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Estimation of genetic diversity of sweet sorghum (*Sorghum bicolor* (L.) Moench) genotypes as a bioethanol source using SSRs markers

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Abstract: Sweet sorghum is a cereal crop that can potentially serve as a source for bioethanol production. This study aims to analyse the genetic diversity of promising genotypes of sweet sorghum at the molecular level. The genetic material consisted of 12 sweet sorghum genotypes. The genetic diversity estimated using 59 SSRs markers showed a polymorphism value of 0.48 and the coefficient of genetic diversity was classified as moderate. The unweighted pair group method arithmetic average (UPGMA) analysis assigned the tested genotypes into three major clusters with a similarity coefficient level of 0.596. This indicates that the genetic similarity of the tested genotypes is moderate to high. Eight unique loci were identified with the SSRs markers in six genotypes, which are considered to control high sugar traits.

Keywords: bioethanol; genetic markers; genotype; germplasms analysis; raw material

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is one of the potential cereal crops used for food and industrial purposes. Sweet sorghum has been studied extensively as an ethanol source (Ratnavathi et al. 2011). Sweet sorghum is suitable as a raw material for bioethanol production since it does not compete with food needs (Khalil et al. 2015). Bioethanol production has an important role as an alternative source of energy which is also considered environmentally friendly and a renewable energy source (Salelign & Duraisamy 2021).

Sweet sorghum is self-pollinating, diploid ($2n = 20$) with a genome size of 730 Mb, about 25 percent the size of sugarcane or maize (Shalini et al. 2019). Sweet sorghum is a C4 plant that has a high sugar content in its stem juice, produces grains, is a lignocellulosic biomass and is a promising source of bioenergy (Rooney et al. 2007; Mathur et al. 2017). Similar to sugarcane, the stalk of sweet sorghum can be squeezed to release a sweet juice with high sugar levels, primarily composed of sucrose, glucose, and fructose (Goshadrou et al. 2011). These sugars serve as excellent substrates for fermentation into ethanol, while the remaining lignocellulosic biomass is known as bagasse (Guilherme et al. 2019). Sweet sorghum exhibits a wide range of adaptability and can thrive in various agroecosystems.

A genetic diversity assessment among the cultivated plants at molecular levels is a fundamental component of crop improvement programmes (Zakaria

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et al. 2023). The assessment provides information on the genetic variations among breeding materials, which is essential for the parental selection for future breeding (Begna 2021). Evaluating genetic diversity in plants can be conducted using morphological, biochemical, and molecular DNA markers (Mehmood et al. 2008). Morphological characterisation has the advantage of being easy to apply and is relatively inexpensive. However, it has the disadvantage of being highly influenced by the environment, resulting in lower accuracy (Afolayan et al. 2019). It often fails to discriminate between accessions with different names, but having the same genetic makeup (Jonah et al. 2011). Conversely, molecular characterisation using molecular markers can accommodate the limitations of the morphological characterisation, and both methods of characterisation (morphology and molecular markers) can complement each other.

Several DNA-based marker systems have been successfully employed to assess the genetic diversity in sorghum (Ng'uni et al. 2011). These markers aid in characterising and developing DNA profiles. The DNA profiles of plants are utilised in the management of plant genetic resources within gene banks. These profiles assist in selecting diverse parent plants to obtain higher genetic variation (Pawar et al. 2019).

DNA-based molecular markers have become very useful for the assessment of diversity among the germplasm collections. Due to their abundance, high polymorphic nature, codominance and amenability to high throughput genotyping, simple sequence repeats (SSRs) are the markers of choice for various genetics and mapping applications in sorghum (Somegowda et al. 2024).

One of the commonly used molecular markers in plant breeding, SSR markers have been proven effective in the genetic characterisation and assessment of the genetic relationships and population structure among genotypes (Amelework et al. 2015). Molecular markers reflect genetic similarities and differences without being influenced by the environment.

Paterson et al. (2009) identified 71 000 SSRs in the sorghum genome. SSRs are reported to be the marker of choice for diversity analyses due to their ability to produce more informative multiallelic locus (Mofokeng et al. 2014), thus representing a significant portion of the eukaryotic genome and providing genetic markers that can detect differences among genotypes. Another advantage of SSR markers is that they are codominant and have a high level of polymorphism, making them widely applicable in genetic diversity analysis, population genetic, and fingerprinting analysis studies (Dossett et al. 2012), and can be conducted relatively quickly, simply, and stably (Swapna & Srivastava 2012).

This study aims to analyse the genetic diversity of potential sweet sorghum genotypes as a bioethanol raw material at the molecular level using SSR markers.

MATERIAL AND METHODS

Plant material and study sites. The genetic material used in the research was 12 sweet sorghum genotypes from the germplasm collection (Table 1) of the Indonesian Cereals Research Institute (ICERI), which were cultivated at Agro Science Park ICERI, Maros, South Sulawesi, Indonesia. The experiments were conducted from January until May 2020. A randomised complete block design (RCBD) with three replications was applied for the experiment. Each replicate had 12 rows corresponding to 12 genotypes. Eighteen plants were planted per row. Recommended cultural practices were followed to grow healthy and vigorous sweet sorghum plants.

Quantitative and morphological characteristic of sweet sorghum. The genotypic factors exhibited significant variations in various plant traits, such as the plant height, leaf area, number of leaves, stem diameter, stem dry weight, Brix content, total sugar content, and ethanol yield from juice (Table 2). An analysis of variance conducted on the quantitative characteristic variables revealed highly significant differences among the genotypes in terms of the plant height, stem diameter, Brix content, total sugar content, and ethanol

Table 1. List of the sweet sorghum used in the study

No	Genotype	Sources/Origin
1	15020B	ICERI, Indonesia
2	1115-C	ICERI, Indonesia
3	4-183-ABEOTO	ICERI, Indonesia
4	61-1-1	ICERI, Indonesia
5	EA-13-1-1	ICERI, Indonesia
6	KL2	ICERI, Indonesia
7	10(1-1)	ICERI, Indonesia
8	23(1-1)	ICERI, Indonesia
9	Buleleng Abang	West Nusa Tenggara, Indonesia
10	WR2	ICERI, Indonesia
11	Super-1 (variety)	ICERI, Indonesia
12	Super-2 (variety)	ICERI, Indonesia

ICERI – Indonesian Cereals Research Institute

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Table 2. Means, standard deviations (SD), ranges and *P*-values of eight quantitative characteristics within 12 genotypes of sweet sorghum

Characters	Mean ± SD	Range	MS genotype	*P*-value	*CV* (%)
Plant height (cm)	288.3 ± 35.3	226.9–367.7	62.99**	< 0.01	12.24
Leaf area (cm)	578.0 ± 32.9	522.9–630.7	5.33*	0.01	5.69
Number of leaves	14.6 ± 2.4	12.33–21.17	20.47*	0.01	16.44
Stem diameter (cm)	2.0 ± 0.2	1.7–2.4	1.56**	< 0.01	10.00
Steam dry weight (g)	196.2 ± 43.3	149.3–309.2	31.00*	0.01	22.07
Brix content (°Brix)	13.1 ± 1.9	10.5–17.1	10.82**	< 0.01	14.50
Total sugar content (%)	15.9 ± 2.8	10.9–19.1	10.29**	< 0.01	17.61
Ethanol 70% yield from juice (L/ha)	789.4 ± 172.2	408.4–913.1	28.74**	< 0.01	21.81

MS – mean square; *CV* – coefficient of variation; significance level *$P < 0.05$, **$P < 0.01$

yield from the juice. The genotypes exerted a significant influence on the observed variations in all the traits. The coefficient of variation (*CV*) values for the observed traits ranged from 5.69% to 22.07%. The lower *CV* values indicate the higher precision in the variables, as they fall below the tolerance of the *CV* value of 20% typically set for field research. A number of morphological traits, such as thick stems, tall stalks, late maturity and high biomass, are regarded as important attributes of the crop for increased bioethanol production (Regassa & Wortmann 2014; Abraha et al. 2015).

The genomic DNA extraction. The performance of the stems and panicles can be seen in Figure 1 and Figure 2. The preparation of genetic material for DNA extraction involved germinating 10–15 seeds for each accession. The plants were considered ready for

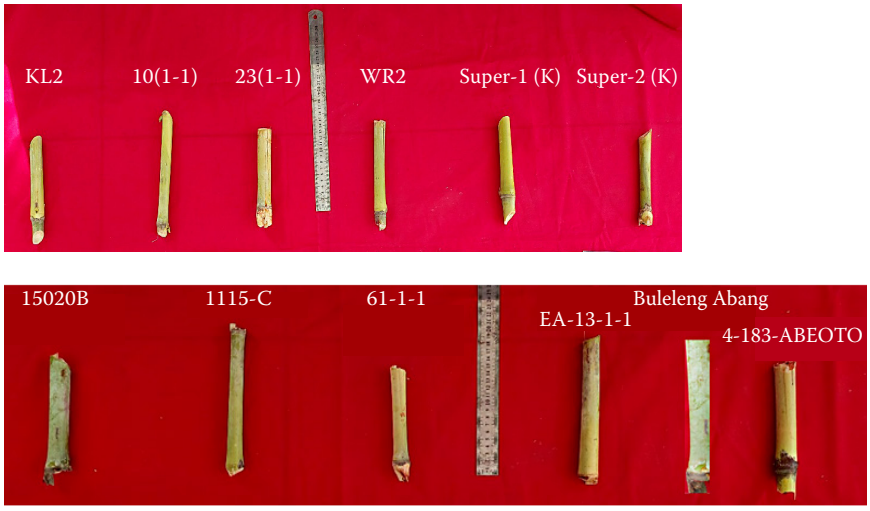

Figure 1. Stem performance of the sweet sorghum lines

Figure 2. Panicle performance of the sweet sorghum lines

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extraction when they reached 7–10 days old after germination. For each genotype, 15 fully opened leaves were randomly selected from 15 individual plants. The DNA extraction was performed following the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) protocol (CIMMYT 2005). Fresh leaf tissue weighing 150–250 mg was used, which was either stored on ice in tubes or frozen at –80 °C in tubes. A total of 1.0 mL of extraction buffer was pipetted out and transferred to a 2 mL tube at the end of the roller. The extract was then incubated in a water bath or an oven at 65 °C for 20 min, with gentle mixing twice or continuous mixing during the incubation. The tube was removed from the heat and allowed to cool for 5–10 min. The sample was further extracted with 1 mL of octanol-chloroform (1 : 24) by mixing through inversion for 5 min. It was then centrifuged at 3 200 rpm for 10 min using a tabletop centrifuge (Digital Angle Centrifuge, RS-0408). The supernatant, which contained the DNA, was transferred into a 2.0 mL Eppendorf tube. Next, 75 µL of 5M NaCl was added, and the DNA was precipitated by adding 1 mL of cold absolute ethanol. The DNA was then pelleted, the ethanol was removed, and the sample was dried under a weak vacuum for 30 min. Finally, the DNA was resuspended overnight in a cold room in 200–500 µL of TE buffer at pH 8.0. Calculations were performed using the gel method, likely referring to gel electrophoresis for visualising and analysing the extracted DNA samples.

Polymerase chain reaction (PCR). Fifty-nine pairs of SSRs primers were procured from Perseroan Terbatas. Genetika Science, Indonesia. The primers' description was assessed using 59 SSRs markers (Table 3). The PCR stage also adhered to the protocol of CIMMYT. The DNA amplification was performed in a reaction of 20 µL containing 12.50 µL of 2× KAPA2G Fast ReadyMix that contains 1.5 nM $MgCl_2$ (1×), 1 µL of the DNA as a template, 1 µL of a 10 µM forward primer, 1 µL of a 10 µM reverse primer and 10 µL of the PCR and 4.5 µL nanopure distilled water to reach a total volume of 20 µL. The utilised *Taq* polymerase was the KAPA2G Fast ReadyMix PCR Kit (Kapa Biosystems, USA). Reaction conditions were used as prescribed for the KAPA 2G Fast ReadyMix PCR Kit (KapaBiosystems, Merck KGaA, Germany).

Specific alleles. Unique alleles detected by each primer were also recorded. The identification of specific alleles required careful visualisation of DNA bands through vertical 8% polyacrylamide gel electrophoresis (PAGE) (Pawar et al. 2019). Specific alleles were marked when they appeared as individual DNA bands without any other bands on the same primer.

Data analysis for SSRs markers. The scoring of the DNA band patterns was based on binary data, where the presence of a band was denoted as 1, the absence of a band as 0, and uncertain band appearances were recorded as 9 (representing missing data). A genotypic data analysis was conducted using the NTSYS-pc program (Ver. 2.1) (Rohlf 2000), and the distance matrix was obtained using the unweighted pair group method with arithmetic averages (UPGMA) constructed using Power Marker (Ver. 3.25). The level of polymorphism (PIC = polymorphism information content) of the used primers was calculated for each SSR marker (Smith et al. 1997), with the formula:

$$PIC=1-\sum_{1}^{n} f_i^2$$

where:

i = 1, 2, 3,.....n;

f_i^2 – allele frequency.

RESULTS AND DISCUSSION

The similarity level of the traits and genetic distance. The level of genetic diversity in plants is crucial for the plant breeding process, as it reflects the adaptability of the plant population (Matus & Hayes 2002). An overview of the amplification in the form of PIC values was obtained from the observed differences in the DNA fragments and scored as the presence or absence of sequence differences, indicating the presence or absence of variation. Based on the PIC value using 59 SSRs markers, a total of 179 alleles were detected among the 12 genotypes (Table 3). The number of alleles ranges from 2–6 per locus, with an average of 3 alleles per locus. The range of the number of alleles observed was fairly similar to the range reported for sorghum by Danquah et al. (2019), but lower than that reported by Mofokeng et al. (2014) and Wang et al. (2013).

The observed heterozygosity (Ho) ranged from 0.0 to 1.00, with an average of 0.29 per locus. Twenty-seven loci had observed heterozygosity values at each locus across all accessions higher than the average (Table 2). Our results are slightly higher than those of Danquah et al. (2019) and Mofokeng et al. (2014). The high degree of heterozygosity observed among the genotypes indicates that the genotypes used in this study came from different geographic origin conditions with different selection rates. The low

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Table 3. Profile of data of 59 polymorphic SSRs markers used for 12 sweet sorghum genotypes

Marker	Major allele frequency	Gene diversity	Hetero-zygosity	PIC	No. of alleles	Size range (bp)	Anealling temperature (°C)	Chr*	Position**
gpsb067	0.58	0.61	0.33	0.61	5	191.83–349.66	54	8	13 744 996
gpsb069	0.42	0.71	0.42	0.58	3	155.90–190.20	52	6	–
gpsb089	0.46	0.64	0.58	0.64	3	200.00–259.33	50	1	43 884 079
gpsb123	0.75	0.40	0.33	0.33	3	180.40–232.66	50	8	52 281 926
gpsb148	0.75	0.38	0.00	0.38	2	210.88–216.33	50	7	327 117
msbcir223	0.71	0.47	0.33	0.35	3	109.00–135.60	51	2	4 657 470
msbcir238	0.67	0.44	0.00	0.44	2	163.25–181.62	53	2	14 746 509
msbcir240	0.96	0.08	0.08	0.08	2	91.00–100.00	53	8	4 467 743
msbcir246	0.46	0.64	0.42	0.64	3	181.40–269.66	50	7	56 279 794
msbcir248	0.42	0.71	0.50	0.71	5	113.50–200.00	53	5	4 746 082
msbcir262	0.58	0.49	0.00	0.49	2	190.20–200.00	51	10	55 324 102
msbcir276	0.83	0.28	0.00	0.28	2	206.12–236.75	51	3	55 555 298
msbcir286	0.38	0.70	0.58	0.70	5	104.50–145.50	55	1	57 452 822
msbcir300	0.25	0.84	0.92	0.79	6	105.99–175.50	61	7	58 286 012
msbcir306	0.83	0.28	0.00	0,28	2	204.90–209.80	50	1	–
msbcir329	0.33	0.73	0.50	0.73	4	180.40–212.25	52	5	1 763 243
Sb6-84	0.33	0.75	0.00	0.75	5	151.00–183.66	54	2	–
sbAGAB02	0.54	0.62	0.33	0.62	4	100.00–148.25	56	7	62 506 705
xcup02	0.58	0.57	0.33	0.57	3	175.50–311.00	57	9	8 143 767
xcup11	0.58	0.57	0.42	0.57	3	190.20–224.50	54	3	1 992 880
xcup14	0.92	0.15	0.00	0.15	2	200.00–209.80	57	3	72 459 931
xcup53	0.83	0.28	0.00	0.28	2	160.80–200.00	55	1	72 905 425
xcup61	0.46	0.68	0.75	0.64	4	151.00–224.50	55	3	2 576 698
xgap084	0.50	0.59	0.25	0.59	3	230.62–427.00	50	–	–
xgap236	0.38	0.77	0.58	0.72	5	75.60–118.00	53	–	–
xgap072	0.50	0.63	0.00	0.63	3	130.57–151.00	67	–	–
xicep0107	0.54	0.50	0.08	0.50	2	193.87–200.00	55	3	3 209 015
Xtxp10	0.50	0.63	0.00	0.63	3	178.99–204.90	63	9	47 916 807
xtxp023	0.83	0.28	0.00	0.28	2	155.08–167.33	50	4	67 961 876
xtxp067	0.67	0.44	0.00	0.44	2	187.75–193.87	65	–	–
Xtxp010	0.46	0.66	0.42	0.66	4	140.00–185.30	50	–	–
xtxp12	0.79	0.36	0.25	0.27	3	159.16–226.13	50	4	48 576 873
xtxp136	0.79	0.33	0.42	0.33	2	249.00–369.00	50	5	–
xtxp145	0.75	0.38	0.00	0.38	2	204.90–241.70	55	6	–
xtxp149	0.92	0.15	0.17	0.15	2	175.50–206.99	55	–	–
xtxp15	0.92	0.15	0.00	0.15	2	185.99–192.99	50	5	42 049 815
xtxp159	0.42	0.69	0.00	0.69	4	175.50–195.10	55	–	–
xtxp176	0.50	0.63	1.00	0.63	3	154.49–249.00	55	–	–
xtxp179	0.92	0.16	0.08	0.16	3	169.37–244.10	55	–	–
xtxp18	0.75	0.38	0.00	0.38	2	209.80–219.60	55	–	–
xtxp114	0.83	0.28	0.33	0.28	2	191.83–242.87	55	3	60 794 263
xtxp201	0.33	0.78	1.00	0.74	6	163.28–311.00	55	–	–

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Table 3 to be continued

Marker	Major allele frequency	Gene diversity	Hetero-zygosity	PIC	No. of alleles	Size range (bp)	Anealling temperature (°C)	Chr*	Position**
xtxp21	0.83	0.28	0.00	0.28	2	160.60–180.40	55	4	67 961 876
xtxp218	0.42	0.66	0.83	0.66	3	290.33–369.00	55	–	–
xtxp217	0.58	0.49	0.00	0.49	2	200.00–209.80	55	–	–
Xtxp141	0.42	0.64	0.42	0.64	3	171.99–256.75	55	10	58 245 266
xtxp238	0.33	0.77	0.92	0.77	5	156.44–219.60	50	–	–
xtxp258	0.50	0.50	1.00	0.50	2	157.99–171.99	55	–	–
xtxp265	0.92	0.15	0.00	0.15	2	204.90–214.70	55	6	51 179 303
xtxp273	0.75	0.38	0.00	0.38	2	187.75–193.87	55	6	156 965
xtxp278	0.75	0.38	0.00	0.38	2	218.37–224.50	50	8	51 120 645
xtxp279	0.63	0.56	0.25	0.48	3	95.50–126.80	55	–	–
xtxp289	0.75	0.38	0.50	0.38	2	295.50–349.66	55	–	–
xtxp295	0.42	0.75	0.67	0.70	5	151.00–239.20	55	7	61 172 112
xtxp030	0.67	0.44	0.00	0.44	2	175.50–200.00	51	–	–
xtxp320	0.38	0.74	1.00	0.73	6	255.20–427.00	55	1	55 381 359
xtxp321	0.50	0.63	0.00	0.63	3	185.99–209.80	55	8	50 508 795
xtxp40	0.92	0.15	0.00	0.15	2	311.00–427.00	51	7	860 855
xtxp57	0.58	0.52	0.08	0.52	3	209.80–239.40	55	6	57 418 801
Total					179				
Mean	0.61	0.49	0.29	0.48	3	75.60–427.00			

PIC – polymorphism information content; *the chromosome number was defined by genetic mapping and BLAST of the primer on the sorghum sequence using Primer-BLAST at the National Center for Biotechnology Information (NCBI) (Billot et.al. 2012); *,**position on the chromosome pseudo-molecule (when a reliable Primer-BLAST result was obtained)

number of alleles in this study may be due to the low genetic variation of the sorghum genotype that was characterised. The frequency of the alleles that appear can be a measure of the polymorphism of a locus (Hartwell et al. 2011).

The polymorphism value is used to determine the level of information provided by a DNA marker (Danquah et al. 2019). A locus is considered polymorphic if its occurrence frequency is less than 0.99. In this study, The PIC values of the SSRs markers ranged from 0.08 (Msbcir 240) to 0.79 (Msbcir 300) with an average polymorphism value of 0.48. This average polymorphism value indicates that there is a moderate level of gene variation among the tested genotypes. This indicates that the sweet sorghum genotype came from a narrow genetic base, potentially derived from a common ancestral gene (Shakoor et al. 2022). The moderate PIC value in this study was similar to that observed by Ali et al. (2008) and Mangena et al. (2018). However, higher polymorphic levels were reported by Tirfessa et al. (2020) and Mamo et al. (2023) in the study of sweet sorghum genotypes analysed with SSRs markers. The polymorphism of multiple genes controls the phenotypic diversity observed within a species (Buckler et al. 2006). This study indicates the possibility of improving the agronomic properties of sorghum through breeding and the importance of this germplasm conservation; however, it requires germplasm exploration to obtain wider genetic diversity.

To evaluate the effectiveness of the markers in differentiating individual genotypes, the PIC values were calculated. Based on the individual PIC values proposed by the reference, a total of 30 SSRs markers used in this study were highly informative (PIC > 0.5) (Danquah et al. 2019). These markers are considered highly informative for analysing the genetic structure of sweet sorghum genotypes, enabling the differentiation of genotypes that may have similar morphology, but are genetically distinct.

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The high range of PIC values shows that populations from each origin are comprised of genetically diverse individuals and their level of differentiation varies across the origins (Mengistu et al. 2020). Additionally, there were 24 markers with PIC values ranging from 0.25 < PIC < 0.5, which can be classified as moderately informative. On the other hand, five markers had a PIC value below 0.25, indicating that they are less informative markers. The narrow differences among PIC values exhibited by the different populations suggest that the discriminatory power of the markers to distinguish between the populations is limited since the markers exhibit low polymorphism among the populations (Smith et al. 1997; Mengistu et al. 2020). This indicates that the marker pair used as a genetic tool in this study is highly valuable for elucidating the genetic diversity of sweet sorghum. The obtained PIC values can be compared with similar studies conducted on sweet sorghum plants. Danquah et al. (2019) reported PIC values ranging from 0.05 to 0.78, while Mofokeng et al. (2014) reported PIC values ranging from 0.01 to 0.81. These findings indicate that microsatellite loci have a significant resolution capacity to demonstrate the variation between sorghum accessions (Salih et al. 2016). The PIC value of the markers can give better or more information on the variability in the germplasm by considering the number of alleles and then the relative frequencies of the individual allele (Smith & Frederiksen 2020).

The level of genetic similarity is shown from the construction of the dendrogram of 12 sweet sorghum genotypes. The results of the cluster analysis show that the genetic similarity coefficient ranges from 0.56 to 0.92 with a correlation matrix value coefficient (r) of 0.81. Based on a genetic similarity coefficient of 0.596, the 12 genotypes form 3 clusters (Figure 3). Cluster 1 consisted of 8 genotypes: 15020B, 10(1-1), 23(1-1), 1115-C, EA-13-1-1, Buleleng Abang, WR2 and Super-1. In this cluster, 10(1-1), 23(1-1) and 15020B were similar to 1115-C, and these four genotypes were grouped close to each other in a sub-cluster. EA-13-1-1 and Buleleng Abang showed similarities with WR2 and Super-1 were grouped together in the same sub-cluster. Cluster 2 consisted of 3 genotypes; 4-183-ABOETO, 61-1-1 were similar to KL2. While cluster 3 only consists of 1 genotype; Super-2 formed an out-group as it is not included in any of the main clusters and appeared as a separate branch at a similarity coefficient of 0.56 as the most genetically distant from the other genotypes. The dendrogram in Figure 4 shows that all the genotypes were separated into three main groups. This shows that the genotypes are the same, the main clusters are closely related and can be derived from the same parental genes (Chen et al. 2020).

The genetic distances and heterosis probabilities for the 12 genotypes of high soluble sugar content sweet sorghum are provided in Table 4. The genetic distance value is 0.08–0.50 with an average genetic distance of 0.33. This indicates that the genetic similarity of the tested genotypes is moderate to high. The level of genetic similarity among the genotypes plays a crucial role in selecting parental combinations for segregating populations and maintaining genetic diversity in breeding programmes (Kong et al. 2000).

The results of the analysis conducted using the NTSYS program demonstrate a level of stability and accuracy in grouping the genotypes, as indicated by a correlation matrix value coefficient (r) of 0.81, which is classified as a good fit. This implies that the utilised primer effectively and accurately forms dendrogram clusters. According to Rohlf (2000), the use of more polymorphic primers leads to the more

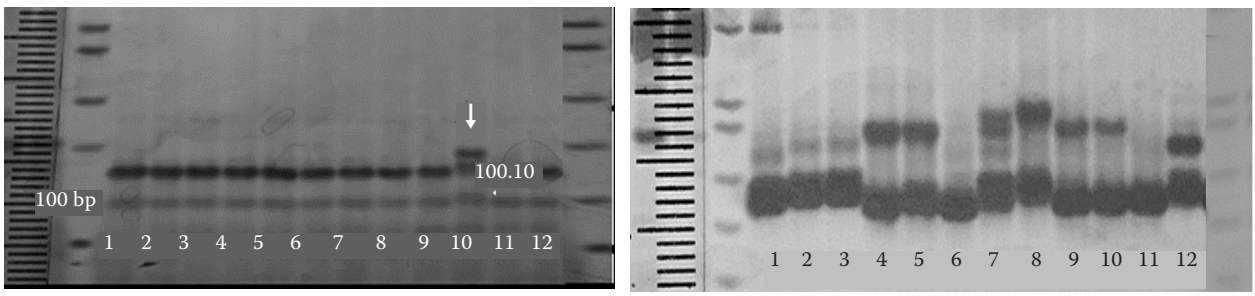

Figure 3. DNA banding profiles of SSR primers Xtxp 265 and Msbcir 286
1 – 15020B; 2 – 1115-C; 3 – 4-183-ABEOTO; 4 – 61-1-1; 5 – EA-13-1-1; 6 – KL2; 7 – 10(1-1); 8 – 23(1-1); 9 – Buleleng Abang; 10 – WR2; 11 – Super-1; 12 – Super-2

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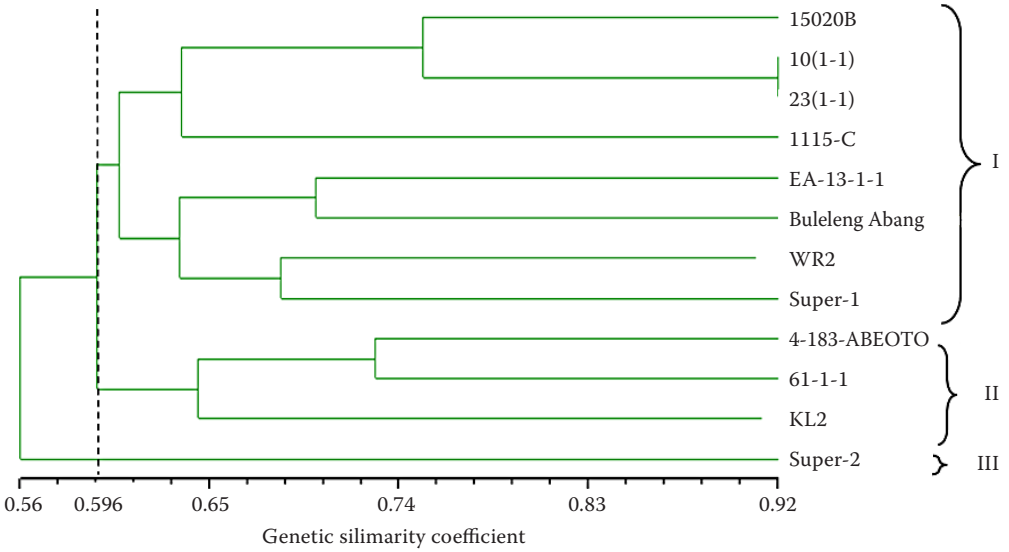

Figure 4. Construction of a dendrogram of 12 sweet sorghum genotypes with high soluble sugar content using the NTSYS pc-2 program/

precise formation of dendrogram clusters, resulting in a higher value of *r*. The coefficient of cophenetic correlation (*r*) reflects the accuracy of genotypic clustering, which is determined based on the estimation of genetic similarity among the characterised lines using the number of primers employed (Billot et al. 2012).

Based on the visualised DNA bands, a total of 8 specific alleles were detected in the SSR markers among the 6 sweet sorghum genotypes (Table 5). These specific alleles are characterised by DNA bands that appear alone without any accompanying DNA bands. Furthermore, each specific allele has a distinct size,

Table 4. Genetic distances among the 12 genotypes of high-sucrose sweet sorghum based on their soluble sugar content character

	15020B	1115-C	4-183-ABEOTO	61-1-1	EA-13-1-1	KL2	10(1-1)	23(1-1)	Buleleng Abang	WR2	Super-1	Super-2
15020B	0.00											
1115-C	0.27	0.00										
4-183-ABEOTO	0.36	0.40	0.00									
61-1-1	0.34	0.37	0.27	0.00								
EA-13-1-1	0.39	0.42	0.38	0.30	0.00							
KL2	0.45	0.46	0.30	0.40	0.31	0.00						
10(1-1)	0.23	0.39	0.41	0.38	0.38	0.45	0.00					
23(1-1)	0.26	0.41	0.41	0.41	0.36	0.46	0.08	0.00				
Buleleng Abang	0.39	0.42	0.39	0.45	0.30	0.41	0.34	0.34	0.00			
WR2	0.44	0.47	0.47	0.46	0.44	0.42	0.37	0.40	0.33	0.00		
Super-1	0.36	0.40	0.34	0.37	0.36	0.38	0.38	0.36	0.32	0.31	0.00	
Super-2	0.42	0.50	0.41	0.45	0.47	0.46	0.39	0.39	0.47	0.47	0.36	0.00
Mean	0.36	0.43	0.38	0.40	0.37	0.43	0.31	0.37	0.37	0.39	0.18	0.00
General mean	0.33											

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Table 5. Specific alleles detected 12 genotypes of sweet sorghum

Marker	PIC	Unique bands	Genotype
Xtxp 265	0.15	f1 (214.70)	KL2
Xtxp 40	0.15	f1 (427.00)	Super-2
Xtxp 159	0.69	g1 (195.10)	Super-1
Xtxp 15	0.15	k1 (100.10)	Super-2
SB6-84	0.75	g4 (155.08) h1 (151.00)	Super-1 Super-2
Xcup 14	0.15	f1 (209.08)	1115-C
msbcir 240	0.08	g3 (180.40)	WR2
msbcir 286	0.70	h1 (145.50)	23(1-1)

PIC – polymorphism information content

indicating the presence of unique genetic characteristics in the tested sorghum genotypes. These primers are associated with sorghum traits, such as high biomass and sugar content, making them valuable markers for analysing the genetic diversity of sweet sorghum (Billot et al. 2012; Gangurde et al. 2017; Pawar et al. 2019). The SSR markers serve to reinforce the identification and characterisation of the observed sweet sorghum genotypes through the presence of these specific alleles. These specific alleles can be considered individual identities or fingerprints of particular genotypes. If a specific characteristic represented by a specific allele is of interest, it is crucial to maintain and preserve the genotype carrying that allele.

CONCLUSION

The SSRs markers used in this study were effective in providing information about the genotypic variability. Of the 59 markers that have a polymorphism value of 0.48, the genetic diversity coefficient is classified as moderate. The genotypes were categorised into three main clusters based on a genetic convenience coefficient of 0.596. This indicates that the genetic similarity of the tested genotypes is moderate to high. This study indicated that the examined genotypes possess a moderate level of variability, highlighting the importance of exploring sweet sorghum germplasm/biodiversity to achieve greater genetic diversity. Furthermore, the SSR markers utilised in this study detected 8 specific alleles in the six sweet sorghum genotypes, offering valuable insights for characterising and identifying genotypes with a high sugar content that have the potential as bioethanol sources.

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