Identification of *SCDR 1* and *P5CS* Genes in Cultivar of Environmental Stress Tolerant Superior Sugarcane (*Saccharum officinarum* L.)

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) is among the important commodities of the commercial sector since it accounts for 65% of the world's sugar production. Indonesia is the world's eighth largest country in terms of productivity, planting area and yield of sugarcane. Environmental stress due to climate change is among the factors that may cause negative impact on the production of sugarcane. The purpose of the present research was to determine genotypic profile of sugarcane cultivars tolerant to environmental stress, as well as lineage based on *SCDR 1* and *P5CS* genes. The methods used in this research were DNA-specific sequence amplification by the PCR method and analysis of analog genetic lineage based on sequences of *SCDR 1* and *P5CS* genes. *SCDR 1* was a gene involved in the responses to drought, salinity and oxidative stresses in sugarcane, while *P5CS* was involved in the responses to drought stress and showed a negative correlation to sucrose productivity under drought stress conditions. The presence of these two genes in the tested sugarcane cultivars indicated a tolerant trait of environmental stress. The research found that the cultivars of PS 41, PS 58, PS 384, PS 851, PS 864, PS 865, PS 881, PS 882, PS 891, PS 921, PS 951, PS 80.910, PS 80.1649, PS CO 902, PS JT 941, Kentung, BZ 132, KK, TLH 2, PSDK 923, BL, PS 862, PSBM 901, and VMC 76-16 were superior sugarcane cultivars due to the presence of those two genes. Sequence similarity level of *SCDR 1* and *P5CS* genes in PSDK 923 and VMC 76-16 cultivars with *Saccharum* genus is high, namely 100% and 61.2%.

Keywords: Environmental-stress, lineage, *P5CS*, *Saccharum officinarum* L., *SCDR1*

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is among the most important commodities of the commercial sector and accounts for 65% of sugar production worldwide (Carson and Botha, 2002). Indonesia is the world's eighth largest country in terms of productivity, planting area and yield of sugarcane (Zhao and Li, 2015). In 2016, the area of sugarcane plantations in Indonesia was 444,220 ha with a total productivity of 2,222,971 tons and predicted to increase in 2017 of up to 453,456 ha for the planting area and 2,465,450 tons for its total productivity (Bambang, 2016). The level of sugar

demand in Indonesia was 4.6 million tons and would continue to rise yearly (Voboril, 2010), but this was not offset by domestic sugar production due to declining conditions of sugarcane planting in Indonesia.

Sugarcane is a unique plant capable of accumulating high sugar levels and is a source of commercial biomass for second-generation bioelectricity and bioethanol (Ferreira *et al*., 2017). Modern sugarcane cultivars are highly productive in the tropics. Environmental stress conditions can affect sugarcane productivity; for example, drought stress can reduce productivity due to a morphophysiological effect that causes water deficiency in sugarcane as well as affecting the inhibition of photosynthetic activity and growth (Ghannoum, 2009). In environment with high salinity, water uptake is difficult, because seawater has a higher concentration than groundwater in general since it binds salt mineral (Nakashima and Suenaga, 2017). Oxidative stress by ROS can be fatal to plants since it can attack cellular metabolic processes, such as the cellular respiration process associated with energy production for cells (Begcy *et al*., 2012).

Sugarcane Drought Related 1 Gene (*SCDR 1*) is among the examples of a gene capable of responding to multiple stresses, such as salinity, drought, and oxidative stresses. *SCDR 1* is strongly expressed in tolerant sugarcane varieties, whereas it is not expressed in susceptible varieties. An over-expression of *SCDR 1* will reduce the harmful effects of environmental stress condition (Begcy *et al*., 2012). In drought stress condition, sugarcane plants expressing *Delta -1-Pyrroline -5-Carboxylate Synthetase* Gene (*P5CS*) will be resistant to the stress conditions (Matin *et al*., 2014). In the present research, tolerant sugarcane cultivars were screened by means of *SCDR 1* and *P5CS* molecular markers to detect cultivars tolerant to drought, salinity and oxidative stress conditions. PCR (polymerase chain reaction) method was used for the screening process to amplify specific fragments in nucleic acids by regulating their temperature to run the reaction (Joshi and Deshpande, 2010). In the present research, the specific fragments in question were *SCDR 1* and P5CS fragments found in the sugarcane genome.

Identification of DNA-sequence-based species is a method that is considered fast, accountable, and consistent, thus it is important for conservation and diversity biological research. Certain genes can be used as markers in the genetic division of species and phylogenetic reconstruction (Irawan *et al.*, 2016). By using the sequences of *SCDR 1* and *P5CS* genes from the sugarcane cultivars under study and analog genetic sequences obtained through data mining from the gene bank, an analysis of lineage between these genes can be identified. The purpose of the present research was to perform early screening and detection of superior sugarcane cultivars resistant to environmental stress conditions in order to increase sugar production in Indonesia and agricultural plant breeding program. In addition, it also aimed to determine the lineage of *SCDR 1* and *P5CS* genes in sugarcane cultivars in Indonesia and genes in other plant species analogous to such genes.

MATERIALS AND METHODS

Genomic DNA Isolation

Samples of sugarcane leaves from 24 sugarcane cultivars were used in the present research, namely PS 41, PS 58, PS 384, PS 851, PS 864, PS 865, PS 881, PS 882, PS 891, PS 921, PS 951, PS 80.910, PS 80.1649, PS CO 902, PS JT 941, Kentung, BZ 132, KK, TLH 2, PSDK 923, BL, PS 862, PSBM 901, and VMC 76-16, obtained from the results of cultivation of seedlings by PT. Madu Baru in Bantul, Special Region of Yogyakarta, and the results of cultivation of seedlings at the Indonesian Sweetener and Fiber Crops Research Institute (*Balittas*) Malang, East Java. Genomic DNAs were isolated using the Nucleon Phytopure reagent kit. The samples were weighed 0.3 grams and added with liquid nitrogen, then grinded. The fine samples were put into a 1.5 mL microtube and gradually added with Phytopure I and II reagents, the samples were then incubated at 65°C for 10 minutes. The samples were incubated at –20°C for 20 minutes, then added with 500 µL cold Chloroform solution and 70 µL Phytopure Resin. Microtube containing samples was then centrifuged

at 3,000 rpm for 10 minutes. The supernatant in the samples were taken and transferred to the new microtube. Each microtube containing supernatant added with 1 : 1 cold isopropanol to the samples. The samples were inverted 8–10 times and then precipitated. The microtube was centrifuged at 10,000 rpm for 10 minutes, the supernatant on the tube was removed and discarded. The washing process was then carried out, pellets were added with 70% ethanol of 100 µL and centrifuged at 10,000 rpm for 5 minutes. The supernatant was removed and discarded, then the washing process was done again. Microtube containing pellets then added with 50 µL 1X TE solution and stored at –20°C.

Amplification of *SCDR 1* and *P5CS* Genes by the PCR Method

Amplification was made using a PCR machine (Bio-Rad T100™ Thermal Cycler). The PCR premixes were made according to the amount of samples to be amplified. The premixes contained sterile ddH_2O, diluted specific primers (Table 1) and Bioline PCR kit. Subsequently, all PCR premix reagents were put into a special 1.5 mL microtube without previously adding DNA templates (isolated genomic DNA) with a composition of 12.5 µL Bioline PCR kit; forward and reverse primers each 1 µL 25 µM; and 6.5 µL ddH_2O. The composition was then homogenized and distributed into each microtube with a volume of 23 µL. 2 µL DNA samples were then added into the microtube and homogenized, thus the total volume in one microtube was 25 µL.

Table 1 *SCDR 1* and *P5CS* (Matin *et al*., 2014) genes specific primer sequences

No.	Primer	Sequence (5'–3')
1	*M.SCDR 1*-Forward	AGAAGAAGGTGGTGGTGGTG
	M.SCDR 1-Reverse	CAGGCTTAGACTTGGGCTTG
2	*P5CS*-Forward	ACAGATGATAAAGTAGCAGAGAC
	P5CS-Reverse	AGACCTTCAACACCCACAG

During amplification of samples, the predenaturation cycle was carried out at 94°C for 1 minute, denaturation at 94°C for 45 seconds, annealing at 62°C for 1 minute, elongation at 72°C for 75 seconds and post-elongation at 72°C for 75 seconds. The denaturation, annealing and elongation cycles were repeated 40 times. The method was used for *M. SCDR 1* and *P5CS* specific primers.

Sequencing of *SCDR 1* and *P5CS* Target Genes

The sequencing method is divided into several stages: purification I, cycle sequencing, purification II, and capillary electrophoresis. The purification I consisted of 10 µL PCR products that were put into the 0.2 µL microtube dome, then added with 4 µL ExoSAP-IT enzyme and homogenized. The tube was put into thermo cycler with ExoSAP-IT activation protocol at 37°C for 15 minutes, ExoSAP-IT inactivation at 80°C for 15 minutes, and endless at 4°C for 15 minutes. In cycle sequencing, mixtures of 1 µL 10 ng DNA samples; 2 µL 3.2 ng reverse or forward primer; 9 µL nuclease-free water, 2 µL BigDye Terminator v3.1; 4 µL buffer 5X; and 2 µL ddH_2O were made in 0.2 mL microtube and then homogenized. The tube was put into thermo cycler with incubation protocol at 96°C for 1 minute, denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds, elongation at 60°C for 4 minutes, and endless at 4°C for 15 minutes. The denaturation, annealing, and elongation cycles were repeated 25 times. In purification stage II, mixtures

of 10 µL DNA samples, 45 µL SAM™ Solution, and 10 µL BigDye X Terminator™ were made in 0.2 mL microtube. The mixtures were then vortexed for 30 minutes and centrifuged at 1000 x g for 2 minutes. Subsequently, the samples were put into Applied Biosystems 3500 Genetic Analyzers for pick readings and DNA sequences. The present research used two representative cultivars namely PSDK 923 and VMC 76–16.

Bioinformatic Analysis

SCDR 1 (Sugarcane drought related 1) target gene has an amplicon size of 315 bp, while the *P5CS* (Delta-1-pyrroline-5-carboxylate synthetase) has an amplicon size of 167 bp (Matin *et al*., 2014). When the results of agarose gel visualization indicate DNA bands similar to the size of the target gene amplicons, a conclusion can be drawn that the tested samples contain the specific target genes desired, namely *SCDR 1* and *P5CS*.

Based on the sequencing results, bioinformatics analysis was then done using Phylip, Clustal X, Phydit, and Tree view offline softwares. The analysis was also done with online softwares such as the BLAST and Clustal W applications on the NCBI and EMBL pages. In addition, data mining from NCBI and DDBJ Gene Bank was also conducted. Based on the formed lineage tree construction, an analysis of lineage between genes was carried out with the Neighbor-joining algorithm. Furthermore, ExPASy online software was use to interpret nucleotide sequences into amino acid sequences. Amino acid sequences was use to know the composition of amino acids in this gene and to see the percentage of each type of amino acid involved and expressed in the process of tolerance to conditions of environmental stress in plants.

RESULTS AND DISCUSSION

The purpose of the method of early detection of sugarcane cultivars was to determine sugarcane cultivars adaptive to environmental stress conditions using molecular markers. The advantage of this method is quick and easy determination of sugarcane cultivars of superior quality that can be planted in various locations. The identification of superior sugarcane cultivars in this research was carried out through DNA amplification by the PCR method. This technique is used in the process of detecting *SCDR 1* and *P5CS* in the genomes of the 24 sugarcane cultivars tested. The primers of these two genes would bind to the corresponding regions in the genomic DNAs of the 24 sugarcane cultivars tested. When the primers were able to bind, the fragments would be amplified.

Based on the electropherogram of PCR products from *SCDR 1* gene amplification on 24 sugarcane cultivars under study, all samples showed DNA bands in the visualization results (Figure 1). *SCDR 1* primer was able to amplify the *SCDR 1* gene sequence of 315 bp in size. The research showed that PS 41, PS 58, PS 384, PS 851, PS 862, PS 864, PS 865, PS 881, PS 882, PS 891, PS 921, PS 951, PS 80910, PS 801649, PSCO 982, PSBM 901, PSJT 941, BL (Bulu Lawang), Kentung, KK (Kidang Kencana), BZ 132, TLH 2, PSDK 923, and VMC 76-16 cultivars were detected to have *SCDR 1* marked by the appearance of a DNA band of 315 bp in size in the electrophoresis results (Figure 1). In addition to the appearance of a DNA band of 315 bp size, the electrophoregram showed an appearance of other DNA band of 166 bp in size in the 24 sugarcane cultivars under study.

In theory, if amplification is done on certain gene sequences with specific primers, only one DNA band should be found on the electropherogram. In the present research, two different DNA bands can be found on the electropherogram in all samples, because the possibility of gene sequences amplified by the primer was not long or specific enough, thus the primer was able to bind to similar sequences but in other regions in the sugar cane genomes. The sequencing of DNA bands on the two cultivars: PSDK 923 and VMC 76–16, found that the sequence of 166 bp in size has similarity to the sequence from the amplification of *SCDR 1* gene of 315 bp in size. Therefore, besides the *SCDR 1* gene region, there was also other region that has similarity to the *SCDR 1* gene sequences in the genomes of 24 sugarcane cultivars under study.

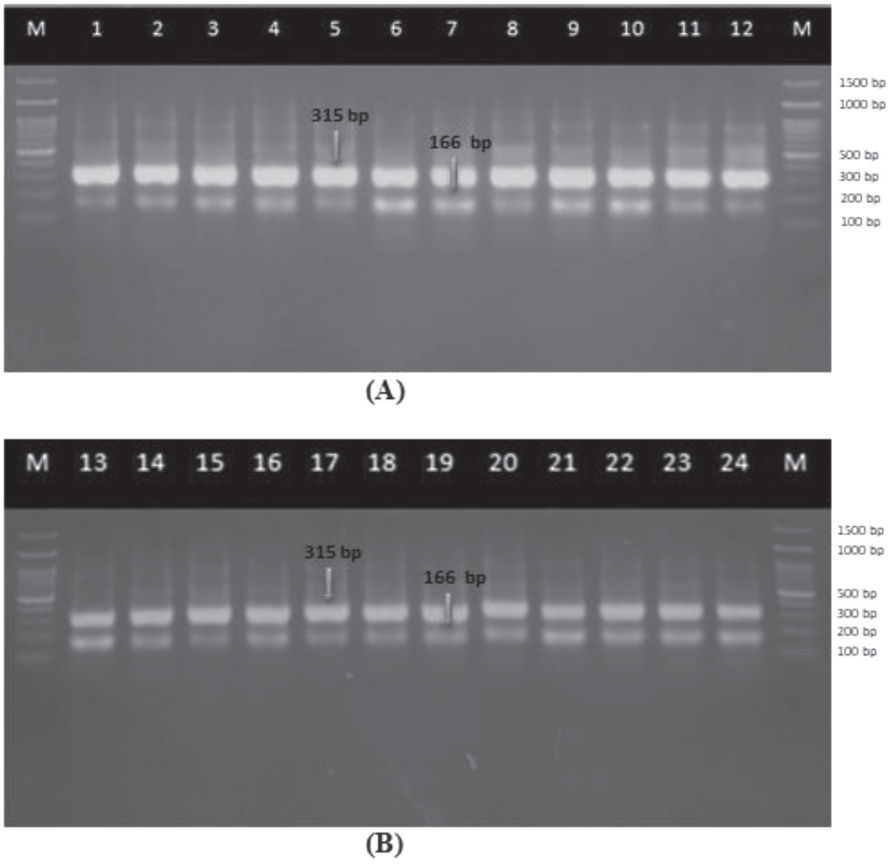

Figure 1 Electropherogram of PCR products with *SCDR 1* target gene in 24 sugarcane cultivars, Note: M : 100bp Marker, 1 : PS41, 2 : PS58, 3 : PS384, 4 : PS851, 5 : PS862, 6 : PS864, 7 : PS865, 8 : PS881, 9 : PS882, 10 : PS891, 11 : PS921, 12 : PS951 (A), 13 : PS80910, 14 : PS801649, 15 : PSCO982, 16 : PSBM901, 17 : PSJT941, 18 : BL, 19 : KENTUNG, 20 : KK, 21 : BZ 132, 22 : TLH 2, 23 : PSDK 923 and 24 : VMC 76–16 (B)

SCDR 1 is one of the genes that play a role in cellular regulation mechanisms of drought, salinity and oxidative stress conditions (Begcy *et al*., 2012). According to Prabawanti (2012), PS 864, PS 865, PSJT 941, PS 881 and Kentung cultivars are known as sugarcane cultivars resistant to drought stress conditions. When *SCDR 1* and *P5CS* were detected in the genomes of the 24 sugarcane cultivars under study, then the sugarcane cultivars had a double defense against drought stress conditions as well as more resistant to environmental stress conditions, such as salinity and oxidative stresses, and they are expected to be able to be planted in various types of land and locations in Indonesia.

SCDR 1 is strongly expressed in tolerant sugarcane varieties, whereas this gene is not expressed in susceptible varieties. This gene is capable of responding to multiple stresses, such as salinity, drought, and oxidative stresses. Photosynthetic parameters, such as transpiration rate, net photosynthesis, stomatal activity, and CO_2 concentration, are not significantly affected by drought and salinity stress conditions due to the activity of this gene. The percentage of carbon fixation is higher

than respiration rate in drought stress conditions since plants will optimize photosynthetic activity, rather than cellular respiration. An overexpression of *SCDR 1* will reduce the harmful effects of environmental stress conditions by reducing the initially high cellular respiration rate (Begcy *et al*., 2012).

The research showed that 24 sugarcane cultivars were also detected to have *P5CS* gene marked by the appearance of a DNA band of 167 bp in size in the electrophoresis results (Figure 2), although the band looked very thin. This thin DNA band indicates that DNA samples containing the gene regions were degraded during sample preparation and treatment, thus the fragments that can be fully amplified were only a small part and resulted in very thin DNA band. Another possibility is the band that appears on the visualization results of ± 90 bp in size is DNA fragment in another region with an initial sequence that is complementary to the target gene primer, hence it can be amplified in the PCR, but it has smaller fragment size than the expected target gene.

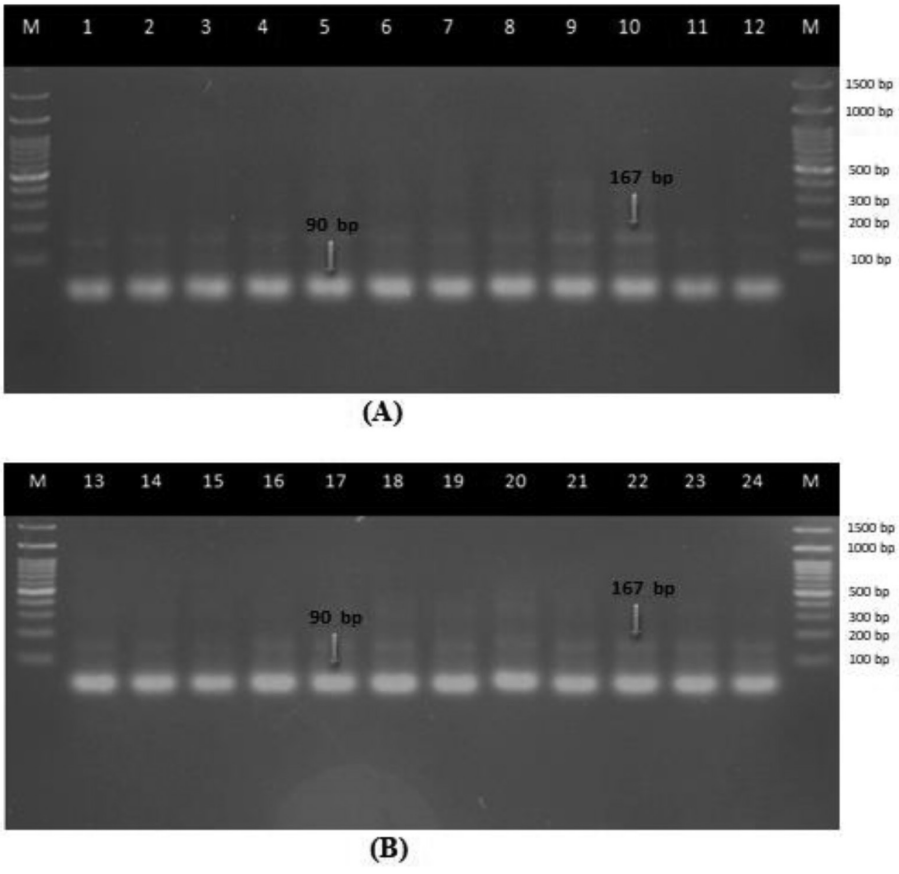

Figure 2 Electropherogram of PCR products with *P5CS* target gene in 24 sugarcane cultivars, Note: M : 100bp Marker, 1 : PS41, 2 : PS58, 3 : PS384, 4 : PS851, 5 : PS862, 6 : PS864, 7 : PS865, 8 : PS881, 9 : PS882, 10 : PS891, 11 : PS921, 12 : PS951 (A), 13 : PS80910, 14: PS801649, 15 : PSCO982, 16 : PSBM901, 17 : PSJT941, 18 : BL, 19 : KENTUNG, 20 : KK, 21 : BZ 132, 22 : TLH 2, 23 : PSDK 923 and 24 : VMC 76–16 (B)

P5CS is a gene that plays a role in the response of plants to drought stress conditions (Matin *et al*., 2014). This gene plays a role in proline amino acid biosynthesis. Proline amino acid is one of the main components that make up enzymes and proteins that play a role in the response to drought stress conditions. The activity of this gene proves to be negatively correlated to sucrose biosynthesis; therefore, in drought stress conditions sugarcane expressing this gene would tend to increase its proline biosynthetic activity and decrease sucrose productivity (Iskandar *et al*., 2011). In drought stress conditions, *P5CS*-expressing sugarcane would resistant to the stress conditions so that it can still grow optimally; however, the side effect is that sugarcane would reduce sucrose productivity in the stem by decreasing the sucrose accumulation in the stem. This is because the sucrose produced would be used to support the metabolic, growth and development processes during the stress conditions, leading to decreased levels of accumulated sucrose in the stem (Matin *et al*., 2014). Based on the foregoing, the 24 sugarcane cultivars under study are found to have *SCDR 1* and *P5CS* in their genomes, thus having multiple defenses in response to drought stress conditions and tolerant to salinity and oxidative stress conditions. The disadvantage is that when these 24 sugarcane cultivars are exposed to drought stress conditions, the accumulation of sucrose in the stem will decrease, resulting in low sucrose productivity in stressful conditions.

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PSDK923           ------------------------------------------------------------      32
VMC76-16          ------------------------------------------------------------      31
BT070164.1        GCCGGAGAAGAAGGAAGAGAAGAAGGAAGAAAAGAAGGACGAAAAGAAGCCGGCGCCGGC     495
EU958393.1        GCCCAAGCCTGAGCCTCCCAAACCAGAGAAATGCTGC-AGTGCCTGTTGTCA--------     503
XM_004975420.3    GCCTAAGACTAAG------------CCTGAGCAAGAGAAAACCAAGCCTCCGACGGCGCC     548
JN979786.1        GCCC------------------AAGTCTAAGCCTGAGGAAAAGCCTAAGCCGGCGCCGCC     464
XM_021462393.1    GCCCAAGCCTAAGCAGGAGCAAAAGCCTAAGCCTGAGGAAAAGCCTAAGGCGGCGACGCC     762

PSDK923           ------------------------------------------------------------      32
VMC76-16          ------------------------------------------------------------      31
BT070164.1        CAAAACGGAGTACAAGTTTGTGCCCTACCCGTACCCGCT---------GCCGAACCCCGC      546
EU958393.1        ---------CTGCAAGCCTAAGCCTGATCCCAAACCGCT---------GATATGTTGTGT      545
XM_004975420.3    CAAGACAGAGTACAAGCTGGTGCCCTACCCGTACCCGTATCCGCTGTCGTATTACCCGGC      608
JN979786.1        CAAGACAGAGTACAAGCTCGTGCCGTACCCGTACCCGGT---------GCCGAACCCCGT      515
XM_021462393.1    CAAAACGGAGTACAAGTTCGTGCCGTACCCGTACCTGGT---------GCCGAACCCTGG      813

PSDK923           --------------------TGCAGTGCTCACAGACACAACAT-----------------      55
VMC76-16          --------------------TGCAGTGCTCACAGACACAACAT-----------------      54
BT070164.1        GATGTGCCCGAGCTGGCCGTGGCAGTGCCCGCCTCAGCAGCAGTGCCAGTGCTGCCAGAA      606
EU958393.1        CTGTTGCC---------------ACTGCAAGCCGGAGAAGAAGGAAGAGAAAAAAAAAAA      590
XM_004975420.3    GATGTGCCCGAGCTGGCCGCGGCAGTGCCCGCCTCAGCAGCAGTGCCAGGGCTGCCAGAA      668
JN979786.1        GATGTGCCAGAGCTGGCCGTGGCAGTGCCCTCCTCACCAGCAGTGCCAGTGCTGCCAGAA      575
XM_021462393.1    GATGTGCCAGAGCTGGCCGTGGCAGTGCCCTCCTCACCAGCAGTGCCAGTGCTGCCAGAA      873
                                      * ***   *      *  *

PSDK923           ------------------------------------------------------------      55
VMC76-16          ------------------------------------------------------------      54
BT070164.1        GCCTCCGCCTCCGCCGCCCAAGTCGCCGCCGAAGCCGCCGCCGTGCACCTGTTCGAGCCA      666
EU958393.1        AAAAAA------------------------------------------------------      596
XM_004975420.3    GCCGCC--------------------GCCACCGCCACCGCCGTGCAGCTGTTCCAACCA      707
JN979786.1        GCAGCCTCCTTTTC---TGCC------GCCGCCGCCACCACCGTGCAGCTGTTCGAGCCA      626
XM_021462393.1    GCCTCCTTCGCCGC---CGCCGGCACCGCCGCAGCGGCCACCGTGCACCTGTTCGAGCCA      930
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Figure 3 Multiple Sequence Alignment of *SCDR 1* gene in PSDK 923 and VMC 76–16 sugarcane cultivars with analog gene sequences from data mining in NCBI and DDBJ. Note: PSDK923 (*SCDR 1* gene in sample sugarcane cultivar PSDK 923), VMC76–16 (*SCDR 1* gene in sample sugarcane cultivar VMC 16–16), BT070164.1 (Predicted protein in *Zea mays* clone ZM_BFc0140H18), EU958393.1 (Predicted protein in *Zea mays* clone 1688888), XM_004975420.3 (*Pi21* gene in *Setaria italica*), JN979786.1 (*SCDR 1* gene in *Saccharum hybrid* 'SP80–3280'), and XM_021462393.1 (*LOC110436138* gene in *Sorghum bicolor*)

Figure 3 and 4 show the difference in the nucleotide base sequences in the *SCDR 1* and *P5CS* genes of PSDK 923 and VMC 76–16 samples of the sequencing results with analog gene sequences in the Gene Bank. Each gene has a different length, in both samples they have the shortest sequence size because the primers used are only able of amplifying with a certain length, thus the overall size of genes cannot be amplified. The difference in base sequences could be due to a mutation that can be caused by insertion, deletion, substitution, transversion, and transition.

```
VMC76-16          ------------------------------------------------------------  0
BK007070.1        -------------------AGCACCAACCCCTCACCCCTTTAA-AACCGCTCGGCTCTAT  40
AY888045.1        ------------------------------------------------------------  0
XM_020310078.1    --------GAGCCGCTGGGGTTTCTGCGCCCCTCGCCCTTTAA-AACCGCTCGGCTCTAT  225
D49714.1          ------------------------------------------------------------  0
NM_001319696.1    ------------------------GTCCGTCTCACCACTTCAA-AAACCCTCTCCTCTCT  35
GQ377720.2        ------------------------------------------------------------  0
EU005373.2        ------------------------------------------------------------  0
PSDK923           -----------------AGCTATTTAATATCCACATCACCCCAGATGATGTCGACA----  93
AK108524.1        CTCCAACGCAGCTACAGGGGTAATTTATCTCTCCTCCCGCCCAAATACCTTCAAAA----  226

VMC76-16          ------------------------------------------------------------  0
BK007070.1        ----------------------------------AAAAAAACACCGCAGATAAGAGAGA  124
AY888045.1        CTGCAGATAAGAGAGAGGGAGGGGCGAGCGAGCGAGACAGGGAGAGGCGGCGCGCCGAGC  83
XM_020310078.1    CTGCAGAT--AAGAGAGGGAGGGGCGAGCGAGAGAGGCAGGGAGAGGCGGCGCGCCGAGC  341
D49714.1          C--GAGACGTGGGAGAGGGATTTACA-GGTAGAGGGA---GAGGGTGGAGGAGGAGAGGC  75
NM_001319696.1    A--CGGACAAGAGAGGCG--GAGGCT-GATAGGGAGAGGAGACGGAGGACCAAGAGCGGC  150
GQ377720.2        ------------------------------------AGACGGAGGACCAGGAGCGGC  21
EU005373.2        ------------------------------------------------------------  0
PSDK923           ----------------T---------------G-------------CAACTGGGGTGGC  113
AK108524.1        CTGGTGCTGATATCGAGC---------------GCGAGGCACACGAGCACCTGGCTCCGC  328

VMC76-16          ----------------------C--TTGCAGTGCTCACAGACACAACATTTCTTCTCAGGC  66
BK007070.1        TGAAAAGAACAGAGGTTGCTGATGGTTTAGTTCTTGAGAAAACATCTTGCCCTTTGGGTG  1370
AY888045.1        TGAAAAGGACAGAGGTTGCTGATGGTTTAGTTCTTGAGAAAACATCTTGCCCTTTGGGTG  1327
XM_020310078.1    TGAAAAGGACAGAGGTTGCTGATGGTTTAGTTCTTGAGAAAACATCTTGCCCTTTGGGTG  1587
D49714.1          TTAAAAAGACAGAGGTTGCTGATGATTTAGTTCTTGAGAAAACATCTTGCCCATTAGGTG  1317
NM_001319696.1    TCAAAAGAACAGAGGTTGCTGAAGACTTAGTGCTCGAAAAAACATCTTGCCCTCTGGGTG  1399
GQ377720.2        TTAAAAGAACAGAGGTTGCTGAAGATCTGGTTCTCGAAAAAACATCTTGTCCTCTAGGTG  1264
EU005373.2        TCAAAAGAACAGAGGTTGCTGAAGATTTGGTTCTTGAGAAAACATCTTGCCCATTAGGGG  1219
PSDK923           ------------------------------------------------------------  113
AK108524.1        ------------------------------------------------------------  627

VMC76-16          TTGCAATCCTCTGGCTTTGGCTTGCATTCATCGCATGGCTTGGTTGGGTTGCAAGGAGTG  126
BK007070.1        TT-CTATTGATTATTTTTGAGTC---CCGACCTGATGCCTTGGTCCAGATTGCGTCTTTA  1426
AY888045.1        TT-CTATTGATTATTTTTGAGTC---CCGACCTGATGCCTTAGTCCAGATTGCGTCTTTA  1383
XM_020310078.1    TT-CTATTGATTATATTTGAGTC---CCGACCTGATGCCTTAGTCCAGATTGCGTCTTTA  1643
D49714.1          TT-CTCTTAATTGTTTTTGAGTC---CCGACCTGATGCCTTGGTTCAGATTGCATCTTTG  1373
NM_001319696.1    TG-CTGTTGATTGTTTTTGAGTC---CAGGCCTGATGCCTTAGTCCAGATTGCGTCTTTA  1455
GQ377720.2        TG-CTATTGATCGTTTTTGAGTC---CAGGCCTGATGCCTTGGTCCAGATTGCGTCTTTA  1320
EU005373.2        TG-CTATTGATCGTTTTTGAGTC---CAGGCCTGATGCCTTGGTCCAGATTGCGTCGTTA  1275
PSDK923           ------------------------------------------------------------  113
AK108524.1        ------------------------------------------------------------  627

VMC76-16          CATGGCTTGGGTGGCAGTGGCATCAG-CCACAC----------------CTCGACG---  165
BK007070.1        GCCATTCGAAGTGGTAATGGTCTTCTCCTAAAAGGTGGAAAAGAAGCGATGAGATCAAAC  1486
AY888045.1        GCCATTCGAAGTGGTAATGGTCTTCTCCTAAAAGGTGGAAAAGAAGCAATGAGATCAAAC  1443
XM_020310078.1    GCCATTCGAAGTGGTAATGGTCTTCTCCTAAAAGGTGGAAAAGAAGCAATGAGATCAAAC  1703
D49714.1          GCAATTCGAAGTGGTAATGGTCTTCTCCTAAAAGGTGGAAAAGAAGCTATCAGATCAAAC  1433
NM_001319696.1    GCAGTTCGAAGTGGCAATGGTCTTCTCCTGAAAGGTGGAAAAGAAGCCATGAGATCGAAC  1515
GQ377720.2        GCAATCCGAAGTGGCAACGGTCTTCTCCTGAAAGGTGGAAAAGAAGCCATGAGGTCAAAC  1380
EU005373.2        GCAATTCGAAGTGGCAACGGTCTTCTCCTGAAAGGTGGAAAAGAAGCCATGAGATCAAAC  1335
PSDK923           ------------------------------------------------------------  113
AK108524.1        ------------------------------------------------------------  627
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Figure 4 Multiple Sequence Alignment of *P5CS* gene in PSDK 923 and VMC 76–16 sugarcane cultivars with analog gene sequences from data mining in NCBI and DDBJ. Note: PSDK923 (*P5CS* gene in sample sugarcane cultivar PSDK 923), VMC76–16 (*P5CS* gene in sample sugarcane cultivar VMC 16–16), AY888045.1 (*P5CS* gene in *Triticum aestivum*), AK108524.1 (*P5CS* gene in *Oryza sativa* ssp. *japonica* 'Nipponbare'), XM_020310078.1 (*P5CS* gene in *Aegilops tauschii* ssp. *tauschii*), BK007070.1 (*Hordeum vulgare* ssp. *vulgare*), D49714.1 (*P5CS* gene in *Oryza sativa* ssp. *japonica* 'Akibare'), GQ377720.2 (*P5CS* gene in *Sorghum bicolor*), EU005373.2 (*P5CS* gene in *Saccharum officinarum*), and NM_001319696.1 (*P5CS* gene in *Zea mays*)

An analysis of *SCDR 1* gene in PSDK 923 and VMC 76-16 sugarcane cultivars shows that they have fairly high gene sequence similarity of 90.22%. Based on the lineage tree (Figure 5), *SCDR 1* gene in PSDK 923 and VMC 76–16 sugarcane cultivars forms clusters with gene sequences in maize (*Zea mays*) and shows close lineage. These results assume that the genomes of maize (*Zea mays*) are also likely to have *SCDR 1* gene sequences, that also plays a role in assisting tolerance to environmental stress conditions (drought, salinity, and oxidative).

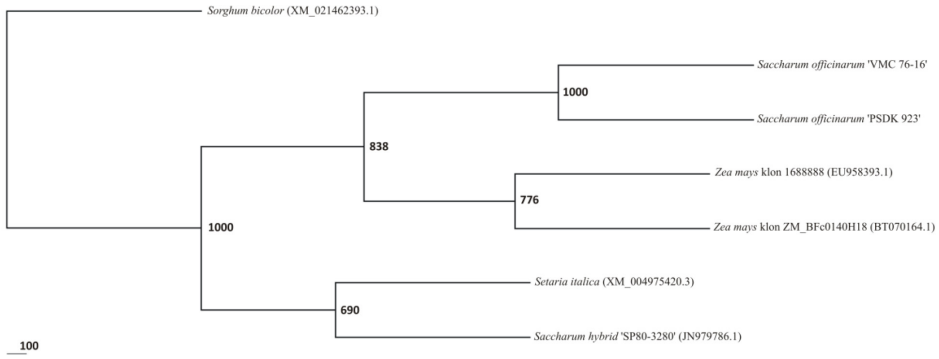

Figure 5 Neighbor-joining tree of *SCDR 1* gene of the sequencing results in PSDK 923 and VMC 76–16 sugarcane cultivars with analogous genes in other plants. The numbers on each node indicate the bootstrap value, from 1000 replications

In addition to forming clusters with close lineage to the genes of maize group, the genes in these two sugarcane cultivars also formed clusters with *Saccharum hybrid* 'SP80–3280' (JN979786.1) and *Setaria italica* (XM_004975420.3) species (Figure 5). *Sorghum bicolor* (XM_021462393.1) species has distant lineage with PSDK 923 and VMC 76–16 sugarcane cultivars compared to other species. In addition to expressing the tolerance to drought, salinity and oxidative stress conditions, the *SCDR 1* gene is also predicted to be able to express tolerance to heavy metal accumulation and pathogenic infection. All of these species belong to the Familia Poaceae, thus *SCDR 1* gene has close lineage to Monocotyledonous plants Class (Begcy *et al.*, 2012). Plants of Familia Poaceae members are tolerant to environmental stress conditions because they have a variety of vegetative reproductions such as tubers, stolons, rhizomes and etc. as well as other tolerance mechanisms. Therefore, it is known that the species of Familia Poaceae members naturally have tolerance to environmental stress conditions, whether morphologically, anatomically, biochemically or molecularly, one of which is the presence of *SCDR 1* gene in sugarcane or other genes that have similar functions or structures.

The lineage tree (Figure 6) shows that the *P5CS* gene sequences in PSDK 923 and VMC 76-16 sugarcane cultivars form clusters with *Oryza sativa* ssp. *japonica* 'Nipponbare' (AK108524.1). PSDK 923 and VMC 76–16 sugarcane cultivars have low level of similarity, which is 38.94%. The level of similarity between PSDK 923 and VMC 76-16 cultivars and *Oryza sativa* ssp. *japonica* 'Nipponbare' is also low, at 44.64% and 30.32%. This low similarity indicates that the *P5CS* gene in both cultivars is quite different. The difference may be caused by the different sources of these two cultivars (different parents, differences in geography, origin of crosses, etc.), thus the inherited gene sequences are also different even though they have similar functions because they adapt to the same conditions.

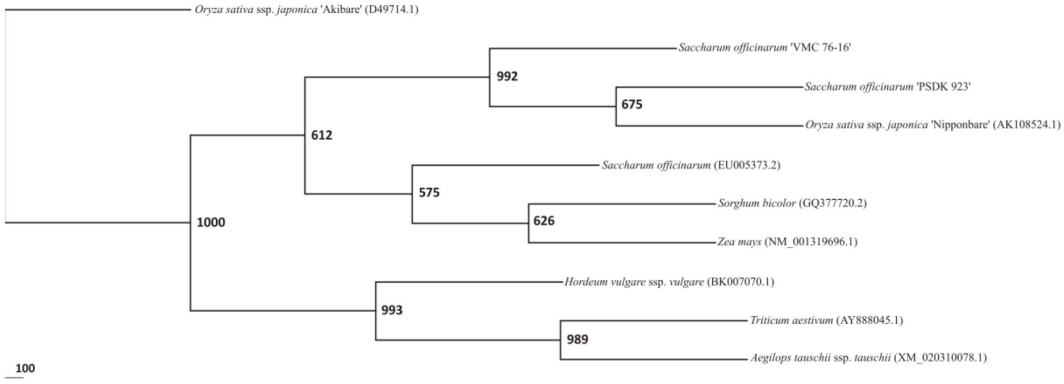

Figure 6 Neighbor-joining tree of *P5CS* gene of the sequencing results in PSDK 923 and VMC 76–16 sugarcane cultivars with analogous genes in other plants. The numbers on each node indicate the bootstrap value, from 1000 replications

The *P5CS* gene in both sugarcane cultivars also has close lineage to *P5CS* gene in *Saccharum officinarum* (EU005373.2), sorghum or *Sorghum bicolor* (GQ377720.2), and maize or *Zea mays* (NM_001319696.1) species (Figure 6). *P5CS* gene in *Triticum aestivum* (AY888045.1), *Aegilops tauschii* ssp. *tauschii* (XM_020310078.1), and *Hordeum vulgare* ssp. *vulgare* (BK007070.1) species also forms a separate cluster (Figure 6). *Oryza sativa* ssp. *japonica* 'Akibare' (D49714.1) has distant gene lineage with PSDK 923 and VMC 76–16 sugarcane cultivars compared to other species.

The present research also created 3D protein construction from amino acid sequences of the nucleotide sequence interpretation results obtained from data mining of *SCDR 1* gene in *Saccharum hybrid* 'SP80–3280' (JN979786.1) species and *P5CS* gene in *Saccharum officinarum* (EU005373.2) species. The purpose of construction was to determine the structure of proteins expressed by these two genes in plants belonging to the *Saccharum* genus.

The *SCDR 1* gene has an amino acid sequence composed of 247 amino acids, while the *P5CS* gene is composed of 716 amino acids. The protein produced by *SCDR 1* gene expression in sugarcane is composed of 19% proline amino acid and 13% cysteine amino acid, but this protein does not have a conserved domain (Begcy *et al.*, 2012). At cellular level, one of the cell response mechanisms to the water deficit condition is by increasing viscosity with production of insoluble substances but not toxic such as mannitol, proline amino acid, lysine amino acid and etc., thus the water remain in the cell. The *SCDR 1* gene mostly expresses the proline and cysteine amino acids, while the *P5CS* gene expresses 3% proline amino acid; 1.3% cysteine amino acid; 5% arginine amino acid; 3% asparagine amino acid; 6.7% lysine amino acid; and 7.7% serine amino acid. In addition to proline and cysteine, the percentage of other amino acids expressed in the *SCDR 1* gene is 1.2% arginine; 1.6% asparagine; 14.2% lysine; and 2.8% serine. These amino acids experience increased biosynthetic activity during environmental stress conditions.

CONCLUSION

Based on the research that has been done, it can be concluded that the 24 sugarcane cultivars studied, PS 41, PS 58, PS 384, PS 851, PS 864, PS 865, PS 881, PS 882, PS 891, PS 921, PS 951, PS 80.910, PS 80.1649, PS CO 902, PS JT 941, Kentung, BZ 132, KK, TLH 2, PSDK 923, BL, PS 862, PSBM 901, and VMC 76–16 sugarcane (*S. officinarum*) cultivars were predicted to be

resistant to environmental stress, particularly drought, salinity and oxidative stresses based on early detection of the presence of *SCDR 1* and *P5CS* genes by the PCR method. With the presence of *P5CS*, the twenty-four cultivars are likely to reduce their sucrose productivity under drought stress conditions. The research was only limited to the early detection of the gene to predict the resistance traits of 24 sugarcane cultivars in Indonesia that were tolerant to environmental stress based on the presence of these genes. This method have not been able to prove accurately whether the resistance traits are really expressed by 24 cultivars that exposed to environmental stress conditions. Further research is needed to look at the resistance traits to environmental stress in the trascriptomic levels to determine the exact of the resistance traits in the 24 sugarcane cultivars studied. Plants belonging to the Familia Poaceae, Classis Liliopsida (Monocot) have close lineage due to the presence of *SCDR 1* and *P5CS* genes. There is a similarity of 83.8% between *SCDR 1* gene sequences in PSDK 923 and VMC 76–16 sugarcane cultivars and *Zea mays* species. There is a similarity of 99.2% between *P5CS* gene sequences in PSDK 923 and VMC 76–16 sugarcane cultivars and *Oryza sativa* ssp. *japonica* 'Nipponbare'. Both sugarcane cultivars show high level of similarity in the *SCDR 1* and *P5CS* gene sequences in plants from *Saccharum* genus, namely 100% and 61.2%.

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