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GRAPHIC ANALYSIS OF NANO-SILICON BY SALINITY STRESS INTERACTION ON GERMINATION PROPERTIES OF LENTIL USING THE BIPLOT METHOD

SUMMARY

Nano particles may be more useful in comparison to bulk materials because they can pass the cell membrane of crops because of their general size. Nano-silicon or SiO_2 is one of the major and frequently used engineered oxide nano particles in deferent fields. Quick germination of cultivated seeds and good seedling establishment are key factors to lentil production under salinity stress soil across arid and semi-arid regions. The present research was aimed at investigating the impacts of various nano-silicon dioxide treatments (0, 1 and 2 mM concentration) on seed germination of lentil under various NaCl concentrations (0, 50, 100 and 150 mM). For better understanding of nano-silicon by salinity stress interaction, the biplot method was used. The first two principal components (PC) axes (PC1 and PC2) explained 86% of the total variation. Results showed that germination significantly delayed by increasing salt stress and application of 1 mM nano-silicon dioxide ($nSiO_2$) could considerably alleviate the adverse effect of salt stress on seed germination percentage, root and shoot length, seedling weight, mean germination time, seedling vigour index and seed reserve mobilization. This investigation results suggested that $nSiO_2$ has favorable effect on lentil seed germination under salinity stress and it can be economic to use suitable concentration of this nano-particle in lentil production under salinity stress. Also, we found that exposure of seeds to SiO_2 nano-particle caused both positive and negative effects on lentil growth.

Key words: Germination rate, nano-particles, salinity stress, seedling vigour, SiO_2

INTRODUCTION

Salinity is one of the most serious problems in planting areas of lentil as it especially grown in arid and semi-arid areas. It is serious problems for crop producers and it is especially true for Iran, where 55% of all the agricultural fields are affected by high soil and water salinities (Haghighi and Pessarakli, 2013). Salinity has destructive effect on the productivity and establishment of most crops in the world and also limits the caloric and nutritional potential of agricultural production (Kumar et al. 2012). It affects water and ion transport throughout the soil–plant–atmosphere and reduces shoot growth and root elongation growth. In addition, the crops must be enable to product satisfactory

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biomass in salinity stresses conditions i.e., should have yield stability. The effect of salinity stress on crops is a reduction in leaf surface area (Parida and Das, 2005) which resulted in decreasing photosynthesis. Salinity decreases photosynthetic rates, but it is not clear whether take places which are responsible for growth reduction through a negative feedback of reduced sink activity causes a lower photosynthetic rate (Heuer, 2005).

Various crops generally need silica to control abiotic stresses such as salinity (Ma, 2004). The silicon reduces toxic metal elevation and increases water-use efficiency as well as photosynthesis rate in crops and also acts as a bioprotectant against biotic stresses such as salinity fungal diseases (Datnoff et al. 1997). Silicon is the second most abundant mineral element in the soil and its ability to ameliorate the negative effect of NaCl on plant growth rate is reported previously (Kafi and Rahimi, 2011). Several studies have reported that salt stressed plants treated with silicon have shown the salt tolerance in different field crops such as barley (Liang et al. 2005) and maize (Parveen and Ashraf, 2010). Recently, different sources of silica are used as fertilizers or applied for promoting the growth of crops while their side effects are still not clear (Epstein, 1999; Savant et al. 1999).

Effective analysis and interpretation of experimental dataset is important at all stages of plant breeding programs. Numerous techniques have been used in the search for an understanding of the data patterns, different methods usually lead to the same or similar conclusions for a given dataset (Sabaghnia, 2012). Yan et al. (2000) developed a genotype main effect plus genotype by environment (GGE) biplot methodology for graphical analysis of multi-environment trials. A biplot is a plot that simultaneously displays the effects of genotypes or entries and the environments or testers and the GGE biplot is a biplot that displays the GGE effects of multi-environment trials. It is constructed by plotting the first two principal components (PC) which are commonly referred to as sites regression models (Yan et al. 2007). However, it can also be equally used for all types of two way data that assume a two-way structure (Yan, 2014). The treatment combinations for example nano-silicon × salinity stress interaction can be generalized as rows and the multiple traits for example germination properties as columns. Yan and Rajcan (2002) used a genotype × trait (GT) biplot, which is an application of the GGE biplot technique to investigate the genotype by trait data and is an excellent tool for visualizing genotype by trait data.

The use of nano-particles has given a lot of attention by the researchers in agricultural subjects, especially by those studying seed properties, although their mechanisms of actions are not well studied (Haghighiet al. 2012). Nano-materials show unique characteristics, they can change physico-chemical properties compared to bulk materials. Nano-particles have greater surface area than bulk materials, and so their solubility tends to be higher (Monica and Cremonini, 2009). Nano-sized silicon enhanced nitrate reductase activity of in soybean (Lu et al. 2002), and reduced salinity stress damages on germination and growth characteristics of tomato (Haghighiet al. 2012). To the our knowledge, this is the first report on the application of nano-silicon as potential agents for generating tolerance in salinity-induced plant stress and improving seed germination characteristics of lentil.

MATERIAL AND METHODS

Seeds of genotypes PI-299127 of lentil (*Lens culinaris* Medik.) from Mexico were used in this research. The average primary seed moisture content was 9.61% and uniform size of them were used in this study. Seeds were sterilized with hypochlorite before germination test and thirty sterilized seeds were transferred onto the two sheets of filter papers inside the petri dishes. Germination evaluated at 20±1 °C in a dark growth chamber with 45% relativity humidity. The experimental design was twice factorial (4×3) arranged in a completely randomized design (CRD) with three replications. Seeds were germinated under three NaCl concentrations (50, 100 and 150 mM) and a control (distilled water) as the first factor Nano-silicon dioxide was procured from Pishgaman of Nanomaterials, Iran, and was applied at three concentrations (0, 1 and 2 mM) at the onset of germination test as the second factor. 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 prticle is displayed in Fig. 1 and the large area TEM image of SiO_2 nano-particles is displayed in Fig. 2.

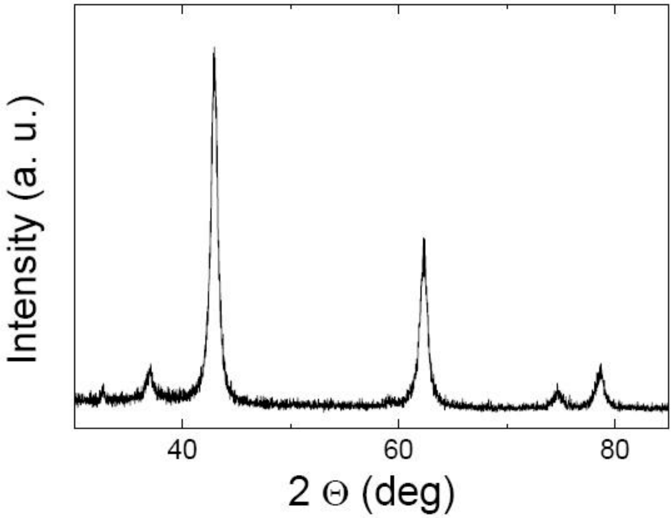


Figure 1. X-Ray diffraction pattern of nano-silicon dioxide particles.

Daily observation for germinating seed continued for ten days and germination percentage (GP) was calculated. The seedlings were evaluated as described in Seedling Evaluation Handbook (AOSA, 1991). Mean germination time (MGT) was calculated according to Ellis and Roberts (1981) as $MGT=\sum T_i N_i/\sum N_i$, where N_i is the number of newly germinated seeds at time T_i.

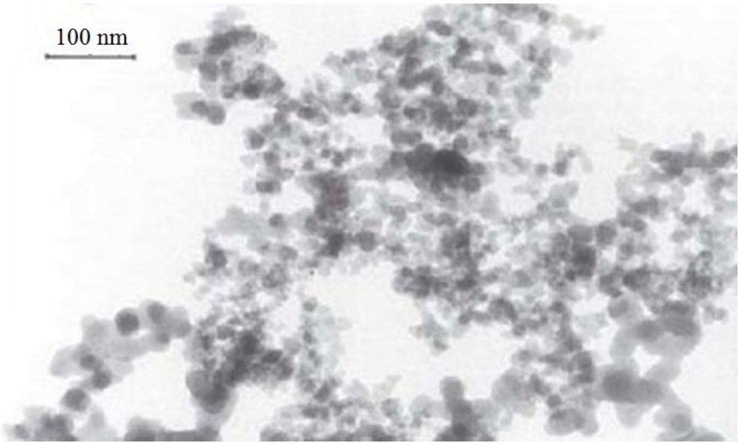


Figure 2. Large area TEM image of SiO_2 nano-particles.

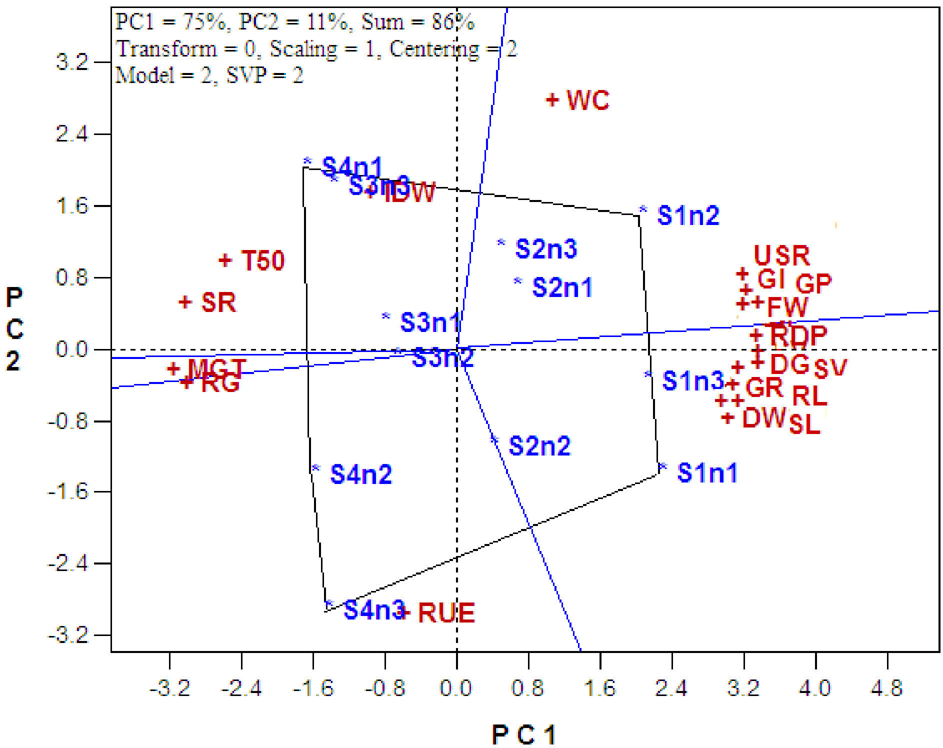
Germination rate (GR) considered as the reciprocal of the mean germination time (Ranal and Santana, 2006). Mean daily germination (DG), calculated as the cumulative percentage of full seed germination at the end of the test, and divided by the number of days from sowing to the end of the test. Temporal distribution of germination can be computed via the germination rate or T_{50} (i.e. the number of days to germination of 50 % of all germinated seeds). T_{50} can be a useful tool for inter-specific comparisons on a quantitative basis. T_{50} was calculated according Coolbear et al. (1984) as $T_{50}= t_i+(t_j-t_i)\times(N/2-n_i)/(n_j-n_i)$, where N is the final number of germinated seeds while nj and n_i are the cumulative number of seeds germinated by adjacent counts at times t_j and t_i, respectively, where $n_i < N/2 < n_j$. The germination index (GI) which expressed as speed of germination was calculated as $GI=\sum(G_t/T_t)$, where G_t is the accumulated number of germinated seeds on day t, and T_t is the time corresponding to G_t in days (Hu et al. 2005). The seed lot having greater germination index is considered to be more vigorous. Also, shoot length (SL), root length (RL), seedling fresh weight (FW), seedling dry weight (DW), initial seed dry weight (IDW) and seed remnant on seedling (SR) were recorded.

Seed reserve utilization was evaluated as delineated by Soltani et al. (2006). After ten days, dry weights of seedlings were determined after drying at 70 °C in the oven to get the constant weight. The weight of utilized reserves of cotyledons (USR) was determined as the primary dry weight of seed minus the dry weight of the seed residue. The seed reserve utilization efficiency (RUE) into seedling tissue was estimated by dividing seedling dry weight by the mobilized stored reserves of seed. The ratio of utilized seed reserve to initial seed dry weight was considered as cotyledons reserve depletion percentage (RDP). Seedling vigour index (SV) was calculated as explained by Abdul-Baki and

Anderson (1973): *VI* = [seedling length (cm) × germination percentage]. Reduction of germination (RG) was determined according to El-Madidi et al. (2004). Seedling water content (WC) was calculated as [(seedling fresh weight-seedling dry weight)/ seedling dry weight].

For better analysis of treatment combination × trait interaction, the biplot analysis as TT biplot (Yan, 2001) was used to determine which treatment combination was best and for what trait. In biplots diagrams the different salinity levels were shown as S1, S2, S3 and S4 (control, 50, 100, 150 mM, respectively), and various concentration of nano-silicon dioxide displayed as n_1, n_2 and n_3 (control, 1 and 2 mM, respectively). The TT biplots were generated using the standardized values of the traits means based on Model 2; dataset was not transformed (Transform=0), within-trait standard deviation standardized (Scale=1), and trait-centred (Centering=2) were used. The polygon pattern were according to treatment-focused singular value partitioning (SVP=2), which is appropriate for visualizing the relationships among traits and treatment combinations. All biplots were generated by the GGEbiplot software (Yan, 2001).

RESULTS AND DISCUSSION



Fiure 3. Lentil treatment combination (salinity × nano-silicon dioxide) by trait biplot showing which treatment combination had the highest values for which traits.

Figure 3 is a TT biplot with a polygon view and it presents the data of four salinity levels on three different nano-silicon dioxide levels (12 treatment combinations) with 18 measured traits. The mentioned polygon view is based on PC axes (PC1 and PC2) which the traits were considered as the tester and the 12 treatment combinations as entries. The two first axes explained 86% of the total variation among the entries due to testers. Fig. 3 shows which treatment combinations (salinity × nano-silicon dioxide) were best at what trait. The treatment combinations at each vertex (vertex treatment combination) of the polygon in the biplot were the best or worst in terms of the trait(s) found within the sector demarcated by any two lines that meet at the origin of the polygon. From Fig. 3, S1n2 (salinity control × nano-silicon dioxide 1 mM) was the best in terms of WC, USR, GI, GP and FW, and therefore it seems that application of low concentration of the nano-silicon dioxide nano-particles at non-stress condition could improve some of lentil seed germination properties and produced strong seedlings. Also, our finding is in agreement with Haghighi et al. (2012) which indicated that application of nano-silicon dioxide nano-particles increased germination parentage of tomato under salinity stress condition.

The S1n1 (salinity control × nano-silicon dioxide 0 mM) was the best in terms of RDP, DG, SV, GR, RL, DW and SL, and therefore it seems that the mentioned traits are sensitive to salt stress and did not influence by the nano-silicon dioxide nano-particles (Fig. 3). From Fig. 3, S4n1 (salinity 150 mM × nano-silicon dioxide 0 mM) was the best in terms of SR, MGT, T_{50} and IDW and so the existence of salinity stress and no application of the nano-silicon dioxide nano-particles caused to delay of seed germination. The S3n3 treatment combination showed similar behavior and this probably refers to inhibitory or toxic effects of nano-silicon dioxide at high concentrations. Wang et al. (2010) reported that high concentration of silicon can resulted in reduction of seed germination. The S4n3 (salinity 150 mM × nano-silicon dioxide 2 mM) was the best in terms of RUE, and therefore it seems that the application of high concentration of the nano-silicon dioxide nano-particles at high salt stress condition could improve seed reserve utilization efficiency (Fig. 3). In contrast, the S4n2 (salinity 150 mM × nano-silicon dioxide 1 mM) was the best in terms of RG, and therefore it seems that the application of the nano-silicon dioxide nano-particles at high salt stress condition increased reduction of germination (Fig. 3).

The vector view of TT biplot is showing the interrelationships among all the measured traits. The lines connecting each trait marker to the origin of the TT biplot are the trait vectors and their length approximates the standard deviation of each trait (Yan et al. 2007). The cosine of the angle between the vectors of any two traits approximates the correlation coefficient between them. On this premise, two traits are positively correlated if the angle between their vectors is an acute angle (< 90°) while they are negatively correlated if their vectors are an obtuse angle (> 90°) and thus they are not correlated if their vectors equal angle = 90°. From Fig. 4, USR, GI, GP, FW, RDP, DG, SV, GR, RL, DW and SL traits were highly positively correlated and it shows they all gave similar information about germination properties among the treatment combinations. The increase of USR caused to increase of seedling characteristics like fresh and dry weight of shoot and root as well as length of shoot and root. Similarly, our findings are in agreement with the report of Soltani et al. (2006). Also, there was strong positive correlation between T50 and SR and between MGT and RG traits.

The WC and RUE traits were not correlated positively or negatively with USR, GI, GP, FW, RDP, DG, SV, GR, RL, DW and SL traits; the T50, SR, MGT and RG were not correlated with WC; and MGT and RG were not correlated with RUE trait (Fig. 4). There was negative correlation between USR, GI, GP, FW, RDP, DG, SV, GR, RL, DW and SL traits with T50, SR, MGT and RG traits. Also, there was negative correlation between WC with RUE traits (Fig. 4). It is clear that with increasing RUE in seed germination process, the seed water content is decreasing and succulent growth took place. This result agreed with Janmohammadi et al. (2008), who reported negative significant correlation between seed reserve utilization efficiency and water content.

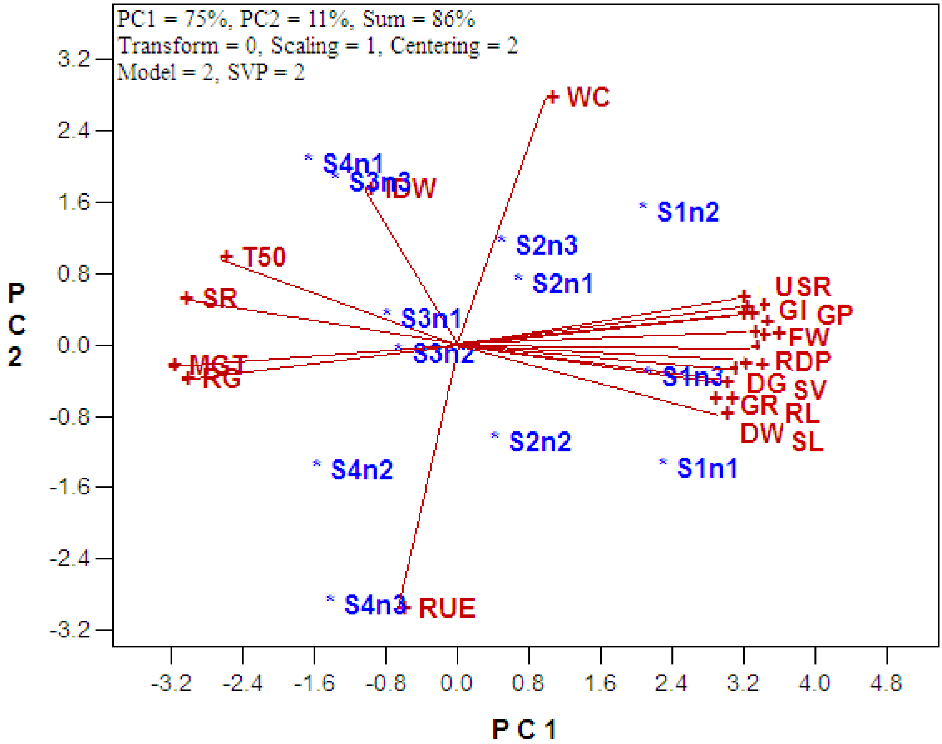


Figure 4. Vector view of treatment combination (salinity × nano-silicon dioxide) by trait biplot showing the interrelationship among all measured traits for 12 different treatment combinations.

In the context of treatment combinations-by-trait analysis, an ideal treatment combination has been defined as the treatment combination that combines several good traits in its genetic composition. In the biplot displayed in

Fig. 4, the single-arrow line that passes through the biplot origin is referred to as the average-tester axis (ATC) abscissa, and on this line is ranked the treatment combinations in terms of their performance. The double-arrow line (ATC ordinate) divides the ATC abscissa into two at the middle and the portion of the ATC towards the right displays the above average treatment combinations and towards the left shows those treatment combinations below average. Based on this biplot, the treatment combinations that performed above average were S1n1, S1n2, S1n3, S2n1, S2n2 and S2n3; while S3n1, S3n2, S3n3, S4n1, S4n2 and S4n3 performed below average in terms of seed germination parameters and seedling growth properties. The poor performance of moderate and high slat treatments in all nano-silicon dioxide nano-particles indicated that the destructive effects of salt stress is more sever than improving influences of nano-silicon dioxide application. Similar to our findings, it has been reported that silicon application could alleviate the adverse effects of salinity stress on seed germination (Haghighi et al., 2012) and increased water-use efficiency and photosynthesis rate in plants (Ma, 2004).

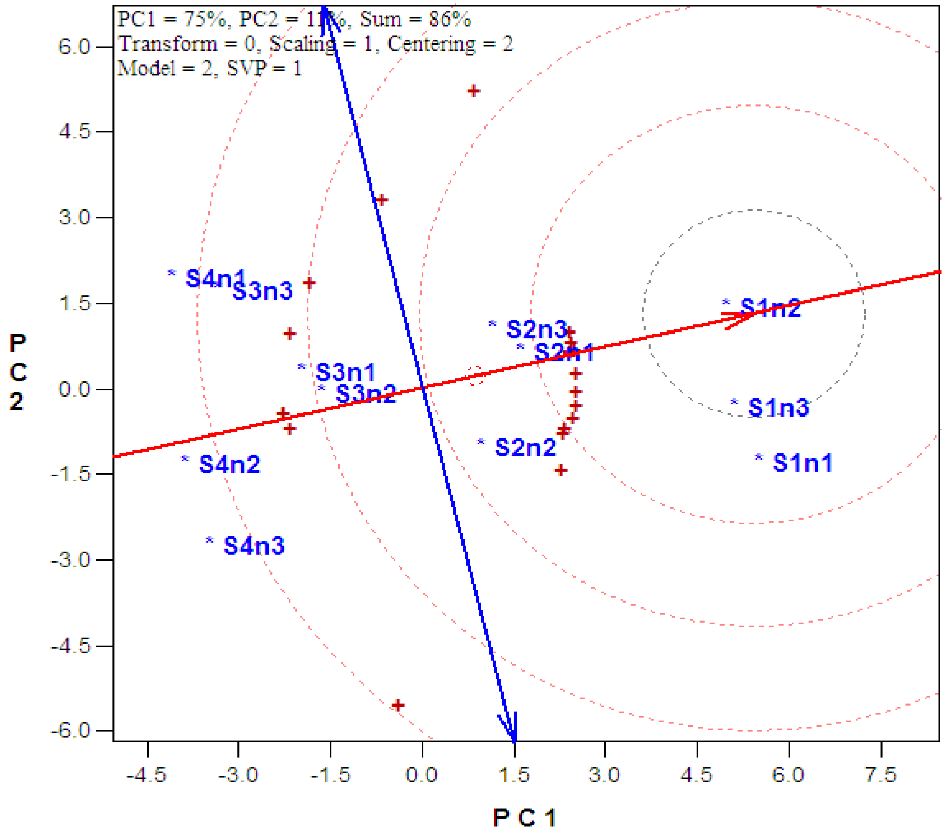


Fig. 5. Ideal tester view of treatment combination (salinity × nano-silicon dioxide) by trait biplot showing the ranking of 12 treatment combinations based on ideal tester.

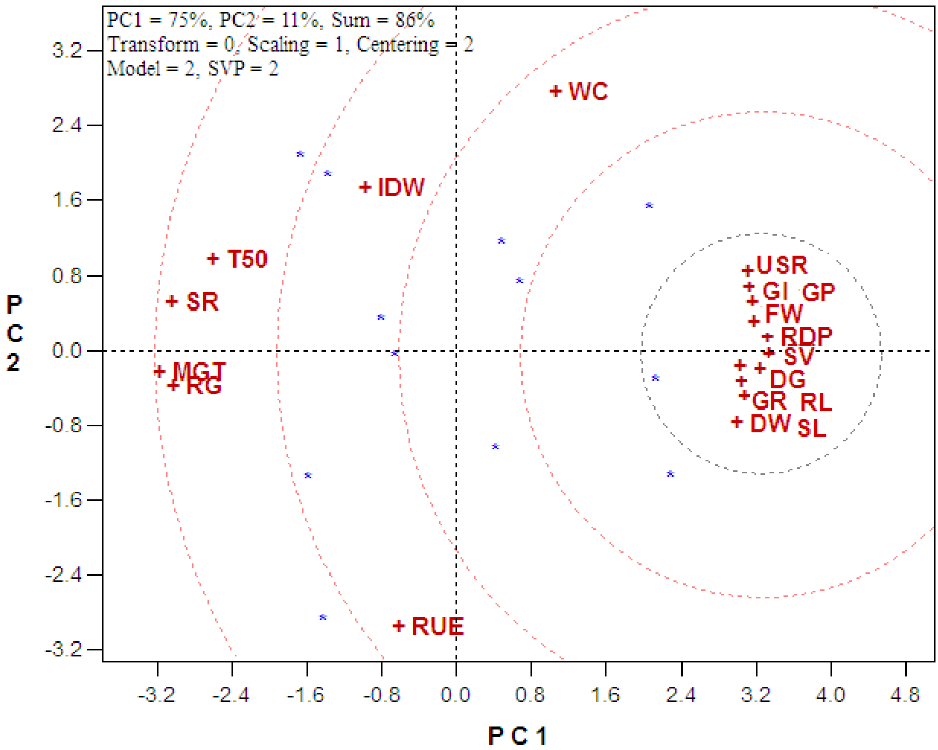


Figure 6. Ranking of different traits according to seedling vigour showing which traits are more related to seedling vigour in 12 treatment combination (salinity × nano-silicon dioxide) by trait biplot.

The seedling vigour index (SV) is an important trait due to representing both seed germination properties as well as seedling characteristics and so the measured traits were rank according to its performance (Fig. 6). The position of SV is comparable to the ideal tester which is highly differentiating of the treatment combination and at the same time representative of the target tester. According to (Yan et al. 2001) ideal tester has small (absolute) PC2 scores (more representative of the overall testers) and large PC1 scores (more ability to discriminate treatment combination in terms of the entry main effect). Fig. 6 showed that USR, GI, GP, FW, RDP, DG, GR, RL, DW and SL traits were the closest to SV and therefore the most desirable of all of them and it the most effective for selecting superior indices. In contrast, T50, SR, MGT and RG traits were the least desirable testers regarding SV.

CONCLUSIONS

The present research aimed to investigate the effect of nano-silicon dioxide nano-particles treatments on germination of lentil under different salinity conditions. Seed germination properties and early seedling growth characteristics are the most susceptible stages to salt stress. The results of revealed that even though salinity delayed germination, higher salt concentrations eventually reduced the percentage of germinated seeds and seedling growth parameters. However, our result suggest that application the of 1 mM nano-silicon dioxide alleviate the adverse effect of salt and improved seed germination properties as well as seedling growth characteristics. The application of favorable amounts of the nano-silicon dioxide could improve seed germination, enhancing seedling growth, and finally influence crop yield under salt stressed condition. The nano-silicon dioxide can be applied by indirect methods such as seed coating. Also, for making practical recommendations, it is essential to investigative the effects of the other nano-particles on different crops under both biotic and abiotic conditions. This interesting topic will be considered in detail in future investigations.

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GRAFIČKA ANALIZA NANO-SILIKONA KROZ INTERAKCIJU STRESA OD SANILITETA NA OSOBINE KLIJANJA KOD SOČIVA KORIŠĆENJEM BIPLOT METODE

SAŽETAK

Nano čestice mogu biti korisnije u poređenju sa rasutim materijalom jer one mogu da prođu kroz membranu usjeva zbog svoje generalne veličine. Nano-silikon ili SiO2 je jedan od glavnih i najčešće korišćenih inženjerskih oksidnih nano čestica u različitim oblastima. Brzo klijanje uzgajanih sjemena i dobro uspostavljanje klijanaca su ključni faktori za proizvodnju sočiva na zemjištu izloženom stresu od saliniteta širom sušnih i polusušnih regiona. Ovo istraživanje je imalo za cilj da ispita uticaj različitih tretmana nano-silikonskog dioksida (koncentracije od 0, 1 i 2 mM) na klijanje sjemena sočiva pod različitim NaCl koncentracijama (0, 50, 100 i 150 mM). Radi boljeg razumijevanja nano-silikona kroz interkaciju na stres od saliniteta, korišćen je bipilot metod. Prve dvije osnovne komponenete (PC) osnova (PC1 i PC2) objašnjavaju 86% ukupne varijacije. Rezultati pokazuju da se klijanje značajno odlaže povećanjem stresa od saliniteta i primjena 1 mM nano-silikon dioksida ($nSiO_2$) može značajno ublažiti negativan efekat stresa od saliniteta na procenat klijanja sjemena, dužinu korjena i izdanka, težinu izdanka, prosječno vrijeme klijanja, vigor indeks klijanaca i mobilizaciju rezervi sjemena. Rezultati ovog ispitivanja pokazuju da $nSiO_2$ ima povoljan uticaj na klijanje sjemena sočiva pod stresom od saliniteta i upotreba odgovarajuće koncentracije ovih nano-čestica kod proizvodnje sočiva pod sresom od saliniteta može biti ekonomično. Takođe, otkrili smo da izloženost sjemena nano-česticama SiO_2 uzrokuje i pozitivne i negativne efekte na rast sočiva.

Ključne riječi: stopa klijanja, nano-čestice, stres usled sliniteta, vigor klijanca, SiO_2