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Genetic Variability Assessment of Sorghum (*Sorghum bicolor* (L.) Moench) Germplasm Accessions using Qualitative Morphological Descriptors

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is considered as the fifth most important cereal crop in the world. It is well adapted to a range of environmental conditions. This study was based on twenty six sorghum germplasm accessions conserved at the seed gene bank of Plant Genetic Resource Center, Gannoruwa, Sri Lanka. The evaluation of the morphological diversity was based on 14 qualitative morphological traits outlined by the International Plant Genetic Resources Institute. Qualitative data recorded from morphological traits were analyzed using PROC CLUSTER procedure of SAS software. The clustering pattern of studied sorghum accessions based on qualitative morphological markers comprised of seven major clusters. Clustering pattern based on the qualitative traits depicts the geographical origin of the studied accessions. This can be explained by the fact that qualitative traits are less influenced by the environment. In principle, qualitative data are expected to provide additional information on hierarchical units. Observation of a considerably high number of clusters consolidates that principle. There were 13 polymorphic qualitative morphological traits with respect to all the studied sorghum germplasm accessions. Cluster I, II, III, IV, V and VI had one or several features shared by all the member accessions those cannot be found in all the members of any other clusters. Also there were unique features restricted to cluster II, III, V and VI. This study reveals sufficient genetic relatedness of studied sorghum germplasm accessions which will meaningful in the conservation and breeding programs of the crops.

Keywords: Sorghum bicolor, germplasm accessions, morphological diversity, qualitative traits

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a monocotyledon, self-pollinating plant, belonging to the family of Poaceae is considered as the fifth most important cereal crop in the world after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*) and barley (*Hordeum vulgare*) in terms of production and area planted. Sorghum is consumed into a wide variety of foods, such as baked products, tortillas, couscous, gruel, steam-cooked products, semi-leavened breads, popped form, fermented or non-fermented porridges and alcoholic or non-alcoholic beverages (Anglani, 1998). Relatively high tolerance to drought and heat makes this crop an ideal crop for human and animal consumption especially in areas with extremely unfavourable temperature and in dry regions receiving minimum precipitation (Ratnavathi et al., 2012).

Therefore, sorghum is particularly important to food security in the arid and semi-arid regions of the world. Furthermore, consumption of sorghum products can be recommended for patients suffering

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from celiac disease or diabetes (Park et al. 2012; Taylor et al., 2006). Nevertheless, being the only known natural food source of the 3-Deoxyanthoxyanins (3-DXA) in significant quantities, sorghum may be useful in helping reduce incidence of gastrointestinal cancer (Yang et al., 2009). Germplasm is the basic material utilized in assessing genetic variability. As defined by Upadhyaya *et al.* (2010), the total gene pool of a species including landraces, advanced breeding lines, popular cultivars, wild and weedy relatives can be considered as a 'germplasm'. Apparently, development of well adapted novel crop varieties with promising high yields through breeding strategies can be recognized as one of the most successful agricultural strategies employed over the past century, particularly with regards to the 'Green Revolution' (Gleadow et al., 2013). Development of such new crop varieties with high yield and desired traits depends on the availability of Plant genetic resources with sufficient information. Identification of crop plants that exhibit exploitable variation for the trait or traits of interest is the first step of any meaningful breeding program. Conservation of plant genetic material using *in-situ* and *ex-situ* conservation strategies play a vital role in breeding programs as well (Shehzad et al., 2014). Sorghum is believed to have a wide range of genetically diverse germplasm (Prajapati et al., 2018).

Sorghum is a diploid (2n=20), self-pollinated crop but capable of out crossing in various frequencies. Wind and insects such as honeybees, wild bees and beetles contribute to the out crossing yet the rate of out crossing is in the range of less than 10% to 73% (Barnaud et al., 2008). Genetic diversity in plants is affected by several factors such as selection, mutation, migration and genetic drift. In fact, domestication or artificial selection favours few alleles over the others resulting in increased frequency of selected alleles (Bhandari et al., 2017). Therefore, plant genetic materials with wide and diverse origins and genetic backgrounds are known to have a genetic diversity. Genetic diversity study is a staggering breakthrough in understanding intra-specific crop performance which will ultimately guide to crop improvement (Aremu, 2005). Moreover it is a step wise process which is performed using specific statistical method or combination of methods through existing variations in the nature with respect to particular crop (Weir, 1996; Warburton and Crossa 2000; Christini et al., 2009).

Assessment of genetic diversity can be accomplished through the use of various data such as morphological and agronomic, pedigree, biochemical and molecular data. Morphological traits are genetically controlled by number of genes and their expression depends on environmental factors in most cases. There are both quantitative and qualitative morphological traits those play vital roles in classification of individuals. Qualitative morphological traits are subjected to comparatively less environmental influence and have mono or oligogenic genetic control (Lima et al., 2017). They do not have quantitative values and might be binary or multicategory. Utilisation of qualitative morphological data is a fruitful strategy in genetic diversity studies not only because it is a practical, low-cost application, with no sophisticated equipment requirements but also they are expected to provide additional information on hierarchical units.

There are number of sorghum germplasm accessions conserved at Plant Genetic Resource Center (PGRC), Gannoruwa, Sri Lanka. Having a better knowledge about the genetic diversity of those germplasm accessions will help in enhancement of breeding and germplasm conservation of this crop. Assessing the genetic diversity of *ex-situ* conserved sorghum germplasm accessions using qualitative morphological markers is the main objective of this study as the morphological characterization is an important measure of assessing genetic diversity in crop plants.

According to Meijaard et al. (2013), knowing more about local people usage of forests (such as fuel, medicine, food and food additives, building construction, etc) is an extremely important factor that could enhance planning of land use and minimise the conflict with them.

2. Methodology

2.1 Plant material and data observation

A total of twenty-six sorghum (*Sorghum bicolor*) accessions including sixteen local, six Italian, two French and two foreign accessions with unknown origins obtained from active seed gene bank of PGRC, Gannoruwa, Sri Lanka (Table 1) was evaluated with regard to morphological descriptors outlined by the International Plant Genetic Resources Institute (IPGRI). This study was based on the evaluation of these germplasm accessions using qualitative traits. Plant colour, synchrony of flowering, grain lustre and grain form were the studied binary traits whereas leaf midrib colour, waxy bloom, inflorescence compactness and shape, inflorescence exsertion, lodging susceptibility, senescence, shattering, glume colour, grain covering and grain colour were counted as multicategorical traits with more than two possible levels.

Table 1: Details of the studied sorghum germplasm accessions.

Accession No.	Accession Name	Origin
93	EDAL ERINGU	MONARAGALA
104	POTH IRINGU	MONARAGALA
110	KARAL IRINGU	MONARAGALA
111	RATHU THIRINGU	MONARAGALA
112	SUDU THIRINGU	MONARAGALA
207	IDAL IRINGU	MONARAGALA
285	EDAL ERINGU	MATALE
310	SORGHUM	MATALE
403	EDAL ERINGU	KURUNEGALA
421	EDAL ERINGU	KURUNEGALA
774	KARAL IRINGU	MATALE
971	KARAL IRINGU	HAMBANTOTA
1531	SORGHUM	ANURADHAPURA
1546	EDAL ERINGU	NUWARA ELIYA
1701	SORGHUM	SRI LANKA
1824	EDAL ERINGU	KANDY
5364	-	UNKNOWN
5365	-	UNKNOWN
6004	ARVAL	FRANCE
6005	ARGENCE	FRANCE
6006	ROCE	ITALY
6007	SOAVE	ITALY
6008	VESPA	ITALY
6010	MN 150	ITALY
6012	SOAVE	ITALY
6013	WONDER DWARF	ITALY

2.2 Experimental site and design

The study was carried out in a plant house at the Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka. The experiment was arranged in a Randomised Complete Block Design (RCBD) with five replications as two individuals from each accession per block. Seeds of each sorghum accession were sown in small plastic pots filled with normal

soil. Pots were maintained in the plant house for germination. Twenty five day-old seedlings were transplanted separately in to the large plastic pots filled with mixture of soil, sand and compost (1:1:1) as each pot contained two plants. All weeds were controlled removing manually.

2.3 Statistical analysis

Qualitative data recorded from morphological traits were analysed using PROC CLUSTER procedure of SAS software (SAS Institute, 2011) by following Ward minimum variance clustering method (Ward, 1963) with the Gower distance matrix (Gower, 1971).

3. Results

According to the pseudo-*F* and pseudo-t^2 criteria obtained from the cluster analysis based on qualitative data, the optimal number of clusters required to represent the genetic diversity among the studied sorghum germplasm accessions was seven (Figure 1).

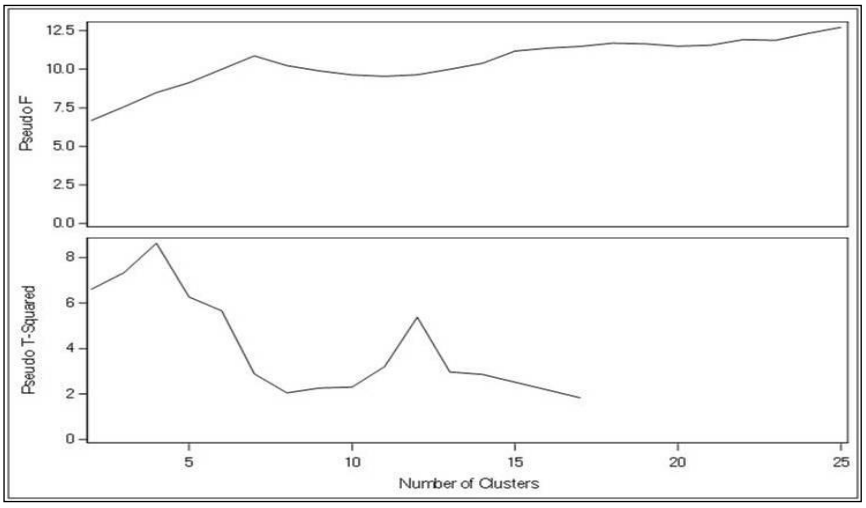

Figure 1: Distribution of pseudo-*F* and pseudo-t^2 statistics according to the number of clusters.

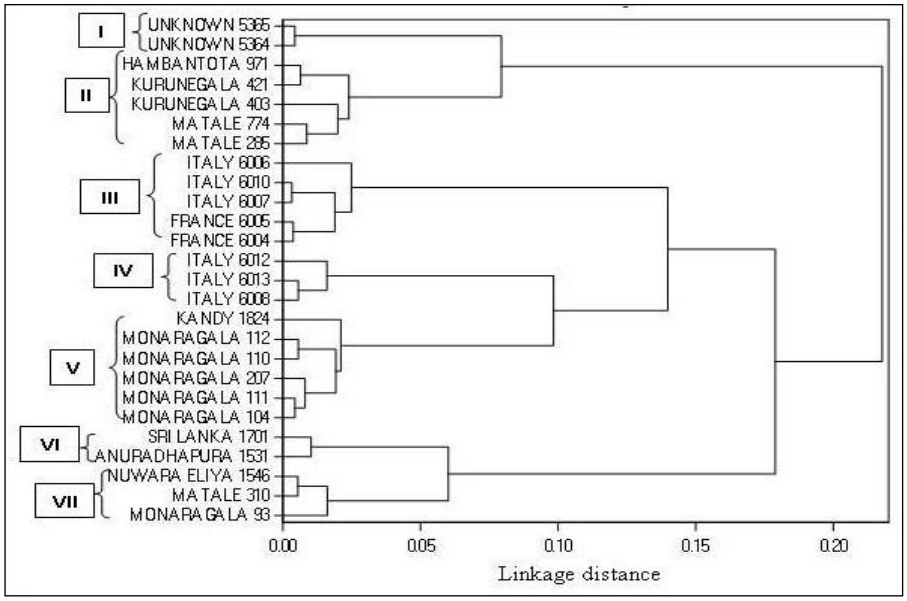

Figure 2: Phenetic dendrogram of 26 sorghum accessions obtained through the algorithm of Gower in the evaluation of 13 qualitative morphological traits

The phenetic dendrogram of dissimilarity obtained through the algorithm of Gower (Gower, 1971) in the evaluation of 13 qualitative morphological traits is represented in Figure 2. The dendrogram shows that seven clusters were formed at dissimilarity co-efficient of 0.06. The number of accessions per cluster ranged from two in 'Cluster I' to six in 'Cluster V'. 'Cluster I' comprised of two foreign accessions (5364 and 5365) with unknown origins. There were five Sri Lankan accessions (285, 403, 421, 774 and 971) grouped into 'Cluster II'. Three Italian (6006, 6007 and 6010) and two French accessions (6004 and 6005) were clustered in 'Cluster III' while the remaining three Italian accessions (6008, 6012 and 6013) were grouped into 'Cluster IV'. Sri Lankan accessions (104, 110, 111, 112, 207 and 1824) coming from Monaragala and Kandy districts made the 'Cluster V'. 'Cluster VI' and 'Cluster VII' comprised of two (1531 and 1701) and three (93, 310 and 1546) Sri Lankan accessions respectively.

Out of 14 studied qualitative morphological traits, one trait namely grain form was recorded as a monomorphic marker with respect to all the studied sorghum germplasm accessions whereas the rest of traits were polymorphic. There are two states of grain form as single and twin. But grains from each and every accession were recorded as single form. Distribution of the states of polymorphic qualitative morphological traits among studied sorghum germplasm accessions and observed clusters of the dissimilarity dendrogram is summarised in Table 2. Two different colours were observed for leaf midrib as dull green and white. Majority of accessions had white midribs whereas only seven accessions belonging to cluster I, II and III were observed to have dull green midribs. Stems of all the studied accessions were covered with the waxy bloom and majority of them were mostly bloomy. Apparently one Italian accession (6006) was observed with completely bloomy stems. When the compactness and shape of the inflorescence were considered at the same time, there were six types of inflorescences as very loose erect, semi-loose erect, loose erect, loose drooping, compact elliptic and semi-compact elliptic. Compact elliptic inflorescences were observed in majority of accessions. Loose drooping and semi-compact elliptic type could be observed only in two Sri Lankan accessions from Monaragala (207) and Matale (310) districts respectively. Slightly exserted, exserted and well-exserted inflorescences were recorded. Well-exserted inflorescences were observed in majority of accessions. Pigmented plant was the most frequent plant colour and was observed in 19 accessions. Majority of studied sorghum accessions showed low level of lodging whereas two Sri Lankan accessions (104 and 1824) were observed to be highly lodged. Senescence could be observed in each and every plant. Italian and French accessions showed comparatively low level of senescence. Synchrony of flowering was absent in lot of accessions. One Sri Lankan accession (1531) from Anuradhapura district and four exotic accessions (5364, 5365, 6008 and 6013) were observed with synchronised flowering patterns. Low level of shattering was the most frequent nature of shattering. One French (6004) and four Italian (6007 6008, 6010 and 6013) accessions were observed with highly shattered panicles. Four different colours of glumes as black, mahogany, red and sienna were observed. Grains of all accession were covered by glumes and different extents of covering were observed. Surprisingly, grains of all the accessions (104, 110, 111, 112, 207 and 1824) from Cluster V were fully covered by the glumes. Majority of accessions had brown colour grains whereas two Sri Lankan accessions (285 and 403) had white colour grains. Red and yellow colour grains were also observed. Only two Sri Lankan accessions (1531 and 1701) had lustrous grains.

Cluster I, II, III, IV, V and VI had one or several features shared by all the member accessions those cannot be found in all the members of any other clusters. All the accessions from 'Cluster I' were tan plants and showed intermediate level of senescence with synchronized flowering patterns. Furthermore, well-exserted inflorescences of those plants bore yellow colour grains. 'Cluster II' members had black glumes. Midribs of slightly senescence plants from 'Cluster III' members were dull green. 'Cluster IV' members had 75% covered grains bearing from slightly exserted inflorescences whereas member accessions of 'Cluster V' observed to have fully covered grains. Lustrous red colour grains bearing from very loose erect primary branches of 'Cluster VI' member accessions were covered with red colour longer glumes.

Also there were unique features restricted to particular clusters. White grains (285 and 403) were observed only in 'Cluster II'. Plants those completely covered with waxy bloom (6006) and highly shattered inflorescences (6004 and 6007) could be found only in 'Cluster III'. Highly lodged plants (104 and 1824), loose drooping panicles (207) and fully covered grains (104, 110, 111, 112, 207 and 1824) could be found only in 'Cluster V'. Only 'Cluster VI' members had semi compact elliptic panicles (310) and lustrous grains (1531 and 1701).

Table 2: Distribution of the states of polymorphic qualitative morphological traits among studied sorghum germplasm accessions and observed clusters of the dissimilarity dendrogram.

Qualitative traits	States of the trait	Recorded accessions with the state	Observed clusters
1. Leaf midrib colour	Dull Green	403, 5364, 6004, 6005, 6006, 6007, 6010	I, II, III
	White	93, 104, 110, 111, 112, 207, 285, 310, 421, 774, 971, 1531, 1546, 1701, 1824, 5365, 6008, 6012, 6013	I, II, IV, V, VI, VII
2. Waxy bloom	Slightly	1531, 1546, 1824	V, VI, VII
	Medium	285, 310, 421, 971, 6004, 6005, 6007, 6010	II, III, VII
	Mostly	93, 104, 110, 111, 112, 207, 403, 774, 1701, 5364, 5365, 6008, 6012, 6013	I, II, IV, V, VI, VII
	Completely	6006	III
3. Inflorescence	Very loose erect	1531, 1701, 6004	III, VI
Compactness and	Semi-loose erect	111, 1824, 6010, 6012, 6013	III, IV,V
Shape	Loose erect	93, 104, 110, 1546, 6005, 6007	III, V, VII
	Loose drooping	207	V
	Compact elliptic	112, 285, 403, 421, 774, 971, 5364, 5365, 6006, 6008	I, II, III, IV, V
	Semi-compact elliptic	310	VI
4. Inflorescence	Slightly exserted	112, 774, 1824, 6006, 6008, 6012, 6013	II, III, IV, V
exsertion	Exserted	104, 111, 207, 310, 1546, 1701, 6007	III, V, VI, VII
	Well-exserted	93, 110, 285, 403, 421, 971, 1531, 5364, 5365, 6004, 6005, 6010	I, II, III, V, VI, VII
5. Plant colour	Pigmented	93, 104, 111, 207, 285, 310, 403, 774, 971, 1531, 1546, 1701, 6004, 6005, 6006, 6007, 6008, 6010, 6012	II, III, IV, V, VI, VII
	Tan	110, 112, 421, 1824, 5364, 5365, 6013	I, II, IV, V
6. Lodging susceptability	Low	93, 110, 111, 112, 207, 310, 403, 421, 1531, 1546, 5364, 5365, 6004, 6005, 6006, 6007, 6008, 6010, 6012, 6013	I, II, III, IV, V, VI, VII
	Intermediate	285, 774, 971, 1701	II, VI
	High	104, 1824	V

Qualitative traits	States of the trait	Recorded accessions with the state	Observed clusters
7. Senescence	Very slightly	207, 6004, 6005, 6006, 6007, 6010	III, V
	Slightly	112, 421, 6013	II, IV, V
	Intermediate	110, 971, 5364, 5365, 6008, 6012	I, II, IIII, VII
	Mostly	93, 104, 111, 285, 310, 403, 774, 1531, 1546, 1701, 1824	II, V, VI, VII
8. Synchrony of flowering	Absent	93, 104, 110, 111, 112, 207, 285, 310, 403, 421, 774, 971, 1546, 1701, 1824, 6004, 6005, 6006, 6007, 6010, 6012	II, III, IV, V, VI, VII
	Present	1531, 5364, 5365, 6008, 6013	I, IV, VI
9. Shattering	Low	93, 104, 110, 111, 112, 207, 285, 310, 774, 1531,	I, II, III, V, VI, VII
	Intermediate	403, 421, 971, 5364, 6012	I, II, IV
	High	6004, 6007	III
	Very high	6008, 6010, 6013	III, IV
10. Glume colour	Black	285, 403, 421, 774, 971, 6012	II, IV
	Mahogany	310, 1546, 6006, 6007, 6008, 6010, 6013	III, IV, VII
	Red	93, 1531, 1701	VI, VII
	Sienna	104, 110, 111, 112, , 207, 1824, 5364, 5365, 6004, 6005	I, III, V
11. Grain covering	50% covered	93, 285, 403, 421, 5364	I, II, VII
	75% covered	310, 774, 971, 1546, 5365, 6007, 6008, 6010, 6012, 6013	I, II, III, IV, VII
	Fully covered	104, 110, 111, 112, 207, 1824	V
	Longer glumes	1531, 1701, 6004, 6005, 6006	III, VI
12. Grain colour	Brown	104, 110, 111, 112, 207, 1546, 1824, 6004, 6005,	III, IV, V, VII
	Red	93, 310, 1531, 1701	VI, VII
	White	285, 403	II
	Yellow	421, 774, 971, 5364, 5365	I, II
13. Grain lustre	Present	1531, 1701	VI
	Absent	93, 104, 110, 111, 112, 207, 285, 310, 403, 421, 774, 971, 1546, 1824, 5364, 5365, 6004, 6005, 6006, 6007, 6008, 6010, 6012, 6013	I, II, III, IV, V, VII

4. Discussion

Consideration of pseudo statistic including pseudo-F and pseudo-t^2 values is one of the best ways for judging the optimal number of clusters in a data set. Relatively large values of pseudo-F compared to nearby values indicate statistically significant clustering points. The general rule for identifying the best clustering point which is statically significant with regard to values of the pseudo-t^2statistic is to move along the line starting from the end corresponding to the highest number of clusters until find the first value markedly larger than the previous value and move back along the line by one cluster. When these two statistics, pseudo-F and pseudo-t^2 were taken into consideration, the studied 26 sorghum germplasm accessions can be divided into statistically significant seven clusters based on 13 qualitative morphological traits (SAS Institute Inc, 2011).

Sri Lankan accessions were observed in 'Cluster II', 'Cluster V', 'Cluster VI' and 'Cluster VII'. Apparently 'Cluster I' and 'Cluster II' separated from the rest of clusters at somewhere around the dissimilarity co-efficient of 0.25. Surprisingly, Sri Lankan accessions coming from three different districts as Hambantota, Kurunegala and Matale made the 'Cluster II'. Clustering pattern based on the qualitative traits depicts the geographical origin of the studied accessions. This can be explained by the fact that qualitative traits are less influenced by the environment. In principle, qualitative data are expected to provide additional information on hierarchical units. Observation of a considerably high number of clusters consolidates that principle.

Leaf midrib colour is an important morphological trait as it is used by farmers to distinguish between juicy and non-juicy types of sorghum landraces (Teshome et al., 1997). As reported by Rangaswami (1936), green colour midribs are associated with juicy stems while white midribs are associated with pithy stems. According to Doggett (1988) white or yellow colour midribs can be found in dry pithy varieties whereas sweet juicy types tend to have an opaque green midrib often with the fine white line down the centre. Mutants with brown midribs have also been reported. Ayyangar and Nambiar (1941b) proved that the dark green midrib is dominant to lighter shades of green. In this study, 'Cluster III' members from Italy and France had dull green midribs.

Waxy bloom can be observed in every plant of sorghum in different amounts. Genotypic expression of wax is controlled by a family of genes. The phenotypic expressions of these genes are enhanced by plant age and water status. Presence of wax plays an important role in preventing plants from water loss by reducing the solar energy load on the plant surface through reflectance and reduction the solar energy load on the leaf surface (Hamissou and Weibel, 2004). One Italian accession (6006) from this study found to be completely covered with wax.

The inflorescence of a sorghum plant is a panicle with a central rachis which originates from primary branches. The shape and compactness of the panicle varies from accession to accession. In here, the variation in rachis length, branch length, distance apart of the whorls and angle of branching give rise to a large number of panicle forms in the face of shape and compactness. Ayyangar (1939) reported that loose panicle is dominant to compact panicle. While semi compact panicle is dominant to compact panicle. Usually each panicle contains between 800 and 3000 seeds depending on the genetic background and environmental influence. The immature inflorescence is bloomed through the top leaf sheath after the uppermost leaf has expanded. The mature inflorescence may remain with lower part still surrounded by the uppermost leaf sheath as slightly exserted manner, or it may be carried well clear of the top leaf sheath as well exserted inflorescence (Doggett, 1988). Majority of accessions observed in the current study was compact elliptic and well exserted.

Sorghum plants can be either pigmented or tan in colour. The glumes and sheath of red plants were found to accumulate 3-deoxyanthocyanidins which is the major pigments that can be found in sorghum whereas the glumes and sheath of tan plants accumulated apigenin (*Siame* et al., *1993*).

Growth of the stem of sorghum plants is usually erect, but in some varieties the stem bending until it may be almost parallel to the ground at flowering time. In this study, two Sri Lankan accessions (104 and 1824) were observed to be highly lodged. When the panicle is filled with grains, the stem tends to touch the ground. According to Deggett (1988), one to three concentric root band rings containing primordial can be found immediately above the attachment of leaf sheath at each stem node of sorghum. Therefore, when a plant falls over, roots may develop at the nodes in contact with the ground, and advantage can be taken of this fact to grow sorghums from cuttings.

As described by Downes (1968) even though sorghum is an annual crop, non senescent types can be survived for years through generation of tillers from old plant bases. Even though each and every studied sorghum plants showed some level of senescence, few accessions were slightly senescence.
As suppression of grain shattering is a valuable agronomic trait acquired through the domestication of sorghum, majority of studied accessions had comparatively low level of shattering.

According to Quinby and Martin (1954), glume colour is affected by the same gene which is responsible for plant colour and several other genes as well. The effects of these genes produce a range of glume colours from straw to blackish purple, reddish purple, golden, mahogany and sienna. According to the results coming from this study, all the grains covered with red colour glumes came from pigmented plants.

Usually almost all grains of sorghum are covered by a glume in different extents. In this study, all the fully covered grains had sienna colour glumes. In most cases, the glume can open by itself and expose the grain. However cleistogamy was observed in several varieties of sorghum (Ayyangar and Ponnaiya, 1939e) due to the rolling of the inner glume, so that it claps the internal flower structures and thereby normal opening of the glumes can be prevented (Merwine et al, 1981).

According to Ayyangar and krishnaswamy (1941), grain colour varies from the pale yellow through various shades of red and brown to deep purple brown. The pigment that responsible for the grain colour is confined to the seed coat layer with the exception of yellow, which can be present in the endosperm. In here, majority of seeds were brown in colour.

Lustrous grains conquer consumer preference over non-lustrous grains. According to the study carried out by Audilakshmi and Aruna (2005), for the develoment of a hybrid which will produce lustrous grains, both parents need to be lustrous and homozygous for the alleles conferring grain lustre at a common locus. In fact, there were two Sri Lankan accessions (1531 and 1701) with lustrous grains.

5. Conclusion

Gower distance matrix obtained by following Ward minimum variance clustering method is a suitable method for classifying sorghum germplasm accessions with respect to qualitative morphological traits. This study reveals sufficient genetic relatedness of studied sorghum germplasm accessions which will meaningful in the conservation and breeding programs of the crops.

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