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Synthesis of Silica Nanoparticles and their effect on priming of wheat (*Triticum aestivum* L.) under salinity stress

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ABSTRACT: Synthesis of silica nanoparticle is getting great attention, due to their increased use in multidiscipline in advanced research. In this study ultrasonication based spherical Silica nanoparticles (50-100 nm) have been synthesized by modified Stober method using Tetraethylorthosilicate as a precursor. The synthesized silica nanoparticles have been used to study their effect on the wheat grown under salinity stress. Wheat seeds were primed in distilled water (control) and in two treatments T1 (SiNps 100 nm) and T2 (SiNps 50 nm).The seed germination increased from 68% (control) to 81% (T1) and 88% (T2).The young seedlings were grown in Hydroponics in Hoagland medium in controlled conditions under non-saline and saline (100 Mm NaCl) environments. All the growth parameters (root & shoot length, plants weight and chlorophyll content) that were reduced in the saline environments were increased insilica nanoparticlesprimed seeds, which shows the potential of silica nanoparticlesin breaking the seed dormancy and improving the growth of wheat under salinity stress.

Keywords: Silica nanoparticles, Sol-gel, ultrasonication, wheat, salinity stress, seed priming, pre-chilling

INTRODUCTION

The synthesis and the use of nanoparticles is of pronounced interest in recent research activities due to progress of Nanoscience and Nanotechnology and their broad applications in multidiscipline (Huan and Shu-Qing, 2014). With widespread of Nanotechnology, nanoparticles are synthesized on large scale now-a-days and silicananoparticles are one of the most popular among these.

Silica nanoparticles (SiNps) attained great attention in scientific research due to their informal synthesis and their broad applications in different industries like; agriculture, pharmacy, pigments, catalysis, electronic etc (Rao *et al*., 2005). Stober was the pioneer to prepare silica nanoparticles of 0.05 to 2 micron in diameter (Stöber *et al.*, 1968). Stober's method is the most popular and common method among researchers with little modifications and is now acquaint with ultrasonication. The literature surveys indicated that the Sol-Gel process (Stober's Method) has been evidenced as a promising simplest and the most economical method for the synthesis of spherical, monodispersed and nanosized silica particles by using TEOS (Tetraethylorthosilicate) as a precursor (Rahman and Padavettan, 2012).

As Nanotechnology is bringing revolutions in agriculture, therefore these magical bullets can be used as a trial for the improvement of wheat growth in the saline environment. Salinity is one of the main factor in decline of wheat growth and yield all over the world. Many investigations indicated that salinity tolerance in wheat and barley can be increased by the addition of small amounts of silicon. A research on 25 lentils genotypes showed significant effect of the SiNps on plants under salinity stress by increment in germination% and seedling growth (JANMOHAMMADI et al. 2015). SiNps had beneficial increase in various growth parameters in tomatoes (Siddiqui and Al-Whaibi, 2014) and maize (Suriyaprabha *et al*., 2012) therefore should be used in fertilizes to improve the sustainable agriculture.

Seed priming is a pre-germination technique in which seeds are soaked in the water at an osmotic potential that allows imbibitions, but prevents the extension of radical (Bradford, 1986). This technique is widely used now-a-days to increase germination, decrease mean germination time, improve growth and yield.

Halopriming (inorganic salts) have been useful in saline conditions (Ghabdian *et al*., 2015). Priming with sodium silicate has improved the seed germination and seedling growth in drought and salt stress in wheat (Hameed *et al*., 2013). Seeds pre-chilling with SiNps broke the dormancy of seeds and the application of SiNps enhanced the seed germination, MGT, germination rate, dry weight of root, shoot and seedling in Tall wheat grass (Azimi *et al*., 2014).

Although Nanoparticles of Silica have been efficient in controlling salinity in many plants but never have been used to control salinity in wheat that is a major staple food and important crop cultivated all over the world. As Silicon supplementation is proved to increase salinity tolerance as well as to improve production of wheat therefore silica nanoparticles can also be used to increase silica availability in wheat that is under salinity stress and Si Nps might give better results due to their Nano size. Therefore the purpose of this study is to synthesize silica nano particles and to investigate their effect on priming of wheat that is grown under saline environments in order to increase the yield of wheat in the marginal areas.

MATERIALS AND METHODS

A. Synthesis of Silica Nanoparticles

Silica nanoparticles were prepared by modified Stober method, using tetraethoxysilane (TEOS) as a precursor. 4 ml DI water was mixed in 10 ml ethanol. 1.8 ml of TEOS was added in the above mixture and sonicated for 30-60 minutes at 35°C. 1.4 ml of NH_4OH was mixed in 1.4 ml DI water separately. NH_4OH was added drop wise to the TEOS with constant and vigorous stirring. Grayish colour indicated the formation of the SiO_2 nanoparticles. Centrifuged at 4000 rpm for 20 minutes and filter out the precipitates of Silica nanoparticles. The particle size can be reduced by increase in the sonication time. The silica nanoparticles were prepared and characterized in AIT, Thailand and the particle size was determined by JEM-2100 plus Transmission Electron Microscope with operating voltage of 200 KV in Nanotec center Thailand.

B. Seed Priming

Four genotypes of wheat from Balochistan were collected from ARI, Quetta, Pakistan. Two salt tolerant varieties (Umeed & Raskoh) and two salt sensitive varieties (Zarghoon & Shahkar) were selected after literature survey. 100 healthy seeds of almost same weight and size were rinsed with water and were soaked in the distilled water for the control, while in silica nanoparticles (0.1 g/100ml) for treatment T1 (SiNps 100nm) and T2 (SiNps 50nm) each. Seeds of each variety were soaked on moist paper towel separately and number of seeds germinated daily was recorded regularly till 5 days. The Final Germination Percentage (FGP) was calculated by this formula

FGP= No of seeds germinated on the final day X 100/ Total No of seeds sown

The Mean Germination Time (MGT) was calculated by this formula

$$MGT = \frac{\Sigma F . X}{\Sigma F}$$

F= No of seeds newly germinated at the time of X, X= No of days from sowing (Matthews and Khajeh-Hosseini, 2007; Azimi *et al*., 2014).

C. Growth of Wheat in Hydroponics

Wheat was grown in Hydroponics in a completely randomized factorial fashion with three replicates of each wheat variety grown under two types of treatments (T1 and T2) in Non-saline and saline environment in June 2016. After ten days the young seedlings were shifted to the hydroponic tanks, containing Hoagland Nutrient medium (Hoagland and Arnon, 1950). Seedlings were supported by Styrofoam sheet floating on the water surface under continuous aeration. This study was carried out in BUITEMS Stress Lab with controlled temperature (25/18° C day/night) and light (800 luxes). After appearance of third leaf 100mM NaCl was added in the saline treatments. Plants were harvested 45 days after this treatment and the data was recorded for plants Root and shoot length, Fresh and dry weight and chlorophyll contents. The recorded data was analyzed for comparison of means and ANOVA by SPSS (Standard Statistical Package).

RESULTS AND DISCUSSION

A. Synthesis of Silica Nanoparticles

In this research silica nanoparticles were prepared by modified Stober method with prolonged ultrasonication. With 30 minutes of ultrasonication spherical silica nanoparticles of 100 nm were synthesized (Fig. 1), while with 60 minutes of ultrasonication spherical silica nanoparticles of 50 nm were produced (Fig. 2). Prolonged ultrasonication (1-2 hours) eradicates the initial structure of silica gel and modifies the size and the shape of nanoparticles, therefore ultrasonication is a helpful tool in reducing the size during the synthesis of silica nanoparticles. The morphology of the produced silica nanoparticles is very similar to the colloidal nanosilica that consists of small particulates arranged into clustures after sonication assisted synthesis (Jafari *et al*., 2014).

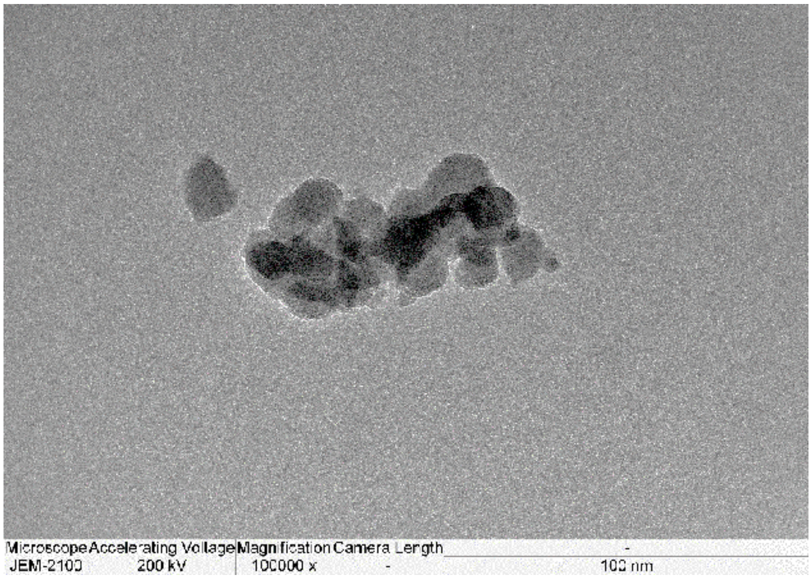

Fig. 1. TEM image of Silica Nanoparticles (100 nm).

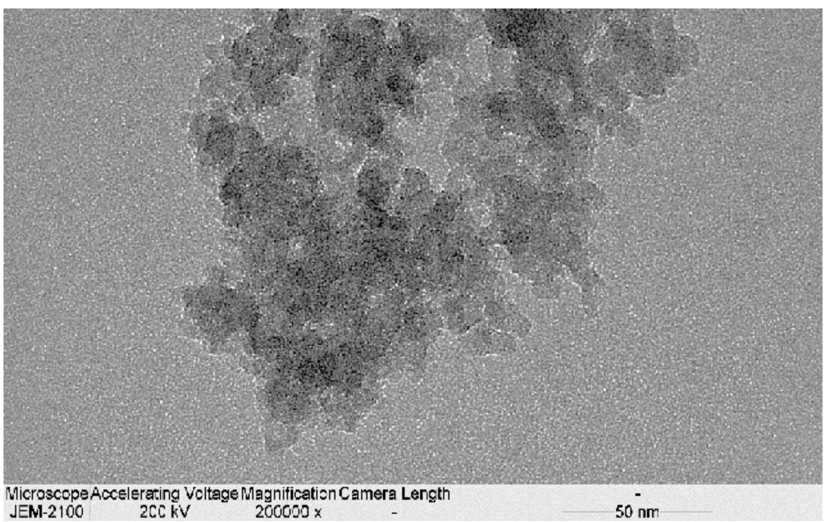

Fig. 2. TEM image of Silica Nanoparticles (50 nm).

B. Effect of SiNps priming on FGP

In our study we used two types of priming techniques Hydropriming (in water) and Halopriming (inorganic salts). The FGP (Final Germination Percentage) that is the average number of seeds that germinated after five days was calculated for each variety under 2 treatments (Table 1). The FGP for the seeds soaked in water (control) was found lowest, (Table 1) although increased remarkably by soaking in silica nanoparticles in T1 (100 nm) and significantly in T2 (50 nm).

Table 1: Final Germination percentage (FGP) of wheat seeds at fifth day of germination.

	Varieties	Seeds in Water (Control)	SiNps 100 nm (T1)	SiNps 50 nm (T2)
1.	**Umeed**	69%	85%	90%
2.	**Raskoh**	70%	85%	90%
3.	**Zarghoon**	68%	80%	85%
4.	**Shahkar**	64%	75%	85%

Overall the seed germination increased from 68% (control) to81% (T1) and 88% (T2) which proves that seed priming with silica nanoparticles can break the dormancy of seeds and increase the germination rate significantly.

C. Effect of SiNps priming on MGT

As a general rule, lower MGT (Mean Germination Time) denotes faster speed of germination (Azimi *et al*., 2014). The MGT decreased from 2.4 (control) to 1.6 in both T1 and T2 in our results (Table 2) which represent faster and improved germination percentage by silica nanoparticles. The results are very similar to increase in germination of tall wheat grass from 58 % to 85.7 % and decrease in MGT from 6.7 to 5.23 by silica nanoparticles pre-chilling (Azimi *et al*., 2014). The results are also in agreement with 95.5% GP and decreased 2.8 to 1.73 MGT observed in maize by silica nanoparticles application (Suriyaprabha *et al*., 2012) and also with 22.16% increase in germination(Siddiqui and Al-Whaibi, 2014). Therefore it can be concluded that seed priming with silica nanoparticles can be an economic and effective way for improving the seed germination and for breaking seed dormancy. Moreover the seeds pre-chilled in silica nanoparticles has shown better growth and were persistent to salt stress throughout this experiment.

Table 2: Germination percentage and Mean Germination Time (MGT).

Treatments	Germ %	MGT
Water (Control)	68%	2.4
SiNps 100 nm (T1)	81%	1.6
SiNps 50 nm (T2)	88%	1.6

D. Root and Shoot Length

The root length has been significantly improved by silica nanoparticles application (Fig. 3) as they helped the wheat to persist the salinity stress. The results are similar to effect of nano silicon dioxide on Changbai larch in which mean height, root collar diameter, main root length and the number of lateral root increased significantly (Bao-shan *et al*., 2004). Whereas the shoot length that is effected by salt stress in control, has shown remarkable increase in silica nanoparticles treatments (Fig. 4). These findings are in agreement to the effect of SiNps on Basil under salinity stress in which shoot height and weight increased significantly (Kalteh *et al*., 2014).

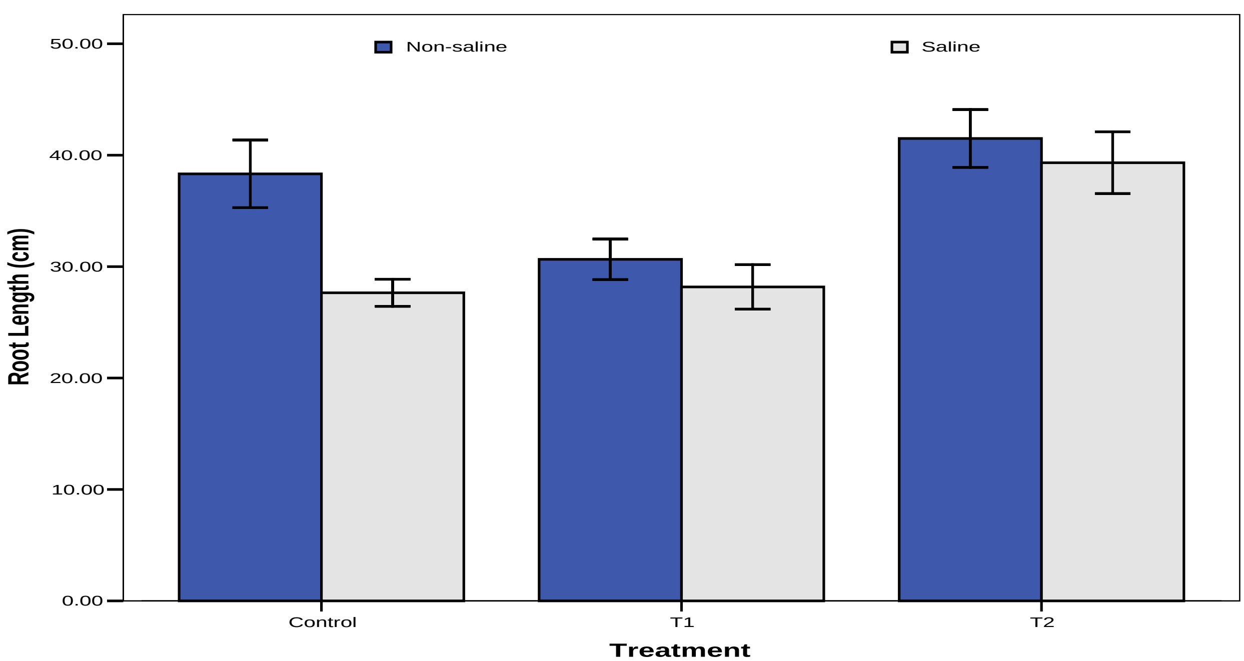

Fig. 3. Effect of silica nanoparticles on the Root length of wheat cultivars.

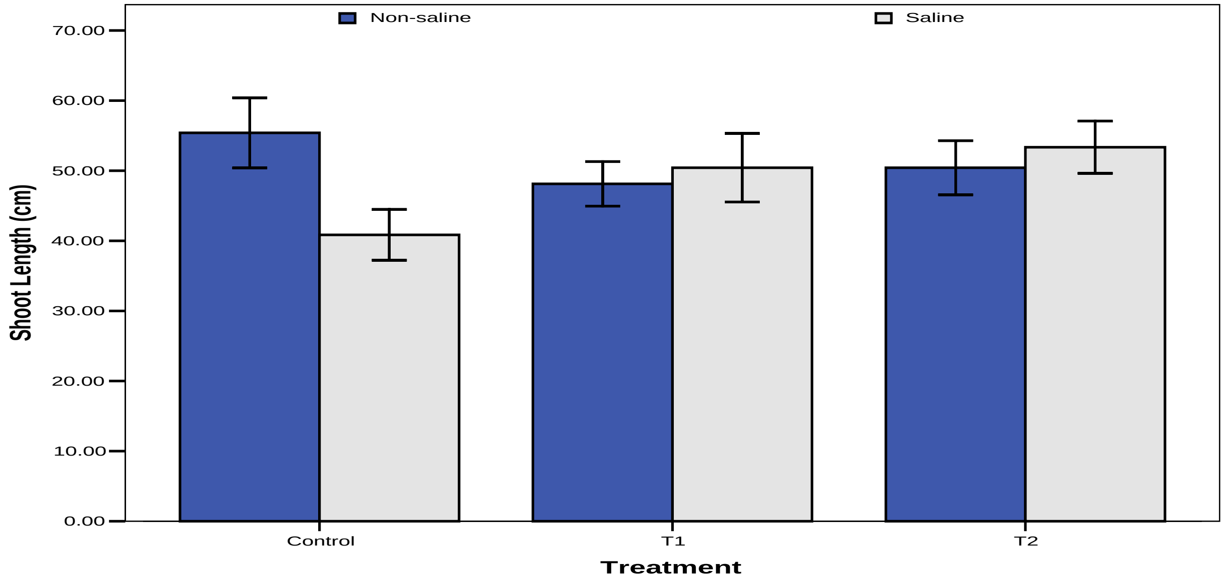

Fig. 4. Effect of silica nanoparticles on the Shoot length of wheat cultivars.

E. Fresh and Dry Weight

Fresh weight of the whole plant had highest values under control (non-saline) that was greatly affected in saline condition (Fig. 5) whereas silica nanoparticles treatments helped plants to survive better in saline environments which agrees with results of SiNps effect on Basil's weight (Kalteh *et al*., 2014). Although there was no significant differences among the dry weight in Non-saline environment but significant differences existed among all the treatments in the saline environment (Fig. 6). The results of all the growth parameters are in agreement to pre-chilling experiment which had increased dry weight of root and shoot and seedling of tall wheatgrass (Azimi *et al*., 2014).

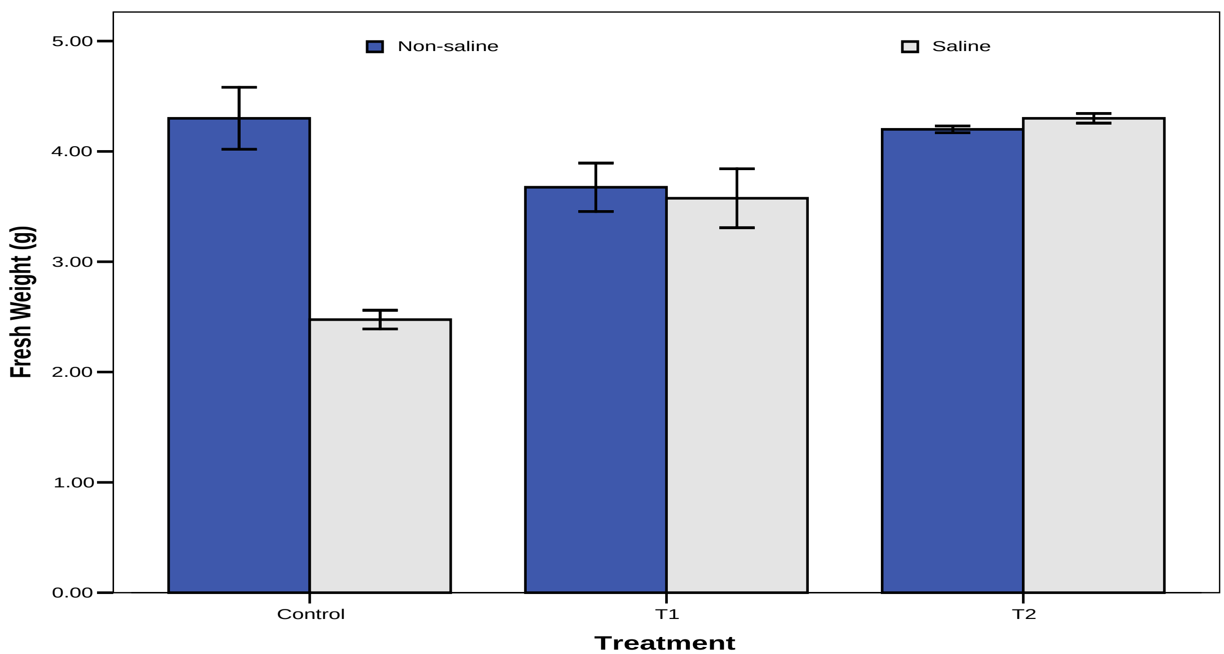

Fig. 5. Effect of silica nanoparticles on the Fresh weight of wheat cultivars.

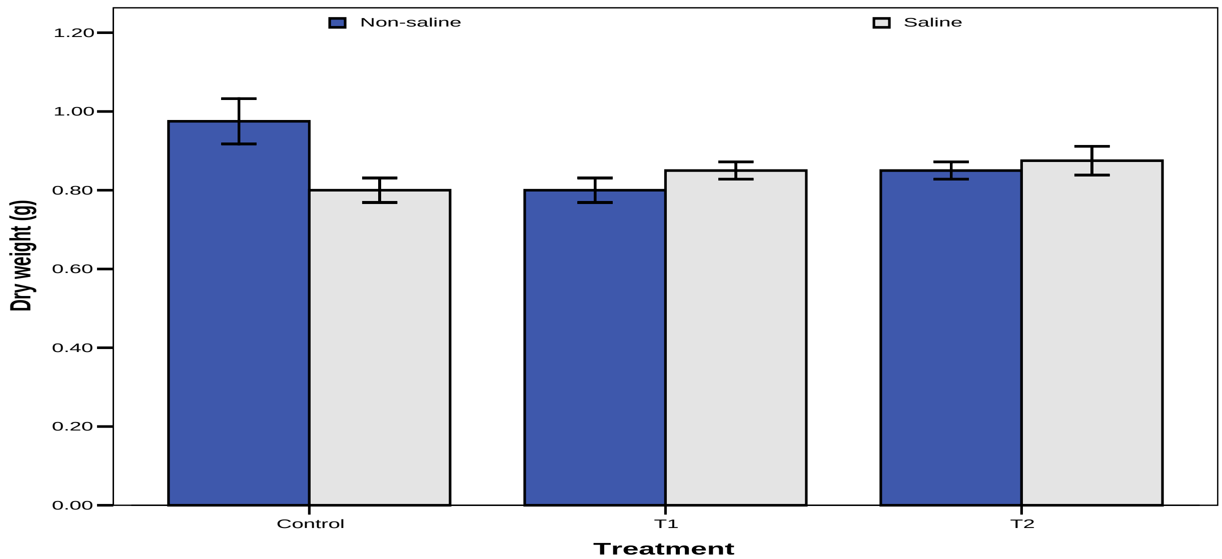

Fig. 6. Effect of silica nanoparticles on the Dry weight of wheat cultivars.

F. Chlorophyll Contents

Chlorophyll contents that was affected by salt stress has been increased significantly by silica nanoparticles priming (Fig. 7) as it has been reported prior that silica can increase the leaf contents of chlorophyll in wheat under saline environments(Hajiboland *et al*., 2016). The results are also in agreement with application of SiNps, that increased chlorophyll content in Basil grown under salinity stress (Kalteh *et al*., 2014).

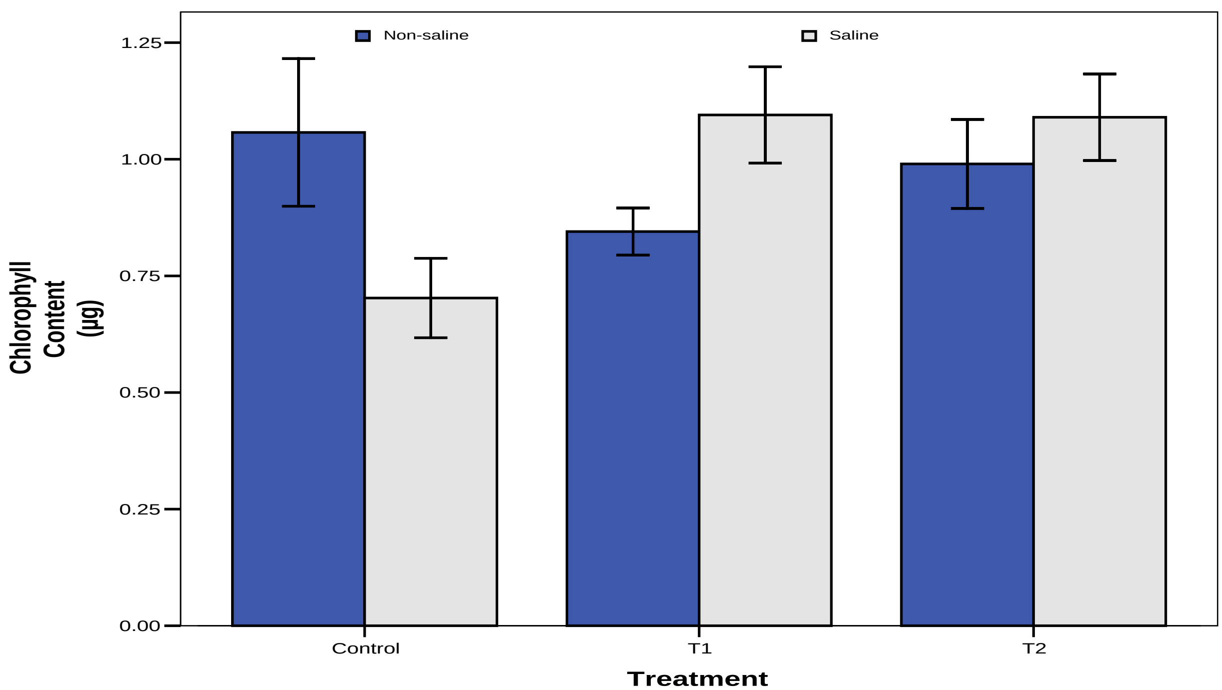

Fig. 7. Effect of silica nanoparticles on the Chlorophyll contents of wheat cultivars.

Table 3: Mean values of three replicates for each variety under each treatment.

Treatments	Varieties	Non-saline (NaCl=0 mM)					Saline (NaCl=100 mM)				
		Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll Content (µg)	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll Content (µg)
Control	**Umeed**	45.3	68.3	5.2	1.1	1.63	26.0	55.0	2.8	0.9	1.02
	Raskoh	38.0	65.0	4.6	1.1	1.09	27.0	36.7	2.4	0.8	0.66
	Zarghoon	27.3	43.3	3.7	0.9	0.73	25.3	36.7	2.3	0.7	0.50
	Shahkar	42.7	45.0	3.7	0.8	0.78	32.3	35.0	2.4	0.8	0.63
T 1	**Umeed**	37.3	57.5	4.5	0.9	0.91	34.7	65.0	4.4	0.9	1.23
	Raskoh	30.0	51.7	3.2	0.8	1.00	26.0	56.7	3.7	0.9	1.38
(SiNps	**Zarghoon**	26.0	38.3	3.4	0.8	0.74	24.7	36.7	2.7	0.8	0.76
100nm)	**Shahkar**	29.3	45.0	3.6	0.7	0.73	23.3	43.3	3.5	0.8	1.01
T 2	**Umeed**	48.0	58.3	4.2	0.9	1.25	48.0	61.7	4.4	1.0	1.36
(SiNps	**Raskoh**	46.7	60.0	4.2	0.9	1.04	42.0	61.7	4.4	0.9	1.11
50nm)	**Zarghoon**	36.0	41.7	4.1	0.8	0.65	36.0	46.7	4.2	0.8	0.77
	Shahkar	35.3	41.7	4.3	0.8	1.02	31.3	43.3	4.2	0.8	1.12

The overall results of this study has shown great improvement in all the growth parameters of wheat cultivars in the saline environments (Table 3). The control that was without silica nanoparticles has been greatly affected by salt stress of 100 mMNaCl while the wheat cultivars under T1 (SiNps 100nm) and T2 (SiNps 50nm) has survived better in Non-saline as well as saline environments, and best results were achieved in T2 as smaller are the nanoparticles they are more affective due their nanosize. The two varieties of wheat "Umeed and Raskoh" were found tolerant to salt stress while the other two varieties "Zarghoon and Shahkar" were found sensitive to salt stress. The efficiency of wheat varieties was observed in this order Umeed> Raskoh>Shahkar> Zarghoon.

CONCLUSION

Silica nanoparticles can be conveniently prepared by modified Stober method in ultrasonication and due to their small size they are immediately utilizable source of silicon for plants. Seed priming by silica nanoparticles broke the dormancy of wheat seeds therefore silica nanoparticles priming that has been introduced for the first time in wheat can be aneconomic and effective technique in breaking the dormancy of seeds and improving their germination rate. Silica nanoparticles significantly enhanced the seed germination and all the growth parameters in the saline environment therefore can be used for improvement of wheat growth and yield in saline areas.

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