E-ISSN: 2664-6773
P-ISSN: 2664-6765
Impact Factor: RJIF 5.6
IJCBS 2024; 6(1): 33-37
www.chemicaljournal.org
Received: 03-12-2023
Accepted: 07-01-2024

Jini Viju PC
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

A Karthikeyan
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

E Sindhu
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Shyama P Nair
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Sushamani K
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Mahalingam L
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Rajesh C
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Corresponding Author:
Jini Viju PC
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, India

Characterization of bacterial surface leached silica using 3D profilometry: Insights and Applications

Jini Viju PC, A Karthikeyan, E Sindhu, Shyama P Nair, Sushamani K, Mahalingam L and Rajesh C

DOI: https://doi.org/10.33545/26646765.2024.v6.i1a.77

Abstract
Four isolates of rhizobacteria that promote plant growth were effectively isolated from the rhizospheric zone of the soil. Among the four isolates, *Bacillus licheniformis* stood out for having the extraordinary capacity to reabsorb a significant amount of silica onto the surface. A key component in promoting plant growth is silica, which is well known for its functions in controlling nutrient intake, lowering metal toxicity, offering structural support, and assisting in photosynthesis. Using the precursor Di Magnesium Trisilicate, *Bacillus licheniformis* demonstrated the ability to synthesize 14 mg of silica in a 50-ml culture. Upon further investigation using a 3D optical profilometer, the extracted silica exhibited a 7.904 µm complex three dimensional structure and 8.06 µm surface roughness. The unique properties of *Bacillus licheniformis* and its ability to generate a significant quantity of silica, position this organism as an excellent candidate for biofertilizer development. The incorporation of the silica precursor highlights its potential as a biofertilizer and emphasizes how it can promote plant growth by providing better structural support and nutrient management.

Keywords: Surface leaching, Rhizobacteria, Silica

1. Introduction

Silica (Si) is one of the major elements in the Earth's crust. The role of silica in soil development and plant growth in agriculture is remarkable. The silicon has the ability to stimulate the transport mechanisms and allowing the plants to uptake nutrients from their surroundings. Si acts at several levels in the plant functioning and in the soil (Guntzer *et al*., 2011) [4]. The silica has the antagonistic activity against the plant pathogens which are known as the biological stresses for the plants. These diseases include powdery mildew, septoria, eyespot, fusarium wilt, tan spot and leaf spots (Rodgers Gray and Shaw., 2004, Ma and Takahashi., 2002, The Savant *et al*., 1997b) [11, 7]. The precipitation of amorphous silica acts a mechanical barrier in plants. (Jones and Handreck., 1967) [5]. Silica plays a major role in alleviating salt stress, drought, phosphorous deficiency, iron, maganese, cadmium, aluminium, zinc toxicity. It also improve calcium, potassium, phosphate uptake, increase resistance to strong wind and rain. Guntzer *et al*., 2011) [4].

The plant growth promoting rhizobacteria (PGPR) have a major role in biofertilization activity. They are living organisms that help the plant to uptake insoluble nutrients and transport them. Microorganisms are integral in the natural phosphorus, carbon and nitrogen cycle. (Girmay Kalayu., 2019) [3]. The imperative role of beneficial microbes such as nitrogen - fixing bacteria, mycorrhizal fungi, and phosphobacteria in soil and plants is inevitable. (Karthikeyan *et al*., 2009) [6]. The biofertilizer itself acts as a better growth promoter. The mixture of both PGPR and silica will be a better treatment for the plants.

There is a rising demand to develop an eco-friendly approach to synthesizing silica. The green technique to synthesize silica with the help of a bacterial template will enhance the scope in various fields. Biological templates like bacteria, DNA, fungi and viruses are widely preferred over artificial templates such as polymer beads and inorganic templates for the synthesis of uniform nanoparticles with good dispersion, defined morphology and tunable sizes (Shilpi *et al*., 2015) [13]. The characterization using 3D optical profilometry describes the surface roughness and topography of the sample.

The present study aims to characterize bacterial surface-leached silica using the precursor Di Magnesium trisilicate, employing 3D optical profilometry for analysis.

Materials and Methods

Bacterial strains and Culture Condition

Using the conventional pure culture method, four distinct types of bacterial isolates were obtained from the rhizospheric region of various tree soils. Through the use of partial 16S rRNA sequencing, the isolates were identified. The isolates included *Bacillus licheniformis* sp. JV, which aids in the intractable phosphates; *Leifsonia* sp. JS, an actinobacterium with astounding remediation properties; *Rhizobium* sp. JV, which aids in nitrogen fixation; and *Rhodobacter* sp. JV02, which imparts excellent photosynthetic activity. 50 ml of nutrient broth was added to 150 ml of Erlenmeyer conical flasks, which were used to cultivate the cultures.

Bacterial surface leaching of silica

Yeast extract 200 ml/L, peptone 5 g/L, sodium carbonate Na_2CO_3 0.33 g/L, ferric hydroxide Fe $(OH)_2$ 0.1 M, and pH 8.0 are the ingredients of the semi synthetic medium used to inoculate bacterial cultures produced in nutritional broth. Subsequent to the inoculation of the stock cultures, 400 mg/L of di Magnesium Trisilicate precursor was applied. In the absence of a forerunner, the media exercises control. The broth was incubated at 65 °C for 6-7 days. Following this time, the cultures in the media were centrifuged at 3000 rpm for 10 minutes. After washing the pellet in an aqueous solution of 0.9%w/v sterile NaCl, the cell biomass was cleaned in an ethanol–ID solution. Following that, the harvested cell biomass was placed on the mica coverslips and stored. (Shilpi *et al*. 2015) [13].

Characterization

Topography of the leached silica was analyzed using the 3D optical profilometer. The analysis helps to find out the surface features of the extracted silica. The 3D optical profilometer was conducted in Dr. C.N. Rao lab, Avinashilingam University, Coimbatore.

Results

Quantitative measurement of leached silica was done. The amount of silica leached was noted. The *Bacillus licheniformis* produced more silica than other stock cultures with the help of precursor (table 1) in 50 ml of media. The leaching of silica was in the range of *B.licheniformis>Rhizobium>Rhodobacter>Leifsonia.*

Table 1: The amount of silica produced in mg quantity for a 50 ml culture was recorded

Cultures	With precursor (mg)	Without precursor (mg)
Rhodobacter sp. JV02	4.00	0.30
Rhizobium sp. JV	7.00	1.10
Leifsonia sp. JV	4.00	0.10
Bacillus licheniformis sp. JV	14.00	2.00

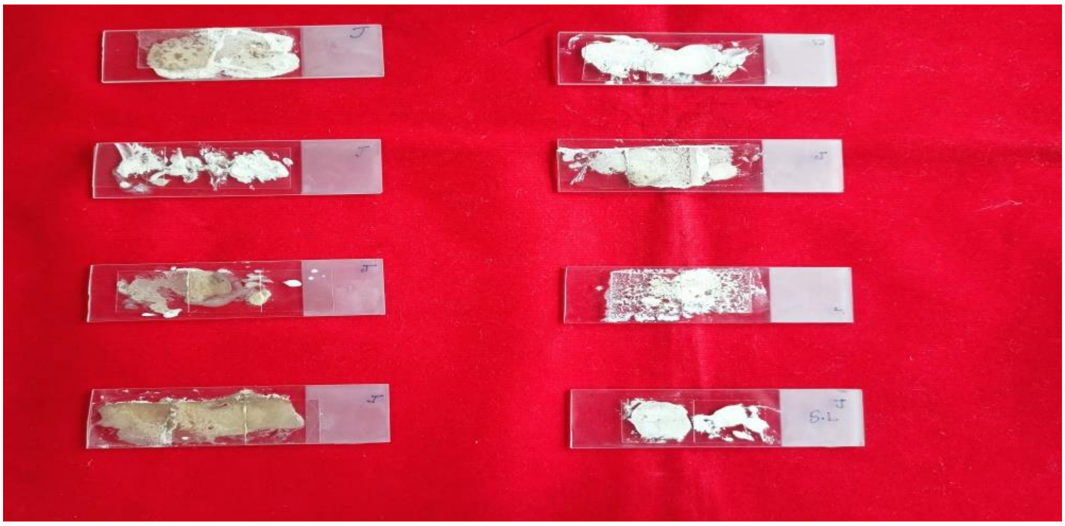
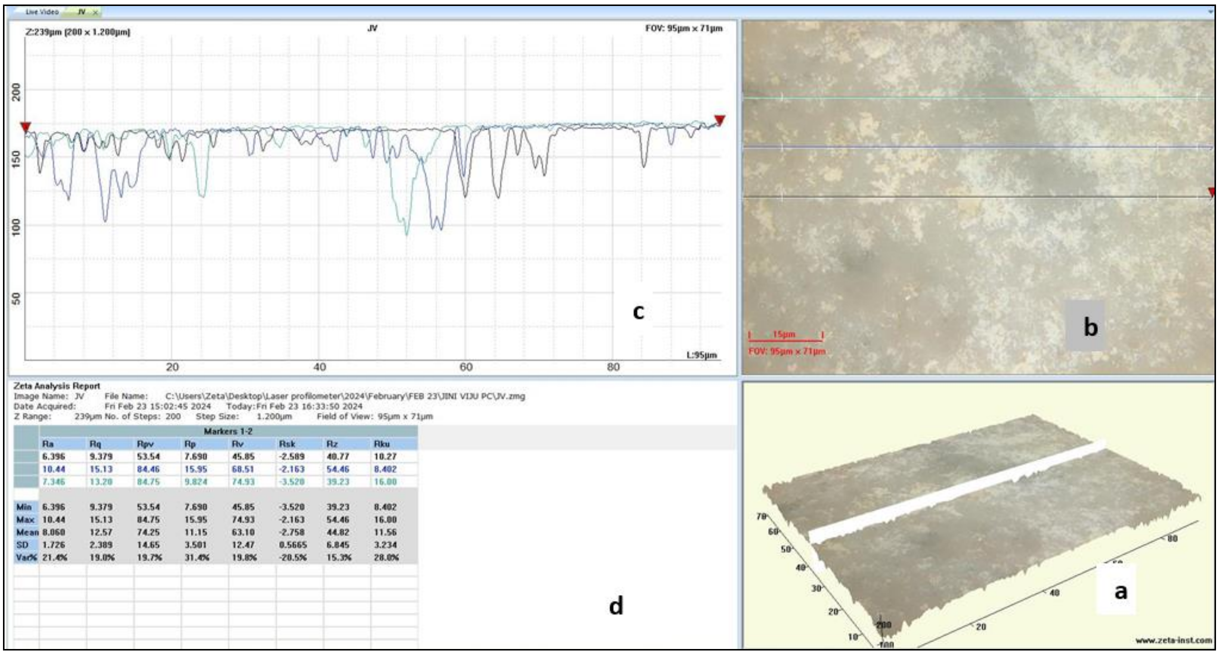


Fig 1: The synthesized silica particles from the surface of the *Bacillus licheniformis.*

3 D Optical Profilometer

A single case of raw data in the form of 3D profilometer images, with a consequent analysis in terms of 1D cross-section profile, is shown in Fig 2. The average roughness of the synthesized silica was 8.060 µm. (Fig 2.d). The Ra value indicates that the produced silica is rough and can be used for surfaces with stress requirements and for forming a design. The total roughness of the silica is 25 microns. According to the New ISO Grade, the scale number for the silica is 9. The silica has 250 microinches. The mean peak width was 275 microinches. The center line average of the silica was 250 numbers. The cut off lengths seem to be 0.1 inches (Fig 2.c). The distance between the tallest profile and the mean line was calculated as 11.15 µm. The distance between the lowest valley profile and the mean line was calculated as 63.10 µm. the maximum height of the sample was 44.82 µm.

It can be seen that the Fig 5.d has the quantitative data of the silica. The average height and width in the right direction were 171.5 µm and 7.904 µm. The left direction implied 158.42 µm height and 7.99 µm width respectively.



	Ra	Rq	Rpv	Rp	Rv	Rsk	Rz	Rku
	6.396	9.379	53.54	7.690	45.85	-2.589	40.77	10.27
	10.44	15.13	84.46	15.95	68.51	-2.163	54.46	8.402
	7.346	13.20	84.75	9.824	74.93	-3.520	39.23	16.00
Min	6.396	9.379	53.54	7.690	45.85	-3.520	39.23	8.402
Max	10.44	15.13	84.75	15.95	74.93	-2.163	54.46	16.00
Mean	8.060	12.57	74.25	11.15	63.10	-2.758	44.82	11.56
SD	1.726	2.389	14.65	3.501	12.47	0.5665	6.845	3.234
Var%	21.4%	19.0%	19.7%	31.4%	19.8%	-20.5%	15.3%	28.0%

Fig 2: a) Typical 3 D view of the sample. b) Tne typical profilometer image (3D shown as 2D projection here) obtained at one of the positions described in a. c) Typical profiles extracted from each 3D images as in b. d) Tables of quantitative information obtained from the selected profiles on roughness.

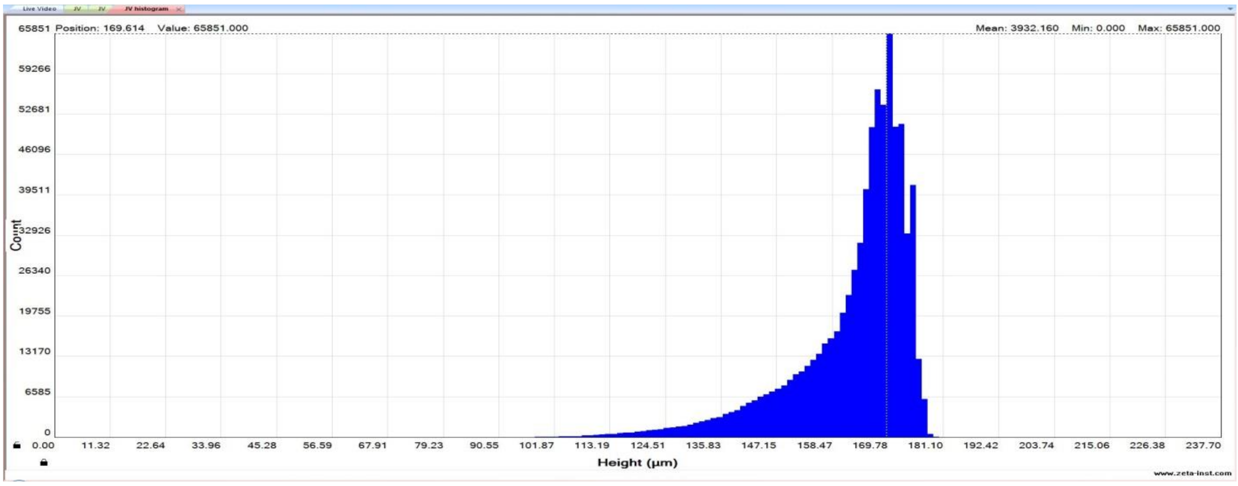


Fig 3: Histogram representing the height of the sample

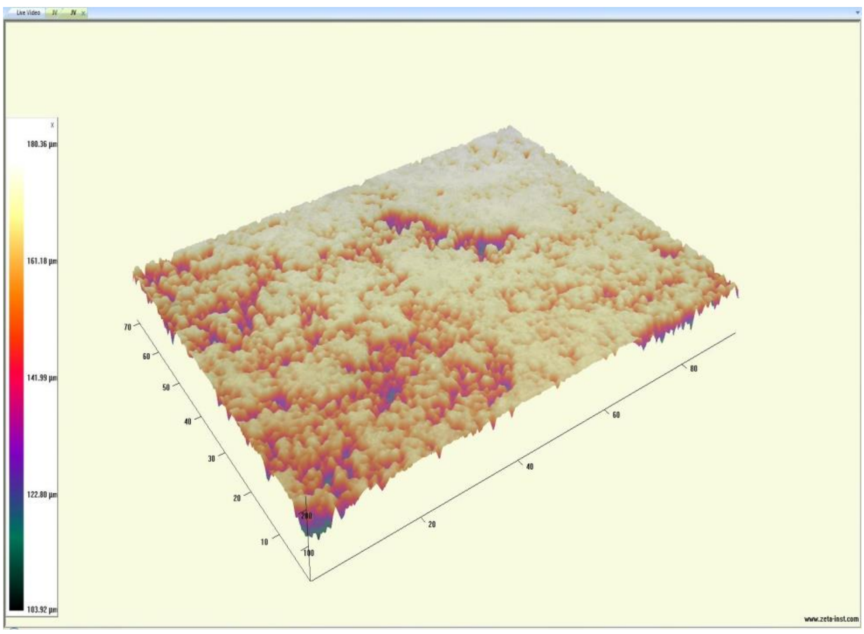


Fig 4: Analytical function of gap depth and width, tentatively describing the ‘intensity’ of the marginal gap.

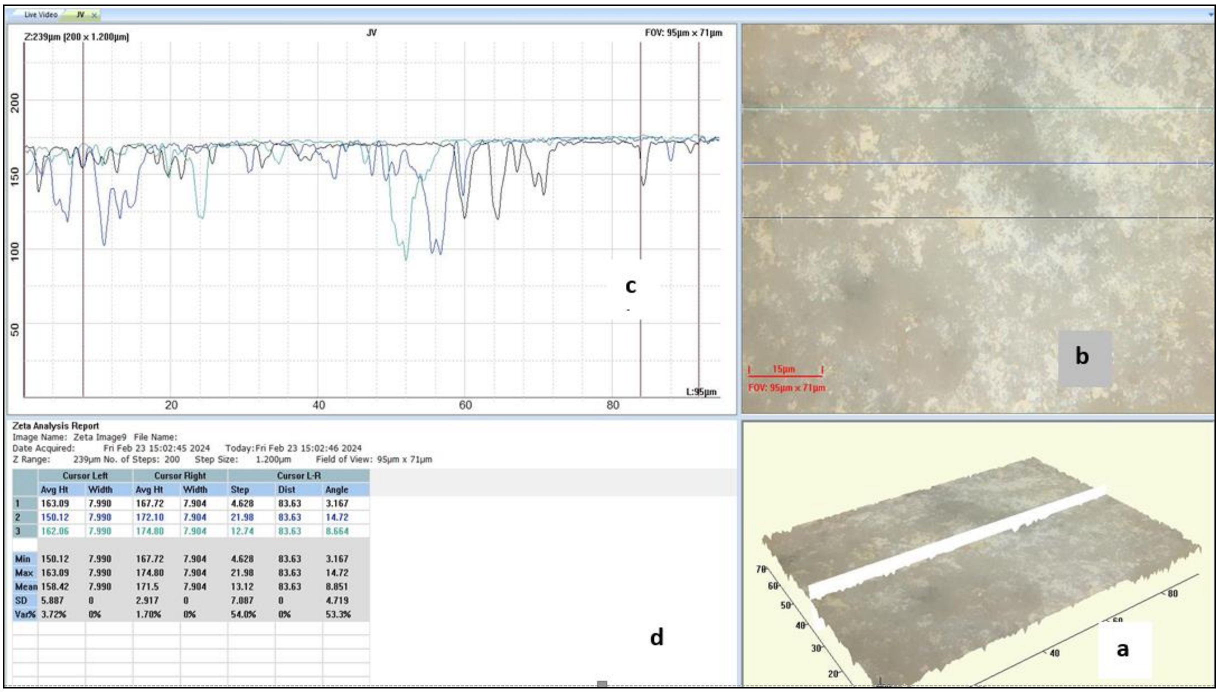


Fig 5: a) 3 Dimensional view of the sample. b) One typical profilometer image (3D shown as 2D projection here) obtained at one of the positions described in a. c) Typical profiles extracted from each 3D images as in b. d) Tables of quantitative information obtained from the selected profiles height, width, distance and angle.

Discussion

Datnoff and Rodrigues (2005) [1] illustrated the positive impacts of silicon in both soil and plants. Shilpi *et al*. (2015) [13] discovered that silica can be produced from the surface of bacteria through organic and inorganic methods utilizing diMagnesium triSilicate and tetra ethyl orthosilicate. Sanjay *et al*. (2008) noted the creation of silica from actinobacterium by exposing it to a precursor K2SiF6. In the current investigation, leached silica was obtained from bacteria with the aid of the precursor diMagnesium triSilicate. The bacterial strain *Bacillus licheniformis*, a phosphate solubilizing bacteria, exhibited higher silica production compared to other bacterial strains.

The investigation conducted by Shilpi *et al*. (2015) [13] focused on utilizing atomic force microscopy to examine the bacterial synthesis of silica. The study revealed that the deposition of silica led to an increase in surface roughness. Additionally, the researchers compared various bacterial strains that were capable of leaching silica. The leached silica was then characterized using 3D optical profilometry. Furthermore, the synthesized silica exhibited a high surface roughness, allowing for versatile design possibilities.

Silica nanoparticles have been discussed in the therapeutic field by Pulicherla *et al*., 2018 [10]. Guntzer *et al*., 2010 [4] conducted a review on the advantages of silicon for plants and soil. Various types of Si fertilizers have been examined in multiple studies (Gascho 2001; Mecfel *et al*., 2007; Meyer and Keeping, 2001) [2, 8, 9]. While PGPRs alone can enhance biofertilizer quality, the addition of silica further increases its appeal. The release of silica from the bacteria's surface significantly improves its effectiveness compared to PGPR alone. Phosphate-solubilizing bacteria are capable of converting insoluble phosphates into soluble ones. These bacteria secrete silica with the assistance of a precursor, aiding in improved nutrient absorption, pathogen resistance, growth enhancement, and heavy metal tolerance in plants. Bacteria that provide silica can serve as superior biofertilizers in agriculture.

Conclusion

The present study found out that *Bacillus licheniformis* is a better bioinoculant for plant growth because it can leach silica with the help of a precursor, so allowing to act as a multitasking organism in the cultivation area.

Acknowledgement

Authors gratefully acknowledge the facilities provided by the Department of Forest Protection, Institute of Forest Genetics and Tree Breeding, Testing facilities are provided by Avinashilingam CNR laboratory.

References

1. Datnoff LE, Rodrigues FA. The role of silicon in suppressing rice diseases. APS net Feature Story; c2005. p. 01-28.
2. Gascho GJ. Silicon sources for agriculture. Studies in plant science. Elseiver; c2008. p. 197-207
3. Girmay Kalayu. Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int. J Agronomy; c2019 Jun. p. 01-07.
4. Guntzer F. Impact de la culture intensive de cereals sur les stocks de silice biodisponible dans le sols europeens. PhD thesis, Aix Marseille University; c2010.
5. Jones LHP, Handreck KA. Silica in soils, plants, and animals. Adv. Argon. 1967;19:104-109
6. Karthikeyan Arumugam, Deeparaj B, Napolean P. Reforestation in bauxite mine spoils with *Casuarina Equisetifolia* frost. and beneficial microbes. Forests, Trees, and Livelihoods. 2009 Jun.
7. Ma JF, Takahashi E. Soil, fertilizer, and plant silicon research in Japan. Elseiver; c2002.
8. Mecfel J, Hinke S, Goedel WA, Marx G, Fehlhaber R, Baeucker E, wienhaus O. Effect of silicon fertilizers on silicon accumulation in wheat. J plant nutrition and soil science. 2007;170:769-772.
9. Meyer JH and Keeping MG. Past, present and future research of the role of silicon for sugarcane in southern

Africa.Studies in plant science. 2001;8:257-275.
10. Pulicherla Yugandhar and N. Savitharamma. Synthesis, Characterization and antimicrobial studies of bio silica nanoparticles prepared from *Cynodon dactylon* L.: A green approach. Bulletin of Material Science.2018 Jun, 41(65).
11. Rodgers Gray BS and Shaw MW. Effects of straw and silicon soil amendments on some foliar and stem base diseases in pot grown winter wheat. Plant Pathology. 2004;53:733-740.
12. Sanjay Singh, Umananda Manjunatha Bhatta, P.V Satyam, Alok Dhawan, Murali Sastri, Prasad Blv. J Material Chem., 2008 May, 18(22).
13. Shilpi show, Abiral Tamang, Trinath Chowdary and Dipankar Mandal. Bacterial (BKH1) Assisted Silica Nanoparticles from Silica Rich Substrates: A Facile and Green Approach for Biotechnological Applications. Colloids and Surfaces B intrefaces. 2015 Jan;126:245-250.
14. Savant NK, Synder GH, Datnoff LE. Silicon management and sustainable rice production. Adv. Agron. 1997b;58:151-199.