Evaluation of Pest Control Based on Morphological Character Variation on 20 Varieties and Genetic Variation Based on RAPD of Sugarcane (*Saccharum officinarum* L.) in Indonesia

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**Abstract.** Sugarcane (*Saccharum officinarum* L.) is the main crop of sugar and one of the main resources for consumption of Indonesian society. Government of Indonesia, which supports self-sufficiency targeting enough amount of sugar, high quality and low price in Indonesia. Indonesian Sweetener and Fibre Crops Research Institute (ISFRI) Malang are one of research site agriculture in Indonesia which specialize in sugarcane start from various researches to increase sugar cane production including sugarcane cultivation. Constraints that often encountered farmers in the process of sugarcane cultivation are a disruption of pest disease. Therefore, early prevention as an effort to control pests and diseases in sugarcane crops needed. Aims of this research were to find character of high quality sugar cane as a breeder of new varieties that are superior and resistant to pests and to analysis genetic variation of 20 varieties sugarcane based on RAPD molecular marker. This research was conducted in KP Karangploso, ISFRI Malang, East Java. The method used is observation and identification on germplasm of sugarcane; each variety was analysed descriptively and numerical phenetic with the similarity of simple matching coefficient (Ssm) and simple Jaccard coefficient (Sj). The character of each variety of germplasm had its own advantages. Genetic variation based on RAPD of 20 varieties shown taxonomical relation between varieties. The results showed that 20 varieties of characterized sugarcane morphology that expected tolerant of pest were in the upstream form of the cylindrical trunk segment, the moderate stem borders, the thick stem wick coat, the thick dorsal leaf hair, and the leaf exfoliate difficult. Variety of sugarcane number accession 475 SOFF 1208 shown the best pest tolerant morphologically and has the lowest percentage of pest attack to 0.29%. The result of ssm and sj shown that breeders suggested in the form of new varieties that are superior and resistant to pests according to most tolerant variety 475 SOFF 1208 are 350 SOFF 1082, 357 SOFF 1089, 360 SOFF 1092, 368 SOFF 1100, 454 SOFF 1186, 473 SOFF 1205, and 476 SOFF 1208.
1. Introduction

Sugarcane (Saccharum officinarum L.) belongs to genus Saccharum, tribe Andropogonaceae of family Poaceae or Gramineae [1]. Saccharum is a genetically complex genus of crop and composed of at least six distinct species as S. officinarum, S. barberi, S. sinense, S. spontaneum, S. robustum, dan S. edule[1-2]. Sugarcane is a main crop for sugar production and plays a vital economic role in Indonesia. Hence, Indonesian Sweetener and Fiber Crops Research Institute (ISFRI) as an agriculture research institute focus on improving sugarcane productivity. The main problem of sugarcane productivity in Indonesia are pests attack and low sugar content caused by unwell cultivation treatment that done by farmers where most farmer are more concerned with plant mass [3]. Breeding is widely known as a basic principle to increase most sugarcane industries productivity in the world and contributes to high degree of genetic variety [1, 5]. The choice of the variety is also one of the most important factor because of its ability to increase sugarcane productivity without an increase in growing costs, and pest-resistant crop may even reduce growing costs [1, 4].

Various efforts have been made to improve sugarcane variety which generally divided into three stage: characterization and evaluation of germplasm, cross-pollination, and selection. The final stage of the three stage leads to adaptation of individual environment [1, 5]. The aim of characterization is to record highly heritable characters, can be easily seen by the eye and expressed in all environment. Hence, morphological characterization is still widely used because of its ease to use [1]. Despite of morphology characters, the estimation of genetic diversity done by using morphological markers are preferred as they are based on the genotypes of an individual, show genetic variation in more detail level. Several molecular marker that can be used to detecting differences within and among genotypes are Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Inter-Simple Sequence Repeat (ISSR), Single Nucleotide Polymorphism (SNP) and Isozymes [2-6]. Despite of questions about its reproducibility, its utility in diversity analysis, the selection of RAPD marker was based on its simplicity, level of polymorphism detection, cost effectiveness, easily applicable to any plant species and target those sequences which abundant throughout the genome and are rapidly evolved [6]. The focus of this study is characterization and evaluation of germplasm collection in Indonesian Sweetener and Fibre Crops Research Institute (ISFRI) to gain superior quality and pests-resistant based on morphological characters and Rapid Amplified Polymorphic DNA (RAPD) marker.

2. Materials and Methods

2.1. Plant Material and Morphological Data Collection

This study evaluated twenty sugarcane varieties from Australia and already cultivated in Indonesian Sweetener and Fiber Crops Research Institute (ISFRI). These twenty varieties are 353 SOFF 1085, 472 SOFF 1204, 477 SOFF 1209, 351 SOFF 1083, 474 SOFF 1206, 352 SOFF 1084, 479 SOFF 1211, 350 SOFF 1082, 475 SOFF 1208, 478 SOFF 1210, 454 SOFF 1186, 291 SOFF 1023, 290 SOFF 1022, 289 SOFF 1021, 288 SOFF 1020, 287 SOFF 1019, 286 SOFF 1018, 282 SOFF 1014, 283 SOFF 1015, 296 SOFF 1028. Morphological data collection done in ISFRI at July 2017. Twenty varieties of sugarcane germplasm are breeded and selected with high brix by Indonesian Sugar Research and Development Center (P3GI).

2.2. DNA Isolation

DNA was isolated from young leaf tissues using a CTAB (cetyl-trimethyl-amonium bromide) protocol [11]. 0.2 gram leaf mashed with sterile mortar and pestle, then added PVP 0,02 gram and 2% CTAB 300 μL. Sample putted into microtube1,5 mL and added buffer CTAB 2% 400 μL. Mixed sample added β-mercaptoethanol and incubated 15 min in water bath 65oC. Sample mixed with CIA solution (24:1) 600μL in cold condition and homogenized for 30 min, then centrifuged 10 min in 13.000 rpm by microcentrifuge (Gyro Spin Gyrozen). Supernatant added with cold isopropanol ratio 1:1. Sample inverted for 14 times and precipitated in 60 min then centrifuged 10 min 13.000 rpm. Supernatant thrown and 100μL absolute ethanol added, centrifuged 10 min 13.000 rpm. Supernatant thrown again and 70% ethanol added, then centrifuged 5 min 13.000 rpm. Pellet dissolved in 50 μL buffer TE and stored in freezer -20°C. DNA concentrations were determined comparatively by spectrophotometer (NanoVue
Plus) in lambda 260 nm. Each λ 260 nm absorbance showed 50 μg/mL of DNA concentration. DNA concentration measured with absorbance formula λ 260 x 50 x dissolve μg/mL. Purity of DNA measured in spectrophotometer which best ratio in between 1.8-2.0 [12].

2.3. RAPD Analysis
The RAPD amplification by PCR (T100 Thermal Cycler BioRad) which each reaction contained a kit 12.5 μL, RAPD primer (OPD1 and OPC2) 2.0 μL, ddH2O 8.5 μL, and template DNA 2.0 μL. The amplification reaction was carried out in an Eppendorf Master cycler Gradient or Techno TC-412 thermal cycler. The reaction included an initial denaturation step of 10 min at 94°C, followed by 35 cycles, each consisting of a denaturation step of 1 min at 94°C, annealing of 1 min at 43°C to 44.3°C (depending on the primer) and an extension of 2 min at 72°C. PCR was terminated with a final extension of 10 min at 72°C. RAPD products were separated on 2% horizontal agarose gels, in TBE buffer with staining in 0.5 μg/mL ethidium bromide, processed in a voltage of 50 V for 55 min and visualized under ultraviolet light using Gel Doc and OptiLab. Digital photo documentation was taken for each gel. The 500 bp DNA ladder plus molecular weight marker was used to compare the molecular weight of amplified products. Twenty samples previously selected for S.officinarum varieties were chosen and ordered from ISFRI for application on sugarcane varieties.

2.4. Data Analysis
Sugarcane morphological characters based on International Union for the Protection of New Varieties of Plant [9] showed results of 84 characters measured and scored as binary data: presence (1) or absence (0). Polymorphic RAPD markers were scored as binary data: presence (1) or absence (0) using Paint Shop Pro 5. Only clearly resolved bands were used in the genetic analysis. Genetic similarity among the varieties was calculated by Jaccard Similarity Coefficient and Simple Matching Coefficient using Multi Variate Statistical Package 3.1 software. Both morphological and genetic similarity dendrogram was constructed based on genetic distance using Unweighted Pair- Cluster Method using Arithmetic Averages (UPGMA) with MVSP 3.1 software.

3. Results and Discussion
Characterization of sugarcane varieties based on UPOV [9] S. officinarum categories and standards in 2005 were morphological characters in the form of leaf back feathers, leaf claws, stem section shape, stem segment structure, stem wax layer, eye shape, stem shape, and so on. Based on all the characters observed, 84 different characters were obtained as OTU in the phenetic classification.

![Figure 1](image-url)  
*Figure 1. Phenetic phylogeny based on morphological characters.*
Fig 1 and Fig 2 are showed same structural phylogeny based on morphological similarities, in Fig 1 show similarities index more than 70% which assumed validity in one species, *Saccharum officinarum* L.

**Figure 2.** Phenetic phylogeny sj based on morphological characters.

**Figure 3.** RAPD result on primer OPD1

**Figure 4.** RAPD result on primer OPC2.
Amplification results of the polymorphic RAPD using primers OPD1 in Figure 3 and OPC2 in Figure 4 based on 20 varieties of *S. officinarum*.

![Figure 5. Phenetic phylogeny ssm based on RAPD.](image1)

![Figure 6. Phenetic phylogeny sj based on RAPD.](image2)

Polymorphic RAPD DNA genetic phylogeny are shown in fig 5 using ssm method and fig 6 with sj method based on bands polymorphic DNA.

The aim of characterization is to record highly heritable characters, can be easily seen and expressed in all environment. Morphological characters that allegedly superior and resistant to pests resulted of its morphology is a form of cylindrical stem segments more resistant compared than the concave-convex into concave which easily attacked by pests. In the segment arrangement moderate stem is the most resistant from strong winds and shows a sturdy, rigid stem and self-resistance from the environment. Thick rod wax coating inhibits and reduces the attractiveness of pests to broach sugarcane stems. Root eyes as a form of prospective roots that can later sprout if implanted, 5 rows of root eyes indicate there are 5 rows of potential roots that can grow. Leaf back feathers as personal protection defense from pests and predators, dominate leaf back feathers decreased pest probability to attack
sugarcane. Easy tear up leaf claws can facilitate pests and predators to attack sugarcane, harder leaf to tear up indicate higher defense of sugarcane from pest.

Numerical taxonomy is an objective classification method and is based on as much as possible similarities in character or characteristics (polythetic) [13]. Operational Taxonomical Unit (OTU) is the same function of similarity, different taxa are obtained based on their properties, and similarity is phenetic. In the practice of numerical-phenetic taxonomy, the concept of taxospecies is used, which is grouping into one species if similarities index ≥ 70%. The index similarity value can be calculated by the Ssm or Sj method [7, 8]. The characters obtained are calculated using two similarity calculation methods, namely Simple Matching Coefficient (SSM) and Jaccard Coefficient (SJ). Molecular phenetics from the RAPD results obtained by the same phylogeny tree with phenetic morphology. Based on the criteria of resistance in terms of morphology, it is estimated that from 20 varieties there are 8 varieties that meet the superior character in the form of back feathers, the composition of the stem is moderate, the waxy layer is moderate to thick, these characteristics can be found in varieties 475 SOFF 1208, 350 SOFF 1082, 357 SOFF 1089, 360 SOFF 1092, 368 SOFF 1100, 454 SOFF 1186, 473 SOFF 1205, and 476 SOFF 1208. Based on same varieties researched by Sulistyantini [10] in ISFRI about percentage of pest attack on sugarcane, which showed varieties of 475 SOFF 1208 assumed as superior character compared to other varieties because it has the lowest percentage of pest attacks at 0.29%. Based on similarity of morphological characters that are considered to be superior characters in defending themselves from pests, are 352 SOFF 1084, 475 SOFF 1208, 350 SOFF 1082, 354 SOFF 1086, 478 SOFF 1210, and 351 SOFF 1083. RAPD DNA genetic phylogeny approach showed that 475 SOFF 1208 which were considered superior to other varieties had a closest relation to 287 SOFF 1019 and 286 SOFF 1018 based on ssm. While based on sj of RAPD DNA genetic phylogeny, the closest relation between varieties 475 SOFF 1208 are 474 SOFF 1206, 479 SOFF 1211, and 352 SOFF 1084. Closest relation between assumed superior varieties based on morphological characters are suggested as parental on crossing over between germplasm in ISFRI, and the results of hybrid morphological characters obtained as resistant to pests as expected.

4. Conclusion
The results showed that 20 varieties of characterized sugarcane morphology that expected tolerant of pest were in the upstream form of the cylindrical trunk segment, the moderate stem borders, the thick stem wick coat, the thick dorsal leaf hair, and the leaf exfoliate difficult. Variety of sugarcane number accession 475 SOFF 1208 shown the best pest tolerant morphologically and has the lowest percentage of pest attack to 0.29%. The result of ssm and sj shown that breeders suggested in the form of new varieties that are superior and resistant to pests according to most tolerant variety 475 SOFF 1208 are 350 SOFF 1082, 357 SOFF 1089, 360 SOFF 1092, 368 SOFF 1100, 454 SOFF 1186, 473 SOFF 1205, and 476 SOFF 1208.

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Acknowledgment
We would like to thank Indonesian Sweetener and Fiber Crops Research Institute (ISFRI),and Faculty of Biology UGM, which support our research project ‘KerjasamaPenelitian, Pengkajian, danPengembanganPertanianStrategis (KP4S), no. 2052/UN1/DITLIT/DIT-LIT/LT/2018’ in 2018. Also best regards and cherish to team of researchers in Laboratory of Genetic and Breeding Biology UGM that support and help in this research project.