Range Management and Agroforestry 46 (1) : 56-61, 2025
ISSN 0971-2070 (Print); 2249-5231 (Online)
https://doi.org/10.59515/rma.2025.v46.i1.08

Research article

Classification of sorghum (*Sorghum bicolor* (L.) Moench) germplasm accessions for various agro-morphological parameters

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Received: 09th March, 2024 Accepted: 07th February, 2025

Abstract

Germplasm characterization serves as the base for sustainable conservation and increased use of crop genetic resources. A total of 132 sorghum germplasm accessions were morphologically characterized and grouped based on DUS descriptors suggested by PPV & FRA (2007) to determine the genetic potential of the accessions to be utilized in future breeding programs. Among 33 DUS traits observed, nine traits were dimorphic, seven traits were trimorphic and the remaining 16 were found to be polymorphic, revealing a wide spectrum of genetic variability among studied accessions signifying their potential to serve as genetic markers for varietal identification and characterization. The clustering patterns based on morphological traits revealed five distinct groups, with a majority of released varieties and pre-release cultures being grouped under a single cluster. The promising accessions *viz*., KO5 19, SO3 260, Madurai Kattai Sevappu, SO3 517 and SO3 88 identified based on available marker traits could serve as potential donors for grain mold resistance, whereas accessions such as KO5 116, KO5 69, KO5 108, TNS 682 and RPHT 14 could be used in further improvement of grain and forage sorghum types.

Keywords: Characterization, Clustering, Marker traits, Sorghum germplasm

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop in the world belonging to the grass family Poaceae (Poehlman and Sleper, 1995). It is a climate-smart, nutrient-rich annual diploid crop species that originated and domesticated from North-Eastern Africa. At present, sorghum is cultivated in 100 countries on all continents, covering about 40.88 million hectares with a grain production of 61.46 million metric tons and a productivity of 1.50 metric tons per hectare (USDA, 2025). Sorghum is a multipurpose crop where the whole plant can be used effectively. It serves as a dietary staple food crop for millions of the poorest and most food-insecure people in the semi-arid tropics of Africa and Asia (Hariprasanna and Patil, 2015). Next to pearl millet, sorghum seems to be the best as well as the cheapest source of energy and micronutrients. It is resistant to drought with a well-developed root system and is hence often known as the 'camel crop.' It thrives very well in tropical, subtropical, and temperate regions of the world, extending to as many as six continents as 'great millet' (Shilpa and Kajjidoni, 2019).

Plant genetic resources are the key components to meet the future food security needs of the world. Characterization and quantification of genetic diversity have long been a major goal in evolutionary biology (Losos *et al*., 2013). Characterization reveals the worth of germplasm to incorporate new gene combinations for use in any crop improvement programs (Reddy *et al*., 2006). The amount of genetic variability available in sorghum is immense. Globally, around 1,68,500 sorghum germplasm accessions have been maintained in 19 institutes. International Crop Research Institute for Semi-Arid Tropics (ICRISAT) genebank maintains 39,948 accessions from 93 countries, making it a world repository for sorghum germplasm conservation (Upadhyaya *et al*., 2016). Therefore, efficient utilization of the genetic potential held in germplasm collections requires detailed knowledge about the collections, including characterization, evaluation and classification. Over the years, genetic diversity among sorghum germplasm accessions was predominantly studied using morphological markers (Joshi *et al*., 2015; Chand *et al*., 2017; Chavan *et al*., 2018; Santhiya *et al*., 2020; Sri Subalakhshmi *et al*., 2021 and Shafiqurrahaman *et al*., 2022). Morphological markers have been widely accepted in plant variety protection, registration and patenting as their description is capable of showing both identity and distinctness. Therefore, the present study emphasizes on morphological characterization and classification of sorghum germplasm accessions based on distinctness, uniformity and stability (DUS) descriptors to determine the genetic potential of the accessions to be utilized in future breeding programs.

Materials and Methods

Experimental site and design: A diverse set of 132 sorghum germplasm accessions, including landraces, released varieties and pre-release cultures which are in the advanced stage of yield trials, were collected from the Department of Millets, TNAU, Coimbatore, and Ramaiah Gene Bank, TNAU, Coimbatore. The germplasm accessions along with seven checks (K12, CO S 28, CO 30, CO 32, TNS 670, CSV 33MF and K11) were evaluated in Augmented Randomized Complete Block Design (Augmented design II) with a row-to-row spacing of 45 cm and plant to plant spacing of 15 cm on a 4 m rows during *Kharif*, 2019 at Department of Millets, Tamil Nadu Agricultural University, Coimbatore. The experimental site is located at 11° North latitude and 76° East longitude with an altitude of 427 meters above the mean sea level. Further, to reduce variability among experimental units, each replication was folded into four blocks, each containing 33 germplasm accessions. The crop was maintained by employing standard cultural and management practices.

Observations and analysis: Distinctness of accessions at different growth stages was noted by 33 DUS descriptor traits based on visual observations from ten randomly selected plants as per DUS test guidelines issued by the Protection of Plant Variety and Farmer's Right Authority (PPV&FRA, 2007). Genetic distance was estimated using the Euclidean approach distance. Hierarchical clustering was performed using the Ward D^2 method (Murtagh and Legendre, 2011). The cluster pattern among 132 sorghum germplasm accessions was determined using the Statistical packages "ape" and "dextended" for R studio.

Results and Discussion

Morphological characterization: Plant genetic resources are the key components to meet the future food security needs of the world. Evaluation based on descriptors determines the genetic worth of the accessions. Morphological markers are frequently used in the genus sorghum for descriptive purposes and enable the grouping of genotypes into grain, forage, or sweet sorghum types. Among 33 DUS traits observed, nine traits were dimorphic, seven traits were trimorphic and the rest sixteen were found to be polymorphic, revealing a wide spectrum of genetic variability among the studied accessions. The range of variability and frequency observed in morphological traits were recorded (Fig 1).

Anthocyanin coloration in the coleoptile and leaf sheath was found to be dimorphic, with the prevalence of greyed purple pigmentation on the coleoptile (53%) and yellow-green coloration on the leaf sheath (67%). Bhusal *et al.* (2017) reported that greyed purple pigmentation on seedlings had a significant association with tannin content in seeds. Mohammed *et al.* (2016) reported that purple pigmentation on leaf sheath could serve as an early-stage indicator for screening of shoot fly resistance. The time of panicle emergence determines the maturity

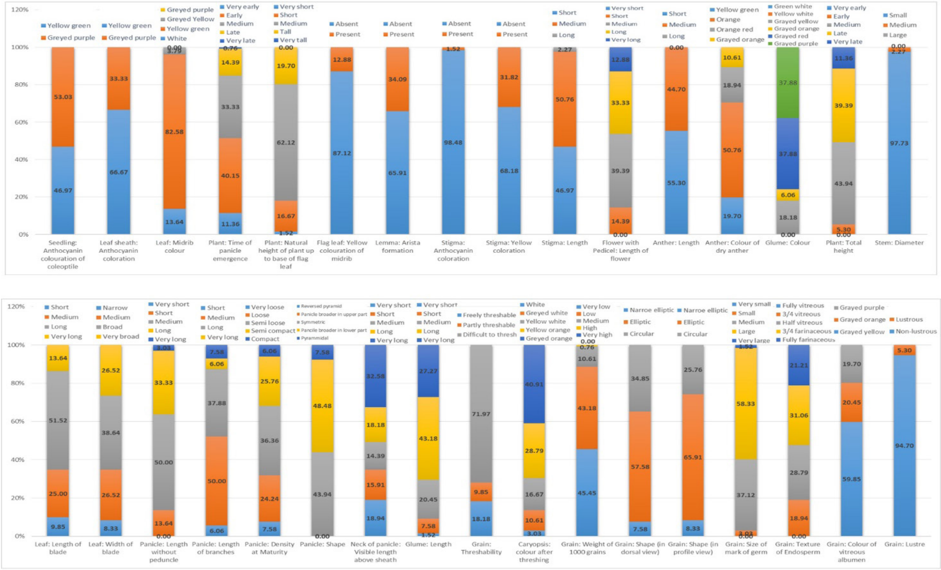


Fig 1. The distribution frequency for various DUS traits in 132 sorghum germplasm accessions

range of genotypes. In the case of drought-affected areas, accessions with earliness are preferred as they escape terminal drought (Noor *et al.*, 2012). Nearly 11% of the accessions were very early flowering with a duration of less than 56 days *viz.*, IS 1219, IS 3336, IS 8528, KO5 93-1, SPV 162, SPV 753, SOR 1096, SO3 162, SO3 202, SO3 210, Madurai Kattai Vellai, Karur Local, SO3 196, KO5 8 and Paiyur 2. Accessions with earliness observed in this study might be utilized for evolving short-duration drought-tolerant cultivars (Khandelwal *et al.*, 2015).

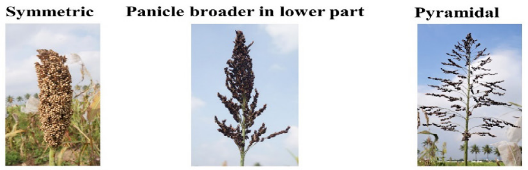
Preferences for grain and fodder types vary based on plant height. Several findings concluded that tall plants are much preferred in forage breeding. Since tall plants are more sensitive to lodging, medium plant types are preferred for grain purposes (Jain and Patel, 2016). Plant height was observed to be polymorphic and exhibited four types of variations. In the present study, the majority of accessions were medium (44%) to tall type (39%). The higher the stem girth, the higher would be the resistance towards lodging *via* enhanced culm strength. The stem diameter was found to be dimorphic. Among the studied accessions, 98% had thin stems (< 2 cm), whereas 2% (TNS 667, CO 26, KO5 69) had medium stem girth (2 - 4 cm).

Leaves are the primary source of assimilates for grain filling and grain yield. Based on leaf length and leaf width, accessions were categorized into four groups. In the present study, the majority of accession had long (52%) and broad (39%) leaves. The results were in agreement with Elangovan *et al.* (2007). The accessions *viz.*, KO5 116, KO5 69 and KO5 108, which are taller with more leaf area, could serve as potential donors for developing high biomass lines.

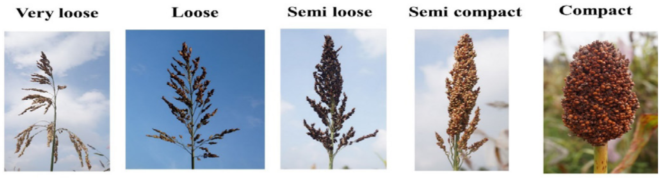
Panicle characters: Lemma arista formation was found to be dimorphic. Sorghum accessions with awns are generally low grain yielders, yet they act as their defensive mechanism from being eaten up by birds (Elangovan *et al.*, 2007; Verma *et al.*, 2017). Almost 34% of the accessions were awned, whereas the rest, 66%, were awnless. Anthocyanin coloration in the stigma was dimorphic. The accessions, *viz.*, KO5 18 and CSV 33MF could be uniquely differentiated from the rest of the accessions by their anthocyanin coloration on stigma. Similarly, the yellow coloration on the stigma was also dimorphic. Yellow-colored stigma was observed in 32% of studied accessions, whereas yellow coloration was absent in the stigma of 68% of studied accessions. Among 132 accessions, 62 had short stigma (<1 mm), 67 had medium stigma (1–2 mm) and 3 had long stigma (>2 mm). The accessions IS 4907, IS 5890 and KO5 8 could be characterized based on longer stigma. Another length was found to be short (<3 mm) in 55% of studied accessions, whereas medium (3–4 mm) in 45% of studied accessions. Bhusal *et al.* (2017), Chavan *et al.* (2018) and Shafiqurrahaman *et al.* (2022) also reported a similar kind of variation for these characters.

Panicle shape, compactness and length are important attributes in determining the yielding ability of an individual genotype. According to panicle length *i.e.* without peduncle, the accessions were categorized into four groups *viz.*, 66 accessions with medium-sized panicles (21–30 cm), 44 accessions with long panicle (31–40 cm), 18 accessions with short panicles (11–20 cm) and four accessions (KO5 116, SO3 297, SO3 218 and CSV 33MF) with very long panicle (>40 cm). In the present study, accessions were grouped into three classes based on panicle shape (Fig 2a). Panicles were broader at the lower part in 48% of accessions, symmetric in 44% of accessions and pyramidal in 8% of accessions. Panicle compactness was highly polymorphic and exhibited five categories of variations (Fig 2b). Among the accessions studied, 10 accessions had very loose type panicles, 32 accessions had loose type panicles, 48 accessions had semi-loose type panicles, 34 accessions had semi-compact type panicles and eight accessions had compact type panicles. Panicle shape and compactness could serve as a valid morphological marker for quicker varietal identification. Sorghum genotypes with loose panicle types are much preferred in areas characterized by high rainfall. Chavan *et al.* (2018), Kavithamani *et al.* (2019) and Shafiqurrahaman *et al.* (2022) also reported a similar kind of variation in panicle length, shape and compactness. The findings of this study revealed that the accessions KO5 19, SO3 260, Madurai Kattai Sevappu, SO3 517, SO3 88 and CSV 33MF had a very loose and pyramidal-shaped panicle, which uniquely differentiates them from the rest of the accessions and could offer wide scope in resistance breeding against mold and ergot.

Glume characters: Generally, grain sorghum can be differentiated from forage types based on its glume length. Several findings concluded that fodder sorghum has maximum glume coverage, whereas in grain types, glume coverage is less (Verma *et al.*, 2017 and; Santhiya *et al.*, 2020). In the present study, glume length of accessions was categorized into five groups. Two accessions (SPV 156 and SO3 322) had very short glumes, 10 accessions had



2a. Panicle shape



2b. Panicle density

Fig 2. Variations in panicle characters shorter glumes, 27 accessions had medium-sized glumes, 57 accessions had longer glumes and 36 accessions had very long glumes (Fig 3). Similarly, the length of the glume covering sorghum grains determines its threshability. Threshability becomes harder with the increased percentage of grain coverage. Grain threshability was observed to be trimorphic. Among the studied accessions, 72% were hard to thresh, 10% were partly threshable and 18% were freely threshable. The findings of this study also revealed that almost three-fourths of the accessions with longer glumes showed difficulty in threshing. The variability observed for glume length and grain threshability could be utilized in forage breeding.

Resistance to grain mold is conferred by harder grains or a high level of phenols in seeds. Several findings concluded that the darker glume color and corneous nature of endosperm were associated with grain mold resistance in sorghum (Audilakshmi *et al*., 1999; Jambunathan *et al*., 1992). Based on glume color, accessions were grouped into four classes. Among 132 accessions, the color of the glume was grayed yellow in 24 accessions, grayed orange in eight accessions, grayed red in 50 accessions and grayed purple in 50 accessions. The variability observed for glume color in this study could be utilized in screening for grain mold resistance.

Grain characters: Reddy *et al.* (2010) reported that grain micronutrient content (Fe and Zn) was highly influenced by the endosperm texture and grain size. In the present study, the texture of endosperm in grains was categorized into four groups (Fig 4a). Among the studied accessions, 25 had 75% corneous endosperm, 38 had 50 % corneous endosperm, 41 had 25 % corneous endosperm and 28 had fully farinaceous endosperm. Among the sorghum accessions studied, the expression of color in vitreous albumen was found to be trimorphic with the preponderance of grayed yellow (60%), grayed orange (20%) and grayed purple (20%) (Fig 4b). Similar results pertaining to endosperm texture and color of vitreous albumen were reported by Thangavel *et al.* (2010), Chand *et al.* (2017) and Santhiya *et al.* (2020).

Kernel color has unique significance in food preparations as it determines the color of the end product. White sorghum is preferred for porridge making, whereas red sorghum is famous for brewing traditional beer (Hikeezi, 2010). Caryopsis color was observed to be polymorphic and exhibited five types of variations with the preponderance of grayed orange in 54 accessions, yellow-orange in 38 accessions, yellow-white in 22 accessions, grayed white in 14 accessions and white in 4 accessions (SO3 103, SO3 104, SO3 146 and SO3 216). Similar results were obtained by Bhusal *et al.* (2017), Chand *et al.* (2017) and Chavan *et al.* (2018).

Fig 3. Variations in Glume length

The weight of 1000 grains in sorghum was categorized into four classes *viz*., very low (less than 16 g) in 60 accessions, low (16–25 g) in 57 accessions, medium (26–35 g) in 14 accessions and high (36–45 g) in one accession (TNS 667). Accessions with high grain weight could be the potential source for developing high grain yielders. The unique varietal-specific fingerprint could be obtained at the grain level based on grain shape and germ size. The expression of grain shape among the accession was found to be trimorphic in both dorsal and profile views. The shape of grain in the dorsal view was found to be narrow elliptic in 10 accessions, elliptic in 76 accessions and circular in 46 accessions (Fig 4c). Meanwhile, the grain shape in the profile view was narrow elliptic in 11 accessions, elliptic in 87 accessions and circular in 34 accessions (Fig 4d). Germ size was observed to be polymorphic and exhibited four variations, dominated by 77 accessions with larger germ size, 49 accessions with medium-sized germ, 4 accessions (KO5 135, SO3 230, TNS 676 and CSV 33MF) with small-sized germ and 2 accessions (SOR 1096 and SO3 218) with very large germ size. Thangavel *et al.* (2010) reported that seed characters can be used as marker-trait in distinctness testing as it enables easy discrimination of genotypes.

Clustering pattern: Morphological traits-based cluster analysis showed that 132 sorghum germplasm accessions

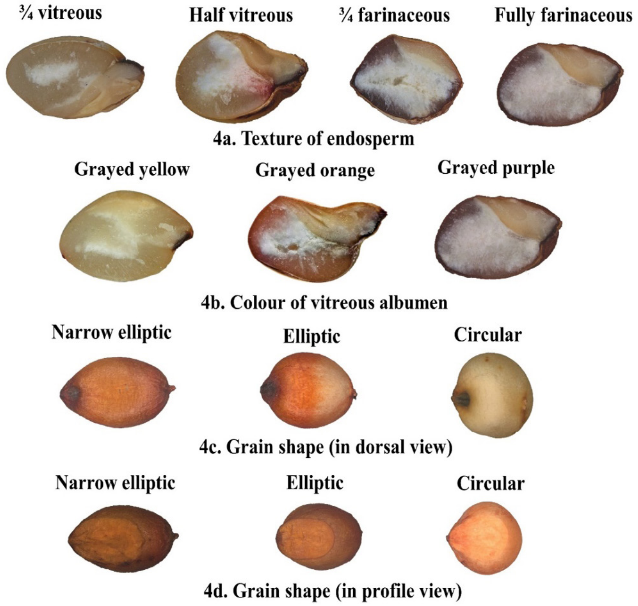


Fig 4. Variations in grain characters

Table 1. Specificity of sorghum accessions for particular traits

Potential source	Marker trait	Promising accessions
Forage breeding	Very tall, very long and very broad leaf blade	KO5 116, KO5 69, KO5 108
Grain purpose	Earliness (< 64 days), grain weight (>31g)	TNS 682, RPHT 14
Ergot and mold resistance	Very loose pyramidal type panicle	KO5 19, SO3 260, Madurai Kattai Sevappu, SO3 517, SO3 88

were grouped under five clusters (Fig 5). The clustering pattern revealed that Cluster I contains thirty-seven accessions, which were mainly characterized by elliptic grain shape. Cluster II contains 23 accessions and a wide range of variability was observed for panicle shape and compactness. Cluster III was the largest with 38 accessions, which have maximum glume coverage, *i.e.*, 100% of grains were covered and they showed difficulty in threshing. Cluster IV consists of 12 accessions, mainly comprising pre-release cultures and released varieties, which were characterized by semi-compact panicle type with symmetric nature. The majority of the accessions in cluster IV are freely threshable with greyed yellow glume color and yellow-white caryopsis color. Cluster V comprises 22 accessions with symmetric panicles with a predominance of semi-compact and compact panicle types. Bucheyeki *et al.* (2009) and Chand *et al.* (2017) also grouped sorghum germplasm accessions based on qualitative traits.

The excellent donors identified based on available marker traits for further sorghum improvement programs are enlisted (Table 1). Hence, these accessions can be well employed in breeding programs concerning grain yield and biomass.

Fig 5. Dendrogram showing the distribution pattern of 132 sorghum germplasm accessions

Conclusion

In the present regime of intellectual property rights, morphological traits serve as a potential marker for DUS (distinctiveness, uniformity and stability) characterization in plant variety registration and protection of breeder's rights. The present study signifies a wide spectrum of genetic variability among studied accessions. The discriminating morphological and DUS criteria can be efficiently used in grouping accessions as grain or forage type and the promising trait-specific accessions identified based on available marker traits could serve as potential donors for the further sorghum improvement program.

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