89.66* 89.66**
8	Cob length	16.96* 17.75**	16.93* 16.63**	13.76* 13.33**	14.20* 14.05**
9	Cob width	12.51* 13.11**	12.66* 13.90**	10.63* 11.41**	10.96* 11.56**
10	Number of grain rows per cob	12.66* 12.66**	13.33* 13.33**	10.66* 11.33**	11.33* 10.66**
11	Grain yield per plant	71.30* 72.00**	72.80* 71.47**	55.69* 64.12**	56.05* 64.63**
12	100 seed weight	20.96* 20.96**	21.51* 20.65**	17.63* 18.10**	19.28* 19.11**

**kharif* 2011 and ***kharif* 2012, 3 replications

Table 3. Pooled physiological characterization of visually assessed characteristics of maize (*kharif* 2011 and 2012).

S. N.	Characteristics	Genotypes			
		AAIMS-1	AAIMS-2	MRM-3777	SUPER-36
1	Leaf: angle between blade and stem	Narrow	Narrow	Wide	Narrow
2	Leaf: attitude of blade	Drooping	Erect	Drooping	Drooping
3	Leaf: anthocyanin colouration of blade & sheath	Present	Absent	Present	Present
4	Leaf: hairs on the blade	Present	Absent	Absent	Present
5	Leaf: anthocyanin colouration of auricle	Present	Present	Absent	Present
6	Leaf: hairs on the auricle	Present	Present	Absent	Present
7	Stem: anthocyanin colourtion of stem	Absent	Absent	Absent	Absent
8	Stem: anthocyanin colouration of brace roots	Present	Absent	Present	Present
9	Tassel: time of anthesis	Early	Medium	Late	Medium
10	Tassel: anthocyanin colouration at base of glume	Present	Present	Absent	Present
11	Tassel: anthocyanin colouration of glumes excluding base	Present	Present	Absent	Present
12	Tassel: anthocyanin colouration of anthers	Absent	Present	Absent	Present
13	Tassel: density of spikelets	Dense	Dense	Sparse	Dense
14	Tassel: attitude of lateral branches	Straight	Straight	Curve	Straight
15	Ear: anthocyanin colouration of silks	Present	Present	Present	Present
16	Ear: time of silk emergence (50% plants)	Early	Medium	Late	Medium
17	Ear: anthocyanin colouration of glumes of cob	White	White	Light purple	Dark purple
18	Ear: type of grain	Flint	Flint	Dent	Semi flint
19	Ear: colour of top of grain	White	White	White with cap	Yellow
20	Ear: shape	Cylindrical	Cylindrical	Conical	Conico-cylindrical
21	Kernel: shape	Shrunken	Round	Flattened	Toothed
22	Kernel: Row arrangement	Straight	Spiral	Irregular	Irregular

Table 4. States of different measurable physiological characteristics with over years mean performance of three replications for four maize genotypes (*kharif* 2011 and 2012).

Genotypes	Characteristics			
	Tassel: length of main axis above lowest side branch (cm)	Plant: ear placement (cm)	Leaf: width of blade (cm)	Ear: diameter without husk (cm)
AAIMS-1	Long (42.25)	Medium (70.20)	Medium (8.07)	Long (7.60)
AAIMS-2	Long (42.27)	Medium (71.18)	Medium (8.30)	Medium (4.95)
MRM-3777	Long (40.40)	Low (58.07)	Narrow (6.55)	Medium (4.86)
SUPER-36	Long (41.86)	High (73.20)	Broad (9.47)	Long (6.90)

Table 5. Pairwise distinctiveness matrix of maize genotypes obtained from COYD analysis.

S. N.	Candidate genotypes	AAIMS-1	AAIMS-2	MRM-3777	SUPER-36
1	AAIMS-1	-	D	D	D
2	AAIMS-2	D	-	D	D
3	MRM-3777	D	D	-	D
4	SUPER-36	D	D	D	-
	Overall distinctiveness	D	D	D	D

D= Distinctiveness

Table 6. Combination over year's uniformity analysis of four measurable characteristics.

Candidate genotypes	Tassel: length of main axis above lowest side branch	Plant: ear placement	Leaf: width of blade	Ear: diameter without husk
AAIMS-1	96	94	112 (1)	126 (1)
AAIMS-2	98	99	83	97
MRM-3777	105	104	99	116
SUPER-36	103	98	102	110 (1)

Symbol: 1 Number of occasions the within years. SD exceeds the UPOV criterian

expressivity is affected by environment due to gene x environment interaction (Law et al 2011a).

The objective of this study was to determine the potential utility of morphological and physiological characters for application in research, product development, seed production, intellectual property right (IPR) and genetic resource conservation management in maize. To accomplish this goal, we assessed the discrimination ability of data obtained from morphology and physiology. The present investigation was conducted to study the comparative evaluation of maize (*Zea mays* L.) genotypes based on distinctness, uniformity

Table 7. Statistical analysis for stability by comparison of respective values of Genetic parameters for four measurable characters based on morphological data (*kharif* 2011 and *kharif* 2012).

S. N.	Character	VG	VP	GCV%	PCV%	h^2 (Broad Sence)	GA
1	Tassel: length of main axis above lowest side branch	0.761*	1.288*	1.351*	1.752*	59.08*	1.37*
		0.742**	1.298**	1.317**	1.763**	57.16**	1.33**
2	Plant: ear placement	74.50*	78.92*	10.375*	10.685*	94.39*	17.26*
		58.33**	59.32**	9.173**	9.251**	98.33**	15.5**
3	Leaf: width of blade	1.263*	1.587*	3.941*	4.418*	79.58*	2.04*
		1.453**	1.596**	18.027**	4.449**	91.04**	2.36**
4	Ear: diameter without husk	2.002*	2.403*	5.733*	6.281*	83.31*	2.66*
		1.558**	2.058**	5.308**	5.827**	75.70**	2.23**

**kharif*2011 and ***kharif* 2012

these genotypes is at the lowest level of number of occasions at which the within years standard deviation exceeds the UPOV criterion and for stability, there was no variation in expression of these characteristics over the years. So, based on these results, it can be concluded that morphological and physiological DUS descriptors can be effectively used for identification and grouping of varieties. The morphological and physiological descriptors used in the present study may be used for DUS criteria for establishment of distinctness of maize varieties and as such more trials should be carried out to validate the findings.

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