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Nutritional composition of millets and its genetic diversity reveal through SRAP markers

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Abstract
Millets are a highly nutritious, non-glutinous, acid-free food. As a result, they are soothing and easy to stomach. The AOAC method was used to determine the proximate composition of fifteen millets, including Banyard millet, Pearl millet, and Sorghum. True protein, total carbohydrate, fat, moisture, ash, fibre, calcium, and iron levels ranged from 5.54-21.47%, 42.58-85.59%, 3.14-6.62%, 7.36-13.97%, 1.07-5.32%, 2.99-12.44%, 10.6936.25 mg/100g, and 3.52-17.60 mg/100g, respectively. SRAP markers were used in genetic diversity studies. A total of 66 bands were generated by 10 SRAP primer combinations, 65 of which were polymorphic, with 56 shared and 9 unique bands. The polymorphism information content values for SRAP markers ranged from 0.60 (SRAP-08) to 0.86 (SRAP04), with an average of 0.78 per primer, and the SRAP primer index ranged from 1.81 (SRAP-08) to 7.82 (SRAP-04). GAFS-11 and BAR-1410 had the lowest similarity, while GHB-732 and GHB-1231 had the most.

Keywords: Proximate composition, srap (sequence related amplified polymorphism), millets

Introduction

Food's nutritional value is the most important variable in preserving human health and overall physical well-being. Due to people's ignorance, some agricultural products are not used as the primary source of nutrition for humans. Types of them are millets. Animal and bird feed is made from millet. Millet serves a variety of dietary and therapeutic purposes. These are underutilized and neglected crop because of little knowledge to people and some critical problems like lower cooking quality, taste and low bioavailability of millets. These issues are resolved, making them beneficial as a source of cash and food for low-income households to fight hunger.

Millets are calming and simple for digestion. They are regarded as the most easily digested and least allergic grains available. Around 8 percent protein and 4 percent fat can be found in millets. They are abundant in minerals and vitamins. In particular, millets are high in calcium.

Millets are essential in maintaining nutritional security for people all over the globe by offering calories and protein (Serna-Saldivar and Espinosa-Ramírez 2019) [22]. Millets are distinguished from cereals by their high calcium levels, dietary fibre, polyphenols, and protein (Amadou *et al*., 2013) [6]. Millets are a nutrient-dense food that contains 60-70 percent dietary carbs, 6-19 percent protein, 1.5-5 percent fat, 12-20 percent dietary fibre, 2-4 percent minerals, and various additional phytochemicals are high in vitamin B, magnesium, and antioxidants (Rathore and Singh, 2016) [20].

Under the National Food Security Mission (NFSM), the Department of Agriculture and Farmers Welfare (DA&FW) is carrying out a Sub-Mission on Nutri-Cereals (Millets) to increase the area, production, and productivity of millets, especially bajra.

The year 2018 marked the National Year of Millets. The Indian government suggested to the UN that 2023 be designated as the International Year of Millets (IYoM) in order to raise demand for millets both domestically and internationally and to provide people with wholesome meals. The United Nations General Assembly (UNGA) designated March 2023 as the International Year of Millets after India's petition received approval from 72 nations. Through funding for research and development, the government is making nutri-cereals more widely known and has set up three Centres of Excellence (CoE). Additionally, start-ups and entrepreneurs receive assistance in creating recipes and value-added products.

Barnyard millet is the fastest growing crop among all millets and can be harvested in a short period of nine weeks. Its grains contain 11.2(g) protein, 10.1(g) crude fibre, 4.4(g) minerals, 15.2(mg) Iron, 11(mg) calcium per 100gm grain. Also, it is a nutritive fodder for animals It provides animals with nutritious forage as well. Research on the nutritional makeup, glycaemic index, and health advantages of barnyard millets revealed that they are good for those with type-II diabetes (Ugare *et al*., 2014) [24]. The crop is prized for its high nutritional content, robust yield, and resistance to drought. The identification of genotypes with increased yield potential without sacrificing nutritional value requires an in-depth analysis of biodiversity and the state of the environment.

Resistant starch, soluble and insoluble dietary fibres, minerals, and antioxidants were discovered to be significantly abundant in pearl millet (Ragaee *et al*., 2006) [19]. According to (Ali *et al*., 2003) [5], it has roughly 92.5% dry matter, 2.1% ash, 2.8% crude fibre, 7.8% crude fat, 13.6% crude protein, and 63.2% starch. Energy: According to the Nutritive Value of Indian foods, pearl millet has a high energy content (361 Kcal/100g), which is comparable to that of major grains like wheat (346 Kcal/100g), rice (345 Kcal/100g), maize (125 Kcal/100g) and sorghum (349 Kcal/100g).

Sorghum grain composition is comparable to maize in many aspects. In terms of nutrition, sorghum has a lot of carbohydrates in the form of starch. Though digestibility is a barrier to the nutritional value, the protein level is substantial and on par with that of wheat and maize. Although its fat content is lower than that of maize, it is

higher than that of wheat or rice. There is a lot of nutritional fibre in certain sorghum cultivars.

Although phytates and polyphenols, two anti-nutritional elements found in millets, are usually restricted to the seed coat, milled millets are often devoid of these elements (Kumar, 2010) [14].

According to Li and Quiros (2001) [15], Sequence Related Amplified Polymorphism (SRAP) is a low-cost, straightforward, and effective method with excellent compliance and consistency. Open reading frames (ORFs) serve as a basis for these identifiers. Due to their impact on functional regions of the genome, these markers are also functional. Because of its unique forward and reverse primer combination (17 or 18 nucleotides long, with core sequences ranging from 13 to 14 bases long), the marker differs from ISSR and SCOT. This combination is employed for two-step amplification. First identified in *Brassica oleracea* L., the sequences CCGG in the forward primer and AATT in the reverse primer follow the primary 10 or 11 bases at the 5' end, which are non-specific and referred to as "filler" sequences. (Li and Quiros, 2001 [15] and Budak *et al*., 2004 [8]).

SRAP markers can be used for linkage map construction (Yeboah *et al*. 2007) [26], genomic and cDNA fingerprinting, gene tagging (Li and Quiros 2001) [15], genetic diversity analysis (Li *et al*. 2009) [16], map-based cloning (Zhang *et al*. 2010) [27], hybrid identification (Kumar Mishra *et al*. 2011) [13], and sex determination (Zhou *et al*. 2011) [28]. SRAP markers are more powerful than SSR, ISSR, or RAPD markers for revealing genetic diversity among closely related cultivars (Budak *et al*. 2004) [8]. SRAP markers were employed in our work to examine genetic diversity in millets' significant cultivars and sophisticated breeding lines.

As a result, this technique is frequently applied to evaluate species diversity and genetic diversity. SRAP markers are recognised to be more stable, less complicated, and more reproducible according to this special primer design.

Materials and Methods

Different types of millets which were grown predominantly in Agricultural Research Station, the seeds of fifteen varieties of millet were used for the present study, in which five varieties of Barnyard millet were received from the Indian Institute of Millet Research, IIMR Hyderabad; five varieties of Pearl millet were received from the Main Pearl Millet Research Station, JAU, Jamnagar; and five varieties of Sorghum were received from the Main Fodder Research Station, AAU, Anand.

1. Crop and Varieties:

Fifteen varieties of millets namely Banyard millet (BAR-1406, BAR-1407, BAR1408, BAR-1409, BAR-1410), Pearl millet varieties (GHB-1129, GHB-1231, GHB-1225,
GHB-732, GHB-905) and Sorghum varieties (GAFS-12, GAFS-11, S-1049, AFS-69, AFS53) were analyzed for their proximate composition like true protein, total carbohydrate, fat, ash, moisture, fibre, iron and calcium.

2. Proximate parameters

2.1. True Protein

True protein was estimated by Folin-Lowry method (Lowry *et al*., 1951) [17] First, 0.2 g of grinded millet seed was extracted with 10 ml of 0.1 N NaOH. A suitable aliquot of 0.1 ml was taken, and a total volume of 3 ml was made with distilled water and 5.0 ml of reagent C [A: 2% Sodium Carbonate in 0.1 N Sodium Hydroxide]. B: 0.5 % Copper sulfate in 1 % Sodium Potassium Tartrate (Prepared fresh) was prepared by mixing 50 ml of reagent A with 1 ml of reagent B (mixed prior to use) in a ratio of 50:1. It was added and mixed properly. After 10 min, 0.5 ml of reagent D (D: Folin-Ciocalteau reagent diluted with distilled water in a 1:1 ratio) was added, thoroughly mixed, and kept for 30 minutes at room temperature for color development. The same protocol was followed for the working standard series. The absorbance was measured at 660 nm and expressed as %. The protein content was calculated by using Bovine serum albumin as a standard. The amount of protein present in the sample was calculated using the appropriate formula.

$$\textbf{True Protein} = \frac{\text{GF} \times \text{OD} \times \text{Toatal Volume} \times 100 \times 10^{-6}}{\text{Sample Aliquot} \times \text{Weight of sample (g)}}$$

2.2. Total Carbohydrates

The determination of total carbohydrate content was done by the Anthrone method as described by Hedge and Hofreiter (1962) [11]. Weigh 1 g of the grinded sample into a flask. It was hydrolysed by keeping it in a boiling water bath for 3 hours with 5 ml of 2 N HCL and cooling at room temperature. Neutralize it with solid sodium carbonate. Centrifuge and volume made up to 100 ml. The supernatant was then collected, and a 1 mL aliquot was taken for analysis. Standard glucose + stock solution is prepared by dissolving 100 mg glucose in 100 ml of water. For a working standard, 10 ml of stock diluted to 100 ml with distilled water Prepare the set of standards by taking 0, 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard; ‘0’ serves as a blank. The volume was made up to 1 ml in all the tubes, including the sample tubes, by adding distilled water. Then, add 4 ml of fresh anthrone reagent (Dissolve 200 mg of anthrone in 100 ml of ice-cold 95% H2SO4). All tubes are heated for ten minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630 nm. A standard graph was drawn by plotting the concentration of the standard on the X-axis versus absorbance on the Y-axis. The total carbohydrate content was calculated with the help of a reference curve prepared by using glucose as a standard.

2.3. Oil Content

Millet having oil content of about 2 to 5 %. Millet oil is extracted from whole ground millet seed using hexane as the solvent. Oil content was determined by Soxhlet extraction (AC, AO, 2005a) [3]. The sample weighed accurately 5 g and put into thimbles filled with 150 ml hexane. These thimbles were plugged with cotton wool. Then the Soxhlet apparatus was assembled and allowed to reflux for 4 hr. Carefully thimble was removed. Then air dried at room temperature until it was without hexane. Per cent oil content was calculated using the formula:

$$\textbf{Oil content (\%)} = \frac{\text{weight of the flask + Oil – Weight of empty flask}}{\text{Weight of the sample (gm)} \times 100}$$

2.4. Calcium and Iron Content

Sample preparation of millet seeds grind in mixture to prepare fine powder and for analysis purpose as per Parakhia *et.al* (2017) [18]. Sample digestion 0.5gm fine

powder of sample in digestion tube then add 7ml HNO_3 Let it pre-digest for 4-6 hour, Digest the sample by Mars-6 Microwave Digestion system. Transfer the solution to 50 ml volumetric flask and make up the solution 50 ml by miliQ water and store this solution to reagent bottle for further analysis. Trace elements analysis was carried out by using MP-AES instrument. This instrument was standardized by NIST (National Institute of Standards and Technology) certified standard. Sample were run on triplicate and its average value was utilize as concentration in sample.

2.5. Moisture Content

The moisture content of millet samples was determined in a hot air oven through drying method according to the procedure described in AC, AO (1997a) [1]. The moisture content of the samples was determined by the differences of weighing 1 g of sample into a petri plate and dried at a temperature of 80°C for 3 hours in hot air oven. The calculation of moisture content was done by using following formula.

$$\textbf{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of oven dried sample}}{\text{Weight of fresh sample}} \times 100$$

2.6. Ash

Ash is a solid and non-aqueous powdery residue which was obtained after the sample was completely burnt at a temperature of 600 °C in a muffle furnace. About 0.2g of finely ground dried sample was weighed into a porcelain crucible and incinerated at 600 °C for 6 hrs. in an ashing muffle furnace until ash was obtained (AC, AO, 2005b) [4]. The ash content in the millet seed sample was calculated as follows:

$$\textbf{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample Taken}} \times 100$$

2.7. Fiber Content

The crude fiber content of the samples was determined by using (AC, AO, 1997b) [2] method. The sample was allowed to boil with 1.25% diluted H_2SO_4, washed with water, further boiled with 1.25% diluted sodium hydroxide and the remaining residue after digestion was taken as crude fiber. 1 g of moisture and fat free sample was weighed and were kept in the fibre bags. The glass spacer was kept into the bags. The bags were loaded in the sample carousel at the previewed positions (positions 1-12). The sample carousel was put into the glass container carefully. The glass container axial was placed on the previewed position of the hot plate. The programme was started in the Fibretherm (Gerhardt). After completion of the program, the fibre bags were removed. The residue was transferred to crucible and weighed (W1) and dried overnight in oven. Later it was Transferred to dessicator for cooling and weighed (W2). The crucible was heated in a muffle furnace at 600˚C for 1.5 hours. Then crucible was cooled in desiccators and weighed (W3).

Weight of Empty Crucible (g)= (W0) g
Weight of the sample = (W1) g
Weight of the crucible + sample before heating at 600ºC = (W2) g
Weight of the crucible + sample after heating at 600ºC = (W3) g

$$\textbf{Fiber (\%)} = \frac{(W2-W0)-(W3-W0)}{W1} \times 100$$

DNA isolation

Total genomic DNA was isolated from young and healthy leaf tissue of 3–4-week-old plants using the modified CTAB method of Doyle, 1990 [10] as (Dhingani *et al*., 2012) [9]. The extracted DNA was quantified using Nanodrop N.D.1000. The quality DNA was determined on 0.8% agarose gel. DNA was diluted to 25 ng/ml and stored at -20 °C for SRAP marker amplification.

1. SRAP marker analysis

PCR was performed in a 15 µl reaction volume containing 10 × PCR buffer (containing 25 mM MgCl2), dNTPs mix (2.5 mM each), 0.6 µM of primers (forward and reverse), 1U Taq DNA Polymerase and 25 ng DNA. 90 SRAP primer combinations were screened by PCR. PCR conditions included initial denaturation at 96 °C for 4 min and 5 cycles of denaturation at 94 °C for 1 min, primer annealing at 35 °C for 1.15 min, and primer extension at 72 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 50 °C for 1.15 min, and primer extension at 72 °C for 2 min. The amplification was completed with a 10 min final extension at 72 °C.

PCR products were subjected to electrophoresis with marker DNA of known molecular weight in 1.5 % agarose gel. After electrophoresis, the gel was carefully taken out of the casting tray and photographed in gel documentation system.

2. Data analysis

Due to multiple bands per primer, the amplified products of SRAP markers were scored as 1/0, where 1 = presence of band and 0 = absence of band. From these 0/1 data, PIC value (Polymorphism information content) and primer index Molecular weights of the bands were estimated by using 100 bp DNA ladder as standard. Genetic similarity between genotypes was calculated using on Jaccard's similarity (J) coefficient by SIMQUAL program and unweighted pair group method average (UPGMA) dendrogram was constructed using SAHN clustering algorithm of NTSYSpc v. 2.20 (Rohlf
1998) [21].

Results and Discussion

Millets namely Banyard millet (BAR-1406, BAR-1407, BAR-1408, BAR-1409,
BAR-1410), Pearl millet varieties (GHB-1129, GHB-1231, GHB-1225, GHB-732, GHB-905) Sorghum varieties (GAFS-12, GAFS-11, S-1049, AFS-69, AFS-53) were analyzed for their nutritive values such as ash, moisture, protein, fat, crude fibre, iron, and calcium. The nutritive composition of millets was presented in Table 1.

Table 1: Proximate composition of millets

Sr. No.	Varieties	True protein (%)	Total carbohy drates (%)	Total fat (%)	Calciu m (mg/100g)	Iron (mg/100 g)	Moist ure content (%)	Ash (%)	Fiber content (%)
1	BAR-1406	5.54	72.37	6.62	22.62	9.48	9.34	4.49	11.33

2	BAR-1407	5.71	67.32	5.70	30.82	3.52	9.28	5.06	12.35
3	BAR-1408	6.63	66.46	4.96	35.57	6.22	7.74	4.63	12.43
4	BAR-1409	6.41	68.35	6.14	27.62	7.51	9.62	5.32	11.69
5	BAR-1410	8.53	68.45	4.47	27.94	6.68	13.97	2.42	12.44
6	GHB-1129	9.59	78.42	4.58	19.81	11.77	7.62	1.28	3.55
7	GHB-1231	7.56	42.58	6.33	13.73	10.79	7.70	1.59	3.86
8	GHB-1225	7.67	78.16	4.43	15.42	17.60	8.03	1.58	2.99
9	GHB-732	7.67	84.14	5.23	36.25	10.68	7.43	1.07	3.82
10	GHB-905	10.44	85.59	5.81	10.69	7.46	8.42	2.03	3.18
11	GAFS-12	10.59	67.83	4.06	25.40	7.21	8.41	3.05	7.10
12	GAFS-11	8.38	78.12	3.58	13.89	14.52	7.73	1.41	3.15
13	S-1049	9.69	84.12	3.54	15.64	6.38	7.36	1.83	3.36
14	AFS-69	21.47	63.48	3.39	22.94	7.77	8.17	3.30	11.79
15	AFS-53	13.42	72.05	3.14	16.63	11.25	7.63	4.38	8.70
S.Em.±		0.14	0.42	0.08	0.16	0.11	0.17	0.04	0.09
C.D. at 5 %		0.42	1.20	0.22	0.46	0.31	0.50	0.10	0.26
C.V. %		3.31	1.69	2.07	1.99	1.71	3.91	3.87	4.06

Proximate composition of Barnyard millet

Proximate composition of barnyard millet (as determine in this work) as follows; protein (6.49%), ash (4.23%), fibre (12.04%), fat (5.52%), moisture (9.8%) and carbohydrate (68.56%). And minerals calcium and iron 28.6 and 6.36 mg/100g respectively (Fig.1 and 2). Verma *et al.,* (2015) [25] discovered that the moisture content of Barnyard Millet 11.93, after analyzing the nutritional composition of grains. The total ash, crude protein, crude fat, crude fiber, and carbohydrate for Barnyard Millet, they were 4.27, 6.93, 2.02, 2.98, and 71.87 %, respectively.

Proximate composition of Pearl millet

Proximate composition of pearl millet (as determine in this work) as follows; protein (8.5%), ash (1.47%), fibre (3.46%), fat (5.23%), moisture (7.83%) and carbohydrate (71.58%). And minerals calcium and iron 17.46 and 11.22 mg/100g respectively (Fig.1 and 2).

Siroha *et al.,* (2016) [23] showed Characterization of Indian Pearl Millet Cultivars for Proximate Composition, and Anti-Nutritional Contents, six Indian Pearl Millet cultivars: HC10, HHB-67, HHB-223, HHB-226, W-445 and GHB-732 were used in this study.

Investigations showed that Pearl Millet contained 6.5-7.7 % moisture, 1.65-1.90 % ash, 5.14-
6.60 % fat, 9.77-11.65 % protein, 2.9-3.83 % crude fibre and 69.49-3.85 % carbohydrates.

Proximate composition of Sorghum

Proximate composition of sorghum (as determine in this work) as follows; protein (11.98%), ash (2.58%), fibre (5.99%), fat (3.53%), moisture (7.85%) and carbohydrate (72.76%). And minerals calcium and iron 18.4 and 8.98 mg/100g respectively (Fig.1 and 2). Jihmo *et al*., (2017) [12] studied proximate analysis of selected Sorghum cultivars to reveal that protein content ranged from 6.23-13.81 %, carbohydrate 65.57-76.28 %, lipid 3.60-10.54 %, fiber 1.65-7.94 %, ash 1.12-1.68 %, and moisture 9.75-16.32 %.

There are a total of eight proximate parameters in this study. For the experiment, 15 different millet varieties were chosen, and the variation AFS-69 of sorghum had the highest true protein content value of 21.47 %, while the lowest value of true protein content was recorded in barnyard millet, the variety BAR-1406, with 5.54%. The total carbohydrate content was recorded at a maximum of 85.59 % in pearl millet, the variety GHB-905, while a minimum of 42.58 % was recorded in pearl millet, the variety GHB-1231. Elements content like calcium was maximum in the variety GHB-732 of pearl millet with 36.25 mg/100g, and the minimum was in the variety GHB-905 of pearl millet with 10.69 mg/100g. Elements content like iron was maximum in the variety GHB-1232 of pearl millet with 17.60 mg/100g, and the minimum was in the variety BAR-1407 of barnyard millet with 3.52 mg/100g. The highest ash content (5.3 %) was observed in the variety BAR-1409 of barnyard millet and the lowest (1.07 %) was in the variety GHB-732 of pearl millet. Maximum oil content was recorded in the variety BAR-1406 of banyard millet with 7.84 %, while the minimum was in the variety AFS-53 of sorghum with 3.14 %. Maximum mean value of fiber was observed in the variety BAR-1410 of barnyard millet with 12.44 % and the lowest 2.99 % was in the variety GHB-1225 of pearl millet (Table 1).

Genetic diversity analysis through SRAP markers

Total 90 combinations of SRAP primer were used for screening, out of which 80 primers did not amplify any PCR product while 10 primer combinations gave satisfactory results. For example, two combinations of primers gave polymorphic bands (Fig. 5). The agarose gel electrophoresis is (1.5 %) was used to separate amplified genomic DNA of fifteen varieties of millets are discussed as under.

Table 2: List of SRAP primer: Forward and Reverse primer combination

Sr. No.	Primer name	Forward primer	Reverse primer
1	SRAP-01	Me-5	Em-3
2	SRAP-02	Me-5	Em-2
3	SRAP-03	Me-4	Em-8
4	SRAP-04	Me-2	Em-1
5	SRAP-05	Me-4	Em-2
6	SRAP-06	Me-4	Em-6
7	SRAP-07	Me-2	Em-12
8	SRAP-08	Me-10	Em-8
9	SRAP-09	Me-10	Em-9
10	SRAP-10	Me-10	Em-10

Total 10 SRAP primer combinations amplified to generate the 66 bands (Table 2). The SRAP 4 primer combination produced maximum numbers of 9 bands, while SRAP 8 produced minimum number of 3 bands. Out of which, 65 bands were polymorphic and 1 band was monomorphic. Among the 65 polymorphic bands, 56 bands were shared polymorphic within two or more varieties, while 9 bands were unique-polymorphic and ranged in size from 108-2103 bp as shown in Table 3.

Table 3: Size, number of amplified bands, per cent polymorphism and PICobtained by SRAP primers in 15 millet varieties.

Sr. No.	SRAP Primer	Band Size (bp)	Total No. of Bands (A)	Polymorphic Bands (B)			Mono- morphic Band	% Polymorphism (B/A)	PIC	SPI
				S	U	T				
1	SRAP-01	118-2103	8	6	1	7	1	87.5	0.85	6.76
2	SRAP-02	108-1168	8	8	0	8	0	100.0	0.85	6.85
3	SRAP-03	114-1459	8	8	0	8	0	100.0	0.85	6.83
4	SRAP-04	118-2061	9	7	2	9	0	100.0	0.86	7.82
5	SRAP-05	213-875	5	3	2	5	0	100.0	0.74	3.72
6	SRAP-06	153-776	7	6	1	7	0	100.0	0.77	5.45
7	SRAP-07	115-1319	8	8	0	8	0	100.0	0.85	6.85
8	SRAP-08	157-399	3	3	0	3	0	100.0	0.6	1.81
9	SRAP-09	244-634	4	3	1	4	0	100.0	0.7	2.8
10	SRAP-10	133-1415	6	4	2	6	0	0.0	0.74	4.48
TOTAL			66	56	9	65	1	-	-	-
AVERAGE			-	-	-	6.5	0.1	98.75	0.78	5.33

The per cent polymorphism obtained for SRAP primers were ranged from 87.5 % to 100 % with an average value of 98.75 % per primer. The Polymorphism Information Content (PIC) values for SRAP markers were ranged from 0.60 to 0.86 with an average value of 0.78 per primer and SRAP primer index (SPI) differed from 1.81 to 7.82 with an average value of
5.33 as presented in Table 3.

Genetic similarity was determined for each pair of fifteen varieties by Jaccard's similarity coefficient which revealed that the lowest similarity of 5 % was noticed between GAFS-11and BAR-1410, while the highest similarity of 73.3 % was noticed between GHB732 and GHB-1231 varieties (Table 4).

Table 4: Jaccard's similarity coefficient of millet varieties based on SRAP data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.000														
2	0.659	1.000													
3	0.617	0.690	1.000												
4	0.512	0.538	0.537	1.000											
5	0.636	0.718	0.591	0.595	1.000										
6	0.400	0.327	0.308	0.357	0.362	1.000									
7	0.396	0.353	0.469	0.386	0.417	0.535	1.000								
8	0.314	0.240	0.327	0.286	0.298	0.595	0.537	1.000							
9	0.286	0.261	0.271	0.282	0.326	0.486	0.475	0.733	1.000						
10	0.224	0.250	0.261	0.237	0.286	0.486	0.400	0.581	0.552	1.000					
11	0.205	0.143	0.133	0.176	0.146	0.286	0.256	0.273	0.267	0.250	1.000				
12	0.116	0.075	0.070	0.129	0.050	0.176	0.128	0.196	0.179	0.200	0.533	1.000			
13	0.190	0.125	0.091	0.088	0.100	0.200	0.150	0.219	0.207	0.185	0.471	0.667	1.000		
14	0.163	0.125	0.143	0.088	0.100	0.200	0.179	0.258	0.207	0.185	0.389	0.429	0.467	1.000	
15	0.227	0.196	0.156	0.139	0.171	0.278	0.220	0.303	0.258	0.241	0.381	0.500	0.625	0.444	1.000

(1: BAR-1406, 2: BAR-1407, 3: BAR-1408, 4: BAR-1409, 5: BAR-1410, 6: GHB-1129, 7: GHB-1231, 8: GHB-1225, 9: GHB-732, 10: GHB-905, 11: GAFS-12, 12: GAFS-11, 13: S-1049, 14: AFS-69, 15: AFS-53)

Cluster Analysis of SRAP

The cophenetic correlation coefficient value of 0.94 was observed (Fig. 4), demonstrating a high association for these genotypes to a specific cluster represented in the dendrogram.

The dendrogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for SRAP data of fifteen millet varieties (Table 4 and Fig. 3). The 15 millet varieties were grouped into two main clusters: cluster-I and cluster-II, which shared 18 % similarity. The cluster-I was divided into two subclusters-A and B with nearly 32 % similarity and both contained a total of 10 varieties (Fig. 3). Subcluster-A was further bifurcated into two groups A1 and A2 which had nearly 54 % likeness. Group A1 consists of four varieties such as BAR-1406, BAR-1407, BAR-1408 and BAR-1410 having nearly 63 % similarity, while group A2 consisted of only one variety *viz*., BAR-1409 having nearly 54 % similarity. Subcluster-B was further bifurcated into two groups B1 and B2 which had nearly 49 % likeness. Group B1 consisted of two varieties such as GHB-1129 and GHB1231 having nearly 54 % similarity, while group B2 contained of three varieties *viz*., GHB1225, GHB-732 and GHB-905 having nearly 57 % similarity.

The cluster-II was divided into two subclusters C and D with nearly 44 % similarity. Subcluster C consists of four varieties GAFS-12, GAFS-11, GAFS-1049 and AFS-53 while, subcluster D consists of only one variety which was AFS-69.

Conclusion

In this study, 15 millet varieties of three different millets were studied for a total of 8 proximate parameters. Sorghum had the highest true protein and total carbohydrate content, with 11.98% and 72.76%, respectively, compared to the other two millets, barnyard millet and pearl millet. Comparing pearl millet to sorghum and barnyard millet, iron concentration was greatest in pearl millet at 11.22 mg/100g. In addition, compared to pearl millet and sorghum, which have lesser contents, barnyard millet has the highest concentration of total fat, moisture, ash, fibre, and calcium, with 5.52%, 9.8%, 4.23%, 12.04%, and 28.6 mg/100g, respectively (Fig. 1). The nutritional profiles of several millets crops were described, which was helpful for improving the nutritious content of grains through plant breeding techniques.

The outcome of this study suggested that the identified millet varieties—barnyard millet, pearl millet, and

sorghum—are all excellent providers of nutrients that can significantly increase the consumption of certain minerals. Therefore, these chosen millet varieties have the potential to aid in eradicating hunger as well as malnutrition among India's vulnerable groups when appropriately utilized through successful product development programs.

Prior to now, research in millets using molecular markers have only included less repeatable dominant markers. Despite millets' importance, little attention has historically been given to their genetic development. The results of the current study using SRAP demonstrated that this marker system will be an important tool to assess millet's genetic diversity and eventually improve it. Finally, it can be concluded that millets showed moderate diversity as evidenced by SRAP markers. This suggested that in order for markers to be effectively incorporated, millets germplasm's genetic base needed to be increased by introduction, mutagenesis, and distant hybridization.

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