J. Plant Production, Mansoura Univ., Vol. 8(9):913 -916, 2017

Effect of Silicon Dioxide Nanoparticles on Growth Improvement of Banana Shoots *In Vitro* within Rooting Stage

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ABSTRACT

Silicon (Si) is considered as one of the most vital elements for the plant life. Nanoparticles interact with plants causing many changes morphologically and physiologically based on the properties of NPs. Nano silicon dioxide (with diameter 20:40 nm) was synthesized from sodium metasilicate and inclusively characterized. Murashige and Skoog media were supplemented with nano SiO_2 in four levels (0, 50,100 and 150 ppm) in addition to IBA in order to find the interaction mechanism between nanosilica and banana plants under control condition *in vitro*. The efficiency of Nano SiO_2 was evaluated in terms of its impact on some morphological traits, total chlorophyll content and total phenolic compounds were also assessed. Results showed a positive effect of silicon dioxide nanoparticles on rooting rate and photosynthesis pigments. Overall, application of Silicon in the nano scale was beneficial in improving growth of banana.

Keywords: *Musa* sp, growth, nanoparticles, silicon dioxide

INTRODUCTION

Bananas (*Musa* spp., family: Musaceae) are rhizomatous perennial monocot giant herbs that originated in the tropical lowlands of Southeast Asia. The world produces annually about 106.7 million tons of bananas (FAO, 2013-14). Vegetative suckers cultivation is considered the traditional method for banana propagation. *In vitro* micropropagation of banana developed for the last thirty years and now is considered well established(Asmare *et al.*, 2012). Moreover, propagation for large scale using tissue culture propagation of banana using shoot tip cultures have been established (Saraswathi *et al*., 2016 and Ghag & Ganapathi, 2017)

Silicon (Si) does not put under the category of essential elements of higher plants, and its essentiality has not been proven yet. It regarded as one of the most beneficial elements for the plant life(Karimi & Mohsenzadeh, 2016). Several studies reported that it is important for various plant species such as wheat, rice, maize and bamboo (Imtiaz *et al.*, 2016).

It was found that Si application improved growth and yield through improving plant water status, modification of ultrastructure of leaf organelles, activation of plant defense systems and mitigation of free radicles (Parveen & Ashraf, 2010). SiO_2 nanoparticles (Nano-SiO_2) are single particles of silica dioxide, an inorganic metal oxide, with a diameter less than 100 nm.

The reasons stand behind the more raising attention using silicon is because of its beneficial impacts on growth and development of plant, especially these plants grown under unfavorable conditions. Due to, its role correlated with plant nutrition remains less focused, the purpose of this research was study the effect of Silicon in nano scale on banana explants development, photosynthesis process and phenols content.

MATERIALS AND METHODS

Silicon Dioxide Nanoparticles synthesis

The method used in this study was according to that described by Essien *et al*. (2011) with some modification. The reagent chemicals used for synthesizing the porous silica network was "sodium meta silicate", Na_2SiO_3 and HCl (37 %). Na_2SiO_3 (5g) was dissolved in deionized water (10 ml) and 2 M HCl (26 ml) was added drop wise under stirring using a magnetic stirrer until a gel was formed at 50°C. The gel prepared above was immediately poured into deionized water and washed successively to remove the NaCl formed during the hydrolysis stage of the reaction. No precipitate was found when the liquid from the last filtration was tested using dilute $AgNO_3$ solution to ascertain the complete removal of NaCl from the gel network.

Thermal Treatment After washing,

The formed gel was put in an oven to dry at 120°C for 24 hrs. then calcined at 600°C for other 3 hours in a furnace and thereafter milled to form powders.

Methods of Characterization

The morphology of the material and particle sizes was determined using scanning electron microscopy (SEM, EVO/MAIO).

Plant materials:

The experiment was conducted at Seed Pathology and Plant Tissue Culture, The Central Laboratory of faculty of Agriculture, Mansoura University, Egypt. Sword suckers were brought from free virus and true to type banana farm "Musa sp" var. Grand Nain. Explants were cut to 3x5 cm and surface sterilized then inoculated in Murashige and Skoog (MS) media containing BAP 3 mg/l. Explants were transferred to multiplication media for two subculturing, 3 cm long shoots were detached from clumps and transferred to the rooting medium.

Rooting media and culture condition:

The explants were cultured on Murashige and Skoog half strength ($MS/_2$) media supplemented with 0.01% Activated charcoal and 3.0% sucrose and 1.0 mg/l IBA in all treatments in addition to the tested materials. Nano SiO_2 was used in four concentrations 0, 50,100,150 mg/l with sonication prior media pouring.

Vegetative parameters

After four weeks, roots number, length, plantlet height, leaves number and fresh and dry weight for both of shoots and roots were measured.

Chemical measurements

Total chlorophyll.

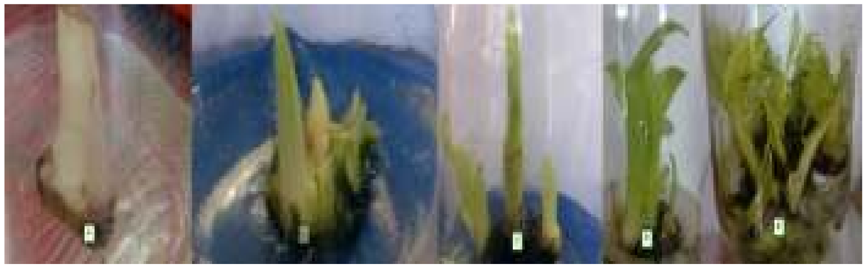
Total chlorophyll was estimated by hand-held chlorophyll meter. Chlorophyll concentration meter makes concentration measurements directly in the leaves. The concentration of total chlorophyll is expressed as Chlorophyll Content Index "CCI ".

Total phenol compounds.

Total phenols amount in the plant tissues was determined as the method proposed by Marfil *et al*. (2011).

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) by using "Genstat 11.1" computer program (2008). The mean comparisons were performed by the least significant difference value (LSD) at 5% level of probability according to the method described by Gomez and Gomez (1984).



A. Establishment of aseptic cultures. B,C,D. Shoot tips growth and development. E. Multiplication stage.

Figure 1. Photo showing development stages of banana explants "Musa sp" Grand nain variety before treatments application described in the method.

RESULTS AND DISCUSSION

Silicon Dioxide Nanoparticles

The synthesized nanosilica is characterized for its morphology and functional groups. It is confirmed as nanometer in size with reference to studies of (Hodson and Evans 1995 and Essien *et al*., 2011). The extracted white hydrous silica precipitate from sodium metasilicate was formed at high temperature. The prepared silica particles are structurally and morphologically characterized for its purity, functional groups, and particle. From SEM results (Fig. 2), the silica nanoparticles are found as aggregates with spherical morphology, it can be concluded that the particle size of silica particles are in the range of 20–40 nm with spherical morphology (Fig.2)

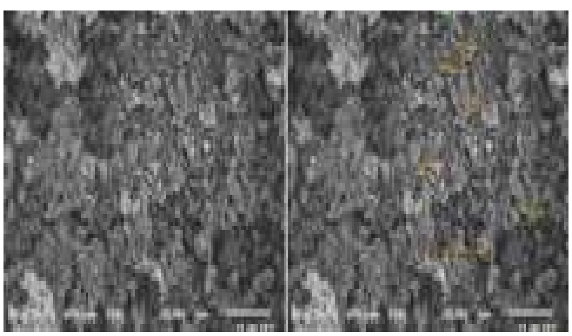


Figure 2. SEM micrograph of the silica powders showing particle distribution and sizes measured at several locations

Table 1. Root parameters in banana shoots treated with SiO_2 nanoparticles after 30 days (average of roots to one shoot)

Treatments	Roots number	Roots length (cm)	Fresh weight (g)	Dry weight (g)
Control	5.67	4.67	0.152	0.0014
SiO_2 at 50 ppm	8.33	12.50	0.242	0.0234
SiO_2 at 100 ppm	9.33	13.17	0.279	0.0250
SiO_2 at 150 ppm	10.00	14.33	0.355	0.0250
LSD 5%	1.33	1.82	0.0096	0.00415

Table 2. Plantlet parameters in banana treated with SiO^2 nanoparticles after 30 days (average of one shoot)

Treatments	Leaves number	plantlet Height (cm)	Fresh weight (g)	Dry weight (g)
Control	3.67	4.17	1.84	0.083
SiO_2 at 50 ppm	5.33	5.83	2.93	0.193
SiO_2 at 100 ppm	6.33	6.67	3.47	0.183
SiO_2 at 150 ppm	7.00	7.50	4.52	0.200
LSD 5%	0.94	0.67	0.44	0.0402

Effect of SiO_2-NPs treatments on root morphology

Changes in banana growth parameters such as number of roots, Root length, leaves number, plantlet height, fresh and dry weight for both of roots and plantlet after 30 days at varying concentrations of nanosilica are presented in Tables 1 and 2 Fig. 3. It is inferred from the observed results that the growth parameters are enhanced in banana with the increasing concentration of SiO_2-NPS.

Results of the present study indicated the positive effect on inducing adventitious roots characteristics on banana due to SiO_2-NPS exposure. Lateral roots number and root length increased obviously in SiO_2-NPS at 150 ppm of (9.33) with 15 length.

Plantlet height and Leaves number are presented in Table 2. Exposure of banana plants to SiO_2-NPs significantly increased the plant height by 7.50 at 150 ppm. In the same line, leaves number increased to (6.67 and 7) leaves in treated plants while, the control shoots formed (3.67) leaves. Studies of Yuvakkumar *et al.* (2011) showed that nano silicon increased growth factors in maize crop. The gradual increase in growth parameters with respect to nanosilica regime provides enlarged leaf area that promotes photosynthetic activity.

Results showed that fresh and dry weights of banana at 50, 100 and 150 mg/L concentrations of SiO_2 nanoparticles had significant higher values compared with the control. In the case of roots biomass, SiO_2 –NPs at 150 ppm treatment recorded the highest values of both fresh and dry weight (0.355 and 0.025 g). In the same line, Plantlet fresh and dry weight recorded the highest values

(4.52 and 0.200). Increasing in root weight proved that silicon oxide nanoparticles facilitated water uptake and its transportation into plant. These beneficial effects of silicon oxide might be associated to its hydrophobicity. The obtained results agreed with these of (Karimi & Mohsenzadeh, 2016) on wheat seedlings, who found significant increase in weight and growth. The higher dry weight percentage in plantlets and roots reflects the increased accumulation of silica in leaf bundle sheath(Suriyaprabha *et al*., 2012). 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).

Table 3. Contents of total chlorophylls, and total Phenols in *Musa* sp shoots treated with SiO_2 nanoparticles after 30 days

Treatments	Chlorophyll Content Index "CCI"	T Phenols µg/g tissue
Control	9.57	67.6
SiO_2 at 50 ppm	15.67	107.3
SiO_2 at 100 ppm	16.83	51.1
SiO_2 at 150 ppm	22.57	34.1
LSD 5%	2.37	8.5

Total chlorophyll content:

Throughout the experiment, control banana plants had lower chlorophyll contents than those under SiO_2-NPs treatments, regardless of Si application (Table 3). Chlorophyll levels of plants increased gradually in the three levels of SiO_2-NPs. After 30 days of culturing on rooting media, total chlorophyll contents were 15.76, 16.83 and 22.57 CCI respectively, higher than in plants under control treatment without Si.

The higher production of chlorophylls in plants grown in the presence of silicon is in agreement with the results of Yao *et al*. (2011). Chlorophylls accumulate mainly in the palisade parenchyma and are the major molecules involved in photosynthesis and the amount of chlorophyll present in leaf tissues, and may be related to photosynthetic capacity. In the same line, Bao-shan *et al*. (2004) tested the nano-SiO_2 particles on Changbai larch (Larix olgensis) seedlings as exogenous application and found that SiO_2-NPs improved growth and seedling quality, including average of seedling height, root collar diameter, main root length, and lateral roots number of seedlings and also enhance the synthesis of chlorophyll .

Total phenol contents:

Total phenol contents in treated and untreated banana shoots are shown in Table3. Results show that total phenol content of leaf decreased at 100 and 150 mg/L concentrations, and increased at 50 mg/L concentrations compared with the control. Phenolic compounds have antioxidant activity that refers to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation ability(Huda-Faujan, *et al*., 2009). Therefore it can be concluded that low concentrations of SiO_2 nanoparticles (50 mg/l) is represent stress to plants, in contrary high concentrations of SiO_2 nanoparticles (100 and 150 mg/l) partly improve plant resistance to stress.

Total phenols are found to be reduced in banana leaves grown in media supplemented with nano silicon. The expression of such compounds is responded more in leaf extract as well as control treatment. These results are related to the study on the defense mechanism in rice through phenols that induced by silicon. (Suriyaprabha *et al*., 2012).

Silicon plays an important role in improvement of the activities of ROS scavenging enzymes in chloroplasts, such as superoxide mutase enzyme(SOD) and enzymes produced in the ascorbate–glutathione pathway (Cao *et al*., 2015).

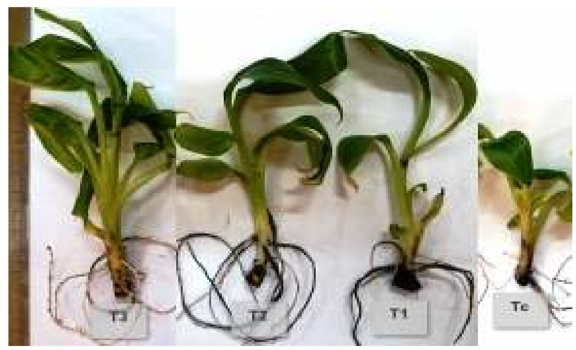


Figure 3. Photo showing the effect of nano silicon dioxide on banana "Musa sp" in the rooting stage under the treatments described in the method.

CONCLUSION

Generally, the role of nano silicon in plant biology is not well developed and the attempts to associate Si with metabolic or physiological activities have been inconclusive. In the current study, it has been demonstrated that SiO_2-NPs can increase plant growth; that may be explained by improvement of photosynthesis rate. Results of this study can be used as a reference for the positive role of SiO_2-NPs on plants growth. Surely, more studies using SiO_2-NPs in a longer period can contribute to more accurate results for the discovered mechanisms of woody plants in response to SiO_2-NPs and finding of the molecular approach mechanism, especially about antioxidant enzymes is necessary.

ACKNOWLEDGEMENT

The authers would like to express special thanks to Prof. dr. Yasser Shabana, Professor of Plant pathology, Mansoura University, for providing most facilities that made this work exist.

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تأثير الجسيمات النانوية لثاني أكسيد السيليكون على تحسين نمو نباتات الموز معمليا ضمن مرحلة التجذير

محمود ابراهيم القاضى ، محمد صلاح سيف البرعي ، أمير محمد ناجي شعلان و لمياء محمود محمد محمود
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أجريت الدراسة بمعمل أمراض البذور و زراعة الأنسجة – المعمل المركزي بكلية الزراعة جامعة المنصورة على نباتات الموز صنف جراندنين الناتجة من زراعة كورمات جمعت من أشجار موز سليمة ظاهريا خالية من الإصابات الفيرسية إنتاج أول عام فى مزرعة خاصة فى مدينة ميت غمر محافظة الدقهلية. تم تجهيز جزيئات تاني أكسيد السليكون في المعمل بإستخدام ميتا سليكات الصوديم وتم الكشف عن حجم الجزيئات بإستخدام الميكرسكوب الإلكتروني وكان حجم الجزيئات من 40:20 نانومتر. تمت التجربة بنقل نباتات الموز الناتجة من مرحلة التضاعف إلي بيئة تجذير مزودة بجزيئات ثاني أكسيد السليكون النانونية بأربع مستويات (150,100,50,0 جزء في المليون) وذلك لمدة أربع أسابيع وذلك بهدف دراسة التفاعل بين جزيئات ثاني أكسيد السليكون وتطور نمو نباتات الموزتحت ظروف متحكم فيها معمليا. وتم تقييم كفاءة جزئيات النانو سليكون من حيث تأثيره على بعض الصفات المورفولوجية، و محتوى الكلوروفيل و المركبات الفينولية الكلية. وأظهرت النتائج تأثير إيجابي من جزيئات ثاني أكسيد السيليكون على معدل التجذير وصفات النبيتات الناتجة وكذلك نسبة الكلوروفيل الكلية.

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