P.ISSN: 0971-8184 II E ISSN: 0976-1926
DOI: https://doi.org/10.56093/ijpgr.v39i1.175909

Indian Journal of
Plant Genetic Resources

RESEARCH ARTICLE

Morphological DUS Characterization and Genetic Diversity Analysis of Maize (*Zea mays* L.) Inbred Lines under Waterlogging Conditions

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Abstract

A comprehensive study was undertaken to characterize 80 maize (*Zea mays* L.) inbred lines for 28 morphological DUS traits under waterlogging (WL) conditions. The descriptors included 20 visually assessed and 8 measurable characteristics. Analysis of variance (ANOVA) for measurable traits revealed highly significant differences among genotypes for all characters studied, indicating the presence of substantial genetic variability. Visually assessed DUS traits showed wide variation among lines for kernel shape, glume colour, and grain colour, except for grain type, since all entries belonged to the field corn group. Cluster analysis based on combined visual and measurable traits grouped the genotypes into two major clusters, indicating clear phenotypic diversity. When clustering was performed using measurable traits alone, SE 610, SE 601, SE 616, I 190 and I 202 formed a separate cluster, highlighting their distinct morphological expression. The combined assessment of qualitative and quantitative DUS descriptors proved efficient in differentiating maize inbred lines. The study provides valuable baseline information for varietal distinctiveness testing, genetic purity maintenance, and selection of potential parents in breeding programs aimed at improving waterlogging tolerance in maize.

Keywords: Cluster analysis, Distinctness, Morphological traits, Stability, Uniformity, Waterlogging duration

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Received: January 6, 2025

Revised: November 14, 2025

Accepted: November 19, 2025

How to cite this article: Garg T, S Thapa and R Ranjan (2026) Morphological DUS Characterization and Genetic Diversity Analysis of Maize (*Zea mays* L.) Inbred Lines under Waterlogging Conditions. *Indian J. Plant Genet. Resour*. 39(1): 48-55.

DOI: https://doi.org/10.56093/ijpgr.v39i1.175909

Introduction

Maize is an industrially important raw product for the production of myriad products such as starch, biofuel, beverages, oils and so on. Attributed to its highest genetic yield potential amongst all cereals, Maize is undoubtedly the Queen of cereals, being an important source of carbohydrates in the diet among developing countries and as a main feed to the livestock in developed countries. Endowed with a wide adaptability habit, this crop of maize is grown in almost every corner of the world, encompassing more than 165 countries and thriving in almost every edaphic and climatic condition. Globally, maize is an important cereal ranking second in the world in terms of acreage, cultivated on nearly 205 million hectares with global production of approximately 1.21 billion metric tonnes in 2023-24 (FAOSTAT 2025). Maize is an important crop for farmers in the northwestern plain (NWP) zone of India, providing both income and food for local communities. The majority of the area under the NWP zone is covered by paddy, and due to the decline in the groundwater table of this region, there is a call from policymakers to diversify the paddy with maize crops. In the northwestern plain zone of India, maize is typically grown during the *kharif* season, which coincides with the rainy season and due to most of the lowland paddy area, waterlogging (WL) is a major problem for maize crops. WL is a condition where the soil is saturated with water for a prolonged period, which results in reduced oxygen availability to the plant roots, leading to several physiological and metabolic disorders (Rana *et al.,* 2025; Kumar *et al.,* 2025a). Maize is a water-loving crop and requires adequate moisture for growth and development. However, prolonged WL can have detrimental effects on maize growth and yield. WL in maize can lead to reduced plant growth and stunted roots, which ultimately result in decreased yield. It can also lead to an increase in disease incidence, such as root rot and fungal diseases, which can further affect the plant's growth and yield. Additionally, WL can also increase nutrient leaching and soil compaction, which can affect the plant's ability to uptake essential nutrients. To mitigate the effects of WL on maize, farmers can employ several strategies (Zaidi *et al.,* 2004; Kumar *et al.,* 2025 b). One of the best strategies is to select maize varieties that are tolerant to WL, or avoid planting maize in areas with poor drainage, which is hardly possible.

Screening is the first and foremost plant breeding activity, and to characterize the screened germplasm based on morphological features is mandatory. For characterization, the DUS (Distinctness Uniformity and Stability) test is carried out for all types of crops. DUS testing involves raising the crops under ambient growth conditions and collecting several morphological parameters to distinguish plants based on several visual and measurement assessments (Yadav and Singh, 2010). In India, the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) has recognised 31 characters as the basic requirement for the characterization of maize genotypes for DUS (Anonymous 2007). Punjab Agricultural University, Ludhiana, is a reservoir of the maize germplasm of different maturity groups along with speciality maize. Maize breeding at PAU started during the 60's and developed several inbred lines with different utilities (Sandhu *et al.,* 2021). This developed germplasm is also classified into different heterotic pools and is being used by different centres in their breeding program. The objective of this study was to characterise the set of inbred lines tested for WL tolerance at different durations morphologically.

Materials and methods

A set of eighty different genotypes of different heterotic pools that were also tested for WL tolerance at different durations, *viz.,* 3, 6 and 9 days of flooding, were sown in the experiment area of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India, in the year 2020-21. All recommended packages of practices were followed. The experimental materials procured for DUS testing are listed in Table S1:

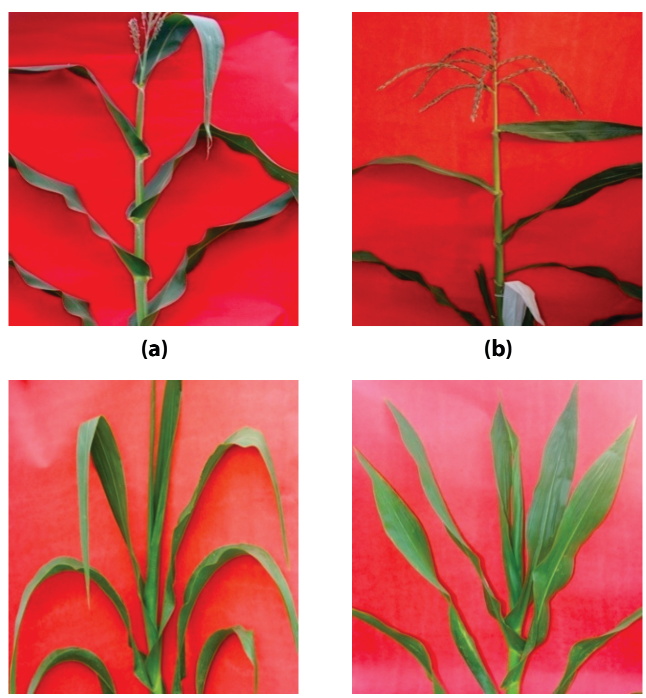
The experiment was conducted in Randomised Block Design (RBD) with three replications, having 2 rows per genotype with row length of 4 m. The plant-to-plant spacing was 20 cm, and row-to-row spacing was 60 cm. Observations for a genotype were made from 18 plants (6 plants selected randomly from each replication) following the guidelines as per PPV&FR authority (Anonymous 2007).

Observations were recorded at different growth stages and taken from the different plant parts. Four types of assessments were followed:

1. **VS:** Visual assessment by observations of individual plants or parts of plants
2. **VG:** Visual assessment by a single observation of a group of plants or parts of plants
3. **MS:** Measurement of a number of individual plants or parts of plants
4. **MG:** Measurement by a single observation of a group of plants or parts of plants

For visually assessed characteristics, no statistical methods were employed, and their expression level was used for assessment of their distinctness.

A total of 28 morphological characteristics (Fig. 1) were taken into account to distinguish 80 maize inbred



(a) (b)

(c) (d)

(e)

(f)

(m)

(n)

(g)

(h)

(o)

(p)

(i)

(j)

(q)

(r)

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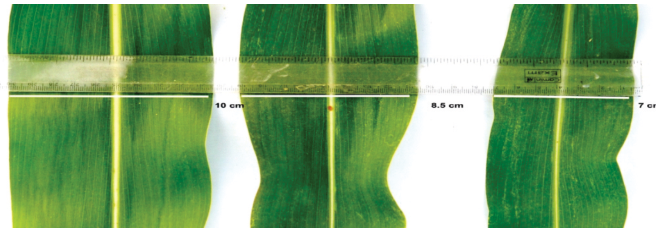
(ab)

(ab)



(ac)

Fig. 1: Different visually assessed DUS characters observed at field condition.

Angle between stem and leaf blade a: acute, b: wide; Attitude of leaf blade c: droopy, d: straight; anthocyanin pigment e: present, f: absent; anthocyanin at base of glume g: present, h: absent; anthocyanin colouration of glumes i: present, j: absent; anthocyanin colouration of anthers k: present, l: absent; spikelet density m: dense, n: sparse; tassel angle o: normal, p: wide; anthesis of tassel q: straight, r: curved; anthocyanin colouration of silk s: present, t: absent; ear placement u: low and high, v: medium, tassel length w: short, x: medium, y: long; plant height z: short, aa: medium, ab: long; ac: different leaf width.

lines, which included 20 visually assessed characteristics and 8 measurable characteristics. Twenty visually assessed and eight measurable DUS traits were recorded in field conditions at their respective crop growth period. Traits including plant height (cm), width of leaf blade (cm), anthesis period (days), length of tassel (cm), silking period (days), cob length (cm), cob diameter (cm) and grain weight (gm) were eight measurable DUS characters. A Vernier Calliper was used for taking cob length and cob diameter, while a long (1-meter) scale was used for plant height and leaf blade width data recording. 1000 grain weight was measured using a standard weighing balance.

Statistical analysis

The lines subjected to DUS characterization were sown in three replications in a Randomized Block Design (RBD). All the measurable characteristics were recorded from six plants for each line and each replication. An average reading was calculated and subjected to one-factor analysis. Significant differences were observed among the inbred lines for all the measurable characters. ANOVA for eight measurable DUS characters and clustering was calculated using the R software package.

Results and Discussion

A total of 28 morphological characteristics were taken into account to distinguish 80 maize inbred lines, which included 20 visually assessed characteristics and 8 measurable characteristics. Data collected during the experiment were analysed, and the results obtained were presented under different sections.

Table 1: Analysis of Variance (ANOVA) statistics for measurable DUS characters

Source of variation	df	Mean squares							
		Cob length	Cob Diameter	Tassel length	Plant height	Silking Days	Leaf blade width	Anthesis days	Kernel 1000 grains
Replication	2	5.064*	0.255^{NS}	0.487^{NS}	75.8165*	2.037^{NS}	0.075^{NS}	2.2625^{NS}	16.559*
Treatment	79	11.95*	0.457*	106.93*	1285.53*	48.881*	4.201*	47.615*	8491.393*
Error	239	1.177	0.072	0.905	17.677	2.379	0.026	2.895	112.560

* = significant at 5% probability level

Measurable DUS Characteristics

The analysis of variance (ANOVA) for measurable DUS characters revealed highly significant differences among treatments for all the traits studied (Table 1). This indicates the presence of substantial genetic variability among the genotypes evaluated under WL conditions. Significant mean squares due to treatments were observed for cob length, cob diameter, tassel length, plant height, days to silking, leaf blade width, days to anthesis, and kernel weight of 1000 grains. The significant variability among genotypes for morphological traits reflects differential genotypic responses to WL stress.

Variability in plant height and tassel length under excess moisture stress indicates differential tolerance mechanisms influencing vegetative growth and reproductive organ development. A marked reduction in plant stature under prolonged WL stress has been reported earlier due to restricted root aeration and impaired nutrient uptake (Zaidi *et al.,* 2010).

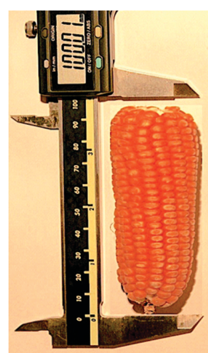
The significant treatment effects for anthesis and silking days suggest that genotypes responded differently in terms of phenological adaptation under stress. A delay in anthesis and silking is often observed under hypoxic conditions as a result of disrupted hormonal signalling and carbohydrate transport (Setter and Waters, 2003; Baishan *et al.,* 2010).

The significant differences in leaf blade width and cob diameter also indicate that genotypes vary in their ability to maintain photosynthetic surface area and ear development under waterlogged environments. Such diversity among genotypes is desirable for the selection of potential parents in breeding programs aimed at improving WL tolerance in maize.

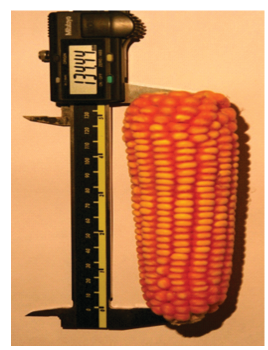
The presence of significant genetic variability, as indicated by the ANOVA, suggests that these morphological traits can serve as reliable selection indices for identifying tolerant genotypes. Similar observations were reported by Zaidi *et al.,* (2003); Kumar *et al.,* (2025c) and Thapa *et al.,* (2025), who highlighted that genotypic variability for morphological and phenological traits under waterlogging conditions can effectively discriminate tolerant and sensitive maize lines.

Visually Assessed DUS Characteristics

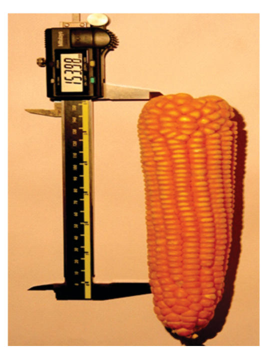
Different visually assessed and measurable traits considered for the experiment were photographed and presented in Fig. 1 and Fig. 2. Data showed significant variation for almost all the traits considered except for the trait, grain type. Since all the maize lines chosen for the experiment were field corn, no entry was recorded for popcorn, waxy corn, opaque corn and sweet corn. The yellow type of colour of grain was recorded for PML 1012, while variegated was recorded for SE 613. I 179



(a) Short



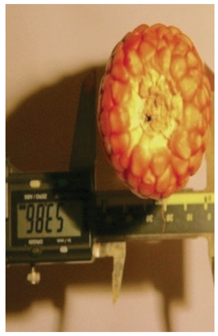
(b) Medium



(c) Long

(d) Small

(e) Medium



(f) Large

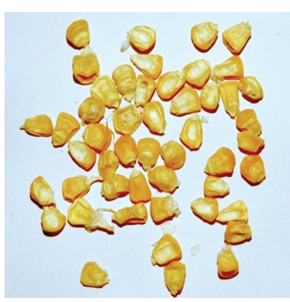
(g) Yellow

(h) White

(i) Red

(j) Orange

(k) Variegated



(l) Shrunken

(m) Round

(n) Indented

(o) Toothed

Fig. 2: Maize inbred lines showing variability for different measurable DUS characters.

Cob length a: short, b: medium, c: long; ear diameter d: small, e: medium f: long; ear shape g: conical, h: cylindrical, i: conical-cylindrical; colour of grain j: yellow, k: white, l: red, m: orange, n: variegated; kernel shape o: shrunken, p: round, q: indented, r: toothed.

and I 202 have red type of grain colour. None of the grain has white or white with a cap state of grain colour. Accordingly, almost every line showed white colouration of glumes of cob except for only two lines, LM 21 and SE 607, which showed light purple colouration. None of the lines showed dark purple colouration. A single line SE 601 showed a shrunken type of kernel shape, while most of the rest were round or toothed, and a few were indented. None of the lines showed a pointed type of kernel shape. These variations are valuable for DUS testing, as visual traits are among the primary descriptors used for establishing varietal distinctiveness (Gethi *et al.,* 2002; PPV&FRA, 2007). Similar patterns of variability for kernel and glume traits have been reported in tropical maize germplasm, emphasizing their importance in genotype differentiation and hybrid parental line identification (Huasheng, 2011; Saha *et al.,* 2024).

Clustering Based on Visually and Measurable Assessed Characters

Twenty different visually assessed and eight different measurable DUS descriptors were employed to generate a Euclidean dissimilarity coefficient matrix, followed by clustering using Ward. D^2 algorithm with the aid of the R Studio software package. The dendrogram thus obtained revealed two major clusters (Fig. 3a). Both Cluster I and Cluster II were further divided into two sub-clusters. Cluster I included the majority of genotypes, consisting of 56 lines, while Cluster II included 24 genotypes. Each sub-cluster of Cluster I was further divided into two major groups, while the first sub-cluster of Cluster II consisted of LM 21 and SE 607, and the second sub-cluster was bifurcated into two major groups. The cluster analysis clustered the

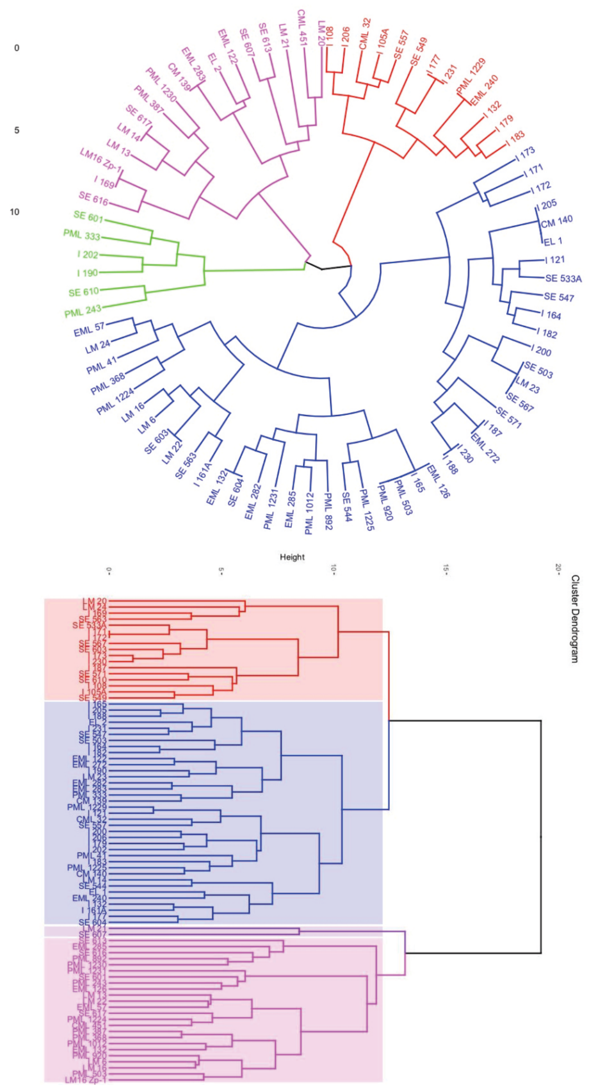


Fig. 3: Clustering of genotypes based on visually assessed and measurable DUS characters.

80 genotypes (Fig. 3b) into two major clusters. Both clusters were further divided into two subclusters. Cluster I consisted of 27 genotypes, while 53 genotypes were included in Cluster II. Each sub-cluster cluster further bifurcated into two major groups. A tanglegram (Fig. 4) was constructed to compare both dendrograms, revealing partial correspondence between visual and measurable trait-based clustering patterns.

Such clustering patterns emphasize that morphological diversity in maize is largely driven by both qualitative and quantitative traits. The clear differentiation of certain lines, such as PML 243, SE 610, SE 601, I 190 and I 202, suggests that they possess unique trait combinations that may serve as reference lines in DUS testing. Similar approaches were employed by Kumari *et al.,* (2025) and Xiao Hong et al. (2006), who used multivariate clustering of maize inbreds based on morphological descriptors to establish distinctness and

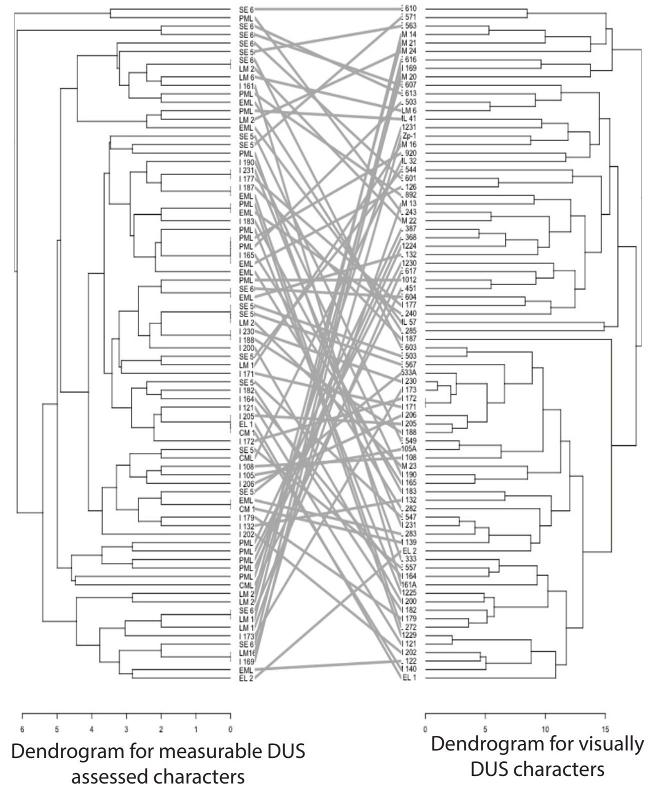


Fig. 4: Tanglegram showing relatedness between different genotypes for measurable and visually assessed DUS characters.

aid in hybrid parent selection.

The observed grouping patterns also align with findings of Tasnim *et al.,* (2025), who reported that measurable and visual DUS traits collectively provide a robust basis for varietal identification and protection under the PPV&FRA framework. The combination of qualitative and quantitative traits thus strengthens the reliability of DUS testing and can contribute to both genetic purity maintenance and stress tolerance breeding, especially under variable environmental conditions such as waterlogging.

Conclusion

The present investigation revealed significant morphological diversity among maize inbred lines evaluated under waterlogging stress using DUS descriptors. Highly significant genotypic differences across measurable and visually assessed traits confirmed the existence of substantial genetic variability. The clustering pattern demonstrated that both qualitative and quantitative traits contribute meaningfully to genotype differentiation. Distinct lines such as SE616, SE610, SE601, I190 and I202 exhibited unique trait combinations, suggesting their potential utility as reference genotypes for DUS testing and as promising parents in waterlogging tolerance breeding.

The integration of DUS characterization with stress-response evaluation thus provides a reliable framework for varietal identification, genetic purity assurance, and sustainable maize improvement under variable environmental conditions.

Acknowledgement

The authors are grateful to the Punjab Agricultural University, Ludhiana, for providing the necessary research facilities.

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