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Interaction of SiO_2 nanoparticles with seed prechilling on germination and early seedling growth of tall wheatgrass (*Agropyron elongatum* L.)

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The effect of six SiO_2 nanosized concentrations (0, 5, 20, 40, 60 and 80 mg L^{-1}) and three seed prechilling treatments (control, seed prechilling before nano SiO_2 treatments, treatments of seed with nano SiO_2 before prechilling) on germination and seedling growth of tall wheatgrass (*Agropyron elongatum* L.) were studied. Results indicated that application of SiO_2 nanoparticles significantly increased seed germination of tall wheatgrass from 58 percent in control group to 86.3 and 85.7 percent in 40 and 60 mg L^{-1}, respectively. Applying SiO_2 nanoparticles increased dry weight of shoot, root and seedling of tall wheatgrass. Increasing concentration of nanoparticle from 0 up to 40 mg L^{-1} increased seedling weight around 49 percent compared to the control, nevertheless decreased under 60 and 80 mg L^{-1} treatments. In conclusion, seed prechilling in combination with SiO_2 nanoparticles largely broke the seed dormancy for *A. elongatum*.

Keywords: seed treatment, seed prechilling, germination rate, nanoparticle.

INTRODUCTION

Efficient seed germination and early seedling establishment are important for increasing forage production in rangeland. Rapid and homogeneous seedling emergence leads to successful establishment, as it produces a deep root system before the upper layers of soil dry out, harden, or pose to adverse temperatures[1]. However, seeds after dry storage often display slow and non-uniform germination due to compromised vigor, especially when stored inappropriately. Moreover, germinating seeds and young seedlings are susceptible to dehydration stress due, in part, to the progressive loss of desiccation tolerance upon seed hydration[2].

Recently, some chemical substances were extremely used for improvement of seed germination and breakdown of seed dormancy in plants. Applications of some nanomaterials can help faster plant germination/production, effective plant protection with reduced environmental impact as compared with the traditional methods[3]. Silicon is a critical element for a number of metabolic and physiological plant activities. Application of silicon fertilizers in silicon-deficient soil can encourage plant growth, improve plant resistance to disease, cold and heavy metals such as manganese, iron, aluminum and copper, and consequently enhance photosynthesis[4, 5]. Silicon fertilization promotes the absorption of potassium and restricts the absorption of sodium, which therefore increases potassium/sodium selection ratio, helps the accumulation of potassium, nitrogen and sulphur in plants, and improves plant nutrition. In addition to the impact of Si on plant protection, various other beneficial effects of Si have been reported, such as amelioration of the adverse effects of Al and Mn toxicity to plants, improvement of water use efficiency[4, 5], as well as enhancement of the salt tolerance. Silicon facilitates water uptake and its transportation into the plant leaves. Beneficial effects of silicon might be related to hydrophilic nature of silicon[6]. It was reported that Si enhanced the stability of lipids in cell membranes of rice plants exposed to drought and heat stresses, suggesting that Si prevented the structural and functional deterioration of cell membranes when rice plants were exposed to environmental stress[7].

The plant growth hormones, such as gibberellins, jasmonic acid (JA) and salicylic acid (SA) play a favorable role in the growth and development of plant. Gibberellins (GAs) influence stem elongation, flower and fruit development and seed germination[8]. Hamayun et al.[9] showed that bioactive GA1 and GA4 contents of soybean leaves increased, when Si was added to control or salt stressed plants. Lin et al.[10] demonstrated that treatment by 500 $\mu L\ L^{-1}$ nanostructured silicon dioxide produced the best result, for which the mean height, root collar diameter, main root length and the number of lateral roots of Changbai larch (*Larix olgensis*) seedlings were increased.

Tahir et al.[11] reported that silicon application significantly increased wheat biomass at both control as well as under saline conditions. Lu et al.[12] shown that a combination of nanosized SiO_2 and TiO_2 could increase nitrate reductase enzyme in soybean (*Glycine max*), increase its abilities in absorbing and utilizing water and fertilizer, encourage its antioxidant system, and actually hasten its germination and growth. Zheng et al.[13] confirmed that nanosized TiO_2 helped water absorption in spinach seeds and consequently accelerated seed germination.

Tall wheatgrass (*Agropyron elongatum* L.) is a non-native perennial bunchgrass. It is extensively used for rangeland rehabilitation on light-textured soils of both shrub lands and grasslands[14]. Previous researchers have shown that seed priming techniques may enhance seed germination speed via inducing uniformity several biochemical changes in the seed. These changes are required to start the germination process such as dormancy breaking, hydrolysis or mobilization of inhibitors, imbibitions and enzyme activation[15]. This research was designed to examine possible beneficial stimulatory effects of nanosized SiO_2 concentrations on seed germination and seedling growth of tall wheatgrass.

MATERIALS AND METHODS

Description of Materials

Tall wheatgrass (*Agropyron elongatum* L.) seeds were harvested from Eshghabad Seed Production Station, Nishabour, in summer 2011. Powder of SiO_2 nanoparticles was supplied by TECNAN Company in Spain. Specific surface area of nanosized SiO_2 was 600 m^2 g^{-1}, average primary particle size was 10–15 nm and purity was more than 99%. The size of SiO_2 nanoparticles (Fig. 1) was determined through Transmission Electron Microscope (TEM) in Central Laboratory of Ferdowsi University of Mashhad.

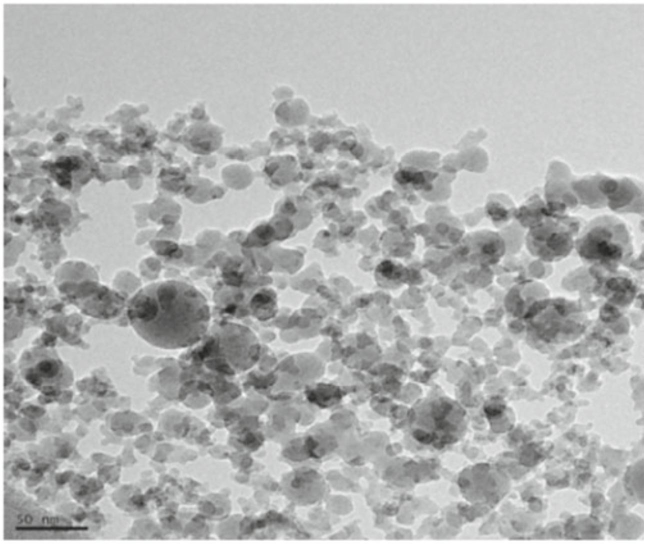

Figure 1. Images of nanosized SiO_2 by Transmission Electron Microscope (TEM)

Treatments

Factorial combinations of six SiO_2 levels (S) and three seed prechilling treatments (P) were applied in a completely randomized design (CRD). SiO_2 concentrations were 0, 5, 20, 40, 60 and 80 mg L^{-1} of nanosized. Prechilling treatments were control (without prechilling), seed prechilling before nano treatments (PS) and treatments of seed with nano treatment and then prechilling (SP). For prechilling treatments, seeds were kept at 4°C for 7 days[16]. The experiment was set in a germinator with an average temperature of 25/15 ± 1°C for 16/8 hours at the College of Natural Resources, Ferdowsi University of Mashhad, Iran.

The procedure for the germination test and preparing the concentrations was adapted from previous studies[17, 18, 19]. Tall wheatgrass seeds were randomly selected and sterilized using NaClO (5%) for 3 minutes and then carefully washed with distilled water for three times. An ultra-sonication treatment was applied to nanoparticles of SiO_2 powders and dispersed in water for 15 minutes which led to full and uniform dispersion and a stable suspension SiO_2 particles. The seeds were located on germination papers in Petri dishes. Four batches of 25 seeds were selected; each of them received 2 ml of SiO_2 suspension right away after ultra-sonication treatment in different concentrations. For control treatment only distilled water was added to the Petri dishes. Germination tests were performed according to the International Seed Testing Association regulations[16]. All concentrations of SiO_2 and the control treatment were tested at the same time to make sure that all treatments are receiving similar light and temperature conditions. Numbers of germinated seeds were recorded in daily basis for 14 days. Seeds were considered germinated when the radicle showed at least 2 mm in length[16]. Mean germination time was calculated based on Matthews and Khajeh-Hosseini[20] (Eq. 1):

$$MGT = \frac{\sum F.X}{\sum F} \quad (1)$$

Where F is the number of seeds newly germinated at the time of X, and X is the number of days from sowing.

Seedling vigors were calculated based on Vashisth and Nagarajan[21] (Eq. 2 and 3):

Vigor index I = Germination% × Seedling length (cm) (2)

Vigor index II = Germination% × Seedling weight (g) (3)

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations[22]:

MDG = Germination% / total experiment days (4)

PV = Maximum germinated seed number at one day / day number (5)

GV= PV × MDG (6)

Data analysis

A two-way ANOVA was applied for the effects of SiO_2 concentrations, prechilling and their interactions by using SPSS 19.0 software. Mean values were compared by using Duncan's multiple range tests at 5% level.

RESULTS AND DISCUSSION

Application of SiO_2 nanoparticles significantly increased seed germination of tall wheatgrass from 58 percent in control group to 86.3 and 85.7 percent in 40 and 60 mg L^{-1}, respectively (Table 2). Nevertheless the highest SiO_2 concentration (80 mg L^{-1}) did not significantly increase seed germination of *A. elongotum*.

Applying SiO_2 nanoparticles increased dry weight of shoot, root and seedling of tall wheatgrass. Increasing concentration of nanoparticle from 0 up to 40 mg L^{-1} increased seedling weight around 49% compared to the control, nevertheless decreased under 60 and 80 mg L^{-1} treatments. Application of 40 mg L^{-1} treatment increased both root and shoot weights by 150 and 14.6% respectively, as compared with the control. However, increasing in root weight had more significant role than shoot weight in the enhancement of total seedling weight. It has been proved that silicon facilitates water uptake and its transportation into plant. Beneficial effects of silicon might be related to its hydrophilicity[6]. Lin et al.[10] demonstrated that treatment of Changbai larch seedlings by 500 μL L^{-1} nanostructured silicon dioxide produced the best result, for which the mean height, root collar diameter, main root length, and the number of lateral roots were increased by 42.5%, 30.7%, 14.0%, and 31.6%, respectively, compared to those of the control.

Response of root, shoot and seedling length to addition of nano SiO_2 was similar to weights data. Treating tall wheatgrass seeds with 40 mg L^{-1} nanoparticle led to the highest increase (two fold) in seedling length. Shoot and seedling length were also increased about 50 and 72% under S40 as compared to the control (Table 1). Tahir et al.[11] also reported that silicon application significantly increased wheat biomass at both control as well as under saline conditions.

As a general rule, lower Mean Germination Time (MGT) represents a faster germination speed. Our results revealed that treating of tall wheatgrass seeds to all nanosized SiO_2 treatments reduced MGT in which the lowest MGT was under 40 mg L^{-1} treatment (5.23 days) whereas the treatment with the highest SiO_2 concentration S80 did not significantly improve seed mean germination speed (Table 2). Activation of respiration and rapid ATP production appears to be most important metabolic events induced by faster seed germination[23]. Zheng et al.[13] found that significant effects of nanosized TiO_2 on spinach seed germination. They referred results to small particle size, which permitted nanoparticles to penetrate into the seed during the treatment period, enhancing their functions throughout the growth period.

Seed treatment with 40 and 60 mg L^{-1} showed the highest germination value, Mean Daily Germination (MDG) and Pick Value (PV). Generally, a vigor index indicates potential of seedling for emergence. All of the SiO_2 treatments improved Vigor Index (VI) e.g. S40 enhanced vigor index I by 181% and vigor index II by 120% as compared to the control (Table 2). Zheng et al.[13] reported that nanosized TiO_2 contributed to water absorption by spinach seeds and as result accelerated seed germination. Feizi et al.[18] found the highest vigor index was under 2 and 10 mg L^{-1} nanosized TiO_2 concentrations.

Our pretest indicated a kind of seed dormancy for tall wheatgrass. For breaking seed dormancy of cold season grasses we may need a period of prechilling at 4°C according to the ISTA rules[16]. According to our results, seed germination was 77.8% under prechilling treatment while under no prechilling (P0) and under seed prechilling simultaneously with nano SiO_2 (PS) were 52.8% and 80.2%, respectively. Khodakovskaya et al.[24] reported that Multi Wall Carbon Nanotubes (MWCNTs) can penetrate to tomato seeds and increase the germination rate by increasing the seed water uptake. The MWCNTs increased the seed germination, up to 90% (compared to 71% in control) in 20 days, and the plant biomass.

Seed prechilling together with SiO_2 nanoparticles (PS) largely broke the seed dormancy for *A. elongatum*. Although prechilling is a major factor for tall wheatgrass seed but seed prechilling jointly SiO_2 nanoparticles hastened seed germination, hence it seems that SiO_2 nanoparticles could be an alternative potential for breaking of seed dormancy. Under PS treatment, seedling weight and length were increased by 53.6 and 47.2% as compared to the control. Also PS accelerated germination speed; i.e. MGT improved from 6.42 in control to 5.26 days under PS. Seedling vigor indices were also increased around 120–130% in PS as compared with control (Table 4). Lin et al.[10] demonstrated that treatment of Changbai larch seedlings by 500 μL L^{-1} silicon dioxide nanoparticles significantly increased mean height and root traits. Higher activities of SOD, GPX, APX, DHAR and GR in salt-stressed cucumber leaves induced by Si addition could protect the plant tissues from membrane oxidative damage under salt stress, thus mitigated salt toxicity and improving the growth of cucumber plants[25].

Several germination-related processes such as gene transcription and translation, respiration and energy me-

Table 1. Influence of nanosized SiO_2 concentrations on seed germination and seedling morphological growth traits of tall wheatgrass seedling

SiO_2 Concentration [mg L^{-1}]	Germination [%]	Seedling weight [mg]	Root dry weight [mg]	Shoot dry weight [mg]	Seedling length [cm]	Root length [cm]	Shoot length [cm]
S0	58d	1.10c	0.28d	0.82b	11.42e	5.21e	6.21d
S5	67b	1.14c	0.46c	0.68c	13.92c	6.57c	7.35c
S20	64.3c	1.07c	0.43c	0.64c	12.15d	5.77d	6.38d
S40	86.3a	1.64a	0.70a	0.94a	19.66a	10.36a	9.30d
S60	85.7a	1.41b	0.57b	0.84ab	17.24b	9.15b	8.10b
S80	59.7d	1.19c	0.41c	0.78bc	12.21e	5.85d	6.35d

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan´s Multiple Range Test.

Table 2. Influence of nanosized SiO_2 concentrations on MGT, GV, MDG, PV and vigor indices of tall wheatgrass seedling

SiO_2 Concentration [mg L^{-1}]	(MGT day)	Germination value	MDG	PV	Vigour index I	Vigour index II
S0	6.7a	2.19c	4.19e	0.54c	618.81e	65.76d
S5	5.64c	4.32b	4.79b	0.90b	948.69c	79.96c
S20	5.68c	4.31b	4.60c	0.93b	803.85d	71.49cd
S40	5.23d	7.61a	6.17a	1.20a	1736.04a	143.91a
S60	5.57c	7.15a	6.12a	1.14a	1514.30b	123.19b
S80	5.98b	3.04c	4.26d	0.68c	762.74d	73.32c

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan´s Multiple Range Test.

Table 3. Effect of prechilling treatments on seed germination and seedling growth parameters of tall wheatgrass

Prechilling	Germination [%]	Seedlin Weight [mg]	Root dry weight [mg]	Shoot dry weight [mg]	Seedling length [cm]	Length Root [cm]	Shoot length [cm]
C	52.8c	0.97c	0.33c	0.64c	11.35c	6.04c	5.30c
PS	77.8b	1.31b	0.50b	0.81b	15.24b	7.31b	7.94b
SP	80.2a	1.49a	0.60a	0.90a	16.71a	8.10a	8.60a

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan´s Multiple Range Test.

Table 4. Influence of prechilling treatments on MGT, GV, MDG, PV and vigor indices of tall wheatgrass seedling

Prechilling	(MGT day)	Germination Value	MDG	PV	Vigour index I	Vigour index II
C	6.42a	3.09b	3.77c	0.79c	624.72c	53.32c
PS	5.72b	5.75a	5.56b	0.99a	1219.95b	103.37b
SP	5.26c	5.47a	5.73a	0.91b	1382.55a	122.13a

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level – using Duncan´s Multiple Range Test.

Table 5. Interaction effects of SiO_2 concentration and prechilling interactions on seed germination, MGT, elongation and vigor index of tall wheatgrass seedling

Prechilling	SiO_2 concentration [mg L^{-1}]	Germination [%]	MGT [day]	Seedling length [cm]	length Root [cm]	Shoot length [cm]	Vigour index I	Vigour index II
C	S0	42g*	7.26a	9.16k	4.45g	4.72i	256.65i	38.83e
	S5	50f	6.25bc	12.08j	5.85f	6.23h	604.27h	35.1e
	S20	52f	6.05bcd	8.67k	4.63g	4.05ij	450.99i	35.51e
	S40	67de	6.44b	15.85d	8.98c	6.87gh	1062de	95.81d
	S60	66e	6.12bc	13.59ghi	7.43d	6.15h	896.43fg	77.67d
	S80	40g	6.41b	8.75k	4.93g	3.81j	349.78i	36.98e
PS	S0	67de	6.43b	12.55ij	5.60f	6.95gh	840.78g	79.23d
	S5	75bc	5.32efg	14.15fg	6.71e	7.45fg	1061.41de	94.12d
	S20	68de	5.99bcde	13.16hij	5.97f	7.20fg	894.36fg	79.03d
	S40	94a	4.7gh	20.28b	10.27b	10b	1905.88b	154.08ab
	S60	94a	5.64cdef	18.22c	9.47c	8.75cd	1712.94c	130.8bc
	S80	69cde	6.17bc	13.10fhij	5.82f	7.28fg	904.3fg	82.95d
SP	S0	67de	6.43b	12.55ij	5.60f	6.95gh	840.78g	79.23d
	S5	76b	5.34efg	15.53de	7.16de	8.37de	1180.38d	110.66cd
	S20	73bcd	4.99fgh	14.61efg	6.71e	7.90ef	1066.21de	99.92cd
	S40	98a	4.51h	22.85a	11.8a	11.03a	2240.23a	181.83a
	S60	97a	4.94gh	19.93b	10.54b	9.39bc	1933.53b	161.09ab
	S80	70bcde	5.37defg	14.78def	6.81de	7.97def	1034.15ef	100.03cd

*Means, in each column, followed by similar le tter are not significantly different at the 5% probability level – using Duncan´s Multiple Range Test.

tabolism, faster reserve transportation and DNA repair may also take place during seed treatment[26], although often limited due to reduced water provide compared to regular germination[2, 27].

Interaction of prechilling and SiO_2 concentration treatments has been shown in Table 5. The highest germination percentage was found when 40 and 60 mg L^{-1} nanoparticles were combined with prechilling. Treatments under seed prechilling before nano treatment (PS), exposing of seeds to 40 and 60 mg L^{-1} improved germination rates by 40% and germination speed by 123% in comparison with control. Similar results were achieved under nano together with seed prechilling (SP) treatments (Table 5). PS treatments improved MGT by 37.9% as compared to control. Clément et al.[28] reported that the soaking of flax seeds in the suspensions of anatase nanoparticles at concentration 100 mg L^{-1} increased seed germination and root growth. These positive effects could be due to antimicrobial properties of anatase crystalline structure of TiO_2 that increase plant resistance to stress[28].

We did not find any interaction effects between SiO_2 concentration and seed prechilling on seedling root and shoot weights. Application of silica nanoparticles with 40 mg L^{-1} largely increased seedling, root and shoot elongation as compared to the control. Use of 40 mg L^{-1} SiO_2 concentration enhanced seed germination of tall wheatgrass around two times more than control groups. Feizi et al.[17] demonstrated that use of TiO_2 nanoparticles extraordinarily enhanced fennel seed germination, while seed germination percentages decreased from exposure to concentrations of bulk TiO_2 particles compared to the control group. We also found the greatest vigor index in 40 mg L^{-1} jointly seed prechilling (Table 5). We believe that high concentration of SiO_2 nanoparticles could be toxic for seed, therefore high concentration adversely affected on seed performance. In previous work, it was demonstrated that using nanosized TiO_2 in low concentration (2 and 10 ppm) could encourage seed germination of wheat in comparison to bulk TiO_2 and untreated control groups, but in high concentrations (100 and 500 ppm) it had an inhibitory or no effect on wheat seed[18].

CONCLUSIONS

Applications of nanomaterial can encourage earlier plant germination, breaking seed dormancy and improve plant production. To our knowledge, this effort is the first information related to the effects of SiO_2 nanoparticles on tall wheatgrass. A significant increase in seed germination, MGT, germination rate, seedling weight and vigor index was found by prechilling and SiO_2 nanoparticles suggest that SiO_2 nanoparticles may be contributed in the metabolic or physiological activity in tall wheatgrass seed exposed to prechilling. Consequently, it has proposed that applying SiO_2 nanoparticles together with prechilling could use as a new alternative potential for seed dormancy breaking in tall wheatgrass.

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