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

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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 SiO<sub>2</sub> nano-particles on plants under salt stress. The experiment was a 3×25 factorial arrangement with three levels of treatment solutions as (T<sub>1</sub>) distilled water as control, (T<sub>2</sub>) 100 mM NaCl concentration and (T<sub>3</sub>) 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 SiO<sub>2</sub> 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 SiO<sub>2</sub> 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-SiO<sub>2</sub>, salt stress, stress response, tolerance, variability

### STRESZCZENIE

Dwadzieścia pięć genotypów soczewicy (*Lens culinaris* Medik.) badano w celu oceny działania nanocząstek SiO<sub>2</sub> na rośliny poddane stresowi zasolenia. Eksperyment przeprowadzono dla 25 genotypów w trzech wariantach doświadczenia: (T<sub>1</sub>) woda destylowana jako kontrola, (T<sub>2</sub>) NaCl o stężeniu 100 mM i (T<sub>3</sub>) 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  $\text{SiO}_2$  może poprawić kiełkowanie i wczesny wzrost siewek podanych stresowi zasolenia, a badane parametry były lepsze u wszystkich genotypów soczewicy. Podsumowując, zastosowanie nanocząstek  $\text{SiO}_2$  było korzystne dla poprawy tolerancji zasolenia siewek soczewicy, a ich zastosowanie może stymulować różne mechanizmy obronne roślin na toksyczność soli.

**Słowa kluczowe:** nano- $\text{SiO}_2$ , 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 ( $\text{TiO}_2$ ) and silicon dioxide ( $\text{SiO}_2$ ). 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,  $20 \pm 2$  °C temperature and relative humidity of 75%).  $\text{SiO}_2$  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  $\text{m}^2/\text{g}$ . 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

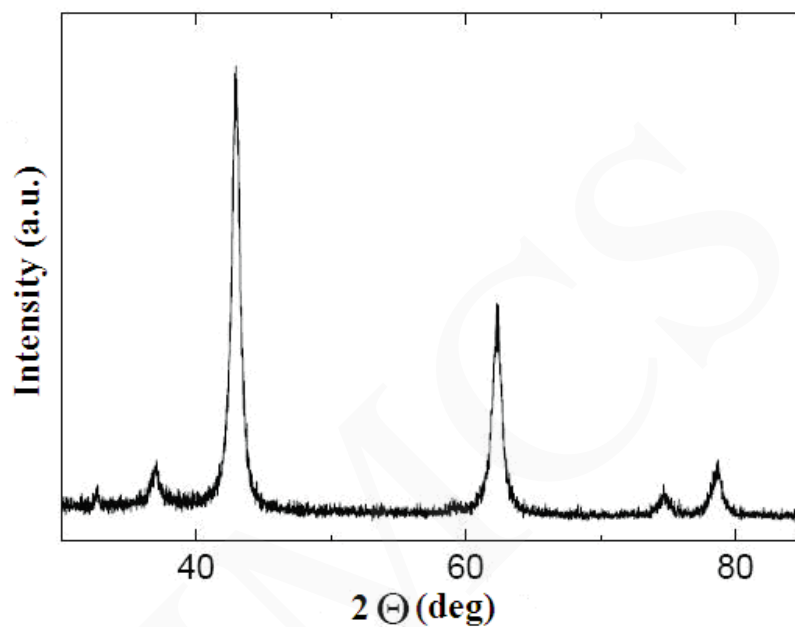


Fig. 1. X-ray diffraction pattern of nano-silicon dioxide particles.

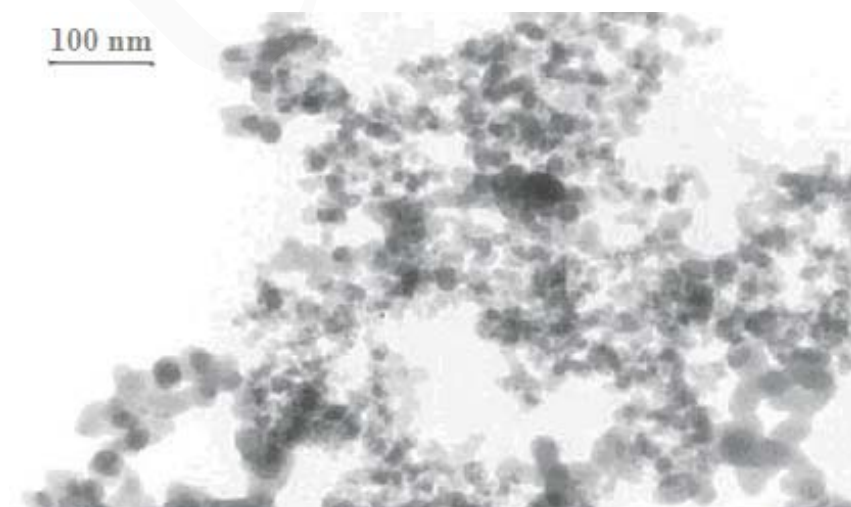


Fig. 2. Large area TEM image of SiO<sub>2</sub> nano-particles.

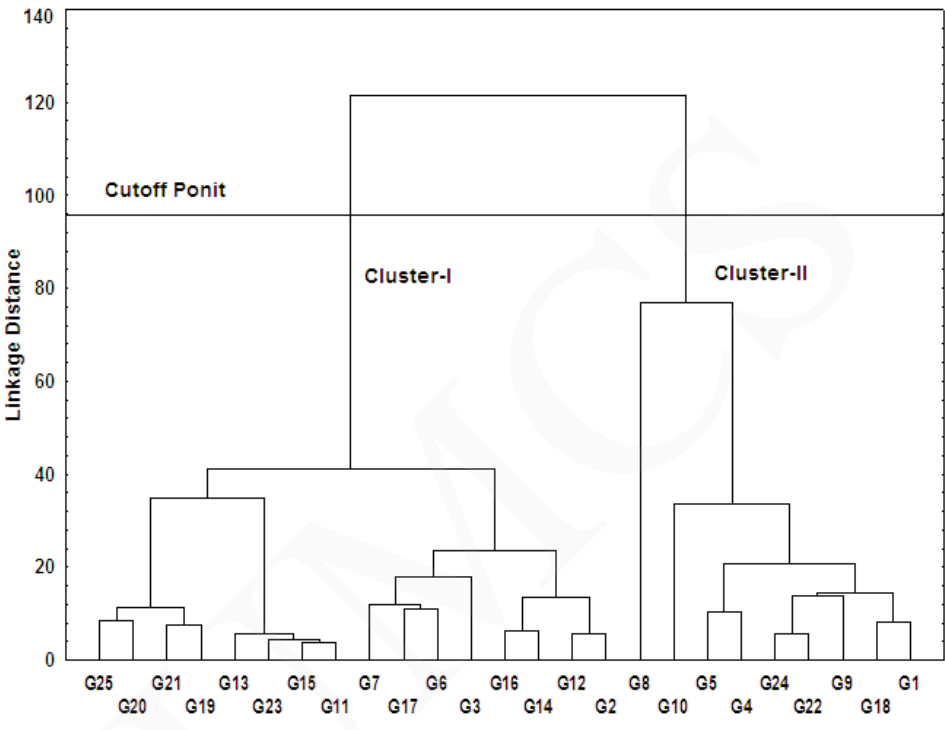


Fig. 3. Dendrogram of cluster analysis for 25 lentil genotypes using the Ward method.

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 SiO<sub>2</sub> 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             |
| G1  | 94.33<br>ABC               | 77.33<br>A      | 92.17<br>A   | 67.33<br>AB       | 40.17<br>BCDE   | 49.45<br>BCD   |
| G2  | 88.67<br>EFGH              | 55.80<br>EFGH   | 72.18<br>DE  | 53.33<br>DEFG     | 37.77<br>CDEF   | 45.87<br>BCDEF |
| G3  | 88.67<br>EFGH              | 46.76<br>GHIJK  | 71.80<br>DE  | 61.33<br>BCD      | 41.67<br>BCDE   | 49.5<br>BCD    |
| G4  | 97.03<br>A                 | 76.80<br>A      | 88.67<br>AB  | 62.21<br>BCD      | 48.79<br>AB     | 50.59<br>BCD   |
| G5  | 87.45<br>FGH               | 72.76<br>AB     | 80.91<br>C   | 54.12<br>DEFG     | 46.1<br>BC      | 49.41<br>BCD   |
| G6  | 85.37<br>HI                | 47.39<br>FGHIJK | 64.01<br>HI  | 48.04<br>FG       | 32.72<br>DEFGHI | 46.68<br>BCDE  |
| G7  | 80.33<br>J                 | 58.81<br>DEFG   | 69.66<br>EFG | 57.37<br>BCDEF    | 35.01<br>DEFG   | 55.45<br>B     |
| G8  | 88.94<br>EFGH              | 59.21<br>CDEF   | 73.21<br>DE  | 55.87<br>CDEFG    | 38.82<br>CDE    | 43.7<br>CDEF   |
| G9  | 90.67<br>CDEFG             | 61.46<br>BCDE   | 73.62<br>DE  | 54.33<br>DEFG     | 37.65<br>CDEF   | 48.46<br>BCDE  |
| G10 | 96.35<br>AB                | 69.76<br>ABCD   | 84.06<br>BC  | 75.79<br>A        | 56.26<br>A      | 65.61<br>A     |
| G11 | 82.33<br>IJ                | 46.83<br>GHIJK  | 65.21<br>GH  | 46.33<br>GH       | 42.33<br>BCD    | 45.74<br>BCDEF |
| G12 | 90.67<br>CDEFG             | 52.00<br>EFGHI  | 83.34<br>C   | 59<br>BCDE        | 33<br>DEFGHI    | 44<br>CDEF     |
| G13 | 87.48<br>FGH               | 43.02<br>IJK    | 70.38<br>DEF | 57.67<br>BCDEF    | 33.67<br>DEFGH  | 43.83<br>CDEF  |
| G14 | 87.48<br>FGH               | 51.99<br>EFGHI  | 70.80<br>DEF | 54.67<br>DEFG     | 37.67<br>CDEF   | 40.67<br>DEFG  |
| G15 | 90.49<br>CDEFG             | 39.24<br>JKL    | 66.54<br>FGH | 57<br>BCDEFG      | 28.33<br>FGHIJ  | 46.33<br>BCDEF |
| G16 | 93.47<br>ABCD              | 50.42<br>EFGHIJ | 75.07<br>D   | 53<br>DEFG        | 22.67<br>J      | 44.33<br>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 | FHIJ   | 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-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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 SiO<sub>2</sub> 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 SiO<sub>2</sub> 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            | AB     | 17.78 | GHI                        | 38.14 | BC    | 139.30 | ABCD   | 100.00 | A       | 115.30 | BCDE  |
| G2  | 27.00            | GHI    | 32.33 | BC                         | 24.39 | GH    | 114.80 | EF     | 82.28  | ABCDEFG | 99.53  | GHI   |
| G3  | 42.67            | ABC    | 25.92 | CDEFG                      | 34.29 | CDE   | 114.10 | EF     | 62.38  | G       | 97.60  | HI    |
| G4  | 42.66            | ABC    | 29.92 | BCD                        | 32.94 | CDE   | 132.50 | ABCDEF | 79.73  | BCDEFG  | 111.80 | CDEF  |
| G5  | 31.25            | DEFGHI | 34.57 | AB                         | 35.22 | BCD   | 112.90 | F      | 89.50  | ABCD    | 103.00 | FGHI  |
| G6  | 36.03            | CDEFGH | 24.54 | CDEFGH                     | 35.01 | BCD   | 127.80 | ABCDEF | 71.38  | DEFG    | 95.49  | I     |
| G7  | 43.03            | ABC    | 26.26 | BCDEF                      | 41.59 | AB    | 122.00 | DEF    | 84.63  | ABCDEF  | 109.00 | DEF   |
| G8  | 41.90            | ABC    | 26.82 | BCDE                       | 27.61 | EFGH  | 141.10 | ABCD   | 67.92  | FG      | 133.30 | A     |
| G9  | 41.08            | ABCD   | 19.67 | EFGHI                      | 28.50 | DEFGH | 145.90 | ABC    | 91.07  | ABCD    | 119.10 | B     |
| G10 | 51.00            | A      | 42.33 | A                          | 47.00 | A     | 148.50 | A      | 87.76  | ABCDEF  | 117.80 | BCD   |
| G11 | 27.00            | GHI    | 18.33 | FGHI                       | 28.00 | EFGH  | 127.70 | ABCDEF | 93.48  | ABC     | 116.80 | BCD   |
| G12 | 36.67            | CDEFGH | 17.00 | HI                         | 30.83 | DEFG  | 126.00 | BCDEF  | 68.67  | EFG     | 112.00 | BCDEF |
| G13 | 29.67            | EFGHI  | 26.33 | BCDEF                      | 23.33 | HI    | 138.30 | ABCD   | 97.59  | AB      | 110.90 | CDEF  |
| G14 | 31.00            | DEFGHI | 17.67 | GHI                        | 24.67 | GH    | 125.20 | CDEF   | 83.00  | ABCDEF  | 112.70 | BCDE  |
| G15 | 37.50            | BCDEF  | 14.67 | I                          | 30.63 | DEFG  | 137.00 | ABCD   | 78.50  | BCDEFG  | 119.80 | BC    |

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 SiO<sub>2</sub> 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 SiO<sub>2</sub> 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-SiO<sub>2</sub> can decrease its toxicity and cause the improvement of crop growth (Savvas et al. 2009). However, application of nano-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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-SiO<sub>2</sub>. Also, improving of seed germination behavior and seedling properties as a result of nano-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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-SiO<sub>2</sub> by seeds (Suriyaprabha et al. 2012b). Data presented in Tables 4 and 5 reveal that the application of nano-SiO<sub>2</sub> had a significant effect on seedling fresh and dry weight

and increased them under salinity stress. Suriyaprabha et al. (2012a) reported that nano-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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-SiO<sub>2</sub> 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-SiO<sub>2</sub> benefits plants under salinity stress and it seems that its beneficial effects are more remarkable for stressed plants. So, nano-SiO<sub>2</sub> 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-SiO<sub>2</sub>, application of nano-fertilizers, seed coating with nanomaterials, nano-water absorbent and etc. may result in enhanced germination and invigorated seedling growth.

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