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Research Article

An image analyser: A rapid and non-destructive method for characterization and diversity assessment of sorghum landraces

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Abstract
Sorghum grown as fodder, food and biofuel crop in about 100 countries is a dietary staple food for more than a million in subtropical and semi-arid regions of Asia and Africa. The reliable characterization of genotypes serves as a base for every crop improvement programme and a machine vision system is a prime option for efficient discrimination. The 39 sorghum landraces collected from various parts of Trichy district were measured for area, perimeter, width, length, aspect ratio, rectangularity, and circularity by Grain Scanner (Grain Scanner RSQI 10A). The maximum area, perimeter, length and width were recorded in Ammapatti local 2 (12.68 mm^2), Keezhapuliyur local 1(13.27 mm), Muthiyampalayam local 3 (4.90 mm) and Ammapatti local 2 (3.82mm), respectively. Among the genotypes observed, Ammapatti local 2 and Keezhapuliyur local 1 showed significance for all the traits except aspect ratio. The cluster analysis grouped genotypes into four clusters and number of genotypes in each cluster was 15, 8, 3 and 13 in clusters I, II, III and IV, respectively. Based on aspect ratio, the members of cluster I and III were elliptical and II and IV were circular. The members of cluster II have high germination percentage, threshability and grain yield due to its boldness. Hence circular bold seeded high yielding sorghum varieties can be developed from members of cluster II. Relatively small-seeded genotypes come under cluster IV. The major contributors for the cluster formation were the length, width and area. The genotypes collected from different locations of the same geographical area were grouped into different clusters and this is due to minor variation that happens in same species during evolution. The minor variation which is not detected by general characterization can be easily scanned by Grain Scanner and so, it is an evident tool in characterizing genotypes based on grain morphology.

Keywords
Sorghum, Grain scanner, Morphological traits, cluster analysis, discrimination

Introduction

Sorghum is a versatile crop and is a principle food crop for people in subtropical and semi-arid regions. Sorghum member of Poaceae family is a C_4 plant with high photosynthetic efficiency. Sorghum is naturally a hardy crop and can grow even in marginal lands with high biotic and abiotic stress resistance. Besides, its importance the total consumption as food crop gradually decreases in the past few decades due to change in life style and consumer preference. Sorghum is a superior choice for this climate changing era and it ensures food and nutritional security. Red Sorghum landraces of Sorghum is rich in anthocyanin and cultivated primarily in Southern districts of Tamil Nadu. These landraces are noted for nutritional quality, antioxidant activity, stress tolerance, and other industrial application as a natural dye (3-DOA highly stable) (Dykes *et al*. 2009; Dykes and Rooney, 2006). The peculiar character of anthocyanin present in red sorghum landraces was that it lacks a hydroxyl group at the C-3 position, which increases the stability of the 3-DOA molecule. Considering its importance, the research work to develop a potential variety has to start and the genotypic discrimination is the primary step.

Morphological, biochemical and molecular level characterization are being routinely used for this purpose. However, it has its own limitations *viz.*, time-consuming, destructive, environmentally influenced, stage specific, expensive and skill-oriented. These drawbacks are overcome by the machine vision system which is easy, non-destructive and hence a better replacement for manual based analysis that provide information about the grain morphological traits with great accuracy (Vithu and Moses, 2016). In grain quality aspect physical characters such as colour, size, shape *etc*., are important. This method is computerised with fully automated quality evaluation system and cost effective. The morphological traits observed include area,

perimeter, width, length, aspect ratio, circularity and rectangularity. The morphological traits such as area, width and length were used to catch out bold seeded genotypes and aspect ratio to find circular genotypes respectively. The minor variation that is difficult to detect by general characterization can be easily screened by Grain Scanner and hence the morphological traits assessed by non-destructive image analysis system can be confidently used to understand and characterize the genotypes.

Materials and Methods

The 39 sorghum landraces (31 red sorghum, seven white and one yellow genotype) were collected from various parts of Trichy district were assimilated from Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli. The seeds were scanned with grain scanner available at Centre of Innovation, Agriculture College and Research Institute, TNAU, Madurai.

The instrument used was scanner type grain analyser (Grain Scanner RSQI 10A, a product of SATAKE Corporation) that provides information about grain morphological traits. The imaging method in the device was an LED (White) light source with sensor CCD (Charge Coupled Device) colour. The parameter measured for each individual grain were area, width, perimeter, length, aspect ratio, rectangularity, and circularity was provided by software package of SATAKE.

The Grain Scanner was first self-diagnosed with empty tray along with the background plate which is blue in colour. After the self-diagnosis, the sample was placed over the tray. Each individual seed was measured for area, perimeter, length, width, aspect ratio, circularity and rectangularity get stored in software installed. The individual sample was observed in three replications and each replication with 10 seeds. The following seven morphological traits *viz.*, area, perimeter, aspect ratio, length, width, rectangularity and circularity related to grain morphology were observed.

- Area - extent of a two-dimensional figure or shape, or planar lamina, in the plane.
- Perimeter - length of the outline of a shape. The perimeter of a circle or ellipse is called its circumference.
- Length and Width - the measure of distance from end to end that is commonly measured to understand the extended dimension of an object.
- Aspect ratio - the proportional relationship between its width and its height (Aggarwal and Mohan, 2010).
- Circularity - describe the closeness of an object to be a true circle.
- Rectangularity- the property possessed by a shape that has angles (Rosin, 1999).

The parameters area, length, and width are used to assess grain size and other parameters such as perimeter, aspect ratio, circularity and rectangularity are used to define the shape of the seed. The data obtained were analysed for the test of significance and the critical difference was calculated at 5% probability (Table 1). The analysed genotypes were cluster based on the seven observed traits using the statistical software package STAR 2.0 created by IRRI.

Result and Discussion

The discrimination among landraces and genotypes was a dynamic effort and computer-based image analysis system well ascertains its role (Geetha *et al*. 2011). The grain morphological traits of 39 sorghum landraces such as area, perimeter, length, breadth, aspect ratio, rectangularity and circularity were measured using grain scanner and their mean values were listed in table 1 and images presented in Fig. 1.

The size of the seed depends upon length, breadth and area. The seed size is an effective selection criterion since it directly determines the germination rate, field emergence, seed vigour (Hojjat, 2011) and most importantly grain yield. The maximum area was recorded by Ammapatti Local 2 (12.68mm^2) and least by Kottathur Local 5 (7.2mm^2). The landraces Ammapatti local 2 (12.68mm^2), Keezhapuliyur local 1 (12.63mm^2), Kottathur Local 1 (10.39mm^2), Kottathur Local 3 (10.06mm^2), Kottathur Local 4 (10.08mm^2), Kottathur Local 6 bold (11.43mm^2), Muthiyampalayam Local 2 (10.4mm^2), Muthiyampalayam Local 5 (9.41mm^2), and Paiyur 2 (10.52mm^2) were positively significant and selection within these genotypes is effective.

The outer boundary measurement perimeter is also an important trait while analysing grain morphology. The two genotypes Ammapatti Local 2 (13.26mm) and Keezhapuliyur Local 1 (13.27mm) recorded the highest perimeter and lowest by Kottathur Local 5 (10.11mm). This trait used along with area to classify seed as bold, medium and small. Based on area and perimeter Ammapatti Local 2 noted to be large seed and Kottathur Local 5 was smaller among the observed genotypes.

The length and width of the seed have an impact on both size and shape of the seed. The maximum

length was noted in Muthiyampalayam Local 3 (4.9mm) and minimum in Kottathur Local 5 (3.43mm). The area of Kottathur Local 5 was minimum since it has the lowest length. The maximum width was observed in Ammapatti Local 2 (3.82mm) and minimum in Visvakudi Local 2 (2.57mm). The larger seed area was observed in Ammapatti local 2 as it has the highest width among genotypes observed.

The genotypes Ammapatti Local 1 (4.48mm),Ammapatti Local 2 (4.37mm), Ammapatti Local 3 (4.51mm), Ammapatti Local 4 (4.58mm), Ammapatti local 6 (4.33mm), Keezhapuliyur local 1 (4.32mm), Kottathur Local 6 bold (4.38mm), Muthiyampalayam Local 1 (4.53mm), Muthiyampalayam Local 3 (4.9mm), Muthiyampalayam Local 4 (4.48mm), Muthiyampalayam Local 6 (4.73mm), Nochiyam Local 1 (4.9mm), Sorathur Local 1 (4.55mm), Venbarour local 1(4.41), Venkalam local 1 (4.47), Veppanthattai local 1 (4.6), Visvakudi local 1 (4.56mm), and Visvakudi local 2 (4.56mm) were significant for length and they form an effective selection platform for this trait.

The genotypes Ammapatti local 2 (3.82mm), Anumandhanayarkottai local 1 (3.09mm), Chiddayankottai local 1 (3.12mm), Kalingamudayanpatti local 1 (3.11mm), Keezhapuliyur local 1(3.71mm), Kerambra local 1(3.19mm), Kottathur local 1 (3.42mm), Kottathur local 3 (3.49mm), Kottathur local 4(3.31mm), Muthiyampalayam local 2(3.38mm), Muthiyampalayam local 5 (3.13mm), Paiyur 2 (3.4mm), Settiyapatti local 1(3.16mm), and Vengatesapuram local 2 (3.12mm) were significant for the trait width.

The significance for all the three traits (length, width and area) was noted in Ammapatti local 2 and Keezhapuliyur Local 1. Hence, they can be used as a parental line in breeding programme with an objective to develop bold seeded high yielding varieties.The aspect ratio denotes ratio between width and height of the grain (ratio of the major axis to the minor axis). The shape of the sorghum grain observed was narrow elliptical, elliptical and circular. The aspect ratio clearly distinguish seed into two category circular (aspect ratio -1) and elliptical (aspect ratio -2) using grain scanner. Since shape of most of the sorghum seed comes under circular and elliptical the traits circularity and rectangularity shows only very limited variation.

Among the genotypes observed Ammapatti local 2 and Keezhapuliyur local 1 shows the significance for all the traits except aspect ratio. The genotypes Paiyur 2, Kottathur local 1, Kottathur local 3, and Kottathur local 4 were positively significant for

area, perimeter, width, circularity and rectangularity. The genotypes Kottathur local 6 bold showed significance for area, perimeter, length, circularity and rectangularity.The observed genotypes showed significant variation for the traits observed and hence they can be used for breeding programme involving these traits.

The cluster analysis based on morphological traits observed, grouped the genotypes into four clusters. The number of genotypes in each cluster were 15, 8, 3 and 13 in clusters I, II, III and IV, respectively. The major contributors for the cluster formation were the length, width and area and so they can be used as a marker for identifying a specific genotype. The dendrogram was drawn using Agglomerative clustering method (Fig. 2). The diverse parameters like area, perimeter, width, length, circularity and rectangularity of the members in clusters I, II, III and IV are detailed in the table 2.

Based on aspect ratio the members of cluster I and III were elliptical and II and IV were circular. The threshability is important while considering millet processing. The bold seeded variety retains more threshability, germination percentage and also high vigour compared with small-seeded variety. The members of cluster II (Ammapatti local 2, Keezhapuliyur Local 1, Kottathur Local 1, Kottathur Local 3, Kottathur Local 4, Kottathur Local 6 Bold, Muthiyampalayam Local 2 and Paiyur 2) have more area and perimeter. Hence, breeding programme can procure parent from cluster II to develop circular bold seeded high yielding sorghum varieties. Relatively small seeded genotypes come under cluster IV. The grain scanner is effectively involved in discrimination of genotypes and varieties in barley, wheat and rice (Dubosclard *et al*. 2015) and sesame (Vasanthan *et al.* 2019) by using grain scanner.

It is also noted that genotypes collected from different locations of the same geographical area were grouped into a different cluster which revealed that these genotypes may have originated from same species but during evolution, minor variations would have happened in grain morphology which characterized them as a separate landrace. The minor variation may not be noticed in general characterization but can be easily detected by Grain Scanner thus making it an efficient tool to understand and characterize genotypes even with minor variation.

On the whole, the Grain Scanner is an evident easy and non-destructive tool in discriminating genotypes based on grain morphology.

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Table 1. Measurement of seed morphological traits in 39 sorghum landraces using grain scanner

Sorghum landraces	Area	Perimeter	Width	Length	Aspect ratio	Circularity	Rectangularity
Ammapatti local 1	8.65	11.85*	2.82	4.48*	1.61*	0.77	0.69
Ammapatti local 2	12.68*	13.26*	3.82*	4.37*	1.15	0.91*	0.76*
Ammapatti local 3	8.43	11.83	2.71	4.51*	1.68*	0.76	0.69
Ammapatti local 4	8.47	11.48	2.58	4.58*	1.79*	0.81	0.72
Ammapatti local 5	7.72	11.15	2.69	4.17	1.57*	0.78	0.69
Ammapatti local 6	8.02	11.47	2.70	4.33*	1.62*	0.77	0.69
Anumandhanayarkottai local 1	8.60	11.03	3.09*	3.72	1.20	0.89*	0.75*
Chiddayankottai local 1	9.21	11.39	3.12*	3.95	1.27	0.89*	0.74*
Kalikampatti local 1	8.22	10.71	3.06	3.59	1.17	0.89*	0.74*
Kalingamudayanpatti local 1	8.58	10.93	3.11*	3.66	1.18	0.89*	0.75*
Kalingamudayanpatti local 2	7.93	11.24	2.74	4.16	1.53*	0.79	0.70
Keezhakunnempatti local 1	8.15	11.27	2.90	3.98	1.38	0.81	0.71
Keezhapuliyur local 1	12.63*	13.27*	3.71*	4.32*	1.13	0.90*	0.76*
Kerambra local 1	9.00	11.12	3.19*	3.69	1.16	0.90*	0.75*
Kottathur local 1	10.39*	12.15*	3.42*	4.03	1.19	0.88*	0.75*
Kottathur local 3	10.06*	12.02*	3.49*	3.99	1.19	0.87*	0.75*
Kottathur local 4	10.08*	12.01*	3.31*	4.07	1.24	0.88*	0.75*
Kottathur local 5	7.20	10.11	2.78	3.43	1.24	0.88*	0.75*
Kottathur local 6 bold	11.43*	12.93*	3.52	4.38*	1.25	0.86*	0.74*
Kottathur local 6 small	8.47	11.05	2.93	3.91	1.36	0.86*	0.73
Muthiyampalayam local 1	8.90	12.01*	2.84	4.53*	1.62*	0.77	0.69
Muthiyampalayam local 2	10.40*	12.33*	3.38*	4.13	1.23	0.86*	0.74*
Muthiyampalayam local 3	8.94	12.36*	2.73	4.90*	1.82*	0.74	0.67
Muthiyampalayam local 4	8.67	11.79	2.81	4.48*	1.61*	0.78	0.69
Muthiyampalayam local 5	9.41*	11.76	3.13*	4.10	1.33	0.85*	0.73
Muthiyampalayam local 6	9.14	12.27*	2.85	4.73*	1.68*	0.76	0.68
Narupatti local 1	8.17	10.68	3.03	3.60	1.19	0.89*	0.75*
Nochiyam local 1	8.78	12.33*	2.69	4.90*	1.85*	0.72	0.67
Puliyampatti local 2	8.62	11.21	3.02	3.90	1.30	0.86*	0.73
Settiyapatti local 1	8.90	11.17	3.16*	3.76	1.19	0.90*	0.75*
Sorathur local 1	8.84	11.90*	2.80	4.55*	1.64*	0.78	0.69
Venbarour local 1	8.25	11.70	2.75	4.41*	1.62*	0.76	0.68
Vengatesapuram local 1	7.36	10.33	2.72	3.69	1.37	0.86*	0.73
vengatesapuram local 2	8.79	11.31	3.12*	3.81	1.23	0.86*	0.74*
Venkalam local 1	8.27	11.64	2.72	4.47*	1.66*	0.77	0.68
Veppanthattai local 1	8.47	11.94*	2.72	4.60*	1.72*	0.75	0.68
Visvakudi local 1	8.50	11.93*	2.77	4.56*	1.67*	0.75	0.68
Visvakudi local 2	7.97	11.56	2.57	4.56*	1.79*	0.75	0.68
Paiyur 2	10.52*	12.13*	3.40*	4.11	1.21	0.89*	0.75*
Mean	**8.99**	**11.66**	**3.00**	**4.18**	**1.43**	**0.83**	**0.72**
SEd	**0.16**	**0.09**	**0.04**	**0.05**	**0.03**	**0.01**	**0.004**
CD (P=0.05)	**0.318**	**0.179**	**0.079**	**0.098**	**0.066**	**0.016**	**0.008**

***Positively significant at 5%**

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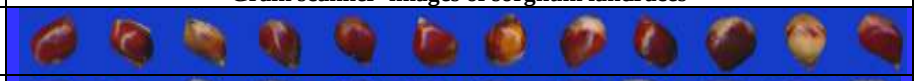
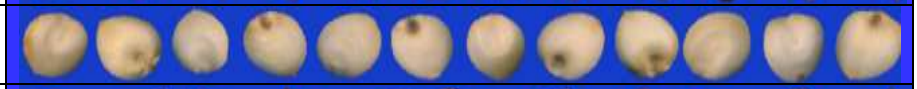
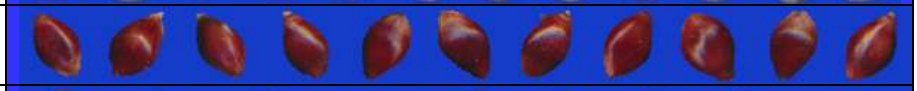
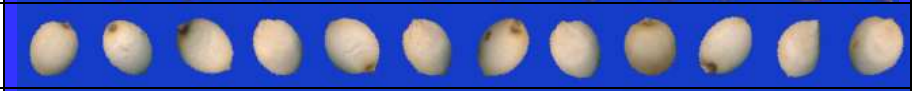
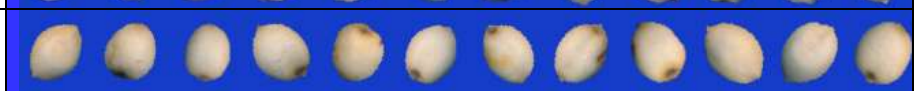
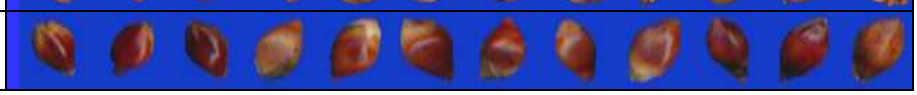
Table 2. Inter cluster and intra cluster range and mean of seven grain parameters

Seed morphological trait	Cluster I			Cluster II			Cluster III			Cluster IV		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Area	7.97	9.14	8.55	10.06	12.68	11.02	7.72	8.15	7.93	7.20	9.41	8.50
Perimeter	11.47	12.36	11.48	12.01	13.27	12.51	11.15	11.27	11.22	10.11	11.76	10.98
Width	2.57	2.85	2.74	3.31	3.82	3.51	2.69	2.90	2.78	2.72	3.19	3.04
Length	4.33	4.90	4.57	3.99	4.38	4.17	3.98	4.17	4.10	3.43	4.10	3.75
Aspect ratio	1.61	1.85	1.69	1.13	1.25	1.2	1.38	1.57	1.49	1.16	1.37	1.25
Circularity	0.72	0.81	0.76	0.86	0.91	0.88	0.78	0.81	0.79	0.85	0.9	0.88
Rectangularity	0.67	0.72	0.69	0.74	0.76	0.75	0.69	0.71	0.7	0.73	0.75	0.74

Sl. No	Genotypes	Grain scanner images of sorghum landraces
1.	Ammapatti local 1	
2.	Ammapatti local 2	
3.	Ammapatti local 3	
4.	Ammapatti local 4	
5.	Ammapatti local 5	
6.	Ammapatti local 6	
7.	Anumandhanayarkottai local 1	
8.	Chiddayankottai local 1	
9.	Kalikampatti local 1	
10.	Kalingamudayanpatti local 1	
11.	Kalingamudayanpatti local 2	
12.	Keezhakunnempatti local 1	
13.	Keezhapuliyur local 1	
14.	Kerambra local 1	
15.	Kottathur local 1	
16.	Kottathur local 3	
17.	Kottathur local 4	
18.	Kottathur local 5	
19.	Kottathur local 6 bold	
20.	Kottathur local 6 small	
21.	Muthiyampalayam local 1	

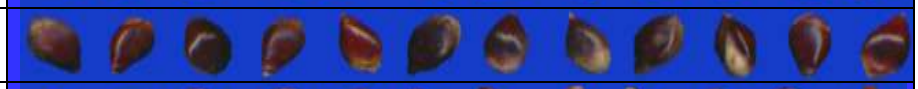
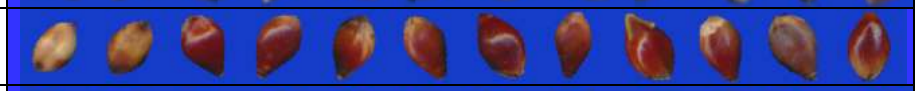
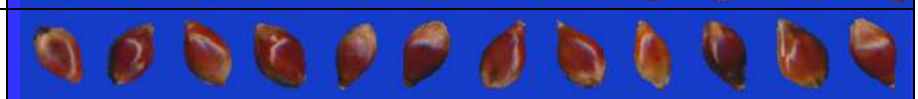
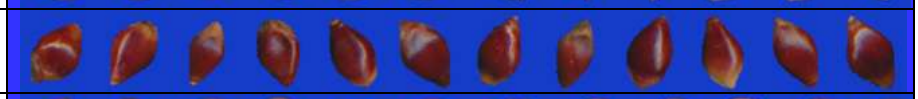
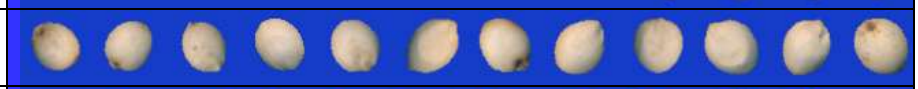
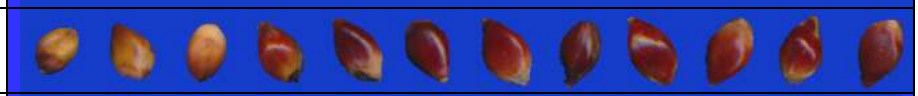
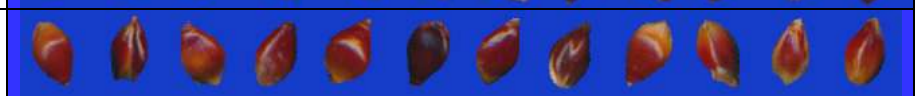
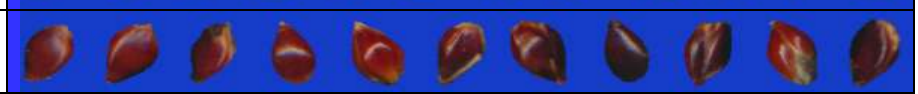
22.	Muthiyampalayam local 2	
23.	Muthiyampalayam local 3	
24.	Muthiyampalayam local 4	
25.	Muthiyampalayam local 5	
26.	Muthiyampalayam local 6	
27.	Narupatti local 1	
28.	Nochiyam local 1	
29.	Paiyur 2	
30.	Puliyampatti local 2	
31.	Settiyapatti local 1	
32.	Sorathur local 1	
33.	Venbarour local 1	
34.	Vengatesapuram local 1	
35.	Vengatesapuram local 2	
36.	Venkalam local 1	
37.	Veppanthattai local 1	
38.	Visvakudi local 1	
39.	Visvakudi local 2	

Fig. 1. Grain scanner images of sorghum landraces

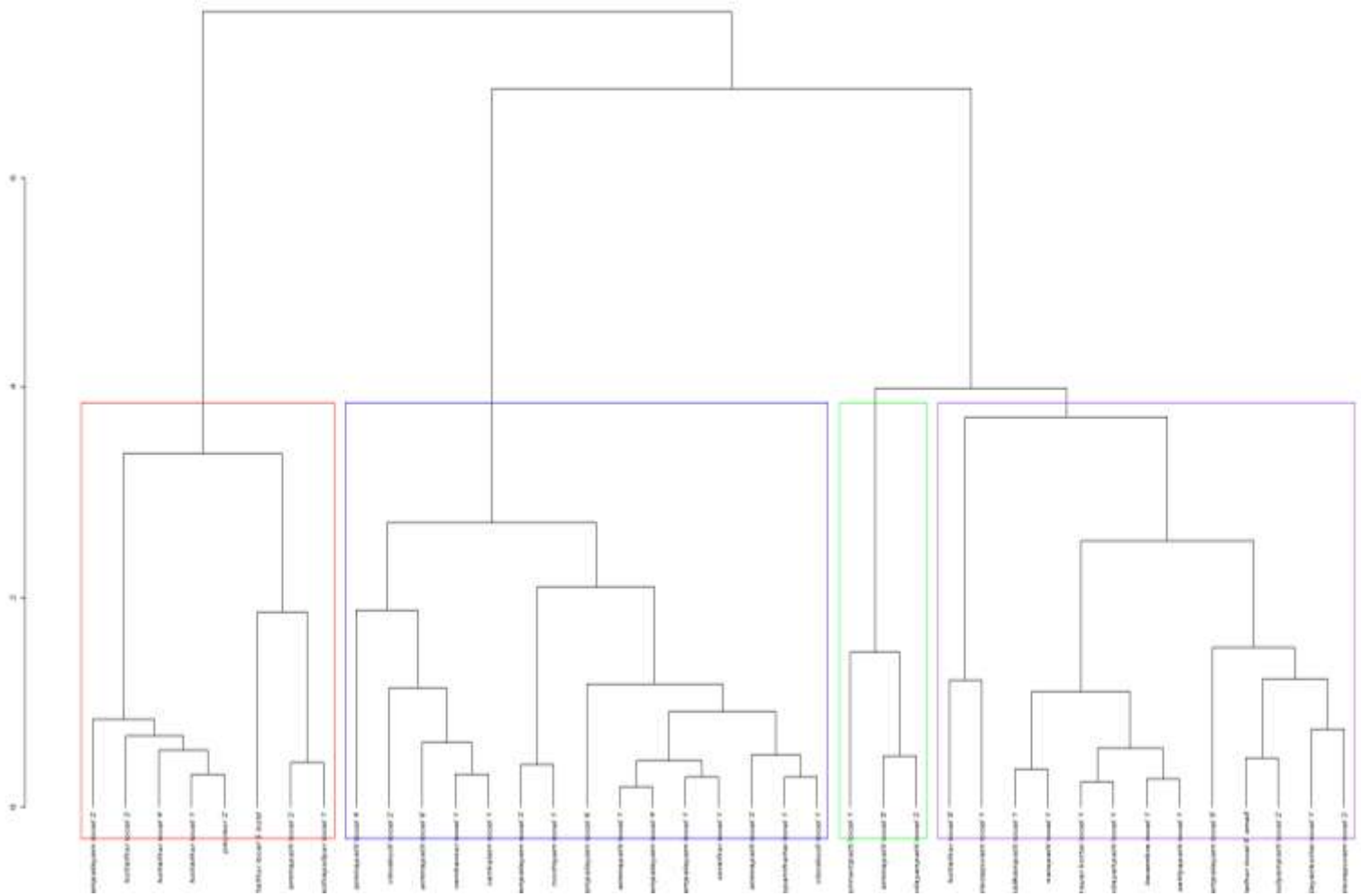


Fig. 2. An Image Analyser based dendrogram of 39 sorghum landraces

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