# Genetic diversity analysis in advanced lines of rabi sorghum based on root, charcoal rot and biochemical components

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**ABSTRACT**

**An investigation was carried out in 23 advanced lines of sorghum along with M-35-1, Muguthi, E-36-1 and SPV-86 as check varieties. Various genetic diversity parameters were studied for 14 quantitative characters *viz*., Root Length, Root Spread, Seminal Roots, Adventitious Roots, Fresh Root Weight, Root Volume, Dry Root Weight, Seed Yield per Plant, Fodder Yield per plant, Charcoal rot Lodging per cent, Mean Node Crossed, Mean Length Spread, Phytic Acid and Inorganic Phosphorus. Twenty Seven sorghum lines were analyzed for $D^2$ analysis. They grouped into 6 different clusters. Cluster I was largest with seven genotypes followed by cluster II and V with six genotypes. Whereas cluster III consisted of four genotypes, while two genotypes were present in cluster IV and cluster VI. The intra cluster distance was maximum in cluster V followed by cluster III and cluster II. Whereas inter clusters distance was maximum between cluster IV and cluster VI. All two genotypes from cluster IV were characterized by high root length, fresh root weight, dry root weight, less charcoal rot infection of both Mean Node Cross and Mean Length Spread. While cluster VI was characterized by root volume and high inorganic phosphorus. These characters are important with respect to overall improvement seed yield combining with charcoal rot resistance and high inorganic phosphorus in sorghum. Crosses among genetically diverse genotypes are likely to throw desirable recombinants. Therefore crossing between the genotypes belonging to cluster IV (GS 21 and Muguthi) and cluster VI (GS-14 and M-35-1) might be useful for identifying recombinants for high yield potential in segregating generations.**

**Key words:** *Sorghum, Roots, Charcoal rot, Phytic acid , Inorganic phosphorous*

Sorghum is an important component in the human food of people at arid and semiarid regional including India. Its stalk is used as cattle feed and also being utilized in a number of traditional preparation of festival importance. It is in grown both during *kharif* and *rabi* season in India. Traditionally *rabi* sorghum produces high quality of grains as it is under influence of winter season which is dry drizzling free and short day weather. Area under *rabi* sorghum is expanding consider to *kharif* season. But genetic improvement of *rabi* sorghum is given importance in recent years (Sameer Kumar *et al.*, 2010). Development of varieties for *rabi* season is mainly governed by the magnitude of genetic diversity available in the base material and extant of the utilization of these *rabi* sorghum available in the germplasm through the breeding programs. The present study was an attempt to as ascertain the nature and magnitude of genetic diversity available in the advanced lines for roots, charcoal rot and biochemical characters. The identified lines will be further utilized as donors in breeding sorghum for *rabi* season.

## MATERIALS AND METHODS

### Physical quality characters

The material comprised of 23 advanced lines and checks *viz.*, M-35-1, Muguthi, E-36-1(Resistant to charcoal rot) and SPV-86(Susceptible to charcoal rot). The origin of 23 lines along with checks is presented in Table 1. Experiment was carried at ARS, Gulbarga during 2012. Lines were

**Table 1.** Origin of 23 advanced lines of sorghum

| Sl. No. | Lines | Pedigree | Region adapted |
|---|---|---|---|
| 1 | GS-1 | Kodikal-3 | North Karnataka |
| 2 | GS-2 | Chincholi-2 | North Karnataka |
| 3 | GS-3 | Mudbal-1 | North Karnataka |
| 4 | GS-4 | M35-1 X Niralkodi-10-14-2 | North Karnataka and Maharashtra |
| 5 | GS-5 | M35-1 X Niralkodi-9-14-1 | North Karnataka and Maharashtra |
| **6** | GS-6 | M35-1 X Bommnahalli-4-1 | North Karnataka |
| **7** | GS-7 | M35-1 X Bommnahalli-4-2 | North Karnataka |
| 8 | GS-8 | M X Bommnahalli-4-3 | North Karnataka |
| 9 | GS-9 | (M35-1 X DSV-4) X M | North Karnataka |
| 10 | GS-10 | (M35-1 X DSV-4) -4-1-29-2 | North Karnataka |
| 11 | GS-11 | (M35-1 X DSV-4)-4-2-1 | North Karnataka |
| 12 | GS-12 | (M35-1 X Sapnapalli) X M35-1 -4-5 | North Karnataka |
| 13 | GS-13 | (M35-1 X Sapnapalli)-4-5 | North Karnataka |
| 14 | GS-14 | JP-1-5 | North Karnataka |
| 15 | GS-15 | M35-1 X Hottigudar-2 -4-5-2 | North Karnataka |
| 16 | GS-16 | (M35-1 X Hottigudar-2) X M35-1 -2-1 | North Karnataka |
| 17 | GS-17 | M35-1 X Hottigudar-2 -4-6 | North Karnataka |
| 18 | GS-18 | Phule Mule X M 35-1 -18-1 | North Karnataka |
| 19 | GS-19 | (IS26779 X M35-1) X M35-1 -1-1-5-1 | North Karnataka |
| 20 | GS-20 | (IS26779 X M35-1) X M35-1 -1-1-5-2 | North Karnataka |
| 21 | GS-21 | (IS26779 X M35-1) X M35-1 -1-1-5-3 | North Karnataka |
| 22 | GS-22 | (IS26779 X M35-1) X M35-1 -1-1-5-4 | North Karnataka |
| 23 | GS-23 | IS26779 X M35-1 -1-2-2-1 | North Karnataka |
| 24 | M35-1 | Popular farmer variety | North Karnataka and Maharashtra |
| 25 | Muguthi | Historical popular variety | North Karnataka and Maharashtra |
| 26 | E-36-1 | Farmers variety | Africa |
| 27 | SPV-86 | Susceptible check | North Karnataka |

grown in the 100 cm length and 30 cm breadth nylon nets with two replications. The following observations were recorded at the time of flowering *viz.*, Root Length (cm), Root Spread (cm), Seminal Roots, Adventitious roots, Fresh Root Weight (g), Root Volume (ml) and Root Dry Weight (g). The field experiments were conducted *rabi* seasons during 2012 and 2013. The materials were sown in RCBD design with three replications. The below mentioned observation were recorded in experimental field on 5 plants in each replication *viz.*, Seed Yield per Plant in gm( SYP), Fodder Yield per Plant in gram(FYP), Charcoal Rot Lodging in per cent (CRL), Mean Node Crossed in number (MNC) and Mean Length Spread in cm (MLS). The biochemical components viz., phytic acid and inorganic phosphorus were estimated from seed as mg/g flour at BARC, Bombay. The mean data was analyzed using Window STAT version 8. Mahalanobis (1936) $D^2$ statistic was used for assessing the genetic divergence between populations. Clustering was carried using Tocher's method as described by Rao (1952). The intra and inter cluster distances were calculated by formula given by Singh and Chaudhary (1977).

**Determination of Phytic Acid (PA)**

The assay of PA is based on modified colorimetric method (Vaintrub and Lapteva, 1988). About 30 mg of ground seed sample was used for extraction of phytic acid in 0.2 N HCl buffer and kept overnight. Crude acid extracts were transferred to fresh tubes containing 20 mg NaCl. The contents were shaken at 350 rpm for 20 min. to dissolve the salt and were allowed to settle at –20°C for 20 min. The mixtures were centrifuged at 8000 rpm at 10°C for 20 min. and clear supernatant was diluted 25 times by mixing with distilled dd$H_2O$. 750 µl of this diluted sample were combined with 250 µl of modified Wade reagent (0.03% $FeCl_3$.6H2O + 0.3% sulfosalicylic acid) in a eppendorf tube, thoroughly mixed on a vortex, and centrifuged at 8000 rpm at 10°C for 10 min. A series of calibration standards containing 0, 0.5, 1, 1.5, 2, 3, 4, 5,7.5, 10 and 12 µg $ml^{-1}$ PA-P were prepared from sodium phytate (Sigma, St. Louis, MO). Absorbance of color reaction products for both samples and standards were read at 500 nm on a UV-Vis spectrophotometer (Jasco, Japan), and calculation of sample PA-P content was estimated by the method described by Latta and Eskin, (1980).

**Determination of Inorganic Phosphorous (IP):**

Inorganic Phosphorus was estimated colorimetrically following extraction of 30-50 mg of a ground sample in 12.5% (v/v) TCA and 25mM $MgCl_2$ buffer (Chen *et al*., 1956). Overnight incubated samples were centrifuged at 10,000 rpm and supernatant was diluted 1:2 with distilled water. A 100µl of the diluted sample was mixed with Chen's reagent and incubated in water bath at 50°C for 1h. After incubation, samples were cooled and absorbance was taken at 660nm in a UV-V is spectrophotometer (Jasco, Japan). A standard curve was plotted by taking the absorbance of known amount of disodium hydrogen phosphate. Based on the calibration curve of the standard IP, the respective OD value of a sample was converted to concentration of inorganic P and expressed in mg/g of sorghum flour.

**Charcoal rot inoculum preparation**

Charcoal rot disease is caused by *Macrophomina phaseolina (Tassi)* Goid. It appears in severe form on the improved varieties like M-35-1 (Kamatar *et al.,* 2000) in hot dry weather with soil moisture stress. However, under normal conditions, it may not appear in the required form and hence, needs artificial inoculation for experimental studies. The pathogen was cultured (Rao *et al*., 1980) on wooden tooth -picks in honeypeptone medium (peptone 1g, honey 5ml, distilled water 94 ml). Tooth-picks were packed into wide mouthed screw-capped bottles and were sterilized at 15 psi for 20 minutes. Two loops of a mycelial-sclerotial suspension made from stock cultures of *Macrophomina phaseolina* were seeded into each 100ml of sterilized cooled honey peptone medium. The medium was shaken thoroughly to allow even inoculums distribution and poured under aseptic conditions into the wide-mouthed bottles (about 20 ml/ bottle), containing the sterile tooth picks so that the level of medium in the bottle covered about one third of the length of the tooth picks. The bottles were incubated at 35$^oC$ for 7 days at which, tooth picks were covered with mycelia and sclerotia of the charcoal rot fungus and ready for use in inoculation.

**Field inoculation**

Plants were inoculated about two weeks after 50 per cent flowering. Irrigation was with held when the majority of the lines were at the boot leaf stage. A fungus infected tooth pick was inserted obliquely into a hole made with an iron pocher into each stalk at its second internode from ground level. Care was taken to ensure that the tooth pick did not emerge through the other side of the stem, for this would promote rapid drying of the inoculum. As mentioned earlier, five plants of each entry in each replication were inoculated.

**Parameters for charcoal rot resistance**

Three normal parameters used in charcoal rot studies were recorded on charcoal rot inoculated plants *viz.*, i) lodging per cent due to charcoal rot, ii) mean number of nodes crossed, iii) mean length of spread in centimeters. Based on the percentage lodging and soft stalk, the genotypes were graded using 0-9 scale (Mayee and Datar, 1986) and grouped into respective categories as follows. Based on mean number of nodes crossed by charcoal rot disease the genotypes were graded using 1- 5 scale (Das *et al.,* 2007) and grouped into respective categories as follows.

## RESULTS AND DISCUSSION

The analysis of variance revealed significant difference among 27 genotypes for 14 quantitative characters indicating considerable variation among genotypes were grouped into 6 clusters (Table 2). The cluster I consisted of highest genotype followed by cluster II and cluster V had 6 genotypes respectively. Cluster III had 4 genotypes, remaining cluster IV and VI had two genotype each. These results are in accordance with the studies conducted by Rahman *et al.* (2004) and Santosh Khadakabhavi (2014).

**Table 2.** Clustering pattern of advance breeding lines of sorghum

| Cluster | No. of genotypes | Name of the genotypes |
|---|---|---|
| I | 7 | GS-1, GS-9, GS-22, GS-2, GS-6, GS-10 and GS- 11 |
| II | 6 | GS-19, GS-20, GS-18, GS-12, GS-16 and GS-3 |
| III | 4 | GS-5, GS-7, GS-4 and GS-23 |
| IV | 2 | GS-21 and Muguthi |
| V | 6 | GS-8, E-36-1, GS-15, SPV-86, GS- 17 and GS- 13 |
| VI | 2 | GS-14 and M-35-1 |

The clustering pattern revealed that there was no perfect relationship between genetic diversity and geographical diversity, since the genotype from heterogeneous geographical location were included in one cluster. There was an absence of correlation between genetic diversity and geographical diversity (Mahalonobis, 1936 and Rao 1952). It may be concluded that genotypes from different background and wide divergence for adaptability.

The intra cluster $D^2$ values ranged from 6.47(Cluster IV) to 14.49 (Cluster V). The maximum inter cluster $D^2$ values was recorded between cluster IV to VI (52.35), while the divergence was minimum between cluster I to IV (14.16) revealing that inter cluster distances were greater than intra cluster distances indicating considerable amount of genetic diversity among genotypes studied (Table 3). Inter cluster distances is the main criterion for selection of genotypes using $D^2$ analysis. Genotypes belonging to the clusters with maximum distance are genetically more divergent and hybridization between such genotypes of divergent cluster is likely to produce wide variability with desirable segregants. These observation are in accordance with the results of Sridhar *et al* (2003). The inter crossing of the genotypes showing diversity should result in generating sufficient variability to operate selection in segregating populations.

**Table 3.** Average intra and inter cluster distance of advanced breeding lines of sorghum

| Cluster | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| **I** | 8.99 | 23.79 | 25.47 | 14.16 | 18.26 | 45.44 |
| **II** | | 9.41 | 17.49 | 33.34 | 23.90 | 26.42 |
| **III** | | | 9.83 | 30.28 | 32.90 | 24.42 |
| **IV** | | | | 6.47 | 27.66 | 52.35 |
| **V** | | | | | 14.49 | 47.57 |
| **VI** | | | | | | 8.81 |

The cluster means for 14 characters on pooled basis is indicated that cluster I had the high means in respect of seminal roots, adventitious roots and dry root weight (Table 4.). The cluster VI exhibited high means for root length, fresh root weight and dry root weight. While low means for MNC. Hence it could be concluded that significant genetic diversity exist among 27 genotypes for most important characters. This could be attributed to long term selection in different direction by both natural and human forces. Similar study on root characters were made by Dhanda *et al*. (2004), Khan *et al.* (2004), Ali *et al*. (2009 a and b). Massive amount of genetic diversity for yield contributing traits exist in sorghum reported by Arun and Audilaksmi, (2008) and Ali *et al*. (2011). The per cent contribution of different characters to total diversity is presented in Table 5. Inorganic phosphorus ranked first (216 times out of 351 total combinations) contributed 61.54 per cent to the divergence of genotypes followed by phytic acid (35.90%), root volume (1.42%), MLS (0.57%) and 0.28 per cent for both seed yield per plant and charcoal rot lodging percent. These are the important characters contributory substantially to the genetic divergence.

**Table 4.** Cluster wise mean performance of advanced breeding lines for root, charcoal rot and biochemical characters

| | RL | RS | SR | AR | FRW | RV | DRW | SYP | FYP | CRL | MNC | MLS | PA | IP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 21.48 | 6.55 | 8.90 | 7.05 | 7.10 | 5.90 | 3.55 | 10.03 | 68.86 | 23.38 | 1.57 | 14.67 | 2.52 | 0.19 |
| II | 21.28 | 7.42 | 7.78 | 6.11 | 6.08 | 5.83 | 2.73 | 8.92 | 65.73 | 28.00 | 1.89 | 18.50 | 1.16 | 0.44 |
| III | 22.33 | 6.58 | 6.50 | 9.08 | 5.83 | 5.88 | 2.88 | 12.15 | 73.62 | 21.33 | 2.08 | 17.42 | 2.90 | 0.47 |
| IV | 28.50 | 5.67 | 8.75 | 5.17 | 8.20 | 6.05 | 4.17 | 5.55 | 70.29 | 32.17 | 1.33 | 16.00 | 3.97 | 0.12 |
| V | 20.83 | 6.11 | 7.94 | 6.67 | 5.51 | 4.75 | 2.69 | 9.43 | 76.11 | 33.94 | 2.00 | 19.83 | 0.92 | 0.20 |
| VI | 17.00 | 6.75 | 7.25 | 5.67 | 4.43 | 6.50 | 2.22 | 6.80 | 60.07 | 26.33 | 1.83 | 17.83 | 1.55 | 0.73 |

RL- Root Length(cm)
RS- Root Spread (cm)
SR- Seminal Roots
AR- Adventious Roots
FRW- Fresh Root Weight (g)
RV- Root Volume (ml)
DRW- Dry Root Weight (g)
SYP- Seed Yield Per Plant (g)
FYP- Fodder Yield Per Plant
CRL- Charcoal rot Lodging (%)
MNC- Mean Node Crossed
MLS-Mean Length Spread (cm)
PA- Phytic acid (mg/g flour)
IP-Inorganic phosphorus (mg/g flour)

**Table 5**. Per cent contribution of characters towards divergence in advanced lines of sorghum

| Sl. No. | Characters | Times ranked 1st | Contribution % |
|---|---|---|---|
| 1 | Inorganic phosphorus (mg/g flour) | 216 | 61.54 |
| 2 | Phytic Acid (mg/g flour) | 126 | 35.90 |
| 3 | Root volume(ml) | 5 | 1.42 |
| 4 | Mean Length Spread (cm) | 2 | 0.57 |
| 5 | Seed Yield Per Plant (g) | 1 | 0.28 |
| 6 | Charcoal rot lodging (%) | 1 | 0.28 |
| | Total | 351 | 100 |

## CONCLUSION

Crosses among genetically diverse genotypes are likely to yield desirable recombinants. Therefore crossing between the genotypes belonging to cluster IV (GS 21 and Muguthi) and cluster VI (GS-14 and M-35-1) might be useful for identifying recombinants for high yield potential as $D^2$ value between these clusters was highest (52.35). All two genotypes from cluster IV were characterized by high root length, fresh root weight, dry root weight less MNC and MLS. While cluster VI was characterized by root volume and high inorganic phosphorus. These characters are important with respect to overall improvement seed yield combining with charcoal rot resistance and high inorganic phosphorus in sorghum.


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