*Indian Journal of Agricultural Sciences* **83** (3): 279–86, March 2013/Article

# Evaluation and classification of sorghum (*Sorghum bicolor*) male sterile lines for fodder traits using multivariate analyses

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Received: 12 June 2012; Revised accepted: 1 February 2013

ABSTRACT

In sorghum [(*Sorghum bicolor* (L.) Moench] numerous male sterile lines for grain sorghum are available. However, there is need of their critical evaluation for important fodder related traits. Therefore, the present study was undertaken to classify the variability available in these male sterile lines for such traits. Statistical analysis was carried out using the SPSS software. Correlation matrix was used to extract the principal components. Unweighted Pair-Group Method using Arithmetic Averages method of hierarchical cluster analysis was utilized with City Block distances to classify the male sterile lines. Significant amount of variability was observed for almost all the traits. Through principal factor analysis fourteen variables were reduced to five principal factors explaining 72.55 per cent variability. The first principal factor (PF) showed high loading for three disease resistance variables and PF 2 ascribed for fodder yield variables, i e plant height, green fodder yield/plant and dry fodder yield/plant. Hierarchical cluster analysis classified ninety one male sterile lines into ten clusters containing one to thirty five lines. The results of hierarchical cluster analysis and principal factor analysis confirmed the findings of each other. The male sterile lines like IMS 9A, 56A, 95A, AKMS 14A, 27A, ICSA 51, ICS 25A, 41251A, 41253A, etc., found to be superior using principal factor analysis were also found to be members of the best performing clusters, i e cluster II, VII and IX.

**Key words:** Cluster analysis, Fodder traits, Male sterile line, Principal factor, Sorghum

In the past few years a plateau has reached as far as the production of fodder is concerned and it has become imperative to break it even. The burgeoning livestock population and depleting land availability for fodder crops and pastures have further aggravated the situation. The availability of required quantity and quality of fodder has direct bearing on animal performance hence it becomes essential to enhance the fodder production. Importance of sorghum as an ideal fodder crop can not be denied due to its quick growing habit, high yielding ability, high dry matter content, better quality, good ratoonability, palatability, digestibility and its suitability in various forms of utilization such as green chop, silage and hay (*karbi*). It is highly adaptable and can be cultivated over wide range of climatic conditions and soil types, except highly alkaline and saline soils. Multicut forage sorghum [(*Sorghum bicolor* (L.) Moench] hybrids may be a very important alternative to meet out the fodder requirement of ever increasing livestock population. Multicut forage sorghum hybrids are advantageous in saving the cost of new/next crop, providing higher yields in shorter period and by offering continuous supply of green fodder for longer duration (Tarumoto 1971). Besides being quantitatively better, multicut forage sorghums are qualitatively superior as well. Satripanon *et al.* (1991) reported improved protein content and digestibility with reduced HCN of multicut forage sorghums because of tenderness and succulence of fodder resulting from frequent cuttings.

The past research efforts in forage sorghum have resulted in development and release of many hybrids, however, only a limited number of male sterile lines are being used for the development of these hybrids though a number of them are available which may lead to genetic vulnerability one day. The improvement in yield potential depends upon the amount of genetic variability available in the gene pool. Finlay (1971) stressed the importance of continuous infusion of new genetic variability in active plant breeding programmes. It is a major asset for initiating a fruitful crop improvement programme. Such a breeding programme should rest not only on the available gene pool, but also on continuous infusion of newer and superior lines to permit fresh genetic variation (Gustafson 1974). In sorghum, numerous male sterile lines are available, however, there is need of their critical evaluation, particularly if the aim is to improve complex quantitative traits like yield. Such work becomes easier if the available male sterile lines

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are classified on the basis of a given set of characters and then pick up them for hybridization to exploit heterosis. Therefore, the present study was undertaken to classify the variability available in male sterile lines of sorghum based on various criteria like morphology and genetic diversity.

## MATERIALS AND METHODS

The experimental material consisted of ninety one male sterile lines of sorghum collected from different sorghum research centres. These were grown at Forage Research area, CCS Haryana Agricultural University, Hisar over three consecutive years (from 2008 to 2010) during *kharif* season in Augmented Design. Each genotype was grown in two rows of 4m length each with 45 and 10 cm row to row and plant to plant spacing, respectively. Each block comprised ten genotypes with three checks (viz. SSG 59-3, HC 308 and HC171) randomized in between. All the recommended package of practices to raise a good crop was followed. The data on five randomly selected plants from each male sterile line were recorded for fourteen important traits which comprised of six fodder yield component traits, viz. plant height (cm), leaf length (cm), leaf breadth (cm), stem girth (cm), number of tillers/plant, number of leaves/plant; two visual fodder quality traits, viz. tan or purple plant type (1-tan and 2-purple) and midrib colour (1-green/2-white); incidence of three most important foliar diseases, viz. grey leaf spot (*Cercospora sorghi*), zonate leaf spot (*Gloeocercospora sorghi*) and sooty stripe (*Ramulispora sorghi*) and infestation by most important insect affecting the crop yield and quality, ie stem borer (*Chilo partellus*). Green and dry fodder yield/plant (g) were also recorded.

Observations for stem borer attack were recorded as prescribed by Mathur *et al*. (1991). Scoring was done at 35 and 45 days after germination on leaf feeding using 1-5 scale (1-resistant to 5-highly susceptible). For Foliar diseases observations were recorded at 35 and 55 days after sowing. Scoring was done using visual standards employing 1-5 scale (1-resistant to 5-highly susceptible) and disease intensity was calculated by using formula:

$$\text{Diseases intensity} = \frac{\text{Sum of all numerical raitngs}}{\text{Total number of leaves observed} \times \text{Highest rating}} \times 100$$

Average of the data on five plants was computed for all the characters. Homogeneity for error variances was tested using Bartlett's test before pooling the data of three years. The data of disease incidence were subjected to angular transformation. Statistical analysis was carried out using the SPSS software. Correlation matrix was used to extract the principal components. The factor axes were rotated using Varimax rotation. Principal factor scores were determined using Anderson-Rubin method. Plotting of different genotypes was done using their individual factor scores taking different principal factors as axes. UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) method of hierarchical cluster analysis was utilized with City Block distances to classify the genotypes and dendrogram was prepared using the rescaled distances. Method suggested by Romesburg (1990) was used to cut the dendogram to form the clusters.

## RESULTS AND DISCUSSION

The improvement in any trait depends upon the amount of genetic variability available in the gene pool. In sorghum, male sterile lines are available but are mainly developed with the aim of improvement in seed yield, whereas the component characters for fodder yield are entirely different than those required for grain yield, therefore, they need critical evaluation for the required traits. Such work becomes easier if the available male sterile lines are classified on the basis of a given set of characters and then pick up the male sterile lines for hybridization to exploit heterosis. In the present investigation, besides various components of fodder yield and resistance, two characters of specific importance, i e midrib colour and plant type, were also evaluated. Green midrib colour is an indicator of sweetness of green fodder and white ones are known to be non-sweet. The tan plant type indicates resistance to foliar diseases like gray leaf spot and zonate leaf spot, however, purple ones are susceptible to these foliar diseases. Thus to tap the fodder yielding potential of forage sorghums, proper evaluation of male sterile lines considering different aspects, will hopefully constitute the liaison characters between the current and future emphatic areas in improvement and exploitation of forage sorghums.

Usually the number of variables included to classify the available lines becomes perceivably large when the above mentioned idea is considered and makes the data matrix perceivably large, complicated, unmanageable and beyond comprehension. Under such circumstances, principal component analysis comes handy to partition the variability into mutually orthogonal components retaining the same amount of variability contained in the original set of variables. In the light of critical comments of Sneath and Sokal (1973), further analysis was carried out using principal factor analysis. In principal component analysis, the total variation contained in a set of variables is considered, whereas in principal factor analysis interest centers on that part of variance only, which is shared by the common factors leaving aside the unique factor (including error) of the variable. Moreover, in contrast to principal component analysis, here the component axes are allowed to interact resulting in distortion of mutual orthogonality. In the present study principal factor analysis was carried out using principal component method, which does not require assumption of multivariate normal distribution of population, in contrast to the other methods like maximum likelihood method (Jaiswal 2000).

The first principal component absorbs and accounts for maximum proportion of total variability in the set of all variables and remaining components account for

progressively lesser and lesser amount of variation. Same trend was observed in the present study as envisaged by Table 1. Significant amount of variability was observed to be present for almost all the traits. Through Principal factor analysis fourteen variables were reduced to five principal components explaining 72.55 per cent variability. The studies of Mathur *et al.* (1991), Mathur *et al.* (1992), Kang and Lee (1996), Katiyar *et al.* (1997), Teshome *et al.* (1997), Greiner *et al.* (2000) and Yadav *et al.* (2003 and 2004) indicated presence of enough variability for various characters in forage sorghum genotypes conforming to the result of the present study. The first five principal components, having Eigen values greater than one and altogether explaining 72.55% of the total variation, were retained (Kaiser 1958). The first principal component accounted for maximum variability, ie 21.39% that reduced gradually to 8.26% in 5$^{th}$ principal component (Table 1). Kang and Lee (1996), Hosoya *et al.* (1997), Ayana and Bekele (1999), Ezeaku *et al.* (1999) and Yadav *et al.* (2003 and 2004) are the few workers who conducted principal component analysis in forage sorghum, though not in male sterile lines, and transferred many correlated variables into a few independent principal components explaining much of the variability of the original set.

Initially the data were analyzed without any rotation to derive clear picture of interaction of variables among themselves and with the principal factors. But it failed to provide much information regarding the idea of correlation between the variables and the principal factors. The failure of principal factor analysis without rotation to draw sensible conclusions prompted to go for analysis with rotation. Varimax method of orthogonal rotation (Kaiser 1958) was utilized in the present study to rotate the factor axes. This is the most commonly used method and can also be placed in a meaningful biological context (Titz 1983). All the fourteen variables showed high loading on different principal factors and none of them was left after rotation of the principal factor axes (Table 2). Moreover, it clearly grouped the similar type of variables by loading them together on a common principal factor.

The first principal factor (PF) showed high loading for three disease resistance variables, i e plant type and gray and zonate leaf spot diseases and can clearly be designated as disease resistance factor. The principal factors 2 ascribed for fodder yield variables, i e plant height, green fodder yield/plant and dry fodder yield/plant. This can be designated as fodder yield factor. The PF 3 showed high loading for stem girth, sooty stripe, leaf length and stem borer infestation. Principal Factor 4 had high loading of leaf breadth, number of tillers/plant and number of leaves/plant and hence can be designated as leafiness factors. Principal Factor 5 was loaded with only one variable, i e midrib colour. The clear cut grouping of similar type of variables by getting loaded on common principal factor elaborates the successful transformation of thirty interrelated variables into nine independent principal factors explaining 73% of the variability of the original set. Application of factor analysis in forage sorghum or grain sorghum could not be traced in the literature to endorse the results of the present investigation.

Principal factor scores were calculated for all the genotypes for all the nine principal factors using Anderson-Rubin method and were utilized in finding genotypes superior for different factors, ie for all the characters cumulatively ascribed to that factor. A high value of principal factor score of a particular genotype in a particular principal factor denotes high values for those variables in that genotype, which that factor is representing. The male sterile lines were plotted on graphs utilizing their principal factor scores based on two factors, ie PF 1 and 2 as these two principal factors are responsible for high fodder yield and resistance to foliar diseases (Fig 1). The male sterile lines which found place towards the better end of both the factors were supposed to be superior for those two factors and hence superior for all

Table 1 Total variance explained by different principal components in forage sorghum male sterile liness

| Principal component | Eigen value | Per cent variance | Cumulative variance |
|---|---|---|---|
| 1 | 2.99 | 21.39 | 21.39 |
| 2 | 2.47 | 18.32 | 39.71 |
| 3 | 1.95 | 13.95 | 53.66 |
| 4 | 1.49 | 10.63 | 64.29 |
| 5 | 1.15 | 8.26 | 72.55 |

Table 2 Factor loadings of different characters with respect to different principal factors (Varimax rotation) in forage sorghum male sterile lines

| Characters/ Principal factors | PF 1 | PF 2 | PF 3 | PF 4 | PF 5 |
|---|---|---|---|---|---|
| Gray leaf spot | 0.932* | 2 0.111 | 0.061 | –0.174 | 0.039 |
| Plant type | 0.895* | –0.043 | –0.151 | –0.162 | 0.067 |
| Zonate leaf spot | –0.786* | –0.069 | –0.234 | –0.206 | 0.068 |
| Dry matter yield/plant | –0.042 | 0.962* | 0.027 | 0.164 | 0.011 |
| Green fodder yield/plant | –0.043 | 0.962* | 0.006 | 0.158 | –0.002 |
| Plant height | –0.008 | 0.699* | –0.069 | 0.034 | 0.176 |
| Stem girth | –0.055 | 0.135 | 0.820* | –0.246 | –0.029 |
| Sooty stripe | 0.130 | –0.235 | 0.735* | 0.067 | –0.069 |
| Leaf length | 0.061 | 0.356 | 0.593* | 0.209 | 0.326 |
| Stem borer | 0.107 | –0.134 | 0.587* | –0.257 | 0.224 |
| Leaf breadth | –0.073 | 0.018 | 0.067 | 0.587* | 0.439 |
| No. of tillers/ plant | –0.110 | –0.043 | 0.049 | 0.851* | 0.081 |
| No. of leaves/ plant | 0.093 | 0.198 | –0.081 | 0.754* | –0.047 |
| Sweet/non-sweet | –0.049 | –0.016 | 0.027 | 0.055 | –0.839* |

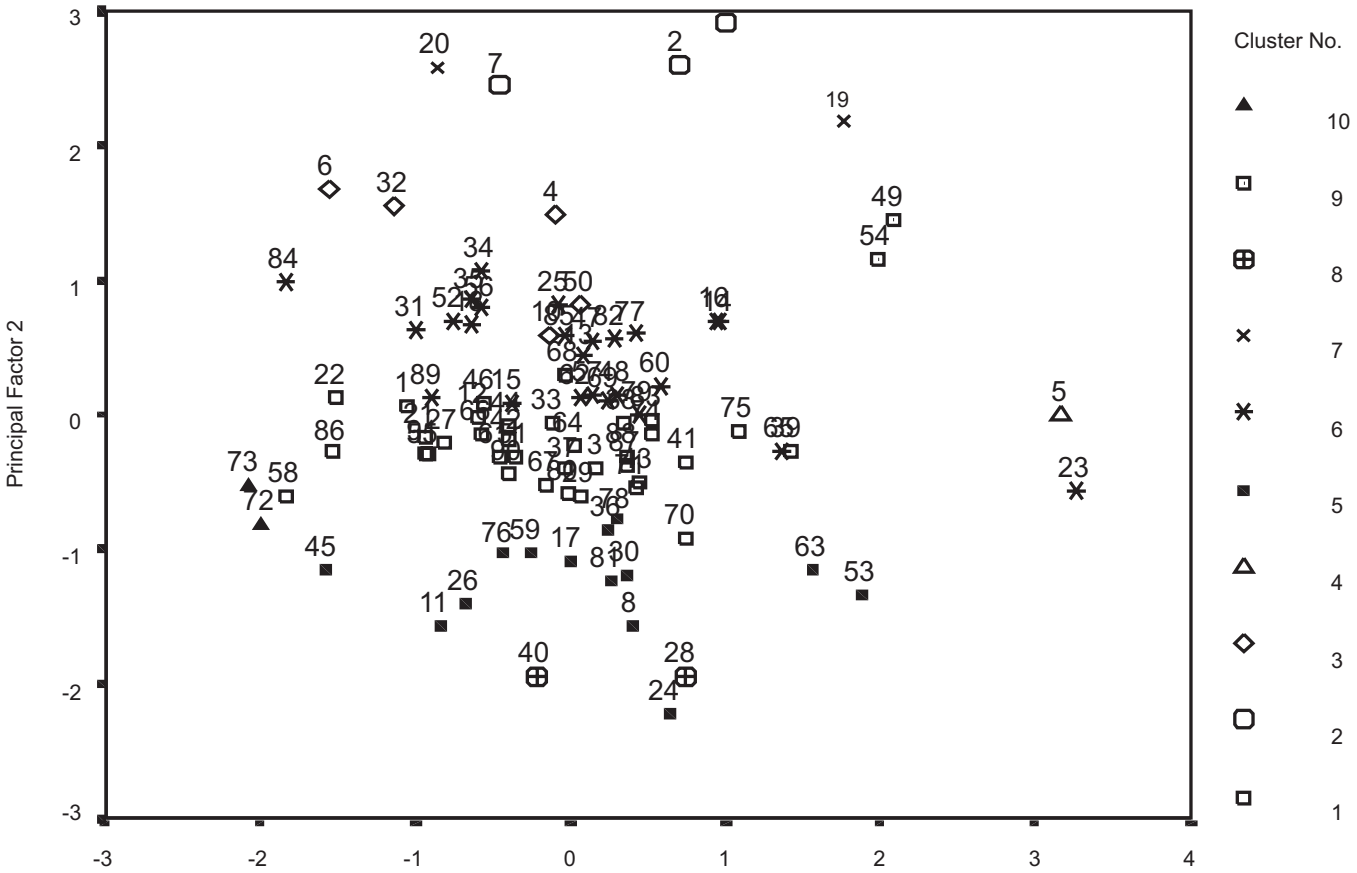


Fig. 1 Location of all ms lines based on their PF Score w.r.t. PF 2 & 4

the characters which both of these factors are defining. The male sterile lines ICS 5A, 46230A, 41251A, ICS 464A, ICS 477A, ICS 60A and ICS 472A found place on the superior end of PF 1 hence they were better fodder yielders and the male sterile lines 41253A, ICS 56A, IMS 9A, ICS 27A, ICS 51A, AKMS 14A, 41251A, ICS 464A, ICS 751A, ICS 90A and ICS 477A got plotted on the superior end of PF 2 hence were resistant to foliar diseases. From the foregoing discussion it is concluded that 41251A, ICS 464A and ICS 477A may prove better male sterile lines in hybridization programme when both the factors are considered together.

Classification studies have special significance in such studies because it is difficult to evaluate large number of lines through breeding plans due to practical limitations and also many of the accessions may be genetically more or less similar due to their common ancestors. Peeters and Martinelli (1989) suggested several advantages of hierarchical cluster analysis to classify such material. In the present study UPGMA (Unweighted pair-group method using arithmetic averages) method of hierarchical cluster analysis was utilized with city block distances to classify 91 male sterile lines and dendogram was prepared using the rescaled distances. Based on the method suggested by Romesburg (1990) the dendogram was cut to form ten clusters containing one (clusters IV) to thirty five (cluster I) lines (Fig 2). Cluster membership of different male sterile lines is presented in Table 3.

No correspondence was observed between the geographical and genetic diversity in the present study. Kang and Lee (1996), Ayana and Bekele (1997) and Lin *et al.* (1998) used cluster analysis to classify different germplasm collections of sorghums and partitioned them into different clusters. In most of the studies no definite association between geographical and genetic diversity were reported, however, Ayana and Bekele (1999) found that a greater proportion of accessions of similar adaptation zones and accessions from regions of origin with similar agro-climatic conditions were grouped together. This implies that geographic diversity is not the only factor determining genetic divergence and is one among the several factors determining the genetic divergence. The grouping of genotypes originating from different eco-geographic regions into one cluster could be attributed to frequent exchange of breeding material and due to operation of similar forces of natural and artificial selection resulting in perpetuation and stabilization of similar genotypes (Murty and Arunachalam 1966). Therefore, parental selection for hybridization should be based on the criteria of genetic diversity.

In the present study, the mean performance of different clusters calculated for different characters revealed wide range of differences among clusters with respect to these

Table 3 Cluster membership profile of different forage sorghum male sterile lines

| Cluster | Genotypes | No. of A-lines |
|---|---|---|
| C I | ICS 5 A, 13 A, 10286 A, 46154 A, 46156 A, 46428 A, ICSA 18, ICSA 79, ICSA 264, ICSA 276, ICSA 323, ICSA 340, ICSA 371, ICSA 400, ICSA 403, ICSA 422, ICSA 467, ICSA 496, ICSA 520, ICSA 570, ICSA 624, ICSA 632, ICSA 633, ICSA 651, ICSA 656, ICSA 670, ICSA 687, ICSA 692, ICSA 734, ICSA 744, ICSA 764, ICSA 766, ICSA 88010, ICSA 90003 and ICSA 24003 | 35 |
| C II | IMS 9 A, 56 A and 95 A | 3 |
| C III | AKMS 14 A, 27 A, 296 A, ICSA 51 and ICSA 465 | 5 |
| C IV | ICS 25 A | 1 |
| C V | 58 A, 2219 A, 41199 A, 46248 A, 46272 A, ICSA 27, ICSA 215, ICSA 415, ICSA 472, ICSA 540, ICSA 609, ICSA 713, ICSA 730 and ICSA 736 | 14 |
| C VI | 88015 A, 467 A, 41130 A, 41152 A,41234 A, 46230 A, 46266 A, ICSA 31, ICSA 90, ICSA 213, ICSA 432, ICSA 444, ICSA 471, ICSA 501, ICSA 504, ICSA 541, ICSA 579, ICSA 631, ICSA 652, ICSA 725, ICSA 733, ICSA 739, ICSA 751, ICSA 753 and ICSA 89003 | 25 |
| C VII | 41251 A and 41253 A | 2 |
| C VIII | ICSA 4 and ICSA 333 | 2 |
| C IX | ICSA 464 and ICSA 477 | 2 |
| C X | ICSA 675 and ICSA 684 | 2 |
| Total | | 91 |

traits (Table 4). The clusters II consisting of only three genotypes (IMS9A, 56A and 95A), was found to be the best with regard to yield and its components followed by cluster VII which was having only two genotypes, i e 41251A and 41253A. The cluster IX (having only two genotypes, i e ICSA 464 and ICSA 477, also showed very good performance for yield and its attributes. Clusters II (IMS 9A, 56A and 95A), III (AKMS 14A, 27A, 296A, ICSA 51and ICSA 465),

Table 4 Cluster means and general mean for different characters in forage sorghum male sterile lines

| Trait/Cluster | C I | C II | C III | C IV | C V | C VI | C VII | C VIII | C IX | C X | Gen. mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 105 | 127 | 101 | 135 | 107 | 122 | 177 | 92 | 195 | 55 | 113 |
| Leaf length (cm) | 67.3 | 85.7 | 72.2 | 88.0 | 64.3 | 64.9 | 83.0 | 70.0 | 78.0 | 46.5 | 67.5 |
| Leaf breadth (cm) | 7.6 | 8.2 | 8.2 | 6.7 | 6.4 | 8.8 | 7.5 | 6.1 | 7.9 | 6.1 | 8.4 |
| Stem girth (cm) | 6.6 | 6.4 | 8.1 | 5.2 | 6.2 | 6.0 | 7.7 | 5.2 | 5.9 | 7.4 | 6.4 |
| No. of tillers/plant | 1.0 | 1.0 | 1.2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| No. of leaves/plant | 13.5 | 14.2 | 15.7 | 15.0 | 13.5 | 14.4 | 14.5 | 13.8 | 17.0 | 10.0 | 13.9 |
| Plant type | 1.2 | 1.0 | 1.0 | 1.0 | 1.1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.1 |
| Sweet/non-sweet | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Stem borer score | 3.4 | 2.3 | 3.2 | 3.0 | 3.4 | 3.4 | 3.0 | 3.5 | 2.0 | 5.0 | 3.3 |
| Gray leaf spot (%) | 14.1 | 9.3 | 11.0 | 12.3 | 14.7 | 12.8 | 10.0 | 10.8 | 11.6 | 21.9 | 12.9 |
| Zonate leaf spot (%) | 10.8 | 8.3 | 11.8 | 13.5 | 11.6 | 10.1 | 12.6 | 12.7 | 10.9 | 15.3 | 11.8 |
| Sooty stripe (%) | 26.3 | 20.0 | 24.7 | 30.3 | 25.2 | 25.8 | 23.1 | 28.3 | 20.3 | 15.8 | 24.0 |
| GFY/plant (g) | 248 | 567 | 412 | 375 | 157 | 333 | 512 | 65 | 425 | 202 | 283 |
| DMY/plant (g) | 81 | 192 | 139 | 130 | 51 | 110 | 172 | 22 | 142 | 65 | 93 |

Table 5 Inter and intra-cluster distances in forage sorghum male sterile lines (UPGMA - City Block Distance)

| Cluster No. | C I | C II | C III | C IV | C V | C VI | C VII | C VIII | C IX | C X |
|---|---|---|---|---|---|---|---|---|---|---|
| C I | *78.98* | | | | | | | | | |
| C II | 487.69 | *101.93* | | | | | | | | |
| C III | 263.14 | 262.44 | *88.66* | | | | | | | |
| C IV | 293.05 | 340.93 | 161.80 | *0.00* | | | | | | |
| C V | 171.96 | 616.56 | 395.07 | 418.57 | *99.50* | | | | | |
| C VI | 163.49 | 374.50 | 159.94 | 172.94 | 291.63 | *90.72* | | | | |
| C VII | 455.82 | 140.48 | 234.03 | 294.35 | 577.32 | 328.51 | *83.30* | | | |
| C VIII | 273.96 | 727.20 | 495.60 | 541.20 | 162.74 | 402.24 | 704.65 | *26.80* | | |
| C IX | 350.46 | 273.23 | 148.76 | 196.80 | 472.38 | 221.08 | 150.20 | 598.70 | *71.40* | |
| C X | 144.72 | 614.07 | 368.44 | 431.60 | 141.86 | 273.43 | 589.80 | 251.15 | 486.30 | *59.20* |

Diagonal values are intra-cluster distances

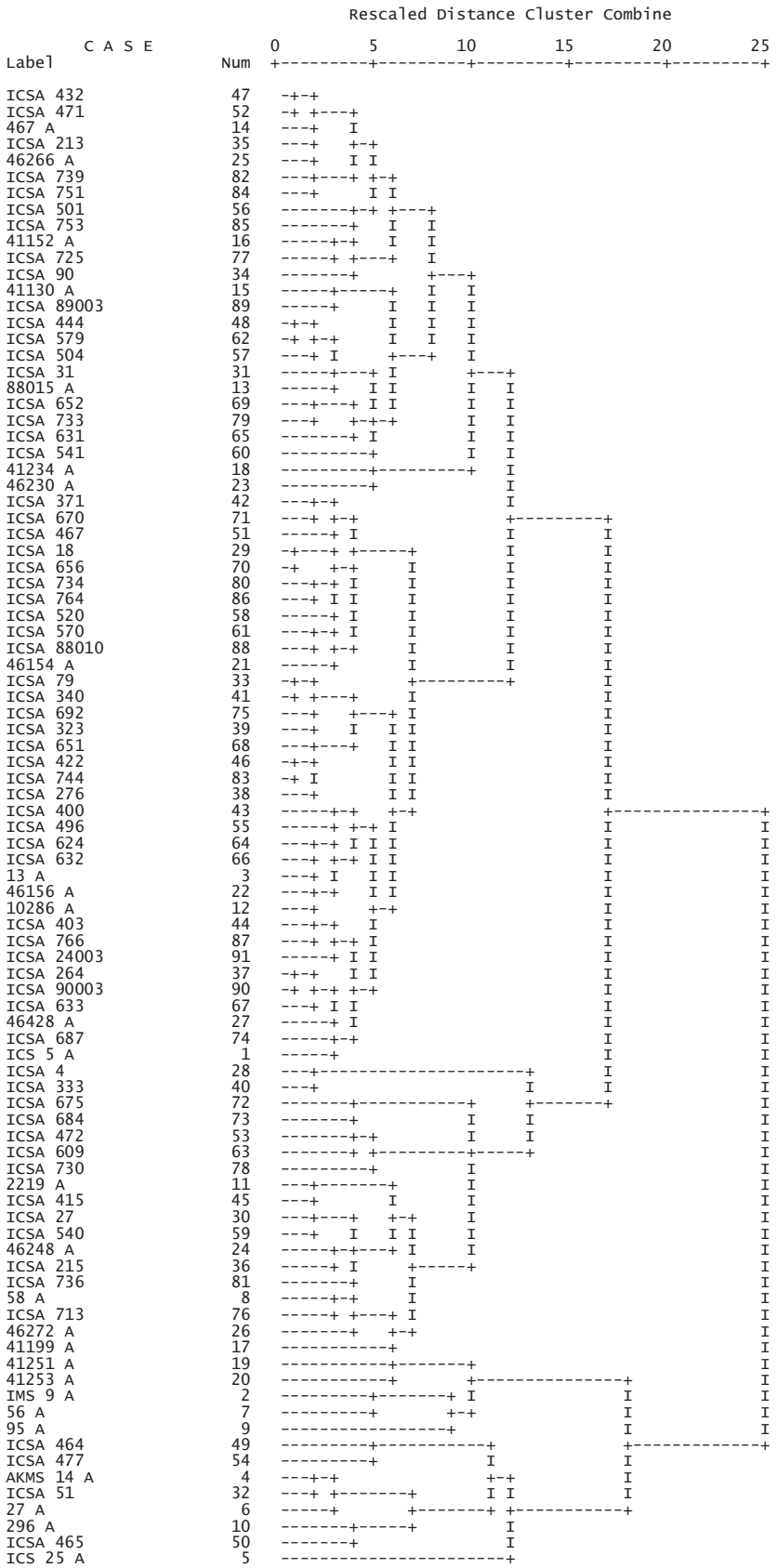


Fig 2 Dendogram portraying clustering pattern of different genotypes

IV (ICS 25A), VII (41251A and 41253A), VIII (ICSA 4 and ICSA 333) and IX (ICSA 464 and ICSA 477) were free from gray and zonate leaf spot diseases. However, the performance of cluster X (ICSA 675 and ICSA 684) was found to be inferior with respect to most of the parameters. The remaining clusters showed more or less moderate performance for different characters. Inter and intra cluster distances were calculated and are presented in Table 5. As the material comprises male sterile lines only, the lines falling in distant clusters were more unrelated. Maximum inter cluster distance was observed between cluster II and VIII followed by cluster VII and VIII meaning thereby that IMS 9A, 56A and 95A not related to the lines ICSA 4 and ICSA 333 and also ICSA 4 and ICSA 333 are not related to the lines 41251A and 41253A. Hence superior lines among these different male sterile lines can be utilized for development of hybrids to broaden the genetic base of forage sorghum hybrids so as to lessen the threat of genetic vulnerability. Romesburg (1990) opined that findings of similar alternatives reduce the decision problem to two stages, i e first, to select the cluster that can best achieve the planning objective, and second select the best alternative within the best cluster.

The results of hierarchical cluster analysis and principal factor analysis confirms the findings of each other. The plots of PF 1 and PF 2 accounting for about 40 per cent variation, shows clear differentiation of genotypes according to their cluster membership denoted by different markers (Fig 2). The male sterile lines like IMS 9A, 56A, 95A, AKMS 14A, 27A, ICSA 51, ICS 25A, 41251A, 41253A, etc., found to be superior using principal factor analysis were also found to be members of the best performing clusters, i e cluster II, VII and IX. Such confirmatory results were also obtained by Yadav *et al.* (2003 and 2004) in forage sorghum. No characterization or classification studies on phenotypic basis could be traced in literature in forage sorghum male sterile lines except Gomashe *et al.*, (2012) for shoot fly, however, Sivaramakrishnan *et al.* (1997) and Arya *et al.* (2006) had done this using RFLP and RAPD markers and have shown the differences among the male sterile lines.

Hence, the present study has proved to be successful in classifying different male sterile lines based on various morphological characters, reducing large number of variables into only five principal factors and identifying different lines better for different combinations of characters. The results of the present study can be used as a stepping stone for evolving well defined approach based on evaluation and characterization of genetic variation in sorghum male sterile lines for fodder traits.

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