Genetic relationships in *Saccharum* complex germplasm collections based on morphological and molecular markers

*Hubungan kekerabatan koleksi plasma nutfah tebu (Saccharum complex) berdasarkan penanda morfologi dan molekuler*

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**Abstract**

Sugarcane has a high degree of heterozygosity and is a cross-pollinator, so information about the genetic relationship between the accessions in germplasm collections is very important for selecting the prospective parent in crossbreeding. This research aims to determine the phylogenetic relationship of 24 Saccharum complex accessions and to verify the grouping of accessions using 37 morphological and three microsatellite molecular markers. Interpretation of morphological and molecular data was obtained from the analysis using the NTYSYpc-2.02i program. The results show that within the 24 accessions analyzed using morphological markers, some accessions did not cluster as the classification at the conservation time. This difference is due to the morphological markers, so the value of genetic similarity among accessions analyzed is increased. In contrast, the grouping of molecular markers shows that each accession was grouped according to the classification at the conservation time. These accessions had a low genetic similarity of 0.20 with a broad genetic distance of 0.80. This broad genetic distance indicates that the twenty-four accessions have a distant genetic relationship with one another, so that the genetic diversity of these accessions is relatively high. The high genetic diversity in germplasm collections improves its potential as a crossing parent to obtain a high heterosis effect.

[Keywords: germplasm collection, heterozygosity, heterosis, microsatellites, Saccharum complex]

**Abstrak**

Tebu memiliki derajat heterozigosis yang tinggi dan menyerbuk silang, sehingga informasi tentang hubungan kekerabatan aksesi dalam koleksi plasma nutfah tebu sebagai calon tetua persilangan merupakan hal yang sangat penting. Penelitian ini bertujuan untuk mengetahui hubungan kekerabatan 24 aksesi *Saccharum* complex dan memverifikasi pengelompokan aksesi tersebut menggunakan 37 penanda morfologi dan 3 penanda molekuler mikrosatelit.

Interpretasi data morfologi dan molekuler diperoleh dari hasil analisis menggunakan program NTYSYpc-2.02i. Hasil penelitian menunjukkan bahwa dari 24 aksesi yang dianalisis menggunakan penanda morfologi, ditemukan beberapa aksesi yang tidak mengelompok sesuai klasifikasi pada saat konservasi. Perbedaan ini disebabkan oleh penanda morfologi, sehingga nilai kemiripan genetik di antara aksesi yang dianalisis meningkat. Sebaliknya, pengelompokan menggunakan penanda molekul menunjukkan bahwa masing-masing aksesi mengelompok menurut spesies sesuai dengan klasifikasi pada saat konservasi, dan 24 aksesi tersebut memiliki kemiripan genetik rendah yaitu 0,20 dengan jarak genetik sebesar 0,80. Jarak genetik yang lebar ini menunjukkan bahwa dua puluh empat aksesi tersebut memiliki hubungan genetik yang jauh antara satu dengan yang lainnya, sehingga keragaman genetik aksesi tersebut cukup tinggi. Keragaman genetik yang tinggi pada koleksi plasma nutfah ini meningkatkan potensinya sebagai tetua persilangan untuk mendapatkan efek heterosis yang tinggi.

[Kata kunci: plasma nutfah, heterosis, microsatelitat, *Saccharum complex*]

**Introduction**

Sugarcane is one of the most important industrial crops in tropical and subtropical regions because of its ability to store high carbohydrates to produce sugar and biofuel. Sugarcane is an economically important agricultural crop cultivated in over 90 countries, contributing to nearly 80% of global white sugar production (Raj et al., 2016; Diederichs et al., 2016; Aitken et al., 2018; Pocovi et al., 2020).

Currently, the national sugar production has not been able to meet consumption needs due to several constraints, including low sugarcane and sugar productivity. One of the efforts to overcome this food problem is to intensify breeding activities to obtain superior sugarcane varieties. The assembly of new superior sugarcane varieties is highly dependent on the availability of genetic
diversity of *Saccharum* spp.

The genetic diversity of *Saccharum* spp. is abundant in Indonesia because this country is known as one of the original centers of *Saccharum* (FAO, 2019). The collection of sugarcane germplasm in the Indonesian Sugar Research Institute (ISRI) began in the 1890s by Dutch researchers through obtaining accessions of sugarcane from within the Indonesian archipelago and introducing clones from abroad (ISRI, 1997). Lamadji (1994) reported that six expeditions conducted in Indonesia to explore wild sugarcane in Kalimantan, Sulawesi, Maluku, North Sumatera, and Irian Jaya (West Papua) in 1976, 1984, 1985, and 1995. In 2014, the volume of sugarcane germplasm collections managed and conserved by ISRI consisted of 5,294 accessions. The collection consists of a group of native cane (*Saccharum officinarum*), wild cane (*Saccharum spontaneum, Saccharum robustum, Saccharum barberi, Saccharum sinensis*, and *Saccharum edule*), wild relatives of *Saccharum* (*Erianthus* and *Miscanthus*), and *Saccharum* hybrid (Mirzawan et al., 2014). The modern sugarcane cultivars originated from the *Saccharum* complex, which consists of two wild *Saccharum* species (*S. spontaneum, and S. robustum*), four cultivated species (*S. officinarum, S. sinense, S. barberi,* and *S. edule*), and four interrelated genera (*Erianthus, Miscanthus, Narenga,* and *Sclerostachya*; Todd et al., 2014).

Sugarcane is a polyplloid plant and has a very complex genome. The *Saccharum* species present large genomes and variations in the number of chromosomes (Liu et al., 2016; Vieira et al., 2018). The complexity of the Saccharum genome is inherited by modern sugarcane cultivars, in which its levels of ploidy, often aneuploidy, and have a large genome size of about 10 GB (Vieira et al., 2018; Zhang et al., 2018).

Pollination of sugarcane is both open-pollinated and cross-pollinated. Individual plants have a high degree of heterozygosity and the appearance of superior varieties caused by heterosis (Birchler & Riddle, 2014). With these types of pollinations, sugarcane will produce genetically distinct offspring: between individual offspring, between offspring and their parents, between generations of one offspring and other offspring generations. To maximize the utilization of the sugarcane germplasm collection, it is necessary to evaluate and analyze genetic relationships between the accessions. Genetic relationships between collected germplasm can be evaluated and analyzed through morphological and molecular markers. This will facilitate the selection process of crossing parents and optimize selection procedures (Khan et al., 2009).

An important step before crossing is the selection of the correct parent cross to obtain superior offspring. The strategy for selecting parents can be carried out by characterizing the germplasm with morphological and agronomical markers. However, errors can occur because the vegetative traits are influenced by the environment and often do not reflect the genetic diversity of actual *Saccharum* accessions (Manechni et al., 2018; Medeiros et al., 2020). Therefore, molecular markers can be used as a complementary tool to characterize morphologically and to identify accessions accurately and reliably so that the combination of accessions as cross parents is suitable for breeding purposes (Marconi et al., 2011; dos Santos et al., 2012; Singh et al., 2018).

Sugarcane breeders and geneticists have discovered the use of numerous DNA markers, including amplified fragment length polymorphisms (AFLP) (Caroll & Curtis, 1996; D’Hont & Glaszmann, 2001), restriction fragment length polymorphisms (RFLP) (Burnquist et al., 1995), single nucleotide polymorphism (SNP), simple sequence repeats (SSRs) (Pandey et al., 2011; Hameed et al., 2012), random amplification of polymorphic DNAs (RAPD) (Ullah et al., 2013), inter simple sequence repeat (ISSRs) (Devaruramath et al., 2012; Rao et al., 2016; Olieviera et al., 2017), r-DNA-ITS sequence and expressed sequence tags-simiple sequence repeat (EST-SSRs) to improve the *Saccharum* breeding (Ali et al., 2019). Among these molecular markers, SSR (microsatellite) markers have been widely used to study sugarcane genetic diversity, genetic mapping, cross-transferability, paternity analysis, segregation analysis, and marker-assisted selection. SSR primer pairs are considered the most capable marker for plant genetics and breeding programs because of their characteristics that are co-dominant, multi-allelic nature, and relatively abundant with an excellent genome coverage (Pan, 2010; Costa et al., 2011; dos Santos et al., 2012; Silva et al., 2012; Lopes et al., 2015; Vieira et al., 2016; Ali et al., 2019).

This study aims to determine the genetic relationship of 24 clones of *Saccharum* complex members based on morphological and microsatellite molecular markers and to verify the grouping of 24 *Saccharum* complex clones based on microsatellite markers.

**Materials and Methods**

This research was conducted in the ISRI’s germplasm collection station and Biotechnology Laboratory in the 2017/2018 planting season. Twenty-four accessions of ISRI’s sugarcane germplasm were members of the *Saccharum* complex, consisting of *S. officinarum, S. barberi, S. sinense, S. robustum, S. spontaneum, Erianthus, Miscanthus*, and *Saccharum* hybrids as an outgroup used in this study (Table 1).

**Morphological characterization**

Morphological characterization was carried out directly in the ISRI’s germplasm collection station when the plants were ± seven months old. Each accession was planted in 2 rows, and each row
consisted of 10 clumps in the germplasm station. Data on measurable morphological characters were recorded in 2017/2018 planting season. Morphological traits were measured on ten random stems for each accession. A total of 37 morphological characters covering three main organs, namely leaves, stems, and buds, were evaluated according to Sastrowijono (1996). Most of these attributes were not subjected to selection in breeding programs; 7 of them related to leaves, 9 of them related to stem traits, while the other seven were subsidiary traits related to buds (Table 2). All measures and observations were carried out in the ISRI’s germplasm station and laboratory by means of metric rule and color chart, by the same operators for each attribute, considered stable enough observations for the different genotypes.

Molecular characterization

Deoxy Nucleic Acid (DNA) extraction was carried out using the CIMMYT Laboratory method. This method followed Saghari-Maroof et al. (1984) with minor modification by reducing one cycle for precipitation. DNA was precipitated without overnight incubation at room temperature but incubated for 60 minutes at -20ºC. This method had been used in the molecular research of sugarcane by Alix et al. (1998). The molecular characterization in this study used three types of microsatellite primers, as shown in Table 3.

The PCR results were visualized by electrophoresis on 1.5% agarose gel with 100 bp DNA ladder (Fermentas) and 1 kb DNA ladder (Promega) as DNA markers. The gel was visualized using Ethidium Bromide by incubated for 45 minutes in a dark room and observed with a UV transilluminator and documented with Doc XR Gel (Biorad).

Data analysis and interpretation

Morphological character data were transferred to the binary data format with number 1 for the character that appeared (present) and number 0 for the character that did not appear (absent). Likewise, molecular data in the form of amplified DNA bands were interpreted as qualitative data. Then the data were presented in the form of binary data as seen from the presence and absence of DNA bands. The presence of DNA bands was marked with the number 1, while the absence of DNA bands as indicated by the number 0 in the same array. The interpretation of this data referred to the research of Pan et al. (2003). The DNA bands generated through microsatellite markers were marked with numbers 1 and 0 and presented in the form of binary data.

Table 1. Sugarcane germplasm accessions were used in the study

| No. | Accession name/ Nama aksesi  | Species/ Spesies  | Locations/ Lokasi  | Years of expedition/ Tahun expedisi |
|-----|-----------------------------|-------------------|--------------------|-------------------------------------|
| 1   | IS 76-202                   | Erianthus         | Sulawesi           | 1976                                |
| 2   | IS 76-205                   | Erianthus         | Sulawesi           | 1976                                |
| 3   | IK 76-149                   | Erianthus         | Kalimantan         | 1976                                |
| 4   | IK 76-150                   | Erianthus         | Kalimantan         | 1976                                |
| 5   | IK 76-153                   | Erianthus         | Kalimantan         | 1976                                |
| 6   | IJ 76-374                   | Erianthus         | Irian Jaya         | 1976                                |
| 7   | IJ 76-375                   | Erianthus         | Irian Jaya         | 1976                                |
| 8   | IJ 76-370                   | Erianthus         | Irian Jaya         | 1976                                |
| 9   | IJ 76-348                   | Erianthus         | Irian Jaya         | 1976                                |
| 10  | IJ 76-396                   | Erianthus         | Irian Jaya         | 1976                                |
| 11  | M 442-51                    | Saccharum spp. Hybrids | B37172 X M213-40  | *)                                   |
| 12  | PS-862                      | Saccharum spp. Hybrids | POJ 2722 (polycross) | *)                                  |
| 13  | TD 50                       | S.officinarum     | Jawa               | *)                                  |
| 14  | TD 53                       | S.officinarum     | Jawa               | *)                                  |
| 15  | 15 OC 18                    | S.sinense         | *)                  | 1915                                |
| 16  | 15 OC 19                    | S.sinense         | *)                  | 1915                                |
| 17  | IK76-36                     | S.spontaneum      | Kalimantan         | 1976                                |
| 18  | IK76-41                     | S.spontaneum      | Kalimantan         | 1976                                |
| 19  | IS 76-175                   | Miscanthus        | Sulawesi           | 1976                                |
| 20  | IS 76-181                   | Miscanthus        | Sulawesi           | 1976                                |
| 21  | IJ 76-412                   | S.rostustum       | Irian Jaya         | 1976                                |
| 22  | IJ 76-415                   | S.rostustum       | Irian Jaya         | 1976                                |
| 23  | X OC 55                     | S.barberi         | *)                  | 1921                                |
| 24  | X OC 56                     | S.barberi         | *)                  | 1925                                |

Notes: *) unknown
Keterangan: *) tidak diketahui
Table 2. Qualitative and quantitative morphological markers are assessed in 24 accessions of the Saccharum complex. Abbreviation, morphological markers/characters, and descriptors were indicated for each observed variable

| Abbreviation | Morphological markers/characters | Descriptors |
|--------------|---------------------------------|-------------|
| D1 | Curved leaf blade of the plant | A. Upright (Tegak)  
Lengkung helai daun | B. Curved <1/2 (Melengkung<1/2)  
C. Curve ≥1/2 (Melengkung≥1/2) |
| D2 | Leaves color | A. Green (Hijau)  
Warna daun | B. Dark green (Hijau tua)  
C. Yellowish green (Hijau kekuningan) |
| D3 | The width of leaf blade | A. Narrow (<4cm) (Sempit (<4cm)  
Lebar daun | B. Medium (4-6cm) (Sedang 4-6cm)  
C. Width (>6cm) (Lebar >6cm) |
| D4 | Auricles position | A. Upright (Tegak)  
Kedudukan telinga daun | B. Oblique (Serong)  
C. Absent (Tidak bertelinga) |
| D5 | The height of auricles | A. 1 cm  
Tinggi telinga daun | B. >1-<3 cm  
C. ≥3 cm  
D. Absent (Tidak bertelinga) |
| D6 | Dewlap color | A. Yellowish green (Hijau kekuningan)  
Warna sendi segiitiga daun | B. Brownish green (Hijau kecoklatan)  
C. Green brownish yellow (Hijau kuning kecoklatan)  
D. Green (Hijau)  
E. Others (Lainnya) |
| P1 | Hairy ligule | A. Absent (Tidak ada)  
Bulu bidang punggung | B. Rarely (Jarang)  
C. Heavy (Lebat) |
| P2 | The length of the hairy ligule | A. Not reaching the top of the ligule (Tidak mencapai puncak bidang punggung)  
Punjang bulu bidang punggung | B. Nearly reaching (≤1cm) the top ligule (Hampir mencapai (≤1cm) puncak bidang punggung)  
C. Reaching the top of the ligule (Mencapai puncak bidang punggung)  
D. Absent (tidak ada) |
| P3 | Hairy ligule width | A. Narrow (<1/4 ligule) (Sempit <1/4 lebar bidang punggung)  
Lebar bulu bidang punggung | B. Width (≥1/4 ligule) (Lebar ≥1/4 lebar bidang punggung)  
C. Absent (Tidak ada) |
| P4 | Hairy ligule position | A. Fall down (Rebah)  
Kedudukan bulu bidang punggung | B. Leaning (Condong)  
C. Upright (Tegak)  
D. Absent (Tidak ada) |
| P5 | Leaf midrib wax | A. Little (Sedikit)  
Lapisan lilin pelepas | B. Medium (Sedang)  
C. A lot (Ranyak) |
| P6 | Removing leaf midribs | A. Hard (Sulit)  
Sifat lepas pelepas | B. Rather easy (Agak mudah)  
C. Easy (Mudah) |
| P7 | Leaf midrib color | A. Dark green (Hijau tua)  
Warna pelepas | B. Yellowish green (Hijau kekuningan)  
C. Green purple (Hijau kuning kecoklatan)  
D. Reddish yellow green (Hijau kuning kemerahan)  
E. Others (Lainnya) |
Table 2. (Continue)

| R1 | Internode color |                      |
|----|-----------------|----------------------|
|    | Warna ruas      | A. Yellowish green   |
|    |                 | (Hijau kekuningan)   |
|    |                 | B. Green (Hijau)     |
|    |                 | C. Green brownish    |
|    |                 | yellow (Hijau kuning |
|    |                 | kecoklatan)          |
|    |                 | D. Green purplish    |
|    |                 | yellow (Hijau kuning |
|    |                 | keunguan)            |
|    |                 | E. reddish yellow    |
|    |                 | green (Hijau kuning  |
|    |                 | kemerahan)           |
|    |                 | F. Others (Lainnya)  |

| R2 | Internode wax |                      |
|----|--------------|----------------------|
|    | Lapisan      | A. Absent (Tidak     |
|    | lilin ruas  | ada)                 |
|    |              | B. Thin (Tipis)      |
|    |              | C. Medium (Sedang)   |
|    |              | D. Thick (Tebal)     |

| R3 | Internode arrangement |                      |
|----|-----------------------|----------------------|
|    | Susunan ruas          | A. Straight (Lurus)  |
|    |                       | B. Zig zag (Berlika) |

| R4 | Internode shape |                      |
|----|-----------------|----------------------|
|    | Bentuk ruas     | A. Cylindrical       |
|    |                 | (Silindris)          |
|    |                 | B. Tumescent         |
|    |                 | (Tong)               |
|    |                 | C. Bobbin (Kumparan) |
|    |                 | D. Conoidal (Konis)  |
|    |                 | E. Obconoidal (Konis |
|    |                 | terbalik)            |
|    |                 | F. Convex-concave    |
|    |                 | (Cembung-cekung)     |

| R5 | Corky path |                      |
|----|------------|----------------------|
|    | Noda gabus | A. Absent (Tidak     |
|    |            | ada)                 |
|    |            | B. Few (Sedikit)     |
|    |            | C. A lot (Banyak)    |

| R6 | Corky cracks |                      |
|----|--------------|----------------------|
|    | Retakan      | A. Absent (Tidak     |
|    | gabus       | ada)                 |
|    |             | B. Few (Sedikit)     |
|    |             | C. A lot (Banyak)    |

| R7 | Growth crack |                      |
|----|--------------|----------------------|
|    | Retakan      | A. Absent (Tidak     |
|    | tumbuh       | ada)                 |
|    |              | B. Rarely (Jarang)   |
|    |              | C. Almost all        |
|    |              | internode (Hampir   |
|    |              | di seluruh ruas)    |

| R8 | Cross section of internode |                      |
|----|----------------------------|----------------------|
|    | Penampang melintang ruas  | A. Round (Bulat)     |
|    |                            | B. Flat (Pipih)      |

| R9 | Bud furrow |                      |
|----|------------|----------------------|
|    | Alur mata  | A. Absent (Tidak     |
|    |            | ada)                 |
|    |            | B. Shallow (Dangkal) |
|    |            | C. Deep (Dalam)      |

| R10| The width of bud furrow |                      |
|    | Lebar alur mata         | A. Absent (Tidak     |
|    |                          | ada)                 |
|    |                          | B. Narrow (Sempit)   |
|    |                          | C. Width (Lebar)     |

| R11| The length of bud furrow |                      |
|    | Panjang alur mata        | A. Absent (Tidak     |
|    |                          | ada)                 |
|    |                          | B. Not reaching the  |
|    |                          | middle of the       |
|    |                          | internode (Tidak    |
|    |                          | mencapai tengah      |
|    |                          | ruas)               |
|    |                          | C. Reach the middle  |
|    |                          | of internode (Mencapai |
|    |                          | tengah ruas)        |

| B1 | Growth ring position |                      |
|----|----------------------|----------------------|
|    | Letak cincin tumbuh  | A. Above the top of  |
|    |                      | the bud (Di atas     |
|    |                      | puncak mata)        |
|    |                      | B. Offends the top  |
|    |                      | of the bud (Menyinggung |
|    |                      | puncak mata)       |
|    |                      | C. Behind the top of |
|    |                      | the bud (Di belakang |
|    |                      | puncak mata)       |
|    |                      | D. Below the top of  |
|    |                      | the bud (Dibawah    |
|    |                      | puncak mata)       |

| B2 | Growth ring shape |                      |
|----|-------------------|----------------------|
|    | Bentuk cincin tumbuh | A. Circular flat    |
|    |                    | (Melingkar datar)   |
|    |                    | B. Curved (Melengkung) |

| B3 | Root band number |                      |
|----|------------------|----------------------|
|    | Jumlah mata akar | A. 1 row (1 baris)  |
|    |                   | B. 2-3 rows (2-3 baris) |
|    |                   | C. 3-4 rows (3-4 baris) |
|    |                   | D. > 4 rows (>4baris) |


Table 2 (Continue)

| No. | B4 | Node shape  | A. Cylindrical (Silindris)  
|     |    | Bentuk buku ruas | B. Conoidal (Konis)  
|     |    |                   | C. Obconoidal (Konis terbalik)  
|     |    |                   | D. Tumescent (Tong)  
|     |    |                   | E. Bobbin (Kumparan)  
| M1  | M2 | Bud position | A. On the former leaf midrib (Pada bekas pangkal pelepah daun)  
|     |    | Kedudukan mata | B. Above the former leaf midrib (Di atas bekas pangkal pelepah daun)  
|     |    |                 | C. Obconoidal (Konis terbalik)  
|     |    |                 | D. Tumescent (Tong)  
|     |    |                 | E. Bobbin (Kumparan)  
| M2  |    | Bud shape | A. Round (Bulat)  
|     |    | Bentuk mata | B. Oval (Oval)  
|     |    |             | C. Obtuse (Bulat telur)  
|     |    |             | D. Rhomboid (Bulat telur terballik)  
|     |    |             | E. Triangular (Segitiga)  
| M3  |    | The widest part of the bud | A. Under the middle of the bud (Di bawah tengah-tengah mata)  
|     |    | Bagian terlebar mata | B. In the middle of the bud (Pada tengah-tengah mata)  
|     |    |               | C. Above the middle of the bud (Di atas tengah-tengah mata)  
| M4  |    | Bud wing size | A. Equal width (Sama lebar)  
|     |    | Ukuran sayap mata | B. Narrow base (Basis sempit)  
|     |    |                 | C. Base width (Basis lebar)  
| M5  |    | Growth center | A. Under the middle of the bud (Di bawah tengah-tengah mata)  
|     |    | Pusat tumbuh | B. In the middle of the bud (Pada tengah-tengah mata)  
|     |    |                | C. Above the middle of the bud (Di atas tengah-tengah mata)  
|     |    |                | D. At the top of the bud (Di puncak mata)  
| M6  |    | The hair on the edge of the bud | A. Absent (Tidak ada)  
|     |    | basal Rambut tepi basal mata | B. Present (Ada)  
| M7  |    | Hair on top of bud | A. Absent (Tidak ada)  
|     |    | Rambut jambul mata | B. Present (Ada)  
| M8  |    | Bud size | A. Small (Kecil)  
|     |    | Ukuran mata | B. Medium (Sedang)  
|     |    |            | C. Big (Besar)  
| M9  |    | The edge of the bud wing | A. Flat (Rata)  
|     |    | Tepi sayap mata | B. Jagged (Bergerigi)  
|     |    |                | C. Notched peaks (Puncak berlekur)  

Table 3. Three types of microsatellite primers were used in this study  
*Tabel 3. Tiga jenis primer mikrosatelit yang digunakan dalam penelitian*

| No. | Primers/Primer | SSR Sequences/Sekuen SSR |
|-----|----------------|---------------------------|
| 1.  | MCSA053C10     | (CAG)5                    |
| 2.  | SMC1047HA      | (GA)26                    |
| 3.  | mSSCIR5        | (GGC)9                    |

Furthermore, binary data were also used to determine the value of genetic similarity and arrange genetic relationship trees. The genetic similarity coefficient used for morphological markers was the Simple Matching similarity coefficient (Sneath & Sokal, 1973). For molecular data, relationships between pairwise accessions were estimated using the Jaccard Coefficient (Cordeiro et al., 2003; Pan et al., 2003; Pocovi et al., 2020).

In this study, the accessions were then clustered by the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) cluster method with the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC 2.02i) program (Schenk et al., 2004; Alwala et al., 2006; Parida et al., 2008; Pocovi et al., 2020). Cophenetics value matrices of the UPGMA clustering were used to test the goodness-of-fit of the clustering to the similarity matrix on which it was based by means of computing the product-moment correlation (r) with 1000 permutations (Mantel, 1967).
Results and Discussion

Morphological characterization

The 24 accessions of the Saccharum complex analyzed, based on 37 morphological characters, showed similarities and differences in morphological descriptors. The most similar morphological characters in the 24 accessions were on the leaves, internodes, and buds. On the leaves, similar traits are curved leaf blade, leaf color, auricle position, auricle height, dewlap color, the length of the hairy ligule, the width of the hairy ligule, hairy ligule position, midrib wax, removing leaf midribs, and leaf midrib color. On the internode, similar characters are internode color, internode shape, corky path, corky cracks, growth cracks, and the cross-section of the internode. In the bud, similar characters are bud furrow, bud furrow width, bud furrow length, growth ring position, growth ring shape, bud position, bud shape, the widest part of the bud, growth center, the hair on the edge of the bud basal, hair on top of bud, bud size and the edge of the bud wing.

The accessions within the Saccharum species show several similar morphological characters. Two S. officinarum accessions (TD50 and TD53) have 27 characters in common, two S. sinense accessions (15OC18 and 15OC19) have 31 characters in common, two S. spontaneum accessions (IK76-36 and IK76-41) have 33 characters in similarity, two S. barberi accessions (XOC55 and XOC56) and two S. robustum accessions (UJ76-412 and IJ76-415) each have 17 characters in common.

The morphological characters distinguished between the accessions analyzed from the genus Erianthus and Saccharum in this study were located in the internode and bud. The distinctive character included the arrangement of internode (Erianthus: straight, Saccharum: zigzag), cross-section of internode (Erianthus: flat, Saccharum: round), the number of root buds (Erianthus: 1 row, Saccharum: 2-3 rows), and the widest part of the bud (Erianthus: under the middle of the bud, Saccharum in the middle of the bud). This resembles the observations reported by Amalraj and Balasundaram (2006), concerning the difference between Saccharum and Erianthus, among others, on the number of root buds. Erianthus had only 1 row of root bud number, whereas in Saccharum the number of root buds was two rows or more.

Qualitative data of morphological characterization were transformed into binary data to make a relationship dendrogram. Figure 1 shows that the phylogenetic relationship among 24 accessions of the Saccharum complex is divided into two large clusters at the similarity value of 0.66 (66%). The first cluster consists of 22 accessions and the second cluster consists of 2 accessions, namely PS 862 (Saccharum hybrid) and XOC55 (Miscanthus).

The greatest value of genetic similarity based on morphological characters was between IK 76-375 and IK 76-205 of 0.9683 (97%). The genetic distance between the two accessions was 0.0317, which means that the phylogenetic relationship between the two accessions is close. This was because IK 76-375 and IK 76-205 are both of the Erianthus genus originating from Kalimantan. In contrast, the lowest genetic similarity values were PS 862 and IJ 76-396 at 0.5714 (57%), and the genetic distance was 0.4286. PS 862 was a commercial sugarcane variety from ISRI's breeding program (Saccharum hybrid), while IJ 76-396 was Erianthus from Papua (Indonesia) so that the genetic relationship between the two accessions was farther than the other accessions.
The clustering based on morphological markers (Figure 1) mainly corresponded to the classification at the conservation time. However, there were several accessions of *Saccharum barberi*, namely XOC 56 and XOC 55, which were distinct from one another, and accessions of *Saccharum robustum*, namely IJ76-412 and IJ76-415, which were not in the same group. Meanwhile, the accessions of *Erianthus, S. spontaneum, S. officinarum*, and *S. sinensis* were clustered according to the classification at the time of conservation.

**Molecular characterization**

The results of DNA amplification of the three microsatellite primers are shown in Figure 2-4. The three primers produced 90 DNA bands with varying sizes of base pairs. In this study, the DNA bands analyzed were DNA bands that could be seen clearly, regardless of the difference in intensity (thickness or thinness of the DNA bands).

Figure 2 shows that the microsatellite amplification of 24 accessions using mSSCIR5 primer resulted in 44 polymorphic DNA bands, with sizes ranging from 83 bp to 1600 bp. Meanwhile, Figure 3 shows that the SMC1047HA primer amplified the DNA of 24 *Saccharum* complex accessions to produce 22 bands. The sizes of the DNA bands ranged from 76 bp to 1642 bp. In Figure 4, it can be seen that the MSCA053C10 primer produced 24 DNA bands with sizes ranging from 98 bp to 950 bp.

Three pairs of microsatellite primers produced 90 DNA bands in the range of 76 bp - 1642 bp. The data generated from the three microsatellite primers were transformed into binary data to form a dendrogram for genetic relationships using the NTSYSpc-2.02i program. Jaccard’s genetic similarity coefficient used a scale of 0.00 - 1.00. The value of 1.00 indicated 100% similarity, which meant that the value of genetic distance or dissimilarity was 0.00 (Indah et al., 2008; Pocovi et al., 2020).

The dendrogram constructed from three microsatellite primers shows in Figure 5 that the 24 accessions of the *Saccharum* complex were divided into two large clusters at a similarity value of 0.02. Large cluster I at a similarity value of 0.19 formed two subclusters, namely I.1 and I.2. Sub-cluster I.1 was divided into four sub-clusters, namely: (A) *S. spontaneum* cluster, (B) *S. officinarum* cluster, (C) *Erianthus* cluster from Kalimantan and Sulawesi; (D) *S. sinensis* cluster.
Figure 3. Electrophorogram of 24 accessions DNA amplification using SMC1047HA primers.

Gambar 3. Elektroforegram amplifikasi DNA 24 aksesi menggunakan primer mikrosatelit SMC1047HA

Remark: IS 76-202, 2. IS 76-205, 3. IK 76-149, 4. IK 76-150, 5. IK 76-153, 6. IJ 76-374, 7. IJ 76-375, 8. IJ 76-370, 9. IJ 76-348, 10. IJ 76-396, 11. M 442-51, 12. PS-862, 13. TD 50, 14. TD 53, 15. 15 OC 18, 16. 15 OC 19, 17. IK76-36, 18. IK76-41, 19. IS 76-175, 20. IS 76-181, 21. IJ 76-415, 22. IJ 76-415, 23. X OC 55, 24. X OC 56, M1. Marker 100 bp and M2. Marker 1kb.

Keterangan: IS 76-202, 2. IS 76-205, 3. IK 76-149, 4. IK 76-150, 5. IK 76-153, 6. IJ 76-374, 7. IJ 76-375, 8. IJ 76-370, 9. IJ 76-348, 10. IJ 76-396, 11. M 442-51, 12. PS-862, 13. TD 50, 14. TD 53, 15. 15 OC 18, 16. 15 OC 19, 17. IK76-36, 18. IK76-41, 19. IS 76-175, 20. IS 76-181, 21. IJ 76-415, 22. IJ 76-415, 23. X OC 55, 24. X OC 56, M1. Marker 100 bp and M2. Marker 1kb.

Figure 4. Electrophorogram of 24 accessions DNA amplification results using MSCA053C10 primer.

Gambar 4. Elektroforegram hasil amplifikasi DNA 24 aksesi menggunakan primer MSCA053C10

Remark: IS 76-202, 2. IS 76-205, 3. IK 76-149, 4. IK 76-150, 5. IK 76-153, 6. IJ 76-374, 7. IJ 76-375, 8. IJ 76-370, 9. IJ 76-348, 10. IJ 76-396, 11. M 442-51, 12. PS-862, 13. TD 50, 14. TD 53, 15. 15 OC 18, 16. 15 OC 19, 17. IK76-36, 18. IK76-41, 19. IS 76-175, 20. IS 76-181, 21. IJ 76-415, 22. IJ 76-415, 23. X OC 55, 24. X OC 56, M1. Marker 100 bp and M2. Marker 1kb.

Keterangan: IS 76-202, 2. IS 76-205, 3. IK 76-149, 4. IK 76-150, 5. IK 76-153, 6. IJ 76-374, 7. IJ 76-375, 8. IJ 76-370, 9. IJ 76-348, 10. IJ 76-396, 11. M 442-51, 12. PS-862, 13. TD 50, 14. TD 53, 15. 15 OC 18, 16. 15 OC 19, 17. IK76-36, 18. IK76-41, 19. IS 76-175, 20. IS 76-181, 21. IJ 76-415, 22. IJ 76-415, 23. X OC 55, 24. X OC 56, M1. Marker 100 bp and M2. Marker 1kb.
The results of this study similar to the results of research conducted by Olieviera et al. (2017) to assess the genetic diversity of 26 accessions of sugarcane from the Active Germplasm Bank of the Embrapa Coastal Tablelands, using inter-simple sequence repeat (ISSR) molecular markers. Sixteen primers were used, resulting in 87 fragments with 91.13% of polymorphism. The similarity of the individuals ranged between 0.22 and 0.87. Four distinct clusters were formed using UPGMA. Despite the large distribution of accessions in most groups, indicating great genetic diversity, these were not separated by a group of the same species in each subcluster, which common alleles could explain among different species, since they are of the same genus. Similar results were found by Raj et al. (2016) that there was a close relationship among the different species that constitute the Saccharum complex; however S. spontaneum, contributing to greater diversity.

Subcluster I.2 consisted of the S. robustum accessions. Large cluster II at a similarity value of 0.04 was divided into two subclusters, namely, subgroup II.1 consisting of accessions of the genus Erianthus originating from Papua and subcluster II.2 consisting of (A) accessions from S. barberi; (B) accession of the genus Miscanthus; and (C) accession of the Saccharum hybrid. Figure 5 shows that the grouping based on the molecular markers of the 24 accessions analyzed were grouped according to species, this is the same as the grouping at the time of conservation. The genetic similarity value for the 24 accessions was very low at around 0.20.

As reported by Aitken et al. (2018), who analyzed the diversity of several accessions to S. spontaneum species from 21 different countries using molecular markers, the results of their analysis revealed that the majority of the accessions were clustered according to the country of origin. Genetic similarity ranged from 0.25 to 0.54, with the highest diversity in accessions collected in Indonesia, followed by China, India, and Thailand, and the lowest in the Philippines. Likewise, the results of the analysis conducted by Manish et al. (2014) on 40 genotypes of Indian sugarcane using microsatellites. The molecular results obtained in the study of Manish et al. (2014) are represented and agree with the possible evolutionary course of sugarcane genotypes. The UPGMA cluster analysis of 40 genotypes in that study produced meaningful grouping based on pedigree or geographical origin of the accessions. The grouping patterns of 40 genotypes are based on their genetic similarity pattern which shows that sugarcane genotypes from the same geographical regions tend to cluster together which may resulting from a shared evolutionary pathway.
Genetic relationship based on morphological and molecular markers

Information about the value of genetic similarity can be used as a reference to determine the value of genetic distance, phylogenetic relationships, and the level of genetic diversity. The value of genetic similarity is directly proportional to the genetic relationship but inversely proportional to the value of genetic distance or dissimilarity. The greater the value of similarity between two accessions, the greater the genetic relationship (closer), but conversely, the value of the genetic distance is small.

The markers used in this study were morphological and microsatellite markers, each of which had advantages and disadvantages. The use of these two markers in this study was to support and complement the analysis of the grouping and phylogenetic relationship of 24 accessions of the Saccharum complex. The data derived from morphological and molecular character were qualitative, so they were transformed into binary data before making the dendrogram of genetic relationship.

The dendrogram of the genetic relationship of the morphological characters was constructed based on the Simple Matching (SM) coefficient similarity (Figure 1). The dendrogram shows that the 24 accessions of the Saccharum complex including the genus Erianthus and Saccharum have a close relationship of 0.5714-0.9683 with a genetic distance of 0.0317-0.4286. This low diversity was due to the 24 accessions analyzed with similar morphological features from the 37 characters observed. Therefore, grouping based on morphological markers was considered insufficient to verify the classification of germplasm collections managed by ISRI, so that molecular markers were still needed to support the validity of existing groupings.

On the other hand, the value of genetic similarity based on molecular markers between 24 accessions ranged from 0.000 to 0.889 (Figure 5). Duplication (similarity value = 1.00) was not found among the 24 clones tested. A high genetic similarity was observed in some accessions because the accessions were included in groups of the same species.

Qualitative data on the characterization of molecular markers were used to construct a dendrogram of genetic relationship with Jaccard’s similarity coefficient. The Jaccard’s coefficient is appropriate for molecular markers because Jaccard's coefficient only considers the characters or bands that appeared (present) as a contributing factor to individual similarity and ignores zero (0) or absence. As in the research of Cordei et al. (2003), Widiasari et al. (2008) and Pocovi et al. (2020) used Jaccard’s coefficient to analyze the genetic diversity of sugarcane which based on molecular markers.

The grouping based on the molecular data shows that of the 24 accessions evaluated, each accession was grouped according to its species. This means that based on molecular markers, the grouping of ISRI’s sugarcane germplasm collections was still in accordance with the grouping at the conservation time.

The results of this study indicate that there are differences in the value of genetic similarity in the 24 accessions analyzed based on the markers used. According to morphological markers, the value of genetic similarity was higher than the value of genetic similarity based on molecular markers. The morphological markers caused the difference because the observed morphological characters were 37 characters with 126 descriptions, while the molecular markers, using three microsatellite primers, could produce 90 DNA bands. In addition, descriptions of morphological characters that were equally absent were considered the same variable, even though their morphological characters were different. It was a cause of increased genetic similarity value when the genetic relationship was analyzed using morphological markers.

Another factor influencing these differences is the difference in the similarity coefficient used on morphological and molecular markers in evaluating the genetic relationship of those 24 accessions. Morphological markers used the Simple Matching similarity coefficient, which involved the absent value (d, 0-0). In contrast, the molecular markers used the Jaccard’s similarity coefficient, which only involved the present values (a, b, c).

However, the two markers gave the same grouping results. This can be seen in the accessions of S. officinarum, TD50 with TD53 into one group; S. spontaneum, IK76-36 and IK76-41 in one group; S. sinense, 15OC18 and 15OC19 in one group; Erianthus clones originating from Irian Jaya (I) also form a separate subgroup of Erianthus originating from Kalimantan and Sulawesi. Meanwhile, the classification of several accessions was different based on morphological and molecular markers. It was shown in the accessions of S. barberi, S. robustum, and Saccharum hybrid. The fact that grouping based on molecular markers is not exactly the same as a grouping based on morphological markers is also found in Pocovi et al. (2020).

Regarding the accuracy of genetic relationships, some researchers argue that molecular markers are considered to be more accurate in phylogenetic analysis than morphological markers. This is because molecular markers analyze individuals at the DNA level, whereas morphological markers had several drawbacks that could affect the accuracy of the data. Bagal et al. (2010) stated that morphological markers are markers that recognize a character according to their natural appearance based on the observer's view (subjectivity). In addition, Martinez et al. (2003) reported that morphological markers were relatively insignificant and inefficient for accuracy in
distinguishing closely related genotypes and analyzing their genetic similarities when compared to DNA fingerprinting techniques. However, morphological markers are helpful at the beginning or early stage, fast, simple, and inexpensive to identify varieties, and could be used as a general approach to measuring genetic diversity between cultivars of different phenotypes. Karuri et al. (2010) stated that it was necessary to combine them with genetic-based markers due to limitations in morphological markers.

**Conclusion**

The grouping of the 24 accessions of the ISRI’s *Saccharum* complex germplasm collection was based on molecular markers according to the grouping at the t conservation time. Meanwhile, the grouping based on morphological markers in several accessions did not match the classification at the conservation time. This occurs in accessions of the *Saccharum robustum, Saccharum barbieri, Saccharum spontaneum*, and the genus *Miscanthus*. Based on molecular markers, the 24 accessions of the *Saccharum* complex had a high degree of diversity. These clones can be used as a combination of crossing parents to produce new superior sugarcane varieties.

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