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RESEARCH PAPER

Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil

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Abstract The present study aims to explore the effect of high surface area (360.85 $m^2\ g^{-1}$) silica nanoparticles (SNPs) (20–40 nm) extracted from rice husk on the physiological and anatomical changes during maize growth in sandy loam soil at four concentrations (5–20 kg ha^{-1}) in comparison with bulk silica (15–20 kg ha^{-1}). The plant responses to nano and bulk silica treatments were analyzed in terms of growth characteristics, phyto compounds such as total protein, chlorophyll, and other organic compounds (gas chromatography–mass spectroscopy), and silica accumulation (high-resolution scanning electron microscopy). Growth characteristics were much influenced with increasing concentration of SNPs up to 15 kg ha^{-1} whereas at 20 kg ha^{-1}, no significant increments were noticed. Silica accumulation in leaves was high at 10 and 15 kg ha^{-1} (0.57 and 0.82 %) concentrations of SNPs. The observed physiological changes show that the expression of organic compounds such as proteins, chlorophyll, and phenols favored to maize treated with nanosilica especially at 15 kg ha^{-1} compared with bulk silica and control. Nanoscale silica regimes at 15 kg ha^{-1} has a positive response of maize than bulk silica which help to improve the sustainable farming of maize crop as an alternative source of silica fertilizer.

Keywords Nanosilica · Bulk silica · Maize · Silica accumulation · Physiological parameters

Introduction

The properties of nanomaterials and their potential applications are increasing in various fields, especially agricultural biotechnology. Transport of certain nanoparticles is involved in a potential pathway of plants for beneficial outcome. Currently, many metal oxide nanoparticles and carbon-based materials are being screened for phyto toxicity as well as environmental toxicity (Dimkpa et al. 2012; Mondal et al. 2011; Monica and Cremonini 2009). However, the investigations on behavior of nanometal oxides such as nanosilica in plants and the mechanism of interaction, its influence, and agricultural application are still under rudimentary stage (Debnath et al. 2011; Ghormade et al. 2011). Generally, the ameliorative effect of bulk SiO_2 sources varies genotypically among plant species (Kulikova and Lux 2010) and hence, we focus to examine the effect of nanosilica accumulation and its influence on maize physiology and metabolic

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defense compounds. Maize (*Zea mays*. L) ranks third in global cereal production and considered as an essential food crop (Mahmood et al. 2005).

Previous studies on the effect of silica nanoparticles (SNPs) in maize reveal improved growth parameters and increased seed stability (Yuvakkumar et al. 2011; Suriyaprabha et al. 2012) but the effect of SNPs on maize physiological components and silica distribution in roots and shoots is essential to gain the detailed functional properties of mineral fertilizers. Determination of essential regulatory and defense compounds in maize such as protein and phenols is necessary to ascertain the biotic and abiotic stress tolerance mechanisms adapted through silica fertilization. Consequently, the above-mentioned nanoparticles-mediated alleviation of abiotic stress may substantially contribute to the adaptation of maize (Miao et al. 2010).

The purpose of the application of SNPs for plant growth improvement is that silicon (Si) is an agronomically important nutrient that accumulated in plants to total concentrations of dry matter similar to other essential macronutrients. Si deficiency causes imbalances of other nutrients resulting in poor growth, if not death of the plant (Epstein 1999; Savant et al. 1999; Rafi et al. 1997). Si also has an active role in the biochemical functions of a plant and may be important in the intracellular synthesis of organic compounds (Matichenkov and Bocharnikova 2001; Mitani and Ma 2005). As amorphous silica particles are widely known to have biocompatibility, application of amorphous nanosilica for food crops is feasible to overcome silica deficiency in soil as well as in plants.

During the application of silica fertilization for plant growth enhancement, the availability of silica source that is economical with increased functional properties for fertilizer applications is currently a demand task. Previous studies of Si application to maize plants have revealed that leaf transpiration rates under water stress, antioxidant processes, and Si deposition are improved (Kaya et al. 2006; Gao et al. 2006). Numerous laboratory, greenhouse, and field experiments have shown the benefits of silicon fertilizers for crops and the importance of silicon fertilizers as a component in sustainable agriculture (Liang et al. 2007; Matichenkov et al. 1999; Lux et al. 1999). However, to benefit from SiO_2, the plant must be able to acquire this element in high concentrations. Approximate requirements of bulk silica for the cultivation of monocots as soil amendment are in the range of 1–100 kg ha^{-1} (Matichenkov and Bocharnikova 2001). Moreover, the degree of Si solubility from siliceous materials depends on particle size and chemical compositions. The silicon transport using metal salts of silicic acid needs their hydrolysis before their uptake, which affects ionic balance of the soil and plant system (Ranganathan et al. 2006).

In order to unravel the role of SNPs, it is preferable to study the physiological changes of maize cultivars differing in nanosilica absorption. The focus of this study is to examine the effects of nanosilica on SiO_2 uptake and deposition, to reveal any patterns of discrimination among maize growth characteristics and biochemical changes such as metabolic compounds (content of protein, chlorophyll, and phenols), and to evaluate the elemental distribution in plant.

Materials and methods

Synthesis and characterization of nanosilica

Nanosilica was extracted from natural source using rice husk ash by acid precipitation followed by alkali extraction method (Kalapathy et al. 2000). The pH was adjusted in such a way to attain the precipitate of silica and then, it was thoroughly washed with double-distilled water to remove the impurities. Finally, the pure silica powder was collected after calcination at 450 °C for 2 h. Synthesized powders were subjected to various characterization studies for identification of their structure and morphology. X-ray diffractometer (XRD) (X' Pert Pro, PANalytical, Netherland) using Cu K_α ($\lambda = 1.5406$ Å) as a radiation source over the 2θ range of 10°–80° at 293 K was employed to explore the crystalline nature of silica nanoparticles. The peaks of silica functional groups from Fourier transform infrared spectra (FTIR) have been obtained in the wavenumber region of 4,000–400 cm^{-1} using FTIR spectrometer (Spectrum 100, PerkinElmer, USA). The morphology and elemental composition of nanosilica was studied by scanning electron microscopy coupled with energy dispersive X-ray analysis (SEM-EDAX) (JEOL JSM-6390LV, Japan). Particle size analysis (Nanophox, Sympatec, Germany) of dispersed silica nanoparticles was done to derive particle size distribution curve. Silica nanoparticles were observed for its morphology and size by transmission electron microscopy (TEM) (CM 200, Philips, USA).

The specific surface area of the prepared silica nanoparticles was analyzed using BET surface area analyzer (Autosorb AS-1MP, Quantachrome, USA). The physisorption analysis was done with N_2 adsorption–desorption measurements at liquid nitrogen temperature (−196 °C). The total pore volume and average pore diameter were calculated according to the Barret–Joyner–Halenda method (Barrett et al. 1951).

Soil amendment with nanosilica and maize culture

Nanosilica was compared with commercial silica (SiO_2, MW 60.08, 40–150 mesh) for field study. The required greenhouse field was prepared in the area of 15 × 10 m of each plots containing sandy loam soil (pH 7.0 ± 0.5) in the agriculture land at Tiruchengode, Tamil Nadu, India. Experimental plots were prepared by adding nanosilica at the concentrations of 5, 10, 15, and 20 kg ha^{-1} (hereafter termed, respectively, as N5, N10, N15, and N20), bulk silica at 15 and 20 kg ha^{-1} (hereafter termed, respectively, as B15 and B20), and control (without silica source). Then, the plots were thoroughly plowed to spread the particles. Maize seeds (*Zea mays* L., TIP TOP) collected from M/s. Rasi seeds Pvt. Ltd, Attur, India, were washed twice with distilled water. Triplicate plot was prepared and then, 25 maize seeds were sown in each plot at a seed interval of 30 cm and appropriate temperature and humidity were maintained.

Growth characteristics

After the seedlings were grown in the four regimes of nanosilica and bulk silica amended soil, the changes in physiological parameters were observed. During this study, the seed germination percentage of each treatment plot was evaluated. Further, the growth characteristics were measured in terms of stem height, number of shoots and roots, stem diameter, root length, and leaf area (third leaf) after 20 and 40 days of maize growth by collecting samples in a randomized block design.

Protein, chlorophyll, and phenol determination

The total protein content of 20-day-old maize leaves was determined according to Bradford method using bovine serum albumin as the standard (Bradford 1976). Chlorophyll was extracted with 80 % acetone and the ratio of chlorophyll *a* to chlorophyll *b* in the leaf samples was determined spectrophotometrically (U-2900; Hitachi, Japan) (Arnon 1949). Total phenolic contents in maize leaves were extracted and estimated according to the method described by Wakabayashi et al. (1997).

Analysis of elemental compositions

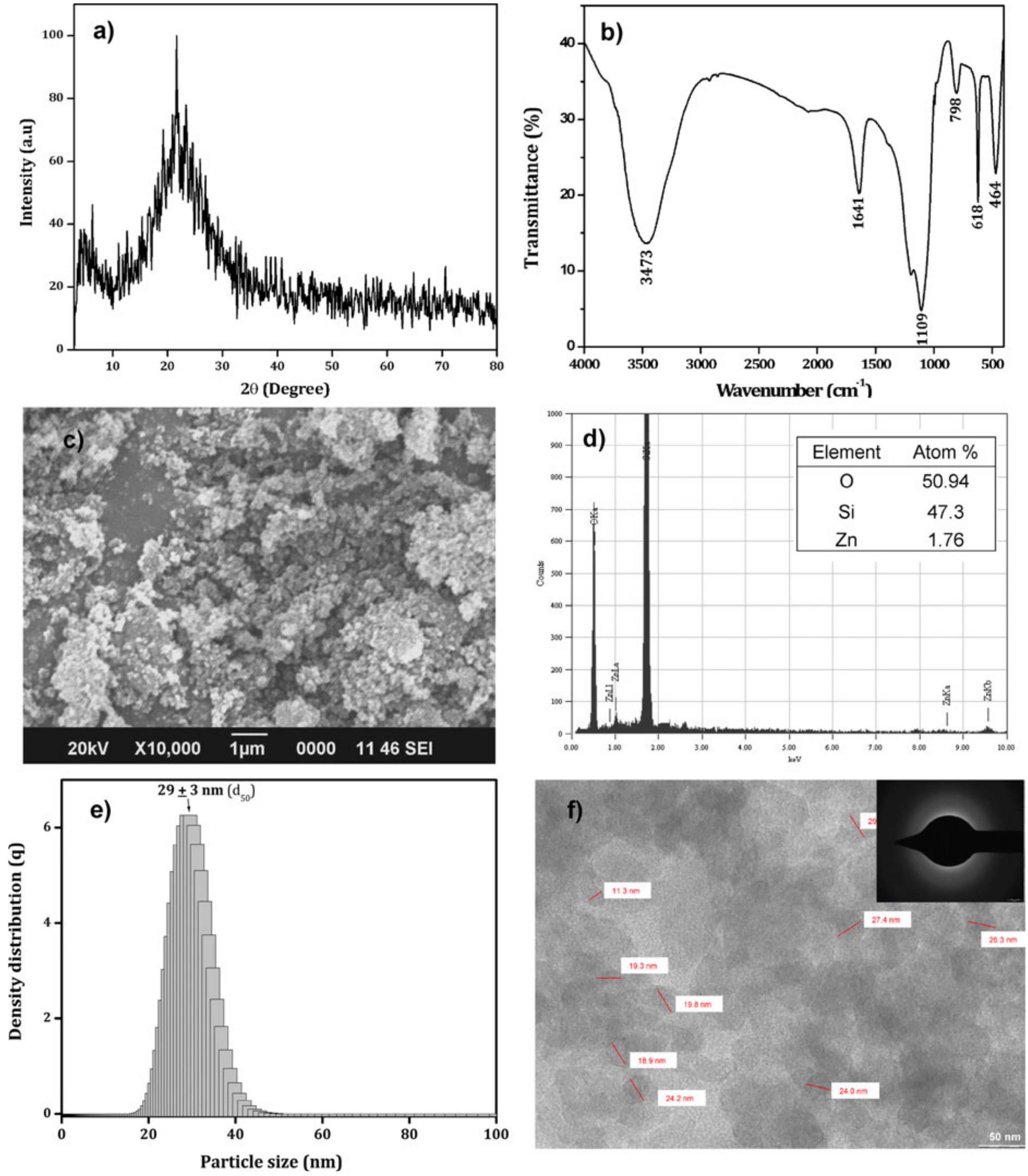
Root and leaf samples were carefully collected after 20 days of maize growth and dried in a hot air oven at 100 °C for 48 h. Then, the samples were burnt in high-temperature muffle furnace at 800 °C for 4 h. The ash obtained from the samples was further used to estimate the total dry weight percentage, silica content (Weimin et al. 2005), and elemental analysis using X-ray fluorescence spectrometer (XRF, EDX-720; Shimadzu, Japan). The differences in Si composition of maize leaf under the nanosilica treatments were also determined in terms of the occurrence of silica functional groups using FTIR.

Gas chromatography–mass spectroscopy (GC–MS) analysis

Shade-dried maize leaves (20 days old) were extracted by methanolic extraction and analyzed by gas chromatography–mass spectroscopy (Thermo GC-Trace Ultra Ver. 5.0; Thermo Scientific MS DSQ II). The GC silica column dimension was 30 m × 0.25 mm at a flow rate of 1 mL min^{-1} at 80 °C and then, the temperature was raised to 240 °C. The volatile compounds present in the control, nano silica and bulk silica treated samples were identified by comparing with the standards or the mass spectrum matched with the inbuilt library (Wiley 9).

Microscopy and X-ray analysis

High-resolution scanning electron microscope (HR-SEM) equipped with EDAX (FEI Quanta FEG 200, The Netherlands) was used for Si accumulation studies. The third leaves of maize were cut to 15 mm in length and leaf blades were examined at an accelerating voltage of 30 kV in a 9 mm working distance with a live detection period of 100 s. For quantification of all samples, 700× magnifications were chosen and the elemental wt% was calculated using ZAF program of the EDAX system. In root anatomical studies, samples of 15-days-old maize roots about 1 mm long were cut at a distance of 10 cm from the apex and the segments were immediately fixed in a formaldehyde solution (40 %) for 24 h. Root sections were cut as thin layer transversely and stained with safranin solution for 1 min and then, mounted on 50 % glycerin. Internal root structures were observed under optical microscope (200×) for anatomical variations.



Element	Atom %
O	50.94
Si	47.3
Zn	1.76

Fig. 1 Characterization results of the prepared silica powders **a** XRD, **b** FTIR **c** SEM, **d** EDAX, **e** PSD, and **f** TEM

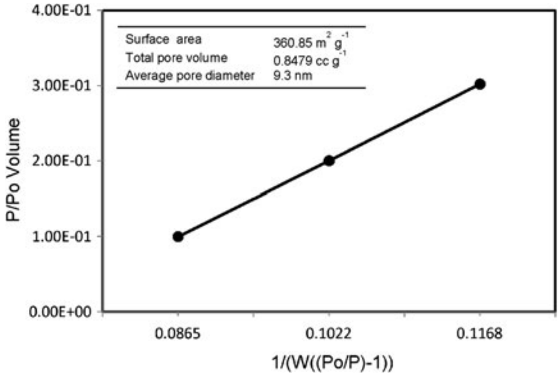


Fig. 2 BET plot of the prepared silica nanoparticles

Statistical analysis

Significant differences in the growth characteristics, total dry weight, content of silica, chlorophyll, and protein due to different concentrations of nanosilica, bulk silica, and control samples of maize were analyzed following one-way analysis of variance. Then, means were compared by Duncan and Tukey's test with the statistical software SPSS version 16.0. All statistical tests were screened for significant variations at the 5 % level ($p < 0.05$).

Results

The synthesized nanosilica is characterized for its morphology and functional groups. It is confirmed as nanometer in size with reference to earlier studies (Hodson and Evans 1995). The extracted white hydrous silica precipitate from rice husk ash is formed at pH 4. The prepared silica particles are structurally and morphologically characterized for its purity, functional groups, and particle. XRD patterns of the prepared silica nanoparticles are amorphous in nature which is confirmed by the observed broad peak at 22° (2θ) (Fig. 1a). FTIR results (Fig. 1b) show that the presence of characteristic peak in the absorption spectra at 1,096 and 451 cm^{-1} corresponds to Si–O–Si and Si–O functional groups. From SEM results (Fig. 1c), the silica nanoparticles are found as aggregates with spherical morphology and EDAX results (Fig. 1d) show 97 % purity of silica with negligible zinc contamination. From particle size distribution study and TEM image analysis, it can be concluded that the particle size of silica particles are in the range of 20–40 nm with spherical morphology (Fig. 1e, f). The selected area electron diffraction (SAED) pattern of TEM image shows an amorphous nature. The obtained surface area for the prepared silica particles is found to have high surface area of 360.85 $\mathrm{m}^2\ \mathrm{g}^{-1}$ (Fig. 2). The total pore volume and average pore diameter is observed to be 0.8479 cc g^{-1} and 9.3 nm, respectively.

Growth characteristics

Changes in maize growth parameters such as seed germination percentage, number of shoots and roots, stem diameter, stem height, root length, and leaf area of 20 and 40 days old plants at varying concentrations of nanosilica and bulk silica are presented in Tables 1 and 2. It is inferred from the observed results that the growth parameters are enhanced in maize with the increasing concentration of nanosilica up to 20 days. But lateral roots and root length are observed to be increased due to nanosilica fertilization. After 20 days, the growth characteristics of maize remains the same in all treated plots including micro silica treatments (Table 1) and plant does not show substantial increment with an increasing concentration of silica sources in the growth characteristics as the mentioned parameters especially stem height and leaf area have not differed significantly with each other (Table 2).

Total soluble silica and chlorophyll content

While estimating the total leaf silica content based on the formation of silicomolybdenum blue complex, increase in silica content with respect to concentration of nanosilica was observed (Table 3). N15 and N20 possess higher silica content (17.83 ± 1.29 and 12.55 ± 1.04 μg mL^{-1}) than any other concentrations including bulk silica (2.50 and 1.85 μg mL^{-1}). Leaf samples collected from experimental plots (20 days) reveal the gradual increase in chlorophyll *a* and *b* content according to the concentration gradient of nanosilica (Table 3) in contrast to bulk counterpart (0.012 and 0.03 μg mL^{-1}) of 20-days-old samples of maize leaves. Moreover, higher chlorophyll content is achieved at N15 and N20 (0.045 and 0.047 μg mL^{-1}, respectively) than other regimes of silica treatments.

Table 1 Effect of nanosilica on growth parameters in maize after 20 days of growth

Growth characteristics	Germination (%)	Root (nos.)	Root length (cm)	Shoots (nos.)	Stem height (cm)	Stem diameter (cm)	Leaf area (cm^2 plant^{-1})
Control	97	11	11	4	13.3	0.8	114.7 ± 2.8
N5	97	20	12	5	14.5	1.1	128 ± 4.1
N10	97.3	11	10.5	5	16.5	1.1	162 ± 6.4
N15	98	18	18	6	19	1.4	260 ± 10.12
N20	98	24	19	7	52	1.6	322 ± 10.26
B15	97	12	9	5	15.5	1	117.6 ± 4.5
B20	97.5	11	7.5	5	18	1.2	180.4 ± 5.9
*$\text{LSD}_{0.05}$	0.0	0.0	0.0	0.0	0.0	0.0	–

*Least significant difference (LSD) at 5 % level

Table 2 Effect of nanosilica on growth parameters in maize after 40 days of growth

Growth characteristics	Root (nos.)	Root length (cm)	Shoots (nos.)	Stem height (cm)	Stem diameter (cm)	Leaf area (cm^2 plant^{-1})
Control	20	18	7	61	1.9	164.3 ± 5.4
N5	22	15	7	74	2.4	294.8 ± 11.3
N10	18	18	11	82	2.9	368.8 ± 21.7
N15	20	12	12	81	3.2	360 ± 21.8
N20	16	20	12	79	3.3	375.1 ± 20.1
B15	10	12	7	62	0.9	199.2 ± 5.4
B20	4	12	11	65	2.4	290 ± 11.62
$\text{LSD}_{0.05}$	0.001	0.0	0.0	0.0	0.0	–

Table 3 Variations in total silica content and chlorophyll (*a* and *b*) content in maize leave samples amended with nanosilica

Sample	Silica content (μg mL^{-1})	Chlorophyll *a* (μg mL^{-1})	Chlorophyll *b* (μg mL^{-1})	Chlorophyll *a* to *b* ratio
Control	2.00 ± 0.52	0.017	0.01	1.647
N5	3.00 ± 0.41	0.01	0.007	1.581
N10	5.75^{b} ± 0.83	0.024	0.01	2.311
N15	17.83^{a} ± 1.29	0.045	0.043	1.049
N20	12.55^{a} ± 1.04	0.047^{a}	0.042	1.121
B15	2.50 ± 0.61	0.012	0.007	1.598
B20	1.85 ± 0.57	0.03	0.02	1.493
$\text{LSD}_{0.05}$		0.006	0.003	0.0

Values within a column followed by single letters (a, b) show significant varietal difference by Duncan's test and least significant difference (LSD) at 5 % level

Dry weight, protein, and phenol determination

Changes in total maize dry weight percentage (%) and essential plant biochemical components such as content of protein and total phenols at 20-day-old maize roots and leaves are presented in Table 4. Root wt% is found to be high at N15 (24.29 ± 0.4 %) and low at N20 in maize than bulk silica (5.67 %) and control (7.73 %). The higher dry weight percentage in roots reflects the increased accumulation of silica in leaf bundle sheath. Influence of silica regimes on the content of total protein and phenol of all the treatment samples are shown in Table 4. Protein content is linearly accelerated according to the concentrations of nanosilica especially at N15 (29.08 mg g^{-1}) with negligible differences in total quantity among all

Table 4 Influence of nanosilica on dry weight percentage and contents of total protein and phenol in maize roots and shoots

Sample	Dry weight (%)		Protein content (mg g^{-1})	Phenol content (µg mL^{-1})
	Root	Shoot	Shoot	Shoot
Control	$7.73^{bc} \pm 0.04$	$5.05^{a} \pm 0.03$	25.28	0.364
N5	$4.4^{a} \pm 0.13$	$6.61^{a} \pm 0.18$	27.31	0.192
N10	$8.77^{bc} \pm 0.05$	$33.96^{ab} \pm 1.42$	27.53	0.202
N15	$24.29^{ab} \pm 0.4$	$21.52^{ab} \pm 0.27$	29.08	0.06
N20	$5.89^{a} \pm 0.01$	$27.68^{ab} \pm 0.34$	27.09	0.422
B15	$5.67^{a} \pm 0.11$	$4.78^{a} \pm 0.29$	26.77	0.323
B20	$11.67^{ab} \pm 0.35$	$9.49^{ab} \pm 0.38$	27.08	0.43
$LSD_{0.05}$	–	–	0.0	0.05

Values within a column followed by combining the letters (ab, bc) show significant varietal difference and single letters indicates non significance by Duncan's test and least significant difference (LSD) at 5 % level

Table 5 Elemental compositions of leaf ash samples treated with nanosilica

Analyte (%)	Control	N5	N10	N15	N20	B15	B20
K_2O	90.35	87.10	87.35	77.39	89.05	91.35	91.01
SiO_2	4.93	8.42	8.02	19.18	7.66	4.39	1.30
Fe_2O_3	0.28	0.49	0.31	0.31	0.43	0.16	0.30
SO_3	0.22	0.48	0.50	0.31	0.48	0.13	0.46
MnO	0.13	0.30	0.20	0.22	0.19	0.10	0.17
CuO	0.05	0.11	0.08	0.06	0.09	0.03	0.07
P_2O_5	4.01	3.01	3.52	2.49	2.05	3.34	5.68
ZnO	0.03	0.02	0.04	0.04	0.05	0.02	0.04

treatments. Total phenol content of 20-days-old maize leaves are drastically reduced at N15 (0.06 µg mL^{-1}) even though irregular modulations occur with other regimes of silica treatments especially higher concentration at B20 (0.43 µg mL^{-1}).

Nutrient variations

Elemental studies on dry leaf ash of maize reflect the nutritional alleviation exerted by nanosilica in comparison with bulk silica sources (Table 5). SiO_2 content of the leaves also varies with the increasing silica regimes. SiO_2 accumulation in plant is not significant with the concentration including control samples except at N15 where SiO_2 content is 19.18 % (Table 5). An FTIR spectrum of leaf ashes for the occurrence of corresponding silica functional groups (Si–O–Si, Si–OH, and Si–O, respectively, at 1,087, 693, and 463 cm^{-1}) are confirmed in all the samples with varying intensity (Fig. 3).

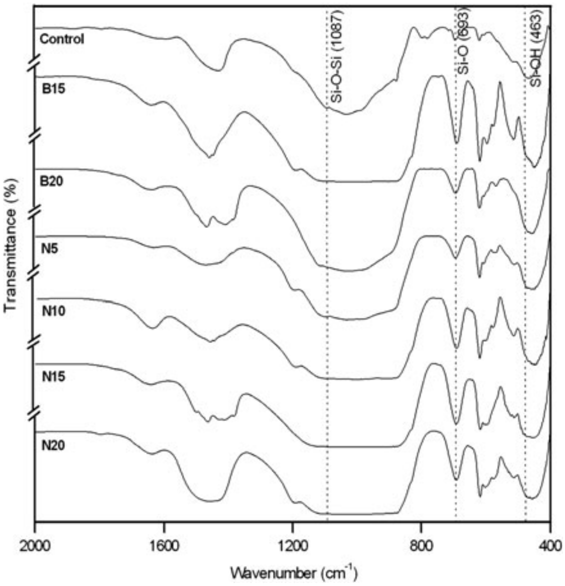


Fig. 3 Occurrence of silica functional groups in terms of transmittance of treated maize leaf samples

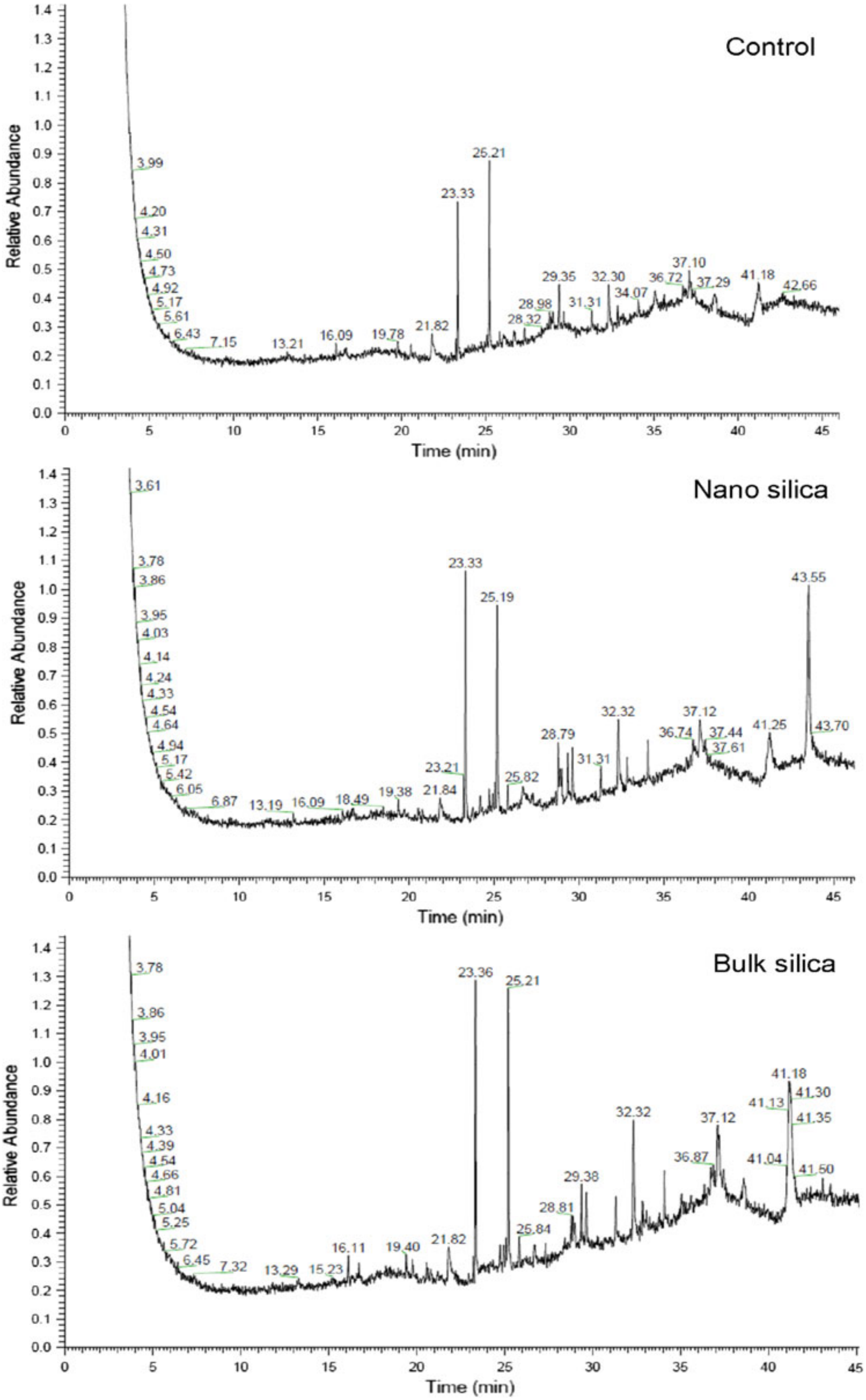


Fig. 4 GC–MS chromatogram of maize leaf extract with respect to silica treatments

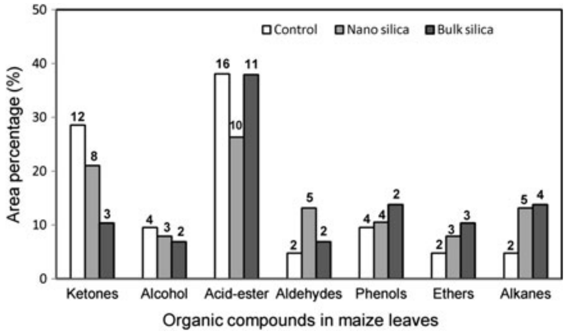


Fig. 5 Total area percentage of organic compounds in maize leaf extract of silica treatments

Variations in organic compounds

The modulations in total organic compounds according to the treatments of nanosilica and bulk silica are elucidated from GC–MS results. A relative abundance of volatile compounds in maize leaf extract with respect to the retention time is presented in chromatograph (Fig. 4). Area percentage (%) of aldehydes, ketones, alkanes, alcohols, acid–esters, and ethers of the bulk and nanosilica-treated leave samples is evaluated using Wiley library 9 and their abundance is plotted in Fig. 5. Specific stress-tolerant mechanisms in the plant are reflected by the abundance of the phenolic compounds in the leaf extract.

Si accumulation in leaves and roots

HR-SEM results exhibited the accumulation pattern of siliceous compounds in leaves and EDXS results reveal the total elemental composition of tested leaves (Fig. 6). The elemental variations of leaves are shown as a table inset in appropriate EDAX images. The third leaf of the maize plants is screened for the variations in the quantity of silica deposition. Interestingly, SEM images at N10 and N15 show wider deposition of silica, which is confirmed through the reflection of silica and corresponding balance in total elemental composition (Fig. 6c, d). From the histological studies of maize roots amended with nano and micro silica treatments (Fig. 7), number of epidermis cells and cell wall extensibility in roots are not influenced with nanosilica treatments. But the cell walls are found to be thick and silica bodies appeared as thick spots (indicated in arrow marks) in maize roots amended with SNPs which shows higher Si accumulation compared to bulk and control.

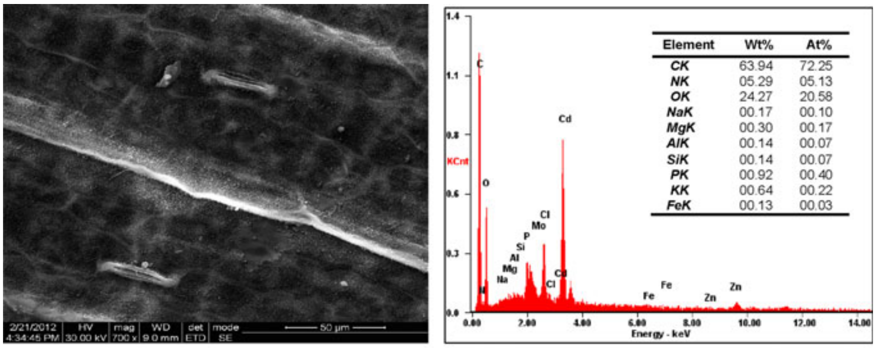
Discussion

Characterization results show that high surface, amorphous nature of silica nanoparticles with porous structure can be proposed as an alternative to bulk silica sources for agricultural applications. Thus, maize plots amended with different concentration of nanosilica and bulk silica are assessed for their discriminations in growth and physiological components. The effect of nanoscale silica particles on the growth and biochemical changes in different concentrations are necessary to evaluate the choice of optimal concentration for soil application.

Experimental maize plots containing four regimes of nanosilica and bulk silica, including control are analyzed for both morphological and physiological discriminations as a function of growth characteristics, plant metabolic compounds, nutrient content, and silica accumulation. Germination percentage of seeds is not affected by any of the plots tested and non-significant differences during germination among the samples are observed. The gradual increase in growth parameters with respect to nanosilica regime provides enlarged leaf area that promotes photosynthetic activity. However, number of shoots and roots do not show considerable variations. After 20 days, plant growth stage starts to stationary phase and hence, role of silica are not influenced at this stage. As silicon is being a nutrient anomaly (Epstein 1999), nanoscale silica is also not involved to contribute nutritive value in enhancing growth parameters rather than conferring physical strength. Similarly, the effect of nano Si on growth characteristics of tomato was studied under salinity levels and has delivered promising results in salt tolerance (Haghighi et al. 2012).

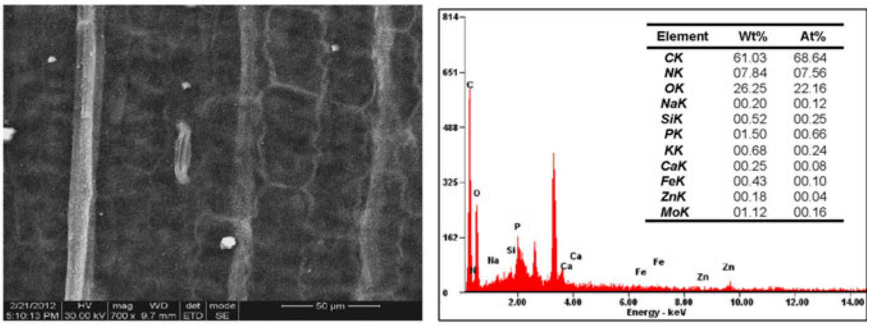
The Si in the plant tissue can be solubilized and indirectly measured in the extracted solution. Increased silica content in nanosilica treatments among other sources reflects the uptake of silica sources from soil. However, the actual action of nano metal/metal oxides on chloroplasts and phyto compounds in plants is not well ascertained (Dimkpa et al. 2012; Mahmood et al. 2005; Kidd et al. 2001) but the nanosilica increases silicon accumulation and provides erected leaves which stimulate the factors that

Fig. 6 Distribution of silica in maize leaves shown in HR-SEM images coupled with EDXS. *Scale bar* 50 μm (×700)



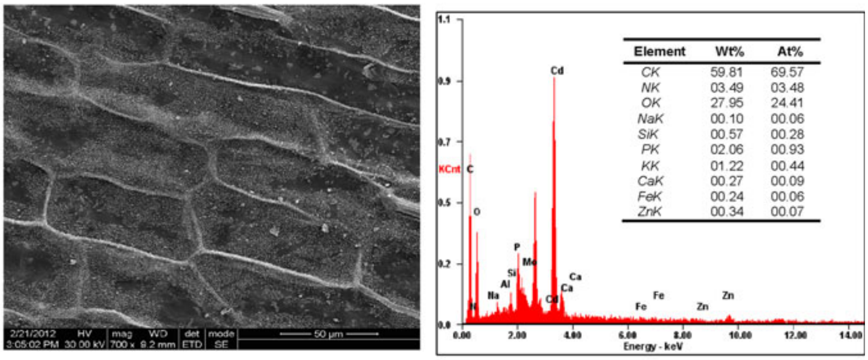
Element	Wt%	At%
CK	63.94	72.25
NK	05.29	05.13
OK	24.27	20.58
NaK	00.17	00.10
MgK	00.30	00.17
AlK	00.14	00.07
SiK	00.14	00.07
PK	00.92	00.40
KK	00.64	00.22
FeK	00.13	00.03

(a) Control



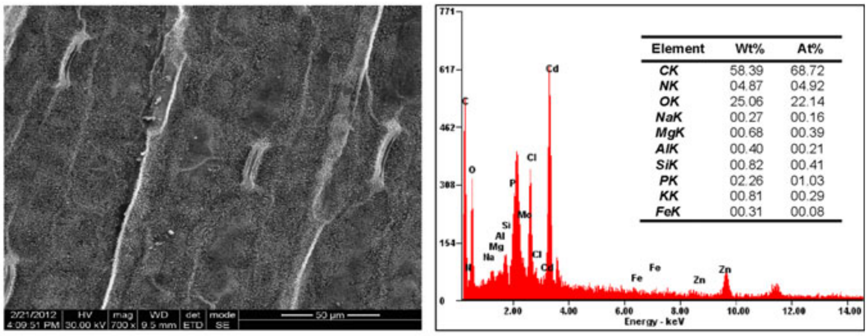
Element	Wt%	At%
CK	61.03	68.64
NK	07.84	07.56
OK	26.25	22.16
NaK	00.20	00.12
SiK	00.52	00.25
PK	01.50	00.66
KK	00.68	00.24
CaK	00.25	00.08
FeK	00.43	00.10
ZnK	00.18	00.04
MoK	01.12	00.16

(b) N5



Element	Wt%	At%
CK	59.81	69.57
NK	03.49	03.48
OK	27.95	24.41
NaK	00.10	00.06
SiK	00.57	00.28
PK	02.06	00.93
KK	01.22	00.44
CaK	00.27	00.09
FeK	00.24	00.06
ZnK	00.34	00.07

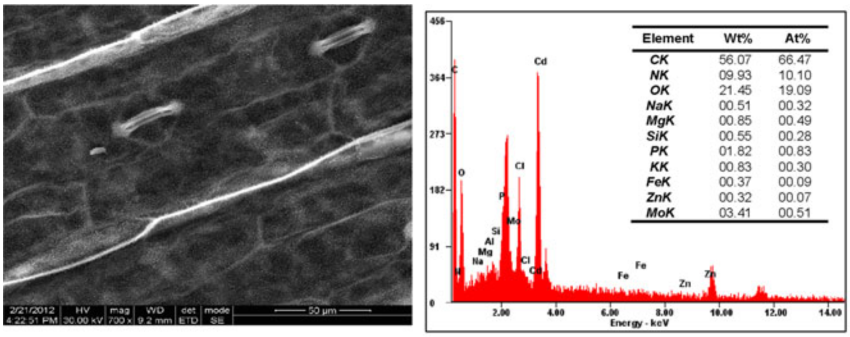
(c) N10



Element	Wt%	At%
CK	58.39	68.72
NK	04.87	04.92
OK	25.06	22.14
NaK	00.27	00.16
MgK	00.68	00.39
AlK	00.40	00.21
SiK	00.82	00.41
PK	02.26	01.03
KK	00.81	00.29
FeK	00.31	00.08

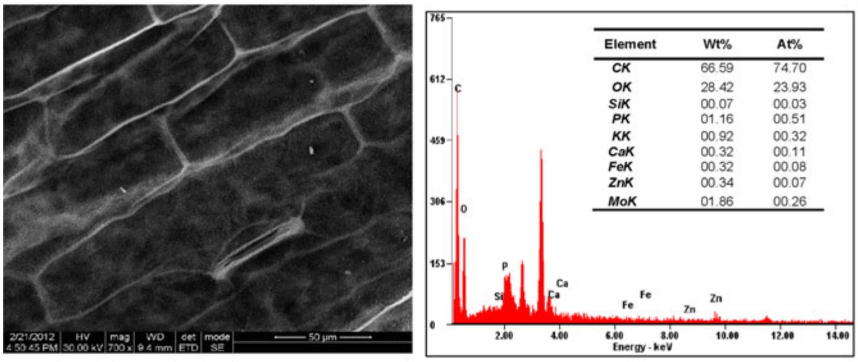
(d) N15

Fig. 6 continued



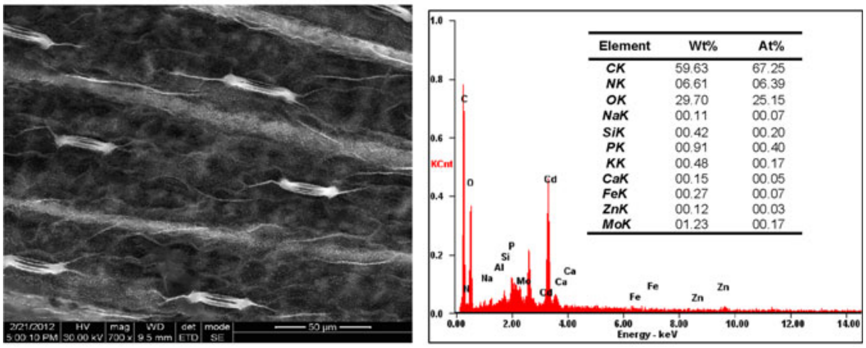
Element	Wt%	At%
CK	56.07	66.47
NK	09.93	10.10
OK	21.45	19.09
NaK	00.51	00.32
MgK	00.85	00.49
SiK	00.55	00.28
PK	01.82	00.83
KK	00.83	00.30
FeK	00.37	00.09
ZnK	00.32	00.07
MoK	03.41	00.51

(e) N20



Element	Wt%	At%
CK	66.59	74.70
OK	28.42	23.93
SiK	00.07	00.03
PK	01.16	00.51
KK	00.92	00.32
CaK	00.32	00.11
FeK	00.32	00.08
ZnK	00.34	00.07
MoK	01.86	00.26

(f) B15



Element	Wt%	At%
CK	59.63	67.25
NK	06.61	06.39
OK	29.70	25.15
NaK	00.11	00.07
SiK	00.42	00.20
PK	00.91	00.40
KK	00.48	00.17
CaK	00.15	00.05
FeK	00.27	00.07
ZnK	00.12	00.03
MoK	01.23	00.17

(g) B20

involved in the synthesis of chlorophyll at an optimal regimes of silica accumulation. The acceleration may be due to increased leaf area that renders better light absorption and photosynthetic activity of chlorophyll *a* and *b*. Shoot dry weight (27.68 ± 0.34 %) at N20 is less among the four concentrations of SNPs. In bulk treatment, there is a less dry weight (4.78 and 9.49 %). While comparing the total dry weight of shoots and roots, roots show lesser in dry wt%. This reduction is correlated with the observation of Hossain et al. (2002) that transition of excess SiO_2 to older leaves is also included in total shoot dry weight. Rather, it did not happen in root absorption.

The increased protein content with the regimes of nanosilica and bulk silica may be attributed to the existence of metabolic balance between induction of proteins such as cell wall transporters and damping off stress-responsive enzyme activities as a function of nanoscale fertilization in maize. Increased phenolic contents in low silica plants are correlated with previous results obtained on Si-deprived plants. The results reveal that the observed silicification may be

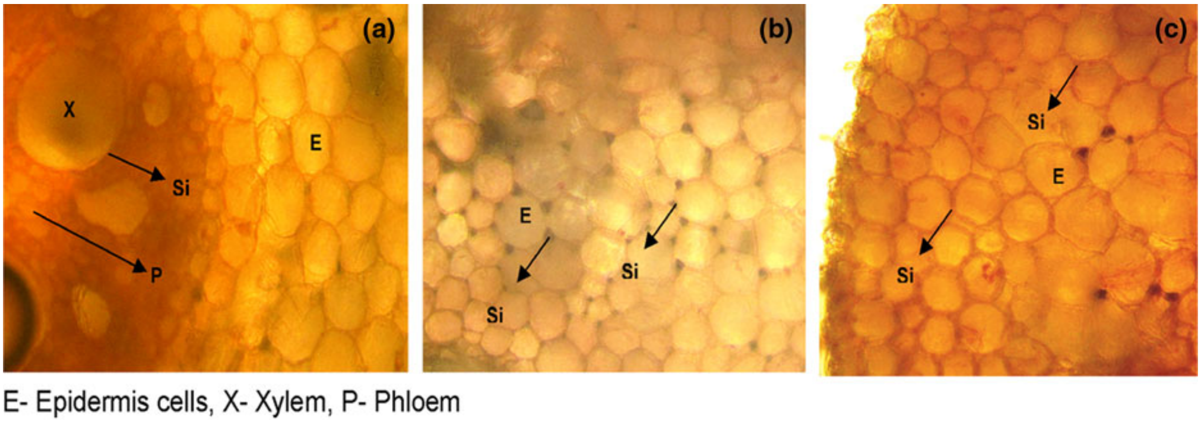


Fig. 7 Microphotograph of maize roots grown in soil amended with **a** control, **b** nanosilica, and **c** bulk silica

substituted by the production of structural phenolic compounds (Carver et al. 1998). In addition, low phenolic content at N15 (0.06 µg mL^{-1}) may reflect the induction of stress tolerance mechanism in maize.

X-ray fluorescence is a direct method of elemental analysis used on oven-dried plant matter. Alleviation of K and P contents has occurred due to increased SiO_2 deposition. Role of silica in ameliorating P and K contents are explained in earlier reports (Miao et al. 2010; Yang et al. 2008) and the same is enhanced by nanosilica than bulk silica. Hence, the accumulation of silica and other elements in maize shoots varies greatly. However, the extent of SiO_2 deposition in maize leaves is found to be maximum at N15. In bulk silica sources, the content of silica is the same as that of control sample (4.4 %). This may be due to increased particle size (micron size) which is unable to be utilized by plant. Other regimes of nanosilica expressed no significant differences among treatment that they greatly vary from that of bulk sources (1–4 %) as well as control. Therefore, maize Si uptake seems to be promoted at the concentration 15 kg ha^{-1} from soil. In bulk silica treatment, the occurrence of silica content during XRF and silica estimation is irregular i.e., high in B15 over B20 with an increasing concentration. These results justify that the corn variety is amenable to SiO_2 fertilization not above 15 kg ha^{-1} for the cultivation. An FTIR spectrum of treated samples implies the gradient of silica accumulation in plants to substantiate the uptake of nanoscale silica by plant. This infers that the occurrence of possible silica functional group is similar in all treatments.

Total organic compounds, such as phenols, aldehydes, ketones, etc., are found to be reduced in maize leaves grown in nanosilica amended soil. The expression of such compounds is responded more in leaf extract of control as well as in bulk silica. From Fig. 5, it is noticed that other organic compounds are not varied considerably. These results are correlated with the study on the protective mechanism of silicon in rice through phenols (Goto et al. 2003). The difference in the optimal usage of SiO_2 by species and organs is due to differences in the endogenous levels of Si in the plant and developmental stage (Hodson and Evans 1995). Hence, it is necessary to clarify the accumulation of silica in roots and leaves through microscopic studies.

The increased SiO_2 accumulation in nanosilica (N10 and N15) treatment helps to avoid water lodging of roots and leaves (Lux et al. 1999). Decrease in silica content during microscopic observation at N20 may be due to the transition of silica to the older leaf blades when abundant Si is absorbed (Hossain et al. 2002). This observation is also substantiated from total dry weight of plant as mentioned elsewhere in this investigation. In contrast to the observation under HRSEM-EDAX study, SiO_2 accumulation in bulk silica decreases with an increase in the concentration of Si content (Table 3, 5). This is may be due to the nature of samples i.e., the samples chosen for elemental composition and silica estimations are 20-day-old leaf ashes including both young and senescence leaves whereas the third leaf of maize is chosen for electron microscopic observation. Hence, the uptake of bulk silica is found to be less and irregular with an employed studies for silica content in this present investigation. Higher accumulation in maize roots is that Si is deposited primarily in the epidermal tissues of roots and leaves in the form of a silica bodies called phytoliths. Hence, nanosilica promotes the induced thickness of epidermal silicon–cellulose layer that supports the mechanical stability of plants thereby resisting lodging (Savant et al. 1999). Moreover, the reflectability of SEM images is in line with the optical microscopic studies. When nanosilica enhances the Si uptake into cells, the dry weight of cells also increases in parallel with the SiO_2 concentration. Thus, Si deposition is linked with the cell wall composition and it can enhance the thickening of the root epidermis than the control plants, which is in agreement with previous reports of bulk silica application studies (Hossain et al. 2002). In contrast, uptake of other nanomaterials like CNTs by root cells is not significant (Mondal et al. 2011). By investigating the responses of maize growth and physiological components to nanosilica regimes in comparison with bulk silica by appropriate methodologies, the concentration of 15 kg ha^{-1} shows an augmented silica accumulation and physiological characteristics.

Conclusions

Silica nanoparticles with high surface area synthesized by economic method are employed for the growth of maize as an alternative source for bulk silica fertilizers. Physiological transformations that are due to nanosilica fertilization considerably enhance the growth characteristics and expression of essential biochemical components in maize than bulk silica. N15 and N20 show the progressive growth characteristics and silica accumulation even though N20 has less phenol content. In fact, elemental analysis and dry weight studies confirm that the quantity of silica accumulation in maize leave samples is higher at N15 than any other treatments. Influence of nanosilica on the regulation of phyto compounds such as proteins and phenols also favored maize. The application of nanoscale fertilizers is found to be superior than bulk silica as soil amendment. Hence, there is a possibility that the nanosilica at 15 kg ha^{-1} may render the optimal regime for improved growth during field application. Promoted Si accumulation in maize roots is believed to contribute drought tolerance and so the present study helps to choose the optimal concentration of nanosilica application to maize crop for better yield.

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References

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Barrett EP, Joyner LG, Halenda PP (1951) The determination of pore volume and area distributions in porous substances. J Am Chem Soc 73:373–380

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72: 248–254

Carver TLW, Robbins MP, Thomas BJ, Troth K, Raistick N, Zeyen RJ (1998) Silicon deprivation enhances localized autofluorescent responses and phenylalanine ammonia-lyase activity in at attacked by *Blumeria graminis*. Physiol Mol Plant Pathol 52:245–257

Debnath N, Das S, Seth D, Chandra R, Bhattacharya SC, Goswami A (2011) Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). J Pest Sci 84:99–105

Dimkpa CO, McLean JE, Latta DE, Manangon E, Britt DW, Johnson WP, Boyanov MI, Anderson AJ (2012) CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. J Nanopart Res 14(9):1125

Epstein E (1999) Silicon. Ann Rev Plant Physiol Plant Mol Biol 50:641–664

Gao X, Zou C, Wang L, Zhang F (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. J Plant Nutr 29:1637–1647

Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnol Adv 29:792–803

Goto M, Ehara H, Karita S, Takabe K, Ogawa N, Yamada Y, Ogawa S, Yahaya MS, Morita O (2003) Protective effect of silicon on phenolic biosynthesis and ultraviolet spectral stress in rice crop. Plant Sci 164:349–356

Haghighi M, Afifipour Z, Mozafarian M (2012) The effect of N–Si on tomato seed germination under salinity levels. J Biol Environ Sci 6:87–90

Hodson MJ, Evans DE (1995) Aluminium/silicon interactions in higher plants. J Exp Bot 46:161–171

Hossain MT, Mori R, Soga K, Wakabayashi K, Kamisaka S, Fujii S, Yamamoto R, Hoson T (2002) Growth promotion and an increase in cell wall extensibility by silicon in rice and some other Poaceae seedlings. J Plant Res 115:23–27

Kalapathy U, Proctor A, Shultz J (2000) A simple method for production of pure silica from rice hull ash. Bioresour Technol 73:257–262

Kaya C, Tuna L, Higgs D (2006) Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. J Plant Nutr 29:1469–1480

Kidd PS, Llugany M, Poschenrieder C, Gunse B, Barcelo J (2001) The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). J Exp Bot 52:1339–1352

Kulikova ZL, Lux A (2010) Silicon influence on maize, *Zea mays* L. hybrids exposed to cadmium treatment. Bull Environ Contam Toxicol 85:243–250

Liang YC, Sun WC, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollut 147:422–428

Lux A, Luxova M, Morita S, Abe J, Inanaga S (1999) Endodermal silicification in developing seminal roots of lowland and upland cultivars of rice *Oryza sativa* L. Can J Bot 77:955–960

Mahmood S, Hussain A, Saeed Z, Athar M (2005) Germination and seedling growth of corn (*Zea mays L.*) under varying levels of copper and zinc. Int J Environ Sci Technol 2(3):269–274

Matichenkov VV, Bocharnikova EA (2001) The relationship between silicon and soil physical and chemical properties. In: Datnoff LE, Snyder GH, Korndorfer H (eds) Silicon in agriculture. Elsevier, Amsterdam, pp 209–219

Matichenkov VV, Calvert DV, Snyder GH (1999) Silicon fertilizers for citrus in florida. Proc Fla State Hortic Soc 112:5–8

Miao BH, Han XG, Zhang WH (2010) The ameliorative effect of silicon on soybean seedlings grown in potassium-deficient medium. Ann Bot 105:967–973

Mitani N, Ma JF (2005) Uptake system of silicon in different plant species. J Exp Bot 56:1255–1261

Mondal A, Basu R, Das S, Nandy P (2011) Beneficial role of carbon nanotubes on mustard plant growth: an agricultural prospect. J Nanopart Res 13(10):4519–4528

Monica RC, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62:161–165

Rafi MM, Epstein E, Falk RH (1997) Silicon deprivation causes abnormalities in wheat (*Triticum aestivum* L.). J Plant Physiol 152:497–501

Ranganathan S, Suvarchala V, Rajesh YBRD, Srinivasa Prasad M, Padmakumari AP, Voleti SR (2006) Effects of silicon sources on its deposition, chlorophyll content, and disease and pest resistance in rice. Biol Plant 50(4):713–716

Savant NK, Snyder GH, Datnoff LE (1999) Silicon management and sustainable rice production. Adv Agron 58:151–199

Suriyaprabha R, Karunakaran G, Yuvakkumar R, Rajendran V, Kannan N (2012) Silica nanoparticles for increased silica availability in maize (*Zea mays*. L) seeds under hydroponic conditions. Curr Nanosci 8(6):1–7

Wakabayashi K, Hoson T, Kamisaka S (1997) Osmotic stress suppresses the cell wall stiffening and the increase in cell wall-bound ferulic and diferulic acids in wheat coleoptiles. Plant Physiol 113:967–973

Wei-min D, Ke-qin Z, Bin-wu D, Cheng-xiao S, Kang-le Z, Run C, Jie-yun Z (2005) Rapid determination of silicon content in rice. Rice Sci 12:145–147

Yang Y, Li J, Shi H, Ke Y, Yuan J, Tang Z (2008) Alleviation of silicon on low-P stressed maize (*Zea mays* L.) seedlings under hydroponic culture conditions. World J Agric Sci 4:168–172

Yuvakkumar R, Elango V, Rajendran V, Kannan N, Prabu P (2011) Influence of nanosilica powder on the growth of maize crop (*Zea mays* L.). Int J Green Nanotechnol 3:180–190