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Analysis of Biosilica in Sugarcane Leaves

Y. Paopun[1*], P. Thanomchat[1], D. Toyen[1]

[1] *Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, 10900,Thailand.*
*Corresponding author's e-mail address: rdiydp@ku.ac.th

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ABSTRACT

Sugarcane is an economic crop of Thailand to produce sugar. The process generates waste in the field more than 10 tons a year. The leaf waste is usually burned and causes many air pollution problems to the environment. Finding ways to utilize the waste will add values and reduce health problems. The aims of this research were to investigate distribution of biosilica on leaves of four most common sugarcane cultivars in Thailand (including KK3, LK92-11, KPS01-12 and UT84-12) and determine biosilica contents in the leaves. Sugarcane leaf surfaces were examined with field-emission scanning electron microscope (FE-SEM) and energy dispersive X-ray spectrometer (EDS). The Si contents were determined with energy dispersive X-ray fluorescence spectrometer (EDXRF). SEM images showed that the distribution of Si on the adaxial was higher than on the abaxial of sugarcane leaf surface. The EDS maps showed that the UT84-12 had the highest Si of 19.77wt% on abaxial leaf surface and the EDXRF results showed that the UT84-12 contained the highest Si of 6.34wt%. In principle, EDXRF is a bulk analysis with large volume of interaction. Therefore, it is more accurate than EDS&FE-SEM. The results also showed that the apical part of the leaves had higher Si content than in the middle and the basal part. This study demonstrated a potential to add value to agricultural-waste sugarcane leaves by transforming it biosilica, which can be used for other functions such as composite materials for rubber products, paints, fertilizers and cosmetics in the future.

INTRODUCTION

Sugarcane belongs to the Poaceae family and *Saccharum* genus. Its scientific name is *Saccharum officinarum* L. It is a native of New Guinea [13]. The sugarcane is grown in the Southeast Asia, India, China, the South America, Islands of the Pacific, Caribbeans, and other tropical regions [8]. In Thailand, sugarcane is a perennial economic crop. It is cultivated for sugar production and is exported to several countries around the world. There are four cultivars of sugarcane that are commonly grown in Thailand including KK3, LK92-11, KPS01-12 and UT84-12. They are popular because they are common, adaptive to various environments and provide high yield [17]. KK3 and KPS01-12 are traditional varieties that have high growth rate and high productivity under flooding condition [2]. UT84-12 is the best cultivar for producing sugar according to the Suphanburi Field Crops Research Center [23]. LK92-11 cultivar grows well and produces high yields [12]. Plants can absorb silicic acid (H_4SiO_4) from the soil and accumulated silica in the structure of cells such as cell wall and cell membrane of grains. Therefore, silica appears in phytolith forms in grass and horsetail [29]. Le Blond *et al*. (2010) reported that the sugarcane leaf contained amorphous silica, which might change to crystalize silica (cristobalite or quartz) when the leaves were burned. Leaves of sugarcane are burned in the fields before or after harvesting for the ease of cultivation. However, the burnt waste causes air pollutions and global warming [25]. The 89.125% of SiO_2 was extracted from sugarcane bagasse by Randy *et al*. (2015) when it was burned at 800°C. Teixeira *et al*. (2020) generated 61.3% of biogenic silica from leaf ash and 52.1% from sugarcane bagasse. Worathanakul *et al*. (2009) reported that calcinated bagasse at 600°C could extract 91.57 % SiO_2. Alves *et al*. (2017) synthesized 99.1 wt % biosilica from sugarcane waste ash. The silica nanoparticles from sugarcane were used to absorb eight acid orange dyes that were used for staining in fiber industry and reused again for 5 cycles [26]. It has been shown that values can be added to the agricultural waste and reduce air pollution. Therefore, we examined further into the distribution and amount of silica in Thai commercial sugarcane leaves used in the sugar industry by using FE-SEM&EDS and EDXRF. This was a feasibility investigation in order to assess a potential to extract silica for further industrial uses, such as Li-ion battery ceramics, paints, rubbers, catalysts, cosmetics and absorbent materials, and membranes for fuel cells [26]. The study was also to increase the value of agricultural waste of sugarcane leaves.

METHODOLOGY

Plant Materials

The sugarcane leaf samples of 4 cultivars were collected from Suphanburi Province, Thailand. They were UT 84-12, KPS 01-12, LK 92-11and KK 3 cultivars. Five leaves of each cultivar were used. The leaf samples were examined by Field Emission Scanning Electron Microscope & Energy Dispersive X-ray Spectrometer (FE-SEM&EDS)

Analysis of Sugarcane Leaves with FE-SEM & EDS

Sugarcane leaf samples were clean with distilled water and dried in a hot air oven at 45°C for 8 hrs. The samples were cut into 1x1 cm^2 and pasted onto a stub with carbon tap and kept in a desiccator for 5 days. The sample were coated with carbon by a sputter coater (Quorum: Q150R). The samples were observed around adaxial and abaxial areas by a FE-SEM (Hitachi: SU8020) which operated at 15kV. The distribution of silicon (Si) and quantitative analysis of elements were determined using arial mode of energy dispersive X-ray spectrometer. Three areas of the leaves (apical, middle, and base) were analyzed by EDS was about 400x510 μm^2.

Analysis of Sugarcane Leaves with Energy Dispersive X-ray Fluorescence Spectrometer (EDXRF)

The sugarcane leaves were 110-155 cm long and 4-6 cm wide. The leaf samples were separated into three parts: apical part, middle part and basal part. Each part of leaves was 37-52 cm long and was ground by a cutting mill (Retsch: SM100). One gram of the powder was put into a sample cup and analyzed with an EDXRF (Rigaku: NEX DE), operated at 60 kV and the measurement time was 12 min. The X-ray detector is silicon drift detector (SDD).

RESULS AND DISCUSSION

Morphologies of the sugarcane leaves of 4 cultivars were analyzed by FE-SEM. The elemental compositions and distributions were determined by EDS. Figure 1A shows a SEM image of the UT 48-12 leaf revealing numerous stomata (S), bulliform cells (BUL) and silica cells (SC). Figure 1B displays the leaf EDS spectrum indicating that the leaf contained silicon (Si), oxygen (O) and carbon (C). Figure 1C is the corresponding X-ray map of Si and Figure 1D is the X-ray map of O. The results indicated that Si and O have similar distributions and were likely to form SiO_2 around stomata, bulliform cells, and silica cells. Figure 2, Figure 3, and Figure 4 were groups of similar data for KPS 01-12, LK 92-11and KK 3 cultivars, respectively. Table 1 listed the Si content in wt% of the four cultivars analyzed in three areas of apical, middle, and base of the leaf. In addition, Si amounts in adaxial and abaxial regions were separately identified. The results showed that UT 84-12 contained the highest Si of 19.77±1.77wt% and KK 3 had the lowest Si of 9.04±2.50wt%. In general, the adaxial contained higher Si than the abaxial area and the apical area had the highest Si, followed by the middle and the basal area. The difference of Si between adaxial area and abaxial area was the highest in the apical part, then the middle and the basal part. The differences were about 2-8wt%, 2-3wt%, and 1-3wt% in the apical, middle, and basal areas, respectively.

The chemical compositions, especially Si contents in three parts of leaves of four cultivars of sugarcanes were also analyzes by EDXRF. The weight percentages of Si found in all cultivars were listed in Table 2. It was found that UT 84-12 cultivar contained the highest Si of 6.34±0.44wt% and KK 3 had the lowest Si of 4.44±0.12wt% in the

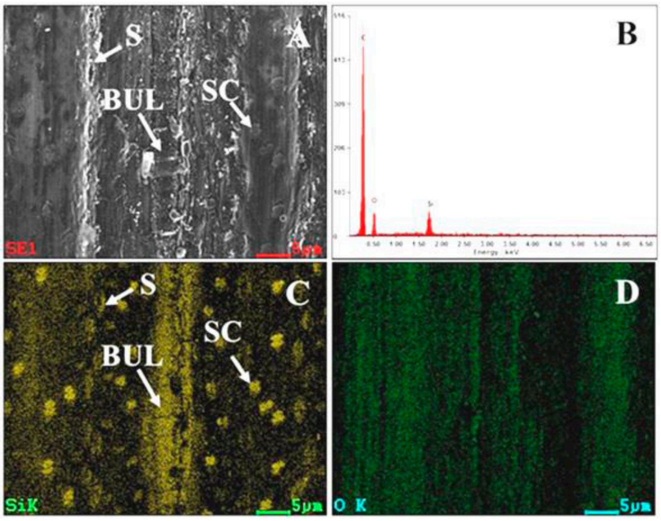

Figure 2. Analysis of KPS 01-12 leaf. A: SEM image on the adaxial area of the apical part of the leaf; B: EDS spectrum of area in A; C: the corresponding X-ray mapping of silicon; and D: X-ray mapping of oxygen BUL= bulliform cell, S=stomata, SC= silica cell.

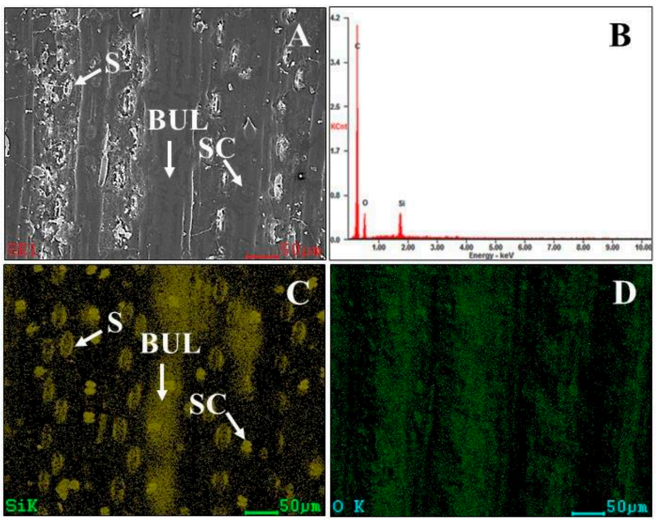

Figure 1. Analysis of UT 84-12 leaf. A: SEM image on the adaxial area of the apical part of the leaf; B: EDS spectrum of area in A; C: the corresponding X-ray mapping of silicon; and D: X-ray mapping of oxygen BUL= bulliform cell, S=stomata, SC= silica cell.

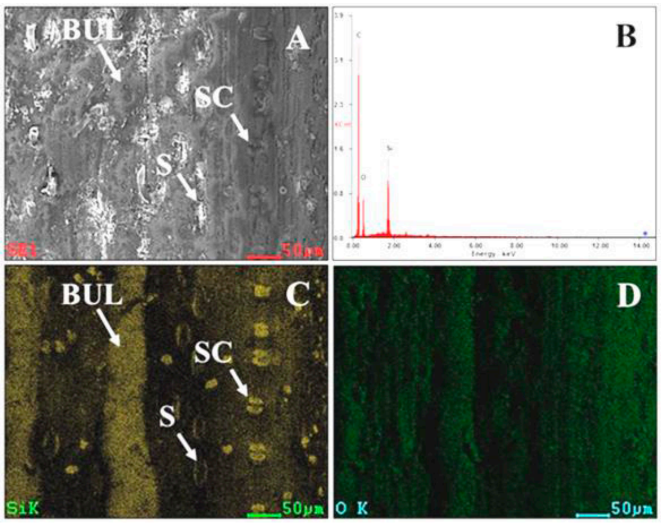

Figure 3. Analysis of LK 92-11 leaf. A: SEM image on the adaxial area of the apical part of the leaf; B: EDS spectrum of area in A; C: the corresponding X-ray mapping of silicon; and D: X-ray mapping of oxygen BUL= bulliform cell, S=stomata, SC= silica cell.

apical part. The trend was the same as EDS results reported in the previous section. The comparison of different wt% is discussed below.

The results agree well with our previous study, Paopun *et al*. (2021) reporting EDS analysis of sugarcane leaf of Supanburi 50 cultivar containing Si on leaf and leaf sheath. Rosello *et al*. (2015) used EDS and found that sugarcane leaf had 5.18 wt% Si. The present study showed that Si accumulates more at the adaxial area of leaf than at the abaxial area. This was different from Si in citrus. Mvondo-She and Marais (2019) reported that Si accumulation only found at the adaxial of citrus leaves. and the older the citrus leaf, the higher Si accumulation. Motomura *et al*. (2008) reported that older leaf of bamboo contained higher Si than young leaf.

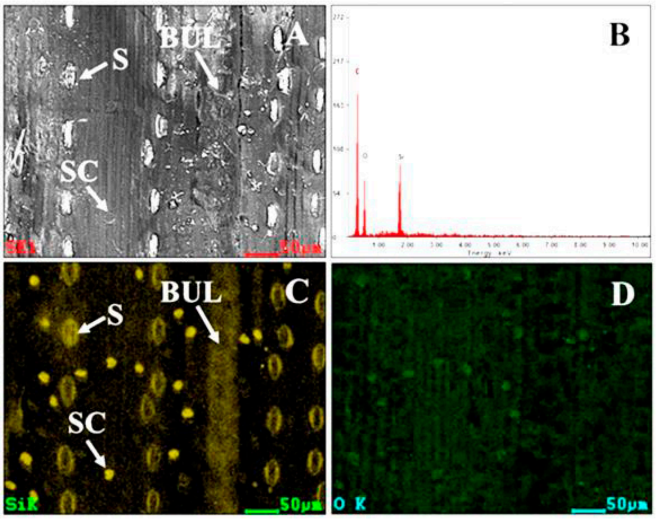

Figure 4. Analysis of KK3 leaf. A: SEM image on the adaxial area of the apical part of the leaf; B: EDS spectrum of area in A; C: the corresponding X-ray mapping of silicon; and D: X-ray mapping of oxygen BUL= bulliform cell, S=stomata, SC= silica cell.

Concerning part of the leaves, we found that the highest Si was accumulated on the apical part of the leaf surface of sugarcane, followed by the middle and the least Si was in the basal part. All four cultivars had the similar results. This was in good agreement with a study by Sangster and Parry (1968) who found that in grass leaves (Rice and Bermuda grass), Si accumulated in the apical zone more than the middle and the basal zone. Typically, Si was accumulated in the cell wall in order to increase resistance to diseases and pathogens [27]. Si could modify properties of soils, in particular, the soils that contain heavy metals such as Al, Mn and Fe. Plants deposit silica in the cell by absorb silicic acid from the soil to increase plant tolerance to salts [15]. The sugarcane leaf surface consists of epidermis cells, silica cells, trichomes and bulliform cells. The bulliform cells appeared only on adaxial area of the leaf and were not found on abaxial area [19]. Mvondo-She and Marais (2019) suggested that monocotyledon had Si deposition in the cell wall, cuticle layer of bulliform cells, silica cells and endodermal cells. SEM in this study revealed that the sugarcane leaf surface had stomata, silica cells and bulliform cells and EDS identified Si in those cells. On both surface of sugarcane leaves appeared stomata and silica cells which adaxial leaf surfaces contained Si more than abaxial. But the bulliform cells only found in adaxial leaf thus EDS analysis show the percentage of Si on higher than abaxial leaf. Sangster and Parry, (1969) reported the highest Si was deposit in grass leaf bulliform cells at the tip zone. Vandegeer *et al*. (2020) reported that plant used Si deposited on the leaf surface to reduce transpiration of leaf and found that Si was accumulated at guard cell, a component of stomata on the leaf. The higher accumulation of Si at adaxial leaf of sugarcane than at the abaxial may be because the adaxial part is exposed to various environmental stress conditions. The adaxial of leaf is exposed to direct sunlight, therefore, sugarcane has Si accumulation on the adaxial area more than abaxial area to reduce transpiration. In addition, Si accumulation on the leaf is part of a mechanism to strengthen the leaf area to increase resistance to diseases and insect damages. Kumar *et al*. (2017) found that the Si deposition in grasses appeared at cell wall, guard cell and silica cell. Silica cell is a special cell in leaf of grass family which is independent of transpiration. In addition, Massey *et al*. (2006) reported the deposition of silica appearing opaline phytoliths (silica cell) in the

Table 1. Silicon content (wt%) in the adaxial and abaxial areas of the apical, the middle, and the basal parts of the leaves of four sugarcane cultivars, analyzed by EDS combined with FE-SEM.

Cultivars of sugarcane	Si (wt%) in sugarcane leaves					
	Apical Part		Middle Part		Basal Part	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
UT 84-12	19.77±1.77a	11.76±2.06a	12.19±0.55a	9.84±2.03a	9.42±1.08a	7.38±1.38a
KPS 01-12	17.41±2.88b	12.71±3.31a	12.65±2.02a	9.34±0.58a	9.26±1.85a	5.76±0.99b
LK 92-11	10.38±2.66c	7.86±2.02b	7.35±1.05b	5.98±0.74b	7.25±2.15b	4.17±0.45c
KK 3	9.04±2.50c	8.16±1.78b	7.25±0.58b	4.24±0.88c	4.27±0.51c	4.11±0.27c

Note: The number followed by the same letters in column (a, b, c) are not significantly different with a confidence level of 0.05.

Table 2. Silicon content (wt%) in the apical part, the middle and the basal part of the leaves of four cultivars, analyzed by EDXRF.

Cultivars of sugarcane	Si (wt%)		
	Apical part	Middle part	Basal part
UT 84-12	6.34±0.44a	5.39±0.21a	3.61±0.21a
KPS 01-12	5.22±0.38b	4.10±0.10b	3.47±0.08a
LK 92-11	5.00±0.46bc	3.62±0.27c	2.74±0.04b
KK 3	4.44±0.12c	4.07±0.16b	2.65±0.07b

Note: The number followed by the same letters in column (a, b, c) are not significantly different with a confidence level of 0.05.

grass leaves were 2-5% of leaf dry weight. The function of silica cells was antiherbivore defense.

Matichenkov and Calvert (2002) reported that typically the Si concentrate in sugarcane leaf was about 0.1-3.2%. Sugarcane belongs to a grass family as well as bamboo and rice. Irzaman *et al*. (2018) found that the percentage of Si extracted from bamboo leave was 10.19%. Chandrasekhar *et al*. (2005) found that rice husk had 88wt% SiO_2. From the present results, Si was found in the sugarcane leaves of all cultivars in different concentrations in three parts of leaves. Currie and Perry (2007) reported that the ability to accumulate of Si was different among plant species. In addition, the difference depended on soil acidity and basidity (pH), which plants could dissolve Si in water at pH below 7 [3]. Therefore, the soluble Si content in soil was another factor affecting the accumulation of silica in sugarcane leaf.

Both FE-SEM & EDS and EDXRF techniques were good in determining Si in sugarcane leaves and in assessing the potential of silica production. FE-SEM & EDS techniques were used to observe Si contents and distributions on the leaf surfaces while EDXRF technique was used to determine Si in bulk. Both techniques identified that the apical part of UT 84-12 contained the highest Si although EDS with FE-SEM provided a much higher Si wt% than EDXRF from the same sample. The EDXRF technique was more accurate than EDS&FE-SEM technique because EDXRF analysis had a larger volume of interaction due to larger incident beam size of 60 kV X-ray compared to a small 15 kV electron beam of EDS & FESEM. EDXRF does not require vacuum. However, EDS&FE-SEM has an advantage to indicate position of element on the sample surface. Therefore, these techniques are complimentary, easy and quick for preliminary assessment of a potential to produce biosilica from sugarcane leaves. They could be used for assessment of Si accumulation of other agricultural wastes in the future.

CONCLUSIONS

The Si accumulated and distributed un-evenly on the leaf surface of four sugarcane cultivars. EDS analysis determined various percentages of Si according to three regions on the leaf surface with respect to adaxial and abaxial areas. UT 84-12 cultivar leaves had the highest Si content. The apex part of leaf contained higher Si than in the middle part and the basal part of the leaf. Therefore, the leaf of UT84-12 cultivar had the highest potential for biosilica production. Both FE-SEM&EDS and EDXRF were good complementary characterization techniques to identify Si in sugarcane and other agricultural wastes.

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REFERENCES

[1] A.G. Sangster, D.W. Parry, Some factors in relation to bulliform cell silicification in the grass leaf, *Ann. Bot*., **1969**, 33, 315-323.

[2] C.H. Palachai, P. Songsri, N. Jongrungklang, Comparison of yield components of sugarcane varities grown under natural short- and long-term water-logged conditions in Thailand, *SABRAO J. Breed. Genet*., **2019**, 51(1), 80-92.

[3] D.A. Barber, M.G.T. Shone, The absorption of silica from aqueous solutions by plants, *J. Exp. Bot*., **1966**, 17(52), 569-578.

[4] F. Massey, A.R. Ennos, S.E. Hartley, Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder, *J. Anim. Ecol*., **2006**, 75, 595-603.

[5] H. Motomura, K. Hikosaka, M. Suzuki, Relationships between photosynthetic activity and silica accumulation with ages of leaf in *Sasa veitchii* (Poaceae, Bambusoideae), *Ann. Bot*., **2008**, 101, 463-468.

[6] H.A. Currie, C.C. Perry, Silica in plants: biological and chemical studies, *Ann. Bot*., **2007**, 100, 1383-1389.

[7] I. Randy, L. Manzano, N. Begum, A. Noorliyana, Silica extraction from sugarcane bagasse as green corrosion inhibitor, *Asian Transactions on Basic&Applied Science*., **2015**, 5(5), 1-3.

[8] I.A. Ross, *Medicinal Plants of the World, Volume3 Chemical Constituents, Traditional and Modern Medicinal Uses*, fifth ed., Humana, Kentucky, **2005**.

[9] Irzaman, N. Oktaviani, Irmansyah, Ampel bamboo leaves silicon dioxide (SiO_2) extraction, *IOP Conf. Series: Earth Environ. Sci*., **2018**, 141, 1-8.

[10] J. Rosello, L. Soriano, M.P. Santamarina, J.L. Akasaki, J.L.P. Melges, J. Paya, Microscopy characterization of silica-rich agrowastes to be used in cement binders: bamboo and sugarcane leaves, *Microsc. Microanal*., **2015**, 21, 1314-1326.

[11] J.S. Le Blond, C.J. Horwell, B.J. Williamson, C. Oppenheimer, Generation of crystalline silica from sugarcane burning, *J. Environ. Monit*., **2010**, 12, 1459-1470.

[12] K. Sumran, N. Kerdsuk, A. Buntoom, Standard yield trail for sugarcane yield and quality: plant cane, The 9th Kasetsart University Kamphaeng Saen Campus conference, **2012**, 2236-2240.

[13] Kew royal botanical garden, plant of the world online, **1753**, https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:419977-1 (cited online 1 November 2022).

[14] L.B. Teixeira, E.G. Moraes, G.P. Shinhe, G. Falk, Obtaining biogenic silica from sugarcane bagasse and leaf ash, *Waste Biomass Valorization*., **2020**, https://doi.org/10.1007/s12649-020-01230-y.

[15] M. Sahebi, M.M. Hanafi, A.S.N. Akmar, M.Y. Rafii, P. Azizi, F.F. Tengoua, J.N.M. Azwa, M. Shabanimofrad, Importance of silica and mechanisms of biosilica formation in plants, *Biomed Res. Int*., **2015**, 1-16.

[16] M.A. Mvondo-She, D. Marais, The investigation of silicon localization and accumulation in Citrus, *Plants*., **2019**, 8(200), 1-12.

[17] P. Boodseephum, A. Wongtamee, Evaluation of yield components and yields of high yielding sugarcane varieties ion plant cane and first ratoon for clay soil area of Phitsanulok province, *Naresuan Phayao J*., **2022**, 15(1), 109-121.

[18] P. Worathanakul, W. Payubnop, A. Muangpet, Characterization for post-treatment effect of bagasse ash for silica extraction, *Int. J. Sci. Res. Innov.,* **2009**, 3(8), 398-400.

[19] P.H. Moore, Anatomy and morphology, in: D.J. Heinz (Ed.), Developments in Crop Science Book series volume11 Sugarcane Improvement through Breeding, Elsevier, Netherlands, **1987**, pp.85-141.

[20] R.H. Alves, T.V. Reis, Green synthesis and characterization of biosilica produced from sugarcane waste ash, *J. Chem*., **2017**, 1-9.

[21] R.K. Vandegeer, C. Zhao, X. Cibils-Stewart, R. Wuhrer, C.R. Hall, Silicon deposition on guard cells increase stomatal sensitivity

as mediated by K^+ Efflux and consequently reduces stomatal conductance, *Physiol. Plant*., **2020**, 1-13.

[22] S. Chandrasekhar, P.N. Pramada, L. Praveen, Effect of organic acid treatment on the properties of rice husk silica, *J. Mater. Sci*., **2005**, 40, 6535-6544.

[23] S. Gonkhamdee, J. Kruangatee, N. Porapagpai, K. Tosena, N. Jungrungklang, P Songsri, Effect of sugarcane varieties on weed control in ratoon sugarcane crop, *Khon kaen Agr J*., **2016**, 44(1), 1119-1124.

[24] S. Kumar, M. Soukup, R. Elbaum, Silicification in grasses: variation between different cell types, *Front. Plant Sci*., **2017**, 8, 1-8.

[25] S. Poltam, S. Kaewrueng, P. Duangpatra, Assessment of biomass loss and air pollution caused by pre-harvest sugarcane burning using the closed loop combustion system model, *EA*., **2018**,11(2), 1-8.

[26] S. Rovani, J.J. Santos, P. Corio, D.A. Fungaro, Highly pure silica nanoparticles with high adsorption capacity obtained from sugarcane waste ash, *ACS Omega*., **2018**, 3, 2618-2627.

[27] T.L. Simson, B.E. Volcani, *Silicon and Siliceous Structures in Biological Systems*, first ed., Springer-Verlag, New York, **1981**.

[28] V.V. Matichenkov, D.V. Calvert, Silicon as a beneficial element for sugarcane, *J. Am Soc. Sugar Cane Technol*., **2002**, 22, 21-29.

[29] W. Kaim, B. Schwederski, A. Klein, *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life an Introduction and Guide*, second ed., Wiley, United Kingdom, **1994.**

[30] Y. Paopun, P. Thanomchat, D. Toyen, D. Changjan, N. Sinbuatong, Silica on leaf surface of supanburi 50 cultivar sugarcane of Thailand, *The 38th MST International Conference*., **2021**, 86-87.