

NASER SABAGHNI, MOHSEN JANMOHAMMADI

Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, Iran. Corresponding author: Naser Sabaghnia, e-mail: sabaghnia@maragheh.ac.ir

Effect of nano-silicon particles application on salinity tolerance in early growth of some lentil genotypes

Wpływ nanocząstek krzemionki na tolerancję zasolenia we wczesnym rozwoju niektórych genotypów soczewicy

ABSTRACT

Twenty-five lentil (*Lens culinaris* Medik.) genotypes were studied to evaluate the effects of the SiO₂ nano-particles on plants under salt stress. The experiment was a 3×25 factorial arrangement with three levels of treatment solutions as (T₁) distilled water as control, (T₂) 100 mM NaCl concentration and (T₃) 1 mM nano-silicon dioxide concentration plus 100 mM NaCl concentration, and 25 levels of lentil genotypes. Results showed a significant reduction in germination percent and seedling growth due to the salinity stress while significantly increased with silicon nano-particles application. The germination percentage, shoot length, root length, seedling fresh weight and seedling dry weight traits showed significant differences among lentil genotypes in treatment solutions. Results indicated that adding SiO₂ nano-particles could improve germination and seedling early growth under salinity stress and the related traits were increased in all of lentil genotypes. Overall, application of SiO₂ nano-particles was beneficial in improving salinity tolerance in the lentil seedling and its application may stimulate the differences defense mechanisms of plants against salt toxicity.

Keywords: nano-SiO₂, salt stress, stress response, tolerance, variability

STRESZCZENIE

Dwadzieścia pięć genotypów soczewicy (*Lens culinaris* Medik.) badano w celu oceny działania nanocząstek SiO₂ na rośliny poddane stresowi zasolenia. Eksperyment przeprowadzono dla 25 genotypów w trzech wariantach doświadczenia: (T₁) woda destylowana jako kontrola, (T₂) NaCl o stężeniu 100 mM i (T₃) nano dwutlenek krzemu o stężeniu 1 mM z dodatkiem 100 mM NaCl. Wyniki wykazały znaczne obniżenie procentu kiełkowania i wzrostu siewek z powodu stresu zasolenia i jednocześnie znaczne zwiększenie badanych parametrów po zastosowaniu nanocząstek

krzemionki. Procent kiełkowania, długość pędów, długość korzeni, świeża i sucha masa siewek wykazały istotne różnice między genotypami soczewicy po traktowaniu roztworami. Wyniki wykazały, że dodanie nanocząstek SiO₂ może poprawić kiełkowanie i wczesny wzrost siewek podanych stresowi zasolenia, a badane parametry były lepsze u wszystkich genotypów soczewicy. Podsumowując, zastosowanie nanocząstek SiO₂ było korzystne dla poprawy tolerancji zasolenia siewek soczewicy, a ich zastosowanie może stymulować różne mechanizmy obronne roślin na toksyczność soli.

Słowa kluczowe: nano-SiO₂, stres solny, odpowiedź na stres, tolerancja, zmienność

INTRODUCTION

Abiotic stresses are the main factor negatively affecting crop growth and productivity worldwide. Salinity is one of the most important environmental stresses, limiting crop production in arid and semi-arid areas of the world and the saline areas are three times larger than the land used for agriculture (20). Over 6% of the world's land is influenced by salinity which accounts for more than 800 million hectares of the land and salinity is one of the key environmental factors that limit agricultural productivity (6). Salt injury depends on several factors like species, cultivar, growth stage and other environmental factors while several physiological processes like photosynthesis, nitrogen fixation and carbohydrate metabolism have been observed to be affected by high salinity (3). Also, salinity stress exposes to secondary osmotic stress, which is invoked by drought stress and the capacity of crop to tolerate salinity is a key factor in successful crop productivity (20). Also, the relationships between salinity stress and mineral nutrition in crops is a complex phenomenon and its complete understanding is essential.

The response of crops to excess salinity (especially sodium chloride) is a complex phenomenon and involves changes in their morphological and physiological characteristics. Seed germination is one of the most critical steps for a crop subjected to salinity stress which fails on saline soils due to accumulation of high salt concentrations in the seed planting zone (29). Seed germination and early seedling growth can significantly affect the emergence. The time from sowing to seedling establishment is of considerable importance in crop production. Any increase in germination vigor may result in early vigorous growth and good crop establishment. Lentil is adapted to low rainfall, predominantly grown in the winter in regions and its seed is rich in protein for human consumption. It is considered an important crop under food point of view, because of its role as a possible component of the cropping systems (in rotation with winter cereals) in the Mediterranean areas (18). In most lentil producing areas yield performances seem to be no more than one-half of potential cultivar yields and are far below theoretical maximum yields (19). This huge difference reflects production potential constraints that prevent the realization of influence of abiotic stresses or true genetic yield potential.

Although salinity problem can be minimized with some pre-sowing treatments (e.g. osmo-priming, halo-priming, hydro-priming), water and drainage, however their cost is very high and an alternative way for overcoming salinity could be an attempt to supplement silicon (27). Silicon is the second most abundant element on the surface of the earth and has not been classified as an essential element while it has been shown to be beneficial for the plant growth (13). It has been shown that added silicon can increase salinity tolerance as well as improve photosynthetic activity of leaf cells in barley (10). However, the role of silicon in alleviating both environmental (heavy metals and salinity) and biotic (diseases and pests) stresses in some crops has been reported (5; 11). Previous investigations showed that salinity tolerance in wheat (1; 27) and barley (10) could be markedly enhanced by the addition of small amounts of silicon.

The use of nano-particles has given a lot of attention by the researchers, especially by those investigating seed properties, although exact mechanisms of nano-particles are not well understood. Nano-particles show unique properties and they can change physico-chemical characteristics in plants to bulk materials. Nano-particles have greater surface area than bulk materials and so, their solubility and surface reactivity tend to be higher (17). Some useful nano-particles which are reported to have a useful effect in plants are titanium oxide (TiO_2) and silicon dioxide (SiO_2). Nano-silicon enhanced NR activity of soybean (12) and had an amelioration effect on salt stressed seedling of tomato in reducing salinity stress on germination of tomato (8). An experiment with 25 lentil genotypes was conducted to study the effectiveness of nano-silicon application in mitigating the adverse effects of salinity and to investigate possible mechanisms of nano-silicon enhancement of salt tolerance in lentil.

MATERIALS AND METHODS

In this investigation, seeds of 25 international lentil genotypes were used whose characteristics including name, pedigree and origin are given in Table 1. Seeds were immersed in a 5% sodium hypochlorite solution for 5 min to ensure surface sterility and washed in distilled water. The primary seed viability of lentil genotypes were greater than 90%. Seeds were soaked in three treatments as: (i) distilled water as T_1 , (ii) 100 mM NaCl concentration as T_2 and (iii) 1 mM nano-silicon dioxide concentration plus 100 mM NaCl concentration as T_3 . One piece of filter paper was put into each 100 mm \times 15 mm Petri dish, and 10 mL of each test solution was added to each experimental sample. Thirty seeds were selected and placed in each Petri dishes and then were covered and sealed with tape, placed in an incubator (with 16/8 h photoperiod, 20 ± 2 °C temperature and relative humidity of 75%). SiO_2 nano-particles (size < 50 nm) were prepared from Pishgaman of Nano-Materials Company, Iran. It has an average primary particle size of 20–30 nm with a corresponding surface area of 180–600 m^2/g . The result of X-ray analysis of the used nano-silicon dioxide particle (Fig. 1) and its large area Transmission electron microscopy (TEM) image (Fig. 2) are displayed.

Daily observation for germinated seed continued for ten days and germination percentage (GP) was calculated. The seeds were considered to be germinating at the moment of radical emergence (1–2 mm in length) based on (2). Also, shoot length (SL), root length (RL), seedling fresh weight (FW), and seedling dry weight (DW) traits were recorded. The datasets were first tested for normality by the Anderson and Darling normality test (14). Each treatment combination was conducted with three replicates, and the results were analyzed employing one-way ANOVA according to factorial experiment (first factor or solution type with three levels and the second factor or genotype in 25 levels) in a randomized completely design layout with SAS 9.1 (21) software. The means were compared by the least significant differences (LSD) method ($P < 0.05$).

RESULTS

Results of the analysis of variance (Table 2) indicated highly significant ($P < 0.01$) differences for the main effect of the first factor (treatment) in germination percentage (GP), shoot length (SL), seedling fresh weight (FW), seedling dry weight (DW) and root length (RL). Also, highly significant ($P < 0.01$) differences in germination percentage and seedling fresh weight, significant ($P < 0.05$) differences in shoot length, and non-significant ($P > 0.05$) differences in seedling dry weight and root length were observed in the main effect of second factor (25 len-

Table 1. The name, pedigree and origins of 25 lentil genotypes

Code	Name	Accession No.	Pedigree	Donor country
G1	PI 299127	ILL-358	RINV-63-64	Mexico
G2	PI 339319	ILL-560	-	Turkey
G3	L 1278	ILL-2580	-	India
G4	Syrian local cultivar	ILL-4400	-	Syria
G5	Line-340	ILL-4404	-	Pakistan
G6	PRECOZ	ILL-4605	-	Argentina
G7	78S 26013	ILL-5588	ILL 16 selection	Jordan
G8	FLIP 84-51L	ILL-5722	ILL 883 × ILL 470	ICARDA†
G9	81S15	ILL-5883	UJL 197 × ILL 4400	Jordan
G10	FLIP 86-35L	ILL-6021	ILL 4354 × ILL 922	ICARDA
G11	FLIP 86-51L	ILL-6037	ILL 4349 × ILL 4605	ICARDA
G12	FLIP 87-22L	ILL-3212	ILL 4349 × ILL 4605	ICARDA
G13	FLIP 89-63L	ILL-3821	ILL 4225 × ILL 4605	ICARDA
G14	FLIP 89-71L	ILL-3829	ILL 4407 × ILL 4605	ICARDA
G15	FLIP 90-25L	ILL-3994	ILL 5588 × ILL 99	ICARDA
G16	FLIP 92-12L	ILL-7177	ILL 5582 × ILL 707	ICARDA
G17	FLIP 92-36L	ILL-7201	ILL 5879 × ILL 5714	ICARDA
G18	FLIP 95-30L	ILL-7686	-	ICARDA
G19	FLIP 96-15 L	ILL-7947	ILL 6209 × ILL 5671	ICARDA
G20	FLIP 96-46 L	ILL-7978	-	ICARDA
G21	FLIP 96-50 L	ILL-7982	-	ICARDA
G22	Bari Masur 4	ILL-8006	ILL 5888 × ILL 5782	Bangladesh
G23	CIFIC	ILL-10837	-	Turkey
G24	Bari Masur 6	ILL-10848	ILL 5888 × ILL 8008	Bangladesh
G25	Local check	-	-	Iran

†ICARDA, International Center for Agricultural Research in the Dry Areas

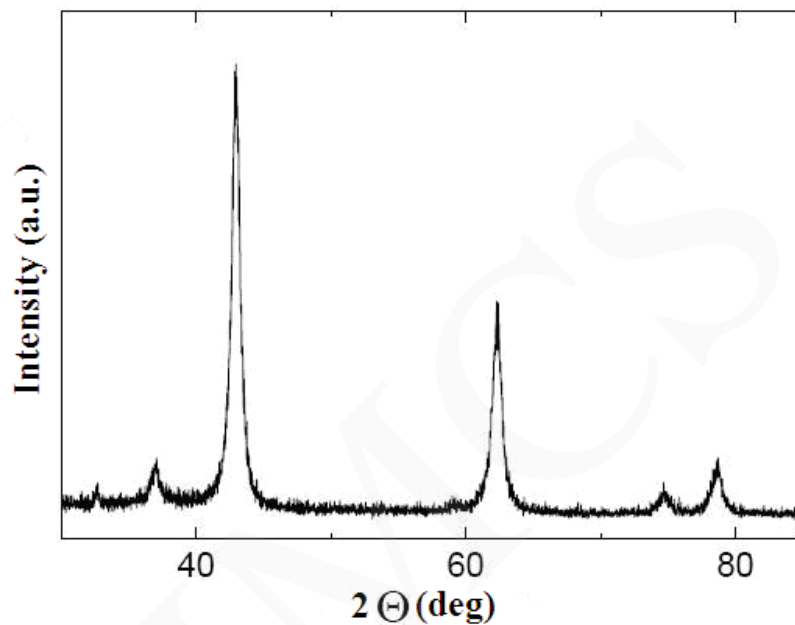


Fig. 1. X-ray diffraction pattern of nano-silicon dioxide particles.

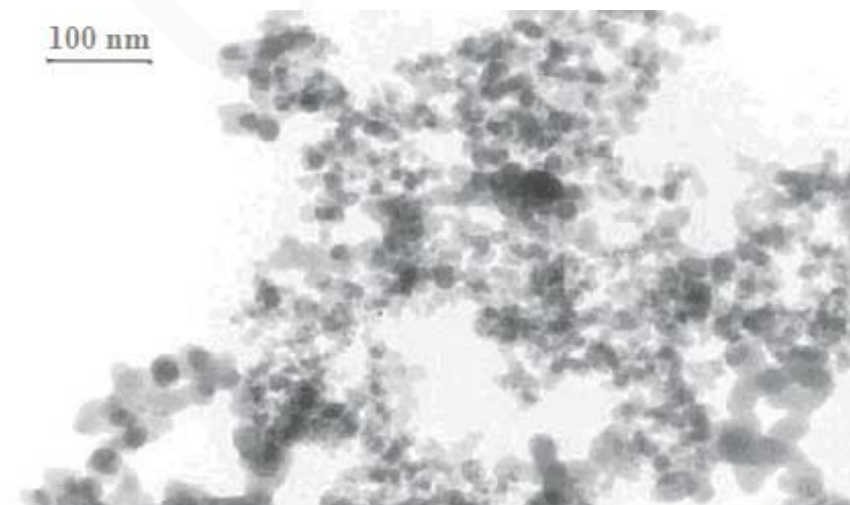


Fig. 2. Large area TEM image of SiO₂ nano-particles.

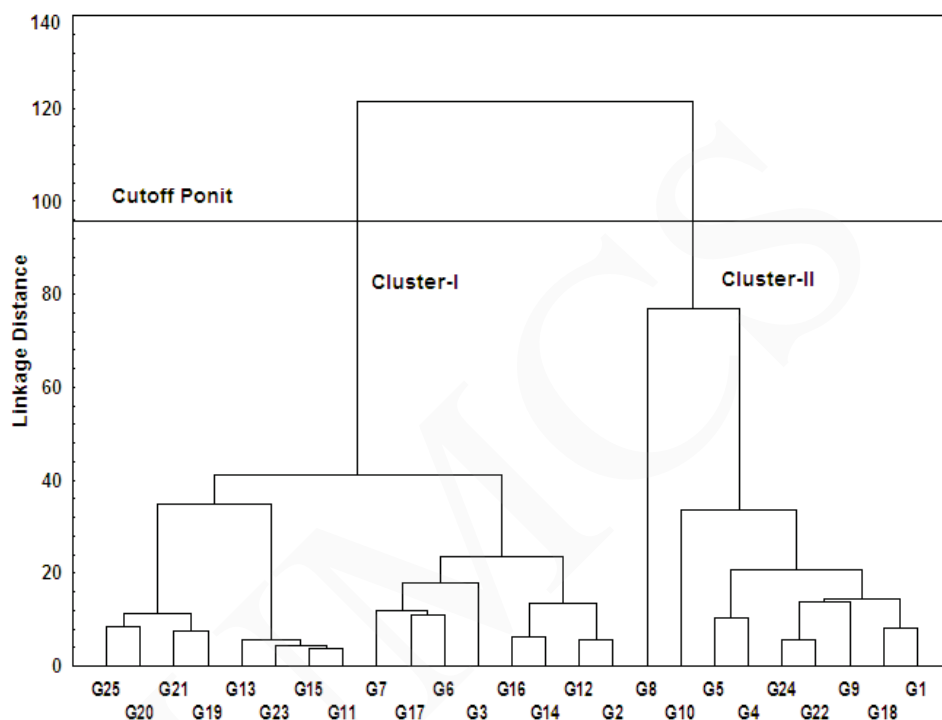


Fig. 3. Dendrogram of cluster analysis for 25 lentil genotypes using the Ward method.

til genotypes) in the present study (Table 2). The interaction effect between two factors (treatment \times lentil genotype or $T \times G$ interaction) was highly significant ($P < 0.01$) in germination percentage and seedling fresh weight, significant ($P < 0.05$) in shoot length, and non-significant ($P > 0.05$) in seedling dry weight and root length (Table 2). Considering the significant $T \times G$ interaction for most of the measured traits, comparisons of means were performed only on combinations of two factors. The results revealed that most of the traits exhibited wide range of variability regarding coefficient of variation (CV) magnitudes (Table 2). The CV values ranged from 6.78 (in germination percentage) to 30.95% (in seedling fresh weight).

The results of means' comparisons further revealed that most of the traits indicated wide range of variability (Table 3). The germination percentage showed significant differences among genotypes in T_1 (distilled water) with genotypes G4 and G24 having the highest percentage (97.03 and 96.59%, respectively). In T_2 (100 mM NaCl), genotypes G1 and G4 having the highest germination percentage (77.33 and 76.80%, respectively) while genotype G17 had the lowest germination percentage (30.35%) under salinity stress. The germination percentage indicated

Table 2. Analysis of variance for measured traits in 25 lentil genotypes

Sources of variation	DF†	GP	SL	RL	FW	DW
Treatment (T)	2	23132.27**	7054.68**	3197.89**	42416.17**	1799.02**
Genotype (G)	24	598.58**	456.52**	276.61**	1391.04ns	27.70**
T×G interaction	48	123.94**	58.39*	81.33**	1252.17ns	8.51ns
Error	150	24.31	38.89	28.18	1171.26	8.38
CV‡		6.78	13.74	17.80	30.95	14.71

†DF, Degrees of freedom, CV‡, Coefficient of variation

** Significant on 0.01 level, * Significant on 0.05 level and ns – Non-significant

Traits are: germination percentage (GP), shoot length (SL), root length (RL), seedling fresh weight (FW) and seedling dry weight (DW).

significant differences among lentil genotypes in T3 (1 mM nano-silicon dioxide plus 100 mM NaCl) with genotype G1 (92.17%) following to genotype G4 having the highest germination percentage (88.67%). It is clear that adding SiO₂ nano-particles improved germination percentage under salinity stress and germination percentage was increased in comparison to T2 conditions (Table 3).

The shoot length (SL) of seedling indicated significant differences among genotypes in T1 (distilled water) with genotype G10 following to genotypes G1 and G18 having the longest shoot (75.79, 67.33 and 65.67 mm, respectively) while genotype G25 had the shortest shoot (33.33 mm) among lentil genotypes (Table 3). Salinity stress existence (T2) decreased shoot length and genotype G10 had the longest shoot (56.26 mm) followed by genotype G4 with 48.79 mm while genotype G16 had the shortest shoot (22.67 mm). Nano-silicon dioxide application under salinity stress (1 mM nano-silicon dioxide plus 100 mM NaCl) alleviated the negative effects of salt and the longest shoot (65.61 mm) was belonged to G10 with 17% increase in comparison with T2 while genotypes G20 (32.5 mm) and G25 (31.67 mm) had the shortest shoot (Table 3).

The root length (RL) showed significant differences among lentil genotypes in T1 (distilled water) with genotype G10 had the highest values (51.00 mm) (Table 4). In T2 (100 mM NaCl), genotypes G5 and G10 having the highest values for root length (34.57 and 42.33 mm, respectively) while genotypes G15 and G16 had the lowest values for root length (37.50 and 38.33 mm, respectively) under salinity stress. The root length indicated significant differences among lentil genotypes in T3 (1 mM nano-silicon dioxide plus 100 mM NaCl) with genotypes G7 and G10 (41.59 and 47.00 mm, respectively) while genotype G20 had the lowest values for root length (16.83 mm) under application of nano-silicon dioxide in

Table 3. Mean values for the germination percentage and shoot length in lentil genotypes via LSD (least significant differences)

	Germination percentage (%)						Shoot length (mm)			
	T1†	T2	T3	T1	T2	T3	T1	T2	T3	
G1	94.33	77.33	92.17	A	67.33	AB	40.17	BCDE	49.45	BCD
G2	88.67	55.80	72.18	EFGH	53.33	DEFG	37.77	CDEF	45.87	BCDEF
G3	88.67	46.76	71.80	GHIJK	61.33	BCD	41.67	BCDE	49.5	BCD
G4	97.03	76.80	88.67	A	62.21	BCD	48.79	AB	50.59	BCD
G5	87.45	72.76	80.91	AB	54.12	DEFG	46.1	BC	49.41	BCD
G6	85.37	47.39	64.01	FHIJK	48.04	FG	32.72	DEFGHI	46.68	BCDE
G7	80.33	58.81	69.66	DEFG	57.37	BCDEF	35.01	DEFG	55.45	B
G8	88.94	59.21	73.21	CDEF	55.87	CDEFG	38.82	CDE	43.7	CDEF
G9	90.67	61.46	73.62	BCDE	54.33	DEFG	37.65	CDEF	48.46	BCDE
G10	96.35	69.76	84.06	ABCD	75.79	A	56.26	A	65.61	A
G11	82.33	46.83	65.21	GHIJK	46.33	GH	42.33	BCD	45.74	BCDEF
G12	90.67	52.00	83.34	EFGHI	59	BCDE	33	DEFGHI	44	CDEF
G13	87.48	43.02	70.38	IJK	57.67	BCDEF	33.67	DEFGH	43.83	CDEF
G14	87.48	51.99	70.80	EFGHI	54.67	DEFG	37.67	CDEF	40.67	DEFG
G15	90.49	39.24	66.54	JKL	57	BCDEFG	28.33	FGHIJ	46.33	BCDEF
G16	93.47	50.42	75.07	EFGHIJ	53	DEFG	22.67	J	44.33	CDEF

ctnd. Table 3

G17	89.16	DEFGH	30.35	L	62.21	HIJ	51.67	DEFG	35.67	DEF	46	BCDEF
G18	93.14	ABCDE	72.95	AB	80.71	C	65.67	ABC	35.33	DEF	50.83	BC
G19	88.73	EFGH	44.27	HIJK	70.53	DEF	36	HI	28.67	FGHIJ	39.33	EFG
G20	88.43	FGH	44.26	HIJK	60.33	IJ	47.67	FG	25.33	GHIJ	32.5	G
G21	86.67	GHI	44.36	HIJK	72.09	DE	49.67	EFG	23.33	IJ	36.67	FG
G22	95.99	AB	71.96	AB	82.79	C	60	BCDE	32.33	EFGHIJ	46	BCDEF
G23	91.95	BCDEF	37.48	KL	66.89	FGH	59.33	BCDE	33.67	DEFGH	48.17	BCDE
G24	96.59	A	71.52	ABC	82.57	C	50	EFG	34.33	DEFGH	46.83	BCDE
G25	87.82	FGH	44.02	HIJK	59.05	J	33.33	I	25	HIJ	31.67	G

†T1, distilled water; T2, 100 mM NaCl; T3, 1 mM nano-silicon dioxide plus 100 mM NaCl

salinity stress condition (Table 4). Similar to germination percentage and seedling shoot length traits, the root length is influenced and improved by application of nano-SiO₂ in salinity stress condition.

The seedling fresh weight indicated significant differences among lentil genotypes in T1 (distilled water) with genotype G10 had the highest weight (148.50 mg) while genotype G5 (112.90 mg) had the lowest weight (Table 4). Salinity stress (T2) decreased seedling fresh weight and genotype G1 (100.00 mg) had the heaviest seedling fresh weight while genotype G3 with 62.38 mg had the lightest seedling fresh weight. Nano-silicon dioxide application under salinity stress (1 mM nano-silicon dioxide at 100 mM NaCl) improved the negative effects of salinity and the heaviest seedling fresh weight (133.30 mg) belonged to G8 and the lightest seedling fresh weight (95.49 mg) belonged to G6 (Table 4). However, negative effects of salinity stress on seedling fresh weight were decreased through adding of nano-SiO₂ particles.

The results of means' comparisons further revealed that seedling dry weight (DW) trait indicated relatively wide range of variability (Table 5). The seedling dry weight showed significant differences among genotypes in T1 (distilled water) with genotype G2 had the highest seedling dry weight (27.33 mg) while with genotype G3 had the lowest seedling dry weight (21.03 mg). In T2 (100 mM NaCl), genotype G1 following to genotypes G6 and G20 had the highest seedling dry weight (22.00, 19.77 and 19.26 mg, respectively) while genotype G3 had the lowest seedling dry weight (10.27 mg) under salinity stress. The seedling dry weight indicated significant differences among lentil genotypes in T3 (1 mM nano-silicon dioxide plus 100 mM NaCl) with genotype G1 (24.66 mg) following to genotypes G2, G8, G17 and G20 having the highest seedling dry weight (22.33, 21.61, 22.29 and 22.62 mg, respectively). Genotype G12 had the lowest seedling dry weight (16.81 mg) under T3 condition (Table 5). Similarly to other traits, the seedling dry weight is influenced positively and improved by application of nano-SiO₂ in salinity stress condition. Dendrogram of cluster analysis using the Ward method (Fig. 3) grouped 25 lentil genotypes into two main groups as; cluster-I including 16 genotypes and cluster-II consisting of 9 genotypes (G1, G4, G5, G8, G9, G10, G18, G22 and G24).

DISCUSSION

Early lentil crop growth decrease in seed germination and important seedling characteristics caused by salinity stress and improvement of the mentioned properties caused by application of the SiO₂ nano-particles can be observed from results of the present research. Analysis of variance indicated that solution treatment type × lentil genotype interaction affected most of the measured traits except seedling fresh and dry weight. The SiO₂ nano-particles application had a signi-

Table 4. Mean values for the root length and seedling fresh weight in 25 lentil genotypes which were tested via LSD (least significant differences)

	Root length (mm)			Seedling fresh weight (mg)		
	T1	T2	T3	T1	T2	T3
G1	47.33	17.78	38.14	139.30	100.00	115.30
G2	27.00	32.33	24.39	114.80	82.28	99.53
G3	42.67	25.92	34.29	114.10	62.38	97.60
G4	42.66	29.92	32.94	132.50	79.73	111.80
G5	31.25	34.57	35.22	112.90	89.50	103.00
G6	36.03	24.54	35.01	127.80	71.38	95.49
G7	43.03	26.26	41.59	122.00	84.63	109.00
G8	41.90	26.82	27.61	141.10	67.92	133.30
G9	41.08	19.67	28.50	145.90	91.07	119.10
G10	51.00	42.33	47.00	148.50	87.76	117.80
G11	27.00	18.33	28.00	127.70	93.48	116.80
G12	36.67	17.00	30.83	126.00	68.67	112.00
G13	29.67	26.33	23.33	138.30	97.59	110.90
G14	31.00	17.67	24.67	125.20	83.00	112.70
G15	37.50	14.67	30.63	137.00	78.50	119.80

ctnd. Table 4

G16	38.33	BCDEF	14.33	I	29.33	DEFGH	130.00	ABCDEF	88.33	ABCDE	109.90	DEF
G17	37.00	CDEFG	26.00	CDEFG	31.92	CDEF	136.80	ABCD	75.72	CDEFG	110.40	DEF
G18	35.33	CDEFGH	30.33	BCD	34.13	CDE	146.90	AB	87.07	ABCDEF	109.00	DEF
G19	31.33	DEFGHI	17.67	GHI	27.33	EFGH	133.90	ABCDEF	86.45	ABCDEF	107.50	EFG
G20	28.33	FGHI	16.33	HI	16.83	I	132.30	ABCDEF	92.08	ABC	109.70	DEF
G21	39.33	BCDE	17.33	HI	25.46	FGH	126.00	BCDEF	77.67	BCDEFG	103.20	FGHI
G22	45.58	ABC	17.33	HI	32.17	CDEF	126.70	BCDEF	97.00	AB	109.50	DEF
G23	26.67	HI	17.00	HI	33.50	CDE	125.40	CDEF	93.55	ABC	113.00	BCDE
G24	25.00	I	23.67	DEFGH	31.17	CDEFG	135.30	ABCDE	88.53	ABCDE	113.90	BCDE
G25	24.50	I	18.67	EFGHI	22.96	HI	126.70	BCDEF	86.59	ABCDEF	106.70	EFGH

†T1, distilled water; T2, 100 mM NaCl; T3, 1 mM nano-silicon dioxide plus 100 mM NaCl

Table 5. Mean values for the seeding dry weight (mg) in 25 lentil genotypes which were tested via LSD (least significant differences)

	T1		T2		T3	
G1	26.00	AB	22.00	A	24.66	A
G2	27.33	A	14.98	CDEF	22.33	AB
G3	21.03	B	10.27	G	19.49	BCDEFGH
G4	25.42	AB	12.61	DEFG	19.68	BCDEFGH
G5	22.64	AB	12.02	EFG	17.42	GH
G6	25.15	AB	19.77	AB	20.68	BCDEF
G7	26.12	AB	15.59	BCDEF	21.24	BCD
G8	26.60	AB	11.91	FG	21.61	ABC
G9	26.14	AB	12.00	EFG	20.32	BCDEFG
G10	22.71	AB	11.93	FG	18.13	DEFGH
G11	26.94	AB	14.04	DEFG	20.86	BCDE
G12	22.96	AB	15.32	CDEF	16.81	H
G13	23.41	AB	12.88	DEFG	17.52	FGH
G14	23.43	AB	14.02	DEFG	20.37	BCDEFG
G15	21.57	AB	16.78	BCD	19.92	BCDEFGH
G16	21.97	AB	16.28	BCDE	19.90	BCDEFGH
G17	25.41	AB	16.38	BCD	22.29	AB
G18	26.45	AB	14.23	DEFG	20.49	BCDEFG
G19	24.70	AB	13.95	DEFG	17.94	EFGH
G20	27.09	AB	19.26	ABC	22.62	AB
G21	25.33	AB	13.48	DEFG	17.84	EFGH
G22	24.56	AB	15.70	BCDEF	17.83	EFGH
G23	21.94	AB	13.88	DEFG	18.23	DEFGH
G24	24.29	AB	12.57	DEFG	21.03	BCDE
G25	22.42	AB	15.04	CDEF	18.55	CDEFGH

†T1, distilled water; T2, 100 mM NaCl; T3, 1 mM nano-silicon dioxide plus 100 mM NaCl

ficant effect on all traits including germination percentage, shoots length, root length, seedling fresh weight and seedling dry weight and caused a significant increase in them under salinity stress. However, a significant improvement of the measured traits was observed when the SiO₂ nano-particles were applied on most of the lentil genotypes.

In this study, seedling shoot and dry weight traits of lentil genotypes were affected by salinity stress. Osmotic potential increases due to accumulation of salt in the plant cells and turgor pressure decreases due to the water absorption reduction and so cells growth decreases. Accordingly, the mineral absorption largely reduces in salinity stress and results in cell size and number reduction and salt stress inhibits growth and division of crop cells and results in cell death (Munns 2002). Although, sodium ion concentration increased in crop shoot as the result of salinity stress, but application of the SiO₂ nano-particles reduces its concentration in plant tissues (Kalte et al. 2014). Also, salinity stress affects crop growth due to toxicity of sodium ion, but adding of the nano-SiO₂ can decrease its toxicity and cause the improvement of crop growth (Savvas et al. 2009). However, application of nano-SiO₂ increased shoot fresh and dry weight under salinity stress and these results are in good agreement with the findings of Gao et al. (2006) in maize and Kalte et al. (2014) in basil.

Silicon increases sustainability of cell wall by forming a layer (Marschner 2011), and its nano-particle form with extra-large surface can affect xylem humidity and water translocation which results in water use efficiency improvement. Also, nano-silicon acts as a delivering agent of genetic material and chemicals into plants as well as animals cell and tissue (Torney et al. 2007). Seed germination prepares a proper base for plant growth and in this study, application of nano-SiO₂ increases seed potential by increasing the seed germination properties under stress condition. These results agree with the findings of Nair et al. (2011), who observed better seed germination of rice in the presence of nano-SiO₂. Also, improving of seed germination behavior and seedling properties as a result of nano-SiO₂ demonstrated that it may act like bulk silicon, whose role and mechanisms on seed germination were investigated previously. The results of the present study coupled with the reports in the literature strongly suggest that silicon and nano-SiO₂ may be involved in the tolerance to biotic and abiotic stresses in higher plants.

In this experiment it has been shown that salinity stress in lentil causes significant reductions in traits germination percentage, shoots length, root length, seedling fresh weight and seedling dry weight. An increase in seed germination under salinity stress may be due to the absorption and utilization of nano-SiO₂ by seeds (Suriyaprabha et al. 2012b). Data presented in Tables 4 and 5 reveal that the application of nano-SiO₂ had a significant effect on seedling fresh and dry weight

and increased them under salinity stress. Suriyaprabha et al. (2012a) reported that nano-SiO₂ increased plant dry weight as well as magnitudes of organic materials such as proteins in maize. We observed high variation among 25 lentil genotypes in salt tolerance as well as response to nano-SiO₂ application which is supported by the present data shown in this study regarding the measured traits and based on Marschner (2011), huge genotypic difference exists in salt tolerance between different plants and plant species. However, inclusion of nano-SiO₂ significantly alleviated the salt toxicity in most of examined lentil genotypes and improved their early growth compared to the corresponding plants treated with salt alone.

Application of silicon enhanced chitinase activity in cucumber after infection with *Pythium* spp. (Cherif et al. 1994), altered metabolic changes in strawberry (Wang and Galletta, 1998), increased antioxidant enzymes activity of bentgrass under drought stress condition (Schmidt et al. 1999), and increased antioxidant enzymes activity of wheat under salinity stress condition (Tuna et al. 2008).

CONCLUSIONS

Generally, results of this research demonstrated that application of nano-SiO₂ benefits plants under salinity stress and it seems that its beneficial effects are more remarkable for stressed plants. So, nano-SiO₂ application must be considered especially in plants faced with stresses and its application under salinity stress significantly increased basil growth characteristics of lentil genotypes. In this experiment, 1 mM nano-silicon dioxide particles application (T3) under 100 mM NaCl salinity stress was as the best treatment for plant growth and improvement. It seems that nano-materials can be potentially applied in the crop production, especially under salinity stress. Pre-sowing seed treatments with some beneficial nanomaterials such as nano-SiO₂, application of nano-fertilizers, seed coating with nanomaterials, nano-water absorbent and etc. may result in enhanced germination and invigorated seedling growth.

REFERENCES

1. Ahmad R., Zaheer S.H., Ismail S. 1992. Role of silicon in salt tolerance of wheat (*Triticum aestivum* L.). *Plant Science*, 85: 43–50.
2. AOSA (1991): Association of Official Seed Analysis (AOSA) 1991. Rules for testing seeds. *Seed Science and Technology*, 12: 18–19.
3. Chen H.J., Chen J.Y., Wang S.J. 2008. Molecular regulation of starch accumulation in rice seedling leaves in response to salt stress. *Acta Physiologiae Plantarum*, 30: 135–142.
4. Cherif M., Asselin A., Belanger R.R. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology*, 84: 236–242.
5. Epstein E. 2009. Silicon: Its manifold roles in plants. *Annals of Applied Biology*, 155: 155–160.

6. FAO 2008. Food and Agriculture Organization. Land and plant nutrition management service. <http://www.fao.org/ag/agl/agll/spush>.
7. Gao X., Zou C.H., Wang L., Zhang F. 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. *Journal of Plant Nutrition*, 29: 1637–1647.
8. Haghighi, M., Afifpour, Z., Mozafarian, M. 2012. The effect of N-Si on tomato seed germination under salinity levels. *Journal of Biological & Environmental Sciences*, 6: 87–90.
9. Kalteh M., Alipour Z.T., Ashraf S., Aliabadi M.M., Nosratabadi A.F. 2014. Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress. *Journal of Chemical Health Risks*, 4: 49–55.
10. Liang Y., Sun W., Zhu Y.G., Christie P. 2007. Mechanisms of silicon mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution*, 147: 422–428.
11. Liang, Y.C., Zhang, W.Q., Chen, J., Ding, R. 2005. Effect of silicon on H⁺-ATPase and H⁺-Pase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt stressed barley (*Hordeum vulgare* L.). *Environmental and Experimental Botany*, 53: 29–37.
12. Lu, C.M., Zhang, C.Y., Wen, J.Q., Wu, G.R., Tao, M.X. 2002b. Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism. *Soybean Science*, 21: 168–172.
13. Marschner H. 2011. Marschner's Mineral Nutrition of Higher Plants. 3rd edition. Academic Press, US.
14. Minitab Inc. 2005. Minitab User's Guide, vers. 14. Minitab Inc, Harrisburg, Pennsylvania, USA.
15. Munns R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment*, 25: 239–250.
16. Nair, R., Poulouse, A.C., Nagaoka, Y., Yoshida, Y., Maekawa, T., Kumar, D.S. 2011. Uptake of FITC labeled silica nanoparticles and quantum dots by rice seedlings: effects on seed germination and their potential as biolables for plants. *Journal of Fluorescence*, 21: 2057–2068.
17. Ruffini, C.M., Cremonini, R. 2009. Nanoparticles and higher plants. *Caryologia*, 62: 161–165.
18. Sabaghnia N., Sbaghpour S.H., Dehghani H. 2008. The use of an AMMI model and its parameters to analyse yield stability in multi-environment trials. *The Journal of Agricultural Science*, 146: 571–581.
19. Sabaghpour S.H., Safikhni M., Sarker A., Ghaffri A., Ketata H. 2004. Present status and future prospects of lentil cultivation in Iran. Proc. 5th European Conference on Grain Legumes, 7–11 June, Dijon, France.
20. Sairam R.K., Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, 86: 407–421.
21. SAS Ins. 2004. SAS/STAT User's Guide. SAS Institute Inc., Cary, NC, USA.
22. Savvas D., Giotis D., Chatzieustratiou E., Bakea M., Patakioutas G. 2009. Silicon supply in soilless cultivations of zucchini alleviates stress induced by salinity and powdery mildew infections. *Environmental and Experimental Botany*, 65: 11–17.
23. Schmidt R.E., Zhang X., Chalmers D.R. 1999. Response of photosynthesis and superoxide dismutase to silica applied to creeping bentgrass grown under two fertility levels. *Journal of Plant Nutrition*, 22: 1763–1773
24. Suriyaprabha R., Karunakaran G., Yuvakkumar R., Prabu P., Rajendran V., Kannan N. 2012a. Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil. *Journal of Nanoparticle Research*, 14: 1294–1296.
25. Suriyaprabha R., Karunakaran G., Yuvakkumar R., Rajendran V., Kannan N. 2012b. Silica nanoparticles for increased silica availability in maize (*Zea mays* L.) seeds under hydroponic conditions. *Current Nanoscience*, 8: 1–7.

26. Torney F., Trewyn B.G., Lin V.S.Y., Wang K. 2007. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nature Nanotechnology*, 2: 295–300.
27. Tuna A. L., Kaya C., Higgs D., Murillo-Amador B., Aydemir S., Girgin A.R. 2008. Silicon improves salinity tolerance in wheat plants. *Environmental and Experimental Botany*, 62: 10–16.
28. Wang S.Y., Galletta G.J. 1998. Foliar application of potassium silicate induces metabolic changes in strawberry plants. *Journal of Plant Nutrition*, 21: 157–167.
29. Yadav S., Irfan M., Ahmad A., Hayat S. 2011. Causes of salinity and plant manifestations to salt stress: a review. *Journal of Environmental Biology*, 32: 667–685.