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# DEVELOPMENT OF MICROSATELLITE MARKERS FROM SUGARCANE (Saccharum Officinarum L.) PHIL 97-3933

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## ABSTRACT

Efficiency of commercial farms can be improved through the application of developed technologies such as the use of microsatellite repeats or simple sequence repeats (SSRs) as genetic markers in plant species. This study developed sets of simple sequence repeat markers (SSRs) from Phil 97-3933 variety, a cultivar known to be highly resistant to sugarcane smut and downy mildew. For the library construction, genomic DNA of Phil 97-3933 was extracted and was digested using methyl-sensitive restriction enzymes *Pst*I and *Aat*II, with six base pair recognition sites. Two hundred sequences were obtained from which 27 contained SSR. A total of 27 SSR primers were developed from sugarcane CV Phil 97-3933 using BatchPrimer3 [1]. SGS P20 had similar sequence identity to *Saccharum* hybrid cultivar R570 clone BAC 227O17, while SGS P141 had similar sequence identity to *S. officinarum* clone LA154P24. Other SSR primers that returned BLASTn similar sequence identities are SGS P131 (*Sorghum* hypothetical protein), SGS P76 (*S. officinarum* clone LA34B02), SGS P112 (*Saccharum* hybrid BAC 235G19), SGS P125 (*Sorghum* hypothetical protein), and SGS P139 (*Sorghum* voucher BTx623 locus pSB1123). The rest of the primers identified did not return any BLASTn result. Phil 97-3933 is a cultivar known to be highly resistant to sugarcane smut. Sugarcane smut caused by *Sporisorium scitamineum* is one of the most serious diseases of sugarcane [2] and has been a long-standing problem in the Philippines. Constructing a genomic library from Phil 97-3933, and developing microsatellite markers from it is a start. Screening and evaluating germplasm collections with SSR markers developed from this local variety could both optimize and facilitate the breeding process in the country.

**Keywords:** Microsatellite markers, molecular markers, Phil 97-3933, Sugar Regulatory Administration

## 1. INTRODUCTION

The Sugarcane Industry Roadmap 2020 of the Sugar Regulatory Administration (SRA) is formulated to serve as guide in the identification and implementation of appropriate programs and interventions to prepare the industry for year 2015 and beyond when tariff of imported sugar will be reduced to 5%, and the full integration of the ASEAN Economic Community (AEC)

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takes effect. It proposes a multi-product sugarcane industry with bioethanol and power as major products other than sugar, and bioplastics, biowater, biofertilizer, and special sugars as sources of revenue. The sugar industry contributes about Php 70 billion to the Philippine economy from the production of raw and refined sugar, molasses, and bioethanol.

The SRA's target outputs are: (1) increase in sugarcane area from 422,384 hectares to 465,000 hectares; (2) increase in farm productivity from 57 tons cane per hectare to 75 tons cane per hectare (57 TC $ha^{-1}$ to 75 TC $ha^{-1}$); and (3) increase in sugar yield from 1.80 bags per ton cane to 2.1 bags per ton cane (1.80 LKg $TC^{-1}$ to 2.1 LKg $TC^{-1}$).

These can be achieved by improving farm productivity and sugar yield. Around 90% of the total sugarcane farms in the country are small farms, and they only produce an average of 50 TC $ha^{-1}$.

The agency, through its programs and interventions, has provided two experimental stations that will cater to the research, development and extension needs of the sugarcane industry in coordination with the Philippine Sugar Research Institute Foundation, Inc. (PHILSURIN), which is the private sector research arm of the sugar industry and University of the Philippines Los Baños. SRA-Luzon Agricultural Research and Extension Center (LAREC) in Floridablanca, Pampanga conducts research and development projects on sugarcane nutrition, while SRA-La Granja Agricultural Research and Extension Center (LGAREC) in La Carlota City, Negros Occidental conducts breeding for variety improvement and maintains the germplasm. LGAREC serves as the only center for sugarcane breeding. The station focuses on the development of varieties that will surpass the performance of control varieties.

Efficiency of commercial farms can be improved through the application of developed technologies such as the use of microsatellite repeats or simple sequence repeats (SSRs) as genetic markers in plant species. Microsatellites reveal co-dominantly inherited multi-allelic loci that can be readily mapped, providing a higher incidence of detectable polymorphisms compared to other techniques like Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) [3].

Phil 97-3933 is a cultivar known to be highly resistant to sugarcane smut. Sugarcane smut caused by *Sporisorium scitamineum* is one of the most serious diseases of sugarcane [2] and has been a long-standing problem in the Philippines. In previous years, losses from smut have been serious and the disease is considered the most destructive in the country. The disease can remain unobserved for a long time and then suddenly break out when a susceptible variety is planted on a large scale. Economic losses could range from negligible proportions to levels serious enough to threaten the sugar production of the area [4].

Developing varieties of sugarcane resistant to sugarcane smut is the only economical method for control of this disease and the best course of action for management [5]. *S. scitamineum* causes high global losses in sugarcane yield and sucrose, yet the information about its genome structure and pathogenic mechanisms is still limited. However, sugarcane smut fungi are biotrophic pathogens that cannot be cultured on artificial media [5]. A better understanding of the host-

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pathogen interaction is necessary for the development and deployment of more effective and durable resistant cultivars.

In the Philippines, the current basis for the evaluation of genetic diversity in sugarcane germplasm is only based on pedigree records and phenotypic traits. The screening and evaluation of available first priority parentals from SRA with simple sequence repeat markers (SSRs) developed from CV-Phil 97-3933 could both optimize and facilitate the development of varieties resistant to sugarcane smut.

This study developed sets of simple sequence repeat markers (SSRs) from Phil 97-3933 variety. Specifically, the study constructed a genomic library of Phil 97-3933 using *Pst*I and *Aat*II restriction enzymes.

## 2. MATERIALS AND METHODS

The study was conducted from June 2015 to May 2017 at the Biochemistry Laboratory and the Molecular Plant Breeding Laboratory, College, Laguna under the Institute of Plant Breeding, College of Agriculture and Food Sciences, University of the Philippines Los Baños.

### DNA Digestion and Library Construction

For the library construction, genomic DNA of Phil 97-3933 was extracted and was digested using *Pst*I and *Aat*II restriction enzymes. Large fractions of repetitive DNA in many plant genomes has complicated all aspects of DNA sequencing and assembly, and thus techniques that enrich for genes and low-copy sequences have been employed to isolate gene space [6]. Methyl-sensitive restriction enzymes *Pst*I and *Aat*II, with six base pair recognition sites, were utilized in this study. Adapters were made by combining 100 µmoles of either *Pst*I or *Aat*II primers with 10 µmoles of 3' overhang primer. The adapters were annealed by placing the 50 µL adaptor solution on a T100 Thermal Cycler (Bio-Rad) and heated for 3 min at 65°C, then stepped down 1.3°C each min for 30 min. One µg of genomic DNA was digested with 10 units of the two enzymes, separately. Included in the 50 µL reaction were 2 µL of adaptor, 10X Ligation Buffer (Invitrogen, Carlsbad, CA), 4 µL of T4 ligase (Invitrogen), and 0.4 µL of bovine serum albumin (Invitrogen). The products were amplified by using 5 µL of the digestion/ligation reaction along with 1 µL of universal primer, 10X Taq reaction buffer (Invitrogen), 50 mM $MgCl_2$, 10 µM dNTPs and 1 µL Taq (Invitrogen). Reactions were placed on a T100 Thermal Cycler (Bio-Rad) with the following cycling pattern: 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 60°C for 2 min, and 72°C for 2 min.

Libraries were constructed using pGEM-T Easy kit according to protocol (Promega, Madison, WI). After ligation, clones were transformed into *E. coli* bacterial strain JM109 (Promega). Colonies were picked and grown in Luria-Bertani medium. Plasmid DNA was isolated via Alkaline-lysis miniprep protocol. Afterwards, the clones were sequenced at the Philippine Genome Center (PGC). Sequences were analyzed using CLC Genomics Workbench (QIAGEN Bioinformatics).

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### Primer Design

Primers, typically 18 to 21 nucleotides in length, were designed when the 5'- and 3'- sequences flanking the repeat motifs for dinucleotide repeats were greater than five, trinucleotide repeats greater than three, and tetra-, penta- and hexa-nucleotide repeats greater than two using BatchPrimer3 [1]. Primer selection criteria were based on the search result of database nucleotide collection using Megablast (Database resources of the National Center for Biotechnology Information). Primers were synthesized at Diamed Enterprise.

## 3. RESULTS AND DISCUSSION

### Bacterial Cell Transformation

Phil 97-3933, after gDNA digestion using *Pst*I and *Aat*II restriction enzymes, were transformed into *E. coli* JM109. The transformed cells were grown in LB/ampicillin/X-gal plates (Fig. 1). Blue and white colonies were observed on the plates indicating successful transformation. White colonies are the ones that contain recombinant plasmid while blue colonies are cells with pGEM-T Easy vector without inserts. White colonies were picked for subsequent plasmid isolation for they are the putative transformants.

### Assessment of Plasmid DNA Quality and Concentration

A total of 434 plasmid DNAs were sent to PGC for sequencing. Only 200 plasmid DNAs were utilized since the other clones were either not sequenced properly, or lacked one of the SP6 or T7 sequence pairs. They were quantified using Nanodrop spectrophotometer. The average absorbance ratio of all plasmid DNA extracted is 1.61, probably due to some protein contaminants during plasmid DNA isolation. It may be because of the proteins that formed a complex with potassium, producing precipitate upon the addition of Solution III. It can be noted that there were times wherein some precipitates got mixed with the supernatant when it was being transferred to a new tube. The average concentration of all the plasmid DNA extracted is 2523.76 ng $uL^{-1}$. Plasmid DNA quality was also verified by running it in a 1% agarose gel electrophoresis (Fig. 2).

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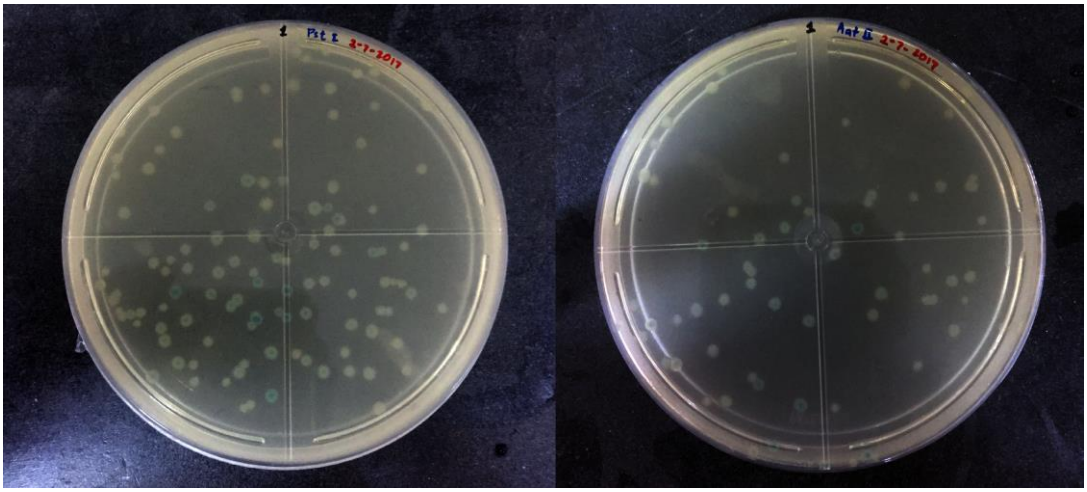

**Figure 1. Plates showing transformed *E. coli* JM109 cells containing digested genomic DNA using *Pst*I (left) and *Aat*II (right).**

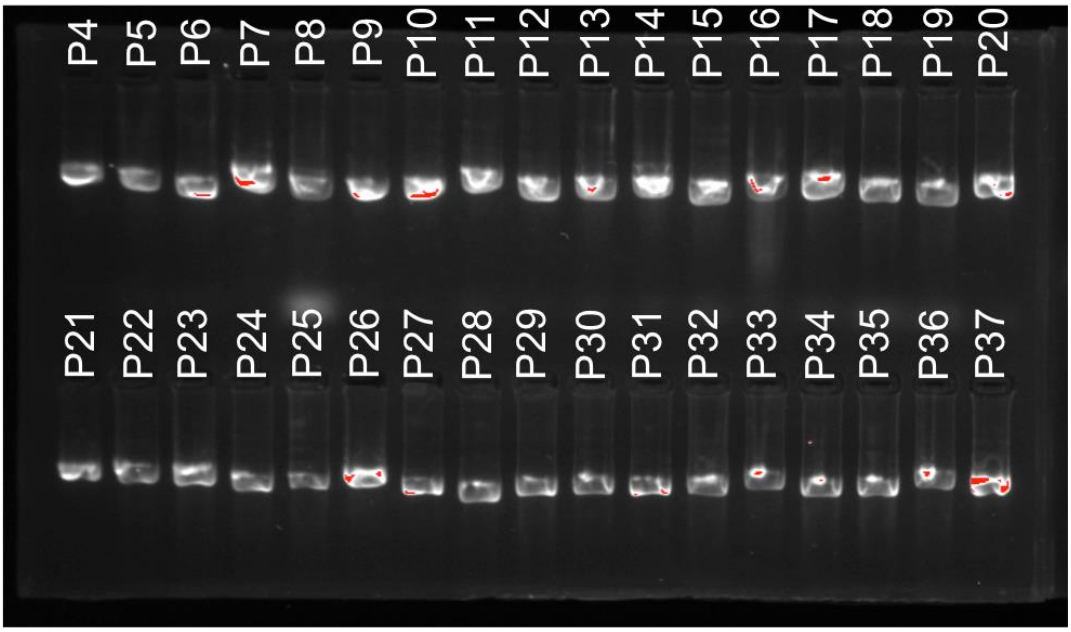


**Figure 2. Representative of the plasmid DNA (clones P4 to P37) containing sugarcane genomic inserts, extracted after being run in a 1% agarose gel electrophoresis.**

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**Sequencing and Bioinformatics Analysis**

Two hundred sequences were analyzed using CLC Genomics Workbench. SP6 and T7 sequences of each clone were trimmed, removing sequences analogous to those of 3' overhang, 3' overhang reverse, *Aat*II, *Aat*II reverse, *Pst*I, *Pst*I reverse, pGEM-T, forward and reverse universal primers. The SP6 sequence of each clone was converted to its reverse-complement and was subsequently aligned to its T7 pair. The sequences that aligned were utilized for bioinformatics analysis.

**NCBI BLASTn Results**

More than half (52%) of the sequences returned a BLASTn result showing "no significant similarity found" (Fig. 3). The highest result came from *Saccharum* hybrid (20%). Modern sugarcane varieties that are cultivated for sugar production are complex interspecific hybrids (*Saccharum* spp.) that have arisen through intensive selective breeding of species within the *Saccharum* genus primarily involving crosses between the species *Saccharum officinarum* L. and *S. spontaneum* L. [7]. Of the 40 sequences that aligned with *Saccharum* hybrid, 36 had gene identity similar to *Saccharum* hybrid cultivar R570, three to *Saccharum* hybrid cultivar RB867515, and one to *Saccharum* hybrid cultivar Q155. *Saccharum* hybrid cultivar R570 returned results ranging from BAC clones to retrotransposons. *Saccharum* hybrid cultivar RB867515 and Q155 returned results showing similarity to their respective chloroplasts.

Sugarcane belongs to the genus *Saccharum* L., of the tribe Andropogoneae in the grass family (Poaceae). This tribe includes tropical and subtropical grasses including the cereal genera *Sorghum* and *Zea*. Sugarcane was included in the Andropogoneae map comparison due to the identification of linkages between maize probes based on a progeny of a modern cultivar [8]. Sugarcane linkage showed syntenic relationships to the duplicated regions of maize and sorghum. Sugarcane and sorghum genomes appear to be more closely related with respect to chromosome organization than either one with maize [9]. *Sorghum bicolor* had 31 similar sequences to this genomic library. All of these sequences returned BLASTn results showing gene identities similar to *Sorghum bicolor* hypothetical proteins, except one that had a gene identity similar to *Sorghum bicolor* voucher BTx623 locus. Thirteen sequences returned a result showing similarities to *Zea mays*. Five sequences had similar gene identity to transcript variants X1, X2 (serine/threonine-protein kinase Nek2), X4 and X7 (homeobox protein Hox-B6-like). Three had similar gene identities to uncharacterized loci (LOC103638481, LOC103645761, LOC103635940), two were similar to clone ZM_BFb0218I13, two were also similar to oligomeric golgi complex subunit 2, and one for *Zea mays* small RNA degrading nuclease 5.

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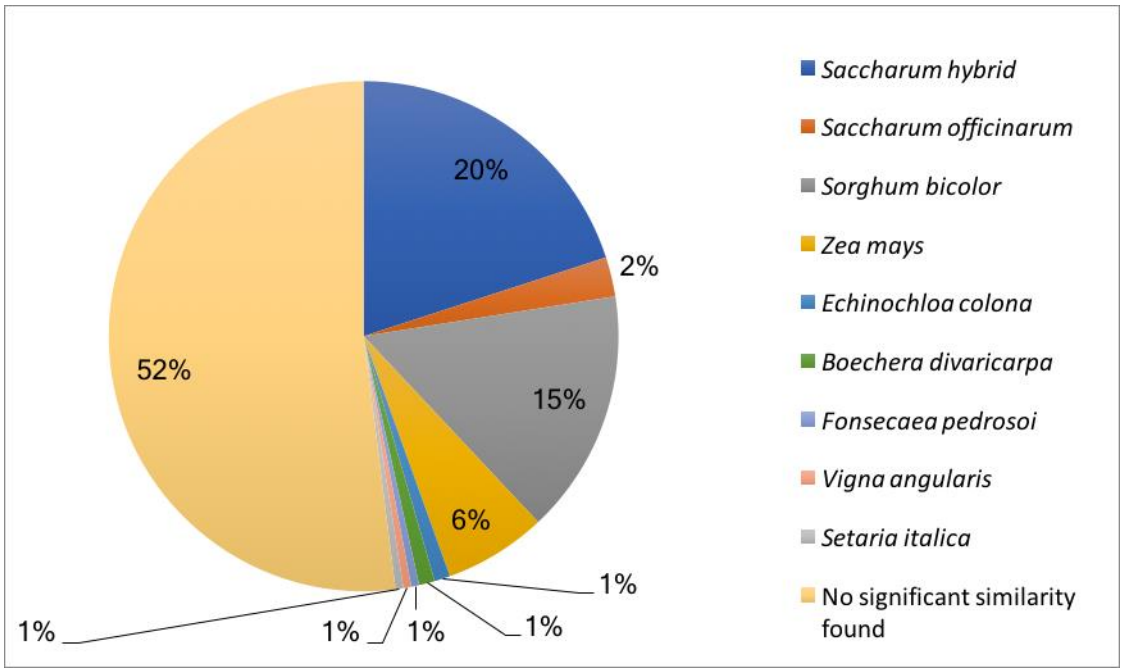


**Figure 3. NCBI BLASTn result. 96 sequences were classified with similarities to *Saccharum* hybrid (40), *Saccharum officinarum* (5), *Sorghum bicolor* (31), *Zea mays* (13), *Echinocloa colona* (2), *Boechera divaricarpa* (2), *Fonsecaea pedrosoi* (1), *Vigna angularis* (1), and *Setaria italica* (1).**

Five sequences were similar to *Saccharum officinarum*. These 'noble canes' accumulate very high levels of sucrose in the stem but have poor disease resistance. *S. officinarum* itself is thought to be the product of complex introgression between *S. spontaneum*, *Erianthus arundinaceus* and *Miscanthus sinensis* [10]. Two sequences were specifically similar to *Saccharum officinarum* cultivar Khon Kaen 3, showing results with gene identities similar to their mitochondrial chromosomes 1 and 2. Three showed their respective similarities to *Saccharum officinarum* clone LA154P24, *Saccharum officinarum* clone LA34B02, and *Saccharum officinarum* clone SoCEND1-2.

Two sequences each had gene identities similar to both *Echinochloa colona* chloroplast and *Boechera divaricarpa* clones (B53- 01-F_I16 and B62-01-F_K17). Samantaray et al. [11] reported that plant regeneration via somatic embryogenesis was achieved in leafbase and leaf tip explants derived from 10-day-old in vitro-grown seedlings of *Echinochloa colona*. A similar pattern of development was also found in *Saccharum* spp. [12]. Likewise, Sunkar and Jagadeeswaran [13] identified miR395 as a conserved miRNA family found in both *Boechera* and *Saccharum officinarum*.

*Fonsecaea pedrosoi* (CBS 271.37 hypothetical protein partial mRNA), *Vigna angularis* cultivar Shumari (chromosome 11), and *Setaria italica* (wall-associated receptor kinase 2-like), each had

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one sequence with similar gene identity. *Fonsecaea pedrosoi* is the principal etiologic agent of chromoblastomycosis, a chronic fungal disease usually limited to the skin and subcutaneous tissues [14]. Sangwan et al. [15] reported that a 35-year-old agriculture worker, presented with complaints of pain, redness, irritation, watering, and photophobia in the right eye (RE) of 20 days duration, following injury by a sugarcane leaf. The history of ocular trauma with vegetable matter (sugarcane leaf) suggested the possibility of fungal infection due to *Fonsecaea pedrosoi*. On the other hand, Mello et al. [16] stated that the Bowman–Birk family (BBI) of proteinase inhibitors is widely distributed in flowering plants, including *Saccharum officinarum* and *Vigna angularis*. Moreover, *Setaria italica*, which exhibits C4 photosynthesis like sugarcane, showed a BLASTn result indicating wall-associated receptor kinase 2-like sequence. Wall-associated kinases, or WAKs, are receptor-like kinases that are linked to the pectin fraction of the cell wall, and have a cytoplasmic protein kinase domain. WAKs are required for cell expansion, are involved in pathogen response, and their expression is activated by numerous environmental stimuli. A recent work supports the idea that WAKs are receptors for both pectin in the cell wall, and for pectin fragments, oligogalacturonic acids (OGs), generated during some pathogen attacks [17].

E-value of all sequences that produced significant alignments ranged from $1x10^{-48}$ to $5x10^{-86}$, while percent identity ranged from 78% to 100%. Parentage of Phil 97-3933 is Phil 91-24-0479 x Phil 87-15. One of its parents, Phil 87-15 also came from the 160 first priority parentals. Parentage of Phil 87-15 is Phil 79-3385 x CP 20-28.

**SSR Mining and Primer Design**

A total of 27 SSR primers were developed from sugarcane CV Phil 97-3933 using BatchPrimer3 [1]. Primers designed were typically 18 to 21 nucleotides in length. Two primers were chosen for screening based on the search result of database nucleotide collection using Megablast (Database resources of the National Center for Biotechnology Information). SGS P20 had similar gene identity to *Saccharum* hybrid cultivar R570 clone BAC 227O17, and SGS P141 had similar gene identity to *Saccharum officinarum* clone LA154P24.

Other SSR primers that returned BLASTn similar gene identities are SGS P131 (*Sorghum bicolor* hypothetical protein), SGS P76 (*Saccharum officinarum* clone LA34B02), SGS P112 (*Saccharum* hybrid cultivar R570 clone BAC 235G19), SGS P125 (*Sorghum bicolor* hypothetical protein), and SGS P139 (*Sorghum bicolor* voucher BTx623 locus pSB1123 genomic sequence). The rest of the primers identified did not return any BLASTn result.

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**Table 1. List of SSR primers designed from sugarcane CV Phil 97-3933.**

| Marker code | | Primers (5' → 3') | $T^a$ | Size | Sequence |
|---|---|---|---|---|---|
| SGS P20 | Forward | AAA ATT CAT GAG AGC ACG TC | 46* | 199 | (GATC)3 |
| | Reverse | TTT GAT CAA TAG TTC CCC TCT | | | |
| SGS P141 | Forward | CTG GCA GGA TAT GAA ATA TGA | 48* | 149 | (TATT)3 |
| | Reverse | CCT TTT ACA GGC GAA ATT TAT | | | |
| SGS P131 | Forward | TAC TGT AGA CTG CGT ACA TGC | 51-54 | 151 | (AAAGA)3 |
| | Reverse | AAG AAA CCA AAA GAG TGG TTG | | | |
| SGS P76 | Forward | TAC ATG CAG ATC ACT CGC TAC | 54-56 | 159 | (CATG)3 |
| | Reverse | CAT GCA TTT GAC TTT GAG AA | | | |
| SGS P112 | Forward | CTG ATG TGA ATC AGG TGT TCT | 55-56 | 161 | (AAGAA)3 |
| | Reverse | CAT TTC CTT TTG CCT TAT TGT | | | |
| SGS P125 | Forward | AAC TGT AAC ACA AGC GAA CAT | 54-55 | 142 | (AAAACA)4 |
| | Reverse | CCA AGG GAA AGA ATG ATA GAT | | | |
| SGS P139 | Forward | GTG CAT TCA ATT TTT CAA GAG | 54-55 | 149 | (CA)12 |
| | Reverse | TTC TTT TAA TCC TGA TGA TGC | | | |
| SGS A50 | Forward | AGA CAA CAA GAC CAC TCT TCA | 54 | 153 | (TCCA)3 |
| | Reverse | GTC ATT CCT GAG GAC ACA TT | | | |
| SGS P4 | Forward | GAT TTC TGT GAG AAA AGC TGA | 54-55 | 139 | (TAT)4 |
| | Reverse | TTG CAA AGA GAA AAG AAA CTG | | | |
| SGS P11 | Forward | TGA TTA TCG TAT GAT GGG AAC | 54 | 161 | (AGCA)3 |
| | Reverse | GCA AAG TGG ATA ATA AGT GGA | | | |
| SGS P24A | Forward | GCA ACA AGG AGG GAT AGA A | 55 | 156 | (GAAG)3 |
| | Reverse | TTA ATT TGA CTC GCT CAC ACT | | | |
| SGS P24B | Forward | CAT TGA AGC AGT CTA CGA CAT | 55-56 | 159 | (GATG)4 |
| | Reverse | CGT CCT CCA ATG TAC CAC TA | | | |
| SGS P110 | Forward | GAG AGA TGA TAG CGA AGA TCC | 54-55 | 171 | (CGGC)3 |
| | Reverse | CAC ATG ACG AGG ATA CGA T | | | |
| SGS P165B | Forward | CTC CTC CTC CTC TCT CTC TCT | 55-56 | 110 | (CT)7 |
| | Reverse | TCG TAG ACT GCG TAC ATG C | | | |
| SGS P165C | Forward | CTC CTC CTC CTC TCT CTC TCT | 52-57 | 110 | (CA)8 |
| | Reverse | TCG TAG ACT GCG TAC ATG C | | | |
| SGS P87 | Forward | GCA ACA ATA GTG ACT TTA GGG | 54 | 152 | (CGC)7 |
| | Reverse | ACG AGC CAT CTC TTT TAT TCT | | | |
| SGS P81 | Forward | TAT AAA GCT ACA GGC TGG CTA | 54-55 | 128 | (TC)7 |
| | Reverse | TTC CAT GCT ATG CTT AAA GAG | | | |
| SGS A51 | Forward | TCA TGC TCA TGT TAA TTT TCC | 54-55 | 153 | (AGCA)3 |
| | Reverse | AGA AAC CTG CAC TGA ATC ATA | | | |
| SGS A16A | Forward | GAC GAT CAC AGA AAG TAG CC | 54-55 | 180 | (AC)7 |
| | Reverse | CAC TTG AGG GTT CAG AGT TTA | | | |
| SGS A16B | Forward | GAC GAT CAC AGA AAG TAG CC | 54-55 | 180 | (CAG)5 |
| | Reverse | CAC TTG AGG GTT CAG AGT TTA | | | |
| SGS A42 | Forward | CAT ACA CAT GCT CTT CGA CA | 54-55 | 144 | (GT)6 |
| | Reverse | TGC GTA CAT AGT TGT TAC GTG | | | |

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| Marker code | | Primers (5' → 3') | Ta | Size | Sequence |
|---|---|---|---|---|---|
| SGS P101A | Forward | TAA TGA CGA GAG GAG AAC GTA | 54-55 | 227 | (GAG)4 |
| | Reverse | GTA CCT AGC TCA GCC GAT T | | | |
| SGS P101B | Forward | TAA TGA CGA GAG GAG AAC GTA | 54-55 | 227 | (CGC)4 |
| | Reverse | GTA CCT AGC TCA GCC GAT T | | | |
| SGS P101C | Forward | TAA TGA CGA GAG GAG AAC GTA | 54-55 | 227 | (GAGGGA)3 |
| | Reverse | GTA CCT AGC TCA GCC GAT T | | | |
| SGS P105 | Forward | AAA CGG GTA CAG ATG TCA TAA | 54-55 | 141 | (TTTCT)3 |
| | Reverse | ACT CCT CTG TAC TCC CAT GTT | | | |
| SGS P129 | Forward | CAT ACA CAT GCT CTT CGA CA | 54-55 | 144 | (GT)6 |
| | Reverse | TGC GTA CAT AGT TGT TAC GTG | | | |

## 4. CONCLUSION

The filtered genomic library created from sugarcane Phil 97-3933 variety, using *Pst*I and *Aat*II restriction enzymes, showed that the obtained sequences from this variety is 20% similar to *Saccharum* hybrid, 15% similar to *Sorghum bicolor*, 6% similar to *Zea mays*, 2% similar to *Saccharum officinarum*, and 1% similar each to *Echinochloa colona*, *Boechera divaricarpa*, *Vigna angularis*, *Setaria italica*, and *Fonsecaea pedrosoi*. More than half of the sequences did not return any significant similarity with BLASTn search, showing that it is really difficult to obtain a genuine assembled monoploid genome of sugarcane. These sequences may not have been deposited yet to the NCBI database. The size and complexity of its genome structure is a challenge in a sugarcane sequencing project. Its highly polymorphic nature represents another challenge. Twenty-seven microsatellite markers were developed from the genomic library. Primers designed were typically 18 to 21 nucleotides in length.

Phil 97-3933 is a cultivar known to be highly resistant to sugarcane smut. Sugarcane smut caused by *Sporisorium scitamineum* is one of the most serious diseases of sugarcane [2] and has been a long-standing problem in the Philippines. Constructing a genomic library from Phil 97-3933, and developing microsatellite markers from it is a start. Screening and evaluating germplasm collections with SSR markers developed from this local variety could both optimize and facilitate the breeding process in the country.

## 5. RECOMMENDATIONS

This study was successful in characterizing the genome of CV-Phil 97-3933. It would enhance the representation of the whole genome if an additional 300 colonies would be extracted and sequenced.

The 25 other microsatellite primers developed from Phil 97-3933 should be synthesized and used in DNA fingerprinting and genetic diversity of the SRA first priority parentals.

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**6. Acknowledgment**

This study was done in collaboration with the Sugar Regulatory Administration La Granja Agricultural Research and Extension Center (SRA-LGAREC) and the University of the Philippines Los Baños Institute of Plant Breeding (UPLB IPB) as part of the Sugarcane Genomics Project of the Philippine Genome Center - Agriculture Program.

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