Indonesian Sorghum Genetic Diversities Based on Simple Sequence Repeat Markers

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***Abstract*— Information on genetic diversity can be obtained by analyzing molecular data using bioinformatics. Molecular data of 15 sorghum varieties based on 38 SSR (Simple Sequence Repeat) markers were analyzed to obtain PIC (Polymorphic Information Content), genetic diversity, and phylogenetic tree. Genetic information in the form of the number of alleles (Na), heterozygosity value observed (Ho), and gene diversity or heterozygosity expected (He). Phylogenetic tree analysis was conducted using the Neighbor-Joining (NJ) method. The analysis showed that the primers used were very informative for detecting sorghum genetic diversity with an average PIC value of 0.54. The number of alleles obtained was 142 from 38 primers and the average number of alleles per primer was 3.82. The phylogenetic tree obtained showed that fifteen sorghum were divided into three clusters, and only the Samurai 2 variety was included in the third cluster. Genetic diversity brought was in the high category with a He value of 0.57. This genetic diversity information can be used to develop breeding strategies and conservation programs of sorghum.**

Keywords*— *genetic variation, molecular markers, microsatellite, Neighbor-joining, Sorghum

I. INTRODUCTION

Bioinformatics and molecular markers are integral to analyzing genetic diversity. Data generated from molecular markers requires bioinformatics analysis to be interpreted. Bioinformatics is used to process, organize, and store biological data. The development of bioinformatics encourages massive biotechnology research, especially research related to sequence data. Many bioinformatics applications are developed for various analyses, such as sequence analysis, alignment, annotation, molecular phylogenetics, and others [1].

Phylogenetics is used to show the relationship among the objects of research. One of the methods used to build phylogenetics is Neighbor joining (NJ). Darwin has been widely used to construct phylogenetic trees using the NJ method. GeneAlex is a general application for analyzing molecular data to obtain genetic diversity information. DARwin generates phylogenetic and genetic distance information from single data, allelic data, and sequences. This software can construct a phylogenetic tree using the Neighbor-joining or UPGMA method [2]. Files are stored in text form for single and allelic data, while sequence data is in FASTA, PHYLIP, NEXUS, and MEGA form [2]. GenAlEx is commonly used to obtain information on the number of alleles (Na), heterozygosity observed (Ho), and genetic diversity (heterozygosity expected (He)). The data can be binary, codominant diploid, DNA sequence, and haploid data [3].

Using molecular markers is one of the best methods to obtain information about genetic diversity. SSR is a molecular marker that has been widely used to analyze genetic diversity. This marker can also be used for genetic purity testing, Marker-Assisted Selection (MAS) QTL mapping, and linkage map construction [4]. SSR is a codominant marker, a highly informative marker-related species, and reproducible [5], [6]. These have made SSR the most widely used molecular marker for over 20 years [6].

SSR marker has been used to analyze the genetic diversity of sorghum. Research conducted [7] used 11 SSR markers to analyze 80 accessions of Ethiopian sorghum. A total of 107 Ethiopian sorghum landraces were analyzed for genetic diversity by [8] using 12 SSR markers. [9] used 15 SSR markers to analyze 100 Ethiopian sorghum's diversity and genetic structure. Research [10] also used 43 SSR markers and morphological variations to analyze the genetic diversity of 32 sweet sorghum from various provinces in China, but the genetic diversity obtained was smaller than the genetic diversity of sorghum from Ethiopia. Ethiopia is the center of origin of sorghum, so the genetic diversity in this country is higher than in China.

In Indonesia, sorghum genetic diversity was analyzed by observing morphological variations. [11] observed agro-morphology and physiology traits of nine sorghum landraces from East Java. The same technique was also used [12]–[14] to analyze the genetic diversity of sorghum, which was then used for breeding programs.

The aims of this are 1) obtaining information on the genetic diversity, 2) constructing a phylogenetic tree using NJ to obtain genetic relationship information, 3) obtaining information on the ability of 50 SSR primers to detect variation for analyzing the genetic diversity of 15 superior sorghum varieties.

II. Literature Review

DARwin and GenAlEx are used by [15] to analyze the genetic diversity of 481 genotypes of *Vitis vinifera* based on 13 SSR primers. Nine primers (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, and VrZAG79) are standard primers used for the identification of grape cultivars in seven European countries (Generes81/GrapeGen06 set) and 4 primers (ISV2, ISV3, ISV4, and VMCNG4b9) are primers routinely used in "Consiglio per la Ricerca in Agricolturae l'analisi dell'Economia Agraria-Uva da Tavola" (CREA-UTV, Turi, Italia). GenomeLabTM GeXP Genetic Analysis System (Beckman Coulter, Brea, USA) was used for PCR product separation and determining the size of the resulting band using manufacturing software. The results showed that the 13 primers used produced numbers of alleles ranging from 11-18 with an average of 12.92. The average Ho and He were 0.84 and 0.82, respectively. The PIC value of each primary ranged from 0.623 (primer ISV3) to 0.859 (primer MD28), with an average of 0.79.

Fifty-seven SSR primers were developed from *Amaranthus hypochondriacus* genome version 2.0. (Amaranth Genomic Resource Database (AGRDB)) using MISA software. These primers were used to extract molecular data for 94 Amaranth accessions consisting of 90 accessions from India and 4 exotic accessions from Russia (1) and USA (3). The samples were obtained from the National Gene Bank, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The number of alleles, Ho, and He were obtained using GenAlex v6.5. The dendrogram was created based on the NJ method using DARwin v6.0.021. Fifty-seven primers were tested, but only 36 primers produced polymorphic bands. The total number of polymorphic bands produced was 138, with an average of 3.83. The highest PIC value was 0.78 (AhySSR29571), whereas the lowest was 0.03 (primer AhySSR31577), and the average value was 0.40. Average genetic diversity (He) and Ho were 0.46 and 0.61, respectively. The phylogenetic tree showed that 94 accessions were divided into three clusters, and clustering was unrelated to the origin of accessions [16].

Bacterial Artificial Chromosome (BAC)-End Sequence (BESs) was used to develop 35 SSR primers. The primers were used to genetically analyze 48 genotypes of pigeonpea [*Cajanus cajan* (L.) Millsp] consisted of 7 varieties, 12 breeding lines, and 29 landraces originating from Tanzania and Kenya. Data were analyzed using GenAlex 6.5 and DARwin, with UPGMA method used to create a phylogenetic tree. Only 33 primers were able to produce polymorphic bands, and the total number of alleles generated was 155 alleles. Primers CcM0443 and CcM0246 detected the highest number of alleles, 11 alleles. Primers CcM0484, CcM0594, CcM0673, CcM0785, CcM1045, and CcM1251 detected only 2 alleles, the smallest number. The Average alleles were 4.78 alleles. The largest PIC was 0.84 (Primer CcM0246), and the smallest was 0.08 (Primer CcM2505), 0.47 was the average of the PIC. The Ho value was 0.28, and the genetic diversity, represented by He, was 0.48. The tested genotype was divided into three clusters, which were undivided by origin or type of genotype [17].

III. Materials and Methods

A. Materials

The genetic materials used were 15 superior varieties of Indonesian sorghum (Bioguma 1, Bioguma 2, Bioguma 3, Soper 9, Soper 7, Soper 6, Kawali, Numbu, Soper 6, Suri 4, UPCA). A total of 50 SSR primers were tested [18], but only 38 were used for genetic diversity analysis (Table 1). Primers that produce monomorphic bands or do not produce bands were excluded from the analysis. DNA Extraction was done using KIT (Plant mini-KIT preparation, GeneAid) following the manufacturer's instructions. Electrophoresis was performed on 0.8% agarose to determine the quality of DNA. Meanwhile, to measure the quantity of DNA, qubit was utilized.

Amplification was performed using a PCR Sensoques thermocycler machine. Each reaction contains primers forward and reverse (10ηg μL^{-1}), DNA (20ηg μL^{-1}), and PCR mix (Promega, USA). The PCR method was carried out with an initial denaturation program of 95°C for 5 min, denaturation at 95°C for 30 sec, temperature-specific aneling of each primer ranging from 50-60°C for 30 sec, extension at 72°C for 1 min, denaturation until the extension was repeated for 35 cycles, final extension at 72°C for 10 sec. Separation of PCR product was carried out using 8% polyacrylamide gel.

B. Methods

Gels were evaluated and given a score, starting from 1 for the smallest bands and increasing accordingly. If no band was present, a 0 score was given. Polymorphic Information Content (PIC) was calculated using equation 1 [19]. Ho and He were calculated with equations 2 and 3 using the GenAlex application [20]. The phylogenetic tree construct was conducted using Neighbor-Joining (NJ) in the DARwin application. Equations 4 and 5 were used to calculate dissimilarity and phylogenetic constructs [2] [21].

$$PIC = 1 - \sum_{i=1}^{n} Pi^2 - \left(\sum_{i=1}^{n} Pj^2\right)^2 + \sum_{i=1}^{n} Pi^4 \quad (1)$$

Where n is numbers of alleles; P_{ij} and P_{ik} are frequency of alellel jth and kth in marker i.

$$H_o = \frac{No.\ of\ Hets}{N} \quad (2)$$

Where N is sample size and *No._of_Hets* is genotype heterozygous sample.

$$H_e = 1 - \sum P_i^2 \quad (3)$$

Where P_i is an allele frequency

$$d_{ij} = 1 - \frac{1}{L}\sum_{l=1}^{L}\frac{m_l}{\pi} \quad (4)$$

Where d_{ij} is dissimilarity beitween unit *i* and *j*, *L* is number of primers, π is number of ploidy samples, and m_l is number of matching alleles for locus *l*.

$$(i,j) = (r-2)d(i,j) = \sum_{k=1}^{r} d(i,k) - \sum_{K=1}^{r} d(j,k) \quad (5)$$

Where *i,j is taxon pair, d*(*i,j*) is distance between taxon, *r* is current number of taxa and sums run on the taxon.

C. Data Set.

The data used was allele data from bands scoring on polyacrylamide gel. The data consisted of 76 variations from 38 primers where each primary consisted of two variables. Each variable is numeric data from 1 - 7. An example of a partial data set is shown in Table 1. The data in Table 1 were used to produce PIC values, phylogenetic and genetic diversity.

TABLE I. PART OF THE DATA SET

Sorghum Varieties	Primer							
	xtxp057		xtxp177		xtxp225		xtxp145	
Bioguma 1	2	4	1	1	2	2	2	4
Mandau	2	4	4	4	1	1	1	5
Super 2	1	4	2	2	2	2	4	7

The data used were derived from the scoring of bands resulting from separating PCR products on polyacrylamide gels. Table 1. shows the scoring data from three varieties on four primers. Each primer consisted of two variables representing the allele or gene variation in varieties. The purpose method is shown in Figure 1

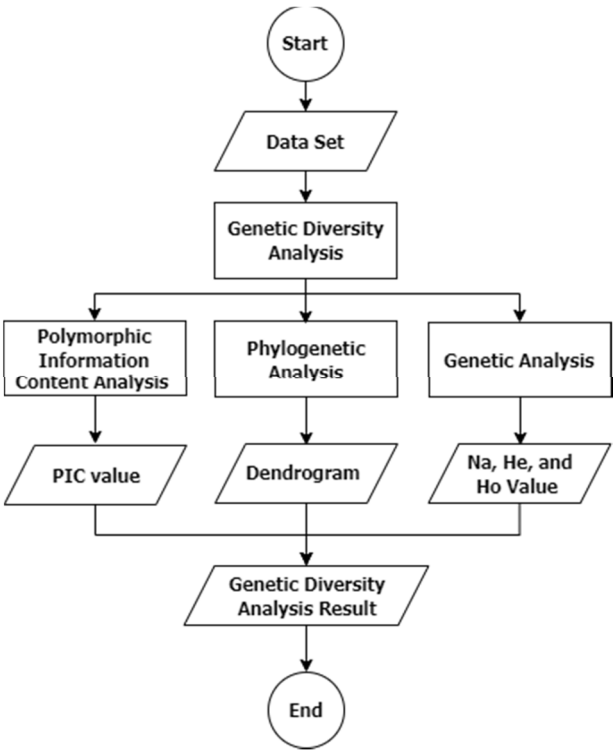

Fig. 1. Purpose method used for genetic diversity analysis of 15 sorghum varieties based on SSR marker

Pseudocode Phylogenetic Analysis

```
varietyDataset ← dataset all variety
ploidy = 2 ← numbe of ploidy
locus = 38 ← number of locus
matrixRange = 2*locus ← range of matrix
columns ← list of content dataset
rows ← list of index content dataset
varieties = []
smlist = []
contentRows = 1
contentColumns = 0
contentIndex = 0
for i in rows
    k = 0 ← increment
    addVarieties() ← add array to varieties
    for j in rangeOfColumns ← range of length columns
        if k % 2 == 0
            lc1 = varietyDataset[j][i]
            lc2 = varietyDataset[j+1][i]
            addVarieties(lc1, lc2)
        Endif
        k+=1
    endfor
endfor
for i in range(105)
    content = 0
    mllist = []
    mlindex = []
    if i == 0
        addSmlist() ← add array to smlist
    endif
    for j in varieties[contentColumns]
        ml = 0
        if j[0] in varieties[contentRows][ content]
            ml+=1
        endif
        if j[1] in varieties[contentRows][ content]
            ml+=1
        endif
        mlphy = ml / ploidy
        addMllist(mlphy) ← add value to mllist
        content += 1
    endfor
    totalml = sum(mllist)
    sm = 1-((1 / locus)*totalml)
    addSmlist[contentIndex] = sm ← add value to list smlist with
                                   index
    contentRows += 1
    mlindex += 1
    if contentRows > 14
        contentColumns += 1
        contentRows += contentColumns + 1
        contentIndex += 1
        if I < 104
            addSmlist()
        endif
    endif
endfor
distanceMatrix = [] ← array of distance matrix values
r = c = r1 = r2 = c1 = rnol = 0
for i range(15)
    c2 = i
    addDistanceMatrix[r]() ← add array to distance matrix with index
    for i range(15)
        if j == rnol
            addDistanceMatrix[r](0)
        elseif j < rnol
            if j == 0
                addDistanceMatrix[r](smlist[0][c1])
                c1 += 1
                c2 -= 1
            else
                c2 -= 1
                addDistanceMatrix[r](smlist[j][c2])
            endif
        else
```

```
            addDistanceMatrix[r](smlist[i][c])
            c += 1
          endif
      endfor
      c = 0
      r += 1
      rnol += 1
endfor
showDendogram(distanceMatrix) ← show result dendogram with
neighbor joining
```

D. Genetic Diversity

Data sets were used to analyze genetic diversity consisting of PIC, phylogenetic, and genetic analysis consisting of the number of alleles (Na), Ho, and He. PIC was calculated to determine the informative level of the primers used. Phylogenetic tree analysis was conducted to cluster varieties based on genetic similarity. The genetic analysis consists of the number of alleles. Ho and He were calculated to get the genetic diversity information.

IV. Result and Discussion

A. PIC and Polymorphic Bands

Thirty-eight of the 50 tested primers could be used for genetic diversity analysis, while the other 12 primers did not produce bands or monomorphic bands. The PIC value obtained ranged from 0.20 (gpsb067) to -0.78 (xcu14), with an average of 0.54 (Table 1). PIC scores are divided into three classes: very informative (PIC > 0.5), moderately informative (0.25 < PIC < 0.5), and low informative (PIC < 0.25) [19]. Based on these classes, the PIC value obtained was included in the very informative category. The result shows that an average of 38 primers were used very well to detect the genetic diversity of sorghum. Twenty-six primers were highly informative, 10 were moderately informative, and two were less informative. (Table 2). This information can be used as a reference for primer selection to analyze the genetic diversity of sorghum.

TABLE II. BANDS NUMBERS AND PIC VALUE

No	Primer Name	Polymorphic Bands	Monomorphic Bands	PIC	Class
1	xtxp043	-	2	-	-
2	xtxp057	4	-	0.64	H
3	xtxp177	4	-	0.59	H
4	xtxp225	3	-	0.44	M
5	xtxp145	7	-	0.76	H
6	xtxp57	2	-	0.27	M
7	xtxp266	5	-	0.56	H
8	xtxp303	4	-	0.53	H
9	xtxp023	6	-	0.73	H
10	xtxp159	2	-	0.31	M
11	xtxp179	3	-	0.54	H
12	xtxp207	2	-	0.27	M
13	sb6-84	3	-	0.57	H
14	xtxp15	4	-	0.55	H
15	xtxp114	3	-	0.42	M
16	xtxp141	3	-	0.52	H
17	xgap84	3	-	0.44	M
18	gpsb067	2	-	0.20	L
19	xgap256	3	-	0.57	H
20	xgap34	-	2	-	-
21	xcup02	4	-	0.61	H
22	xcup53	4	-	0.70	H
23	xcup62	4	-	0.70	H
24	xcu14	6	-	0.78	H
25	xtxp320	-	-	-	-
26	xtxp197	3	-	0.48	M
27	xtxp067	5	-	0.69	H
28	xcup63	4	-	0.68	H
29	xtxp030	-	2	-	-
30	xtxp115	-	2	-	-
31	xtxp149	2	-	0.37	M
32	svpepcaa	3	-	0.44	M
33	xicep0310	-	1	-	-
34	sbsgab02	3	-	0.52	H
35	xtxp312	-	-	-	-
36	xtxp302	-	-	-	-
37	xtxp069	-	2	-	-
38	gpsb123	-	2	-	-
39	xtxp136	-	2	-	-
40	xtxp001	3	-	0.53	H
41	xtxp278	2	-	0.26	L
42	scup11	3	-	0.23	M
43	msbcir300	5	-	0.54	H
44	msbcir286	6	-	0.67	H
45	xicep01076	-	2	-	-
46	xcup61	4	-	0.67	H
47	xtxp168	4	-	0.61	H
48	sb5-206	6	-	0.65	H
49	xgap206	5	-	0.58	H
50	xtxp021	6	-	0.75	H
Total		145	17	-	-
Average		3.82	-	0.54	H

B. Phylogenetic Analysis

The results of phylogenetic tree construction using the NJ method are shown in Fig 2. Figure 2 shows 15 sorghum varieties divided into 3 clusters. The first cluster consisted of 10 varieties divided into two sub-clusters. Sub-cluster one consisted of the varieties Bioguma 1, Bioguma 2, Bioguma 3, Soper 7, Soper 9, Kawali and Numbu. Sub-cluster two consisted of the varieties Suri 4, Mandau, Super 1, UPCA, and Super 2. The second cluster consisted of Suri 3 and Soper 6 varieties, while the third cluster consisted of Samurai 2.

Varieties in one cluster have lower genetic variation compared to other varieties in different clusters. Genetic variation also represents genetic distance, where the smaller the genetic variation, the closer the genetic distance. The varieties with the shortest genetic distance were Bioguma 1 and Bioguma 2, and the longest were Soper 6 and Mandau

(Table 3). Crossing on individuals with a long genetic distance will produce more genetic variation than those with a low genetic distance, so the opportunity to find new variations is high.

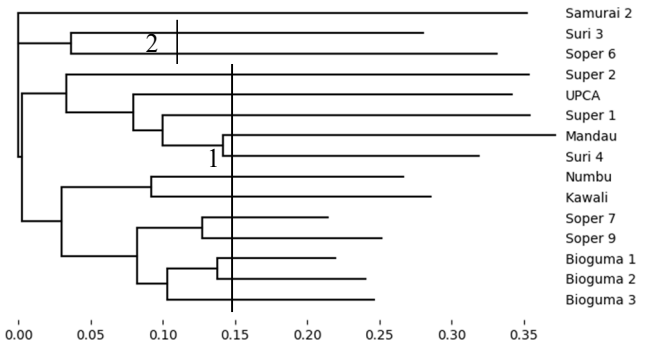

Fig. 2. Phylogenetic fifteen superior sorghum from Indonesia based on SSR markers using the NJ method.

C. Genetic Diversity

The highest number of alleles was obtained from primer xtxp145 with seven polymorphic alleles. The total number of alleles (Na) from 38 primers was 142 alleles, with an allele average of 3,737. The more alleles obtained, the more sensitive the primer detects genetic variation. The number of alleles for each primer is shown in Table 1.

The He value in this study is categorized as high, which was 0.579. This value indicates that the 15 sorghum varieties tested have large genetic diversity. Therefore, it still has the potential to be developed into new varieties as required. [22] states that plant populations that have He values above 0.558 are classified as having a high level of genetic diversity. A high level of He indicates that the species has great genetic diversity. High genetic diversity is required for adaptation to prevent extinction from pests, diseases, and climate change.

Genetic diversity information is needed to design strategies for developing new varieties. High genetic diversity gives breeders more choices and opportunities to develop new varieties that are better genetic and appropriate for their needs. In addition, genetic diversity is necessary in designing conservation programs. This is because species that lack genetic diversity cannot evolve in response to environmental changes, which increases the risk of extinction [23], [24].

TABLE III. GENETIC DISTANCE OF 15 INDONESIAN SORGHUM VARIETIES BASED ON SSR MARKER USING NJ METHOD

Units	UPCA	Mandau	Numbu	Kawali	Super 1	Super 2	Suri 3	Suri 4	Soper 6	Soper 7	Soper 9	Bioguma 1	Bioguma 2	Bioguma 3
Mandau	0.567													
Numbu	0.615	0.644												
Kawali	0.650	0.680	0.368											
Super 1	0.549	0.533	0.626	0.661										
Super 2	0.665	0.694	0.559	0.595	0.676									
Suri 3	0.545	0.574	0.548	0.584	0.556	0.599								
Suri 4	0.515	0.421	0.591	0.627	0.480	0.642	0.522							
Soper 6	0.699	0.728	0.529	0.564	0.710	0.643	0.632	0.675						
Soper 7	0.613	0.642	0.409	0.445	0.624	0.557	0.546	0.589	0.527					
Soper 9	0.658	0.687	0.455	0.490	0.669	0.602	0.592	0.635	0.572	0.211				
Bioguma 1	0.620	0.649	0.417	0.452	0.631	0.565	0.554	0.597	0.534	0.268	0.314			
Bioguma 2	0.640	0.670	0.437	0.472	0.652	0.585	0.574	0.617	0.554	0.289	0.334	0.184		
Bioguma 3	0.647	0.677	0.444	0.479	0.658	0.592	0.581	0.624	0.561	0.296	0.341	0.260	0.280	
Samurai 2	0.647	0.676	0.594	0.629	0.658	0.644	0.580	0.623	0.677	0.592	0.637	0.599	0.619	0.626

V. CONCLUSION

This study used SSR molecular markers to obtain molecular data of Indonesian sorghum to provide genetic diversity information. The SSR primers used were highly informative for detecting genetic diversity. The analysis showed that the genetic diversity of sorghum was classified as high. The phylogenetic tree shows that the varieties were divided into three clusters.

This study was limited to testing the genetic variation of 15 Indonesian sorghum varieties based on 38 SSR markers that produced polymorphic bands. Twelve SSR markers that produced monomorphic bands or did not produce bands were not included in the analysis process. In the future, this information will be used as a reference to select primers for sorghum genetic diversity analysis. Genetic diversity information will be used for breeding strategies and genetic conservation of sorghum.

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REFERENCES

[1] A. Kumar and N. Chordia, “Role of Bioinformatics in Biotechnology,” 2017. [Online]. Available: www.tsijournals.com

[2] CIRAD, “DARwin,” 2014.

[3] R. Peakall and P. E. Smouse, “GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update,” *Bioinformatics*, vol.

28, no. 19, pp. 2537–2539, Oct. 2012, doi: 10.1093/bioinformatics/bts460.

[4] P. Rajendrakumar, "Molecular Marker Development Using Bioinformatic Tools," in *Sorghum Molecular Breeding*, R. Madhusudhana, P. Rajendrakumar, and J. V Patil, Eds., New Delhi: Springer India, 2015, pp. 179–195. doi: 10.1007/978-81-322-2422-8_8.

[5] W. Powell, G. C. Machray, and J. Proven, "Polymorphism revealed by simple sequence repeats," *Trends Plant Sci*, vol. 1, no. 7, pp. 215–222, 1996, doi: 10.1016/1360-1385(96)86898-1.

[6] A. S. Mason, "Plant genotyping," *Plant Genotyp Methods Protoc*, vol. 2015, pp. 77–89, 2015.

[7] B. Nemera, M. Kebede, M. Enyew, and T. Feyissa, "Genetic diversity and population structure of sorghum [Sorghum bicolor (L.) Moench] in Ethiopia as revealed by microsatellite markers," *Acta Agric Scand B Soil Plant Sci*, vol. 72, no. 1, pp. 873–884, 2022, doi: 10.1080/09064710.2022.2117078.

[8] O. Gamachu, F. Tileye, and D. Tesfaye, "Genetic diversity analysis of sorghum [Sorghum bicolor (L.) Moench] races in Ethiopia using simple sequence repeats (SSR) markers," *Afr J Biotechnol*, vol. 20, no. 11, pp. 431–439, Nov. 2021, doi: 10.5897/ajb2021.17409.

[9] W. Mamo, M. Enyew, T. Mekonnen, K. Tesfaye, and T. Feyissa, "Genetic diversity and population structure of sorghum [Sorghum bicolor (L.) Moench] genotypes in Ethiopia as revealed by microsatellite markers," *Heliyon*, vol. 9, no. 1, p. e12830, Jan. 2023, doi: 10.1016/j.heliyon.2023.e12830.

[10] J. Zheng, L. Ting, Q. Yucen, L. Jieqin, L. Yanlong, C. Shuang, X. Zhaoshi, Z. Qiuwen, "Assessment of genetic diversity of sweet sorghum collection using phenotypic variation and ssr markers," *Pak J Bot*, vol. 53, no. 1, pp. 145–153, 2021, doi: 10.30848/PJB2021-1(7).

[11] S. Sulistyawati, Dyah Roeswitawati, Jabal Tarik Ibrahim, and Maftuchah, "Genetic diversity of local sorghum (Sorghum bicolor) genotypes of East Java, Indonesia for agro-morphological and physiological traits," *Biodiversitas*, vol. 20, no. 9, Aug. 2019, doi: 10.13057/biodiv/d200911.

[12] D. Wirnas, N. Oktani, H. Rahmi, D. Andriani, F. Faturrahman, E. Rini, S. Marwiyah, T. Trikoesoemaningtyas, "Genetic analysis for designing an ideotype of high-yielding sorghum based on existing lines performance," *Biodiversitas*, vol. 22, no. 12, 2021.

[13] E. P. Rini, S. Marwiyah, and D. Wirnas, "Genetic Analysis in an F2 Population Derived from Indonesian Local Sorghum with New Superior Variety," *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, vol. 50, no. 3, pp. 275–282, 2022.

[14] Maftuchah, L. Febriana, Sulistyawati, H. A. Reswari, and E. D. Septia, "Morphological diversity and heritability of nine local sorghum (Sorghum bicolor) genotypes in east java, indonesia," *Biodiversitas*, vol. 22, no. 3, pp. 1310–1316, Mar. 2021, doi: 10.13057/biodiv/d220330.

[15] E. Cretazzo, P. M. Sanz, S. Lorenzi, M. L. Benítez, L. Velasco, and F. Emanuelli, "Genetic Characterization by SSR Markers of a Comprehensive Wine Grape Collection Conserved at Rancho de la Merced (Andalusia, Spain)," *Plants*, vol. 11, no. 8, Apr. 2022, doi: 10.3390/plants11081088.

[16] G. Vats, D. Das, R. Gupta, A. Singh, A. Maurya, S. Rajkumar, A. Singh, R. Bharadwaj, S. Kumar, S. Kaushik, V. Gupta, K. Singh, R. Singh, "Validation of Genome-Wide SSR Markers Developed for Genetic Diversity and Population Structure Study in Grain Amaranth (Amaranthus hypochondriacus)," *Agriculture*, vol. 13, no. 2, p. 431, Feb. 2023, doi: 10.3390/agriculture13020431.

[17] A. Bohra, A. Dubey, R Saxena, R Penmetsa, K. Poornima, N. Kumar, A. Farmer, G. Srivani, H. Udpadhyaya, R. Gothalwal, S. Ramesh, D. Singh, K. Saxena, P. Kishor, N. Singh, C. Town, G. May, D. Cook, R. Varshney, "Analysis of BAC-end sequences (BESs) and development of BES-SSR markers for genetic mapping and hybrid purity assessment in pigeonpea (Cajanus spp.)," *BMC Plant Biol*, vol. 11, Mar. 2011, doi: 10.1186/1471-2229-11-56.

[18] D. Bhattramakki, J. Dong, A. K. Chhabra, and G. E. Hart, "An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench," *Genome*, vol. 43, no. 6, pp. 988–1002, Dec. 2000, doi: 10.1139/g00-074.

[19] D. Botstein, R. L. White, M. Skolnick, and R. W. Davis4, "Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms," 1980.

[20] R. Peakall and P. Smouse, "Appendix 1-Methods and Statistics in GenAlEx 6.5," 2015.

[21] O. Gascuel and M. Steel, "Neighbor-joining revealed," *Molecular Biology and Evolution*, vol. 23, no. 11. pp. 1997–2000, Nov. 2006. doi: 10.1093/molbev/msl072.

[22] F. De Maria Russo Godoy, M. Lenzi, B. Henrique Dos Santos Ferreira, L. DA Vicente Silva, C. Martini Zanella, and G. Matos Paggi, "High genetic diversity and moderate genetic structure in the self-incompatible, clonal Bromelia hieronymi (Bromeliaceae)," 2018. [Online]. Available: https://academic.oup.com/botlinnean/article/187/4/672/5055131

[23] M. Govindaraj, M. Vetriventhan, and M. Srinivasan, "Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives," *Genet Res Int*, vol. 2015, pp. 1–14, Mar. 2015, doi: 10.1155/2015/431487.

[24] M. Y. Chung, J. Merila, J. Li, K. Mao, J. L. Pujol, Y. Tsumura, M. G. Chung "Neutral and adaptive genetic diversity in plants: An overview," *Front Ecol Evol*, vol. 11, Feb. 2023, doi: 10.3389/fevo.2023.1116814.