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EFFICIENCY OF SI IN ALLEVIATING NACL-INDUCED STRESS IN OILSEED RAPE

Dragan Lazić[1], Marina Putnik-Delić[1]*, Milena Daničić[1], Milan Župunski[2], Danijela Arsenov[2], Slavica Vuković[1] and Ivana Maksimović[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, Trg Dositeja Obradovica 8, 21000 Novi Sad, Serbia; [2]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovica 2, 21000 Novi Sad, Serbia
***Corresponding author' s e-mail: putnikdelic@polj.uns.ac.rs**

Crop cultivation worldwide is often affected by NaCl excess in soil solution. Silicon (Si) is the second abundant element of Earth crust which may alleviate effects of abiotic stress in plants. In the present study, the effect of Si added to NaCl-containing nutrient solution, on physiological and biochemical traits of oilseed rape (*Brassica napus* L.) was investigated. Silicon (Si), 5 mM, in the form of inorganic fertilizer was applied to 50 mM and 100 mM NaCl -containing nutrient solution, in semi-controlled conditions of a greenhouse. Both salinity levels significantly reduced intensity of transpiration and activities of catalase and guaiacol peroxidase in leaves. Application of Si, on the other hand, significantly increased antioxidant activity (up to 80%). In addition, free proline accumulation was reduced 5 times in the presence of Si. Addition of Si to nutrient solution containing 50 mM NaCl alleviated effect of salts on plant fresh weight, number of leaves per plant, total leaf area and concentration of photosynthetic pigments. Accumulation of N in leaves and roots of plants increased, while concentrations of Na and K were lower in the presence of Si. Concerning growth and biochemical traits of oilseed rape, Si addition was found more effective at lower salt concentrations, while increase in antioxidant enzyme activity was actually caused by both NaCl concentrations.
Keywords: growth, nutrition, oilseed rape, salinity, silicon.

INTRODUCTION

Soil and soil solution salinity are the most prominent problems which affect around 20% of agricultural land. In nature, higher plants inhabit regions rich in salts, such as seashore and estuaries, but more extensive problem in agriculture is accumulation of salts in irrigated soils. In addition, the effect of salinity is most common in arid and semi-arid areas of the world (40% of the earth's surface), where low-quality irrigation water and improper soil management contribute to this issue (Taiz and Zeiger, 2014). Irrigation water in Vojvodina, which is the most important agricultural region of Serbia, belongs to class of III (water unsuitable for irrigation) and its EC is about 1010 $\mu S\ cm^{-1}$ (Savić *et al.*, 2015). Also, trend of climate change is undoubtedly one of the main causes of salinization. The most abundant type of salt in the substrate is NaCl (Flowers and Colmer, 2015). Excessive amounts of NaCl in the growing substrate may lead to many metabolic disorders in crops (Munns and Tester, 2008). Some researchers consider Cl concentrations higher than 20 mM toxic (Marchner, 2012). NaCl is salt that declines soil water potential and then, plants should be osmotically adapted. In addition, basic processes as plant nutrition, photosynthesis and water flow may be disturbed by presence of excessive salt concentrations. Furthermore, reactive oxygen species (ROS) may accumulate in plant tissues in response to salts, enhancing activity of antioxidant enzymes (Noreen and Ashraf, 2009). Many studies indicate that antioxidant system is crucial in plant defense towards salinity (Jovičić *et al.*, 2017).

There are several strategies to improve plant salt tolerance, such as seed priming, application of growth regulators and also plant breeding. It is well-known that silicon may exert several beneficial effects on plant metabolism which are mostly recognized when crops are exposed to abiotic stress (Ma *et al.*, 2001). Silicon (Si) is one of the most abundant elements in earth's crust which is absorbed from soil solution by plants in the form of mono silicilic acid (H_4SiO_4). Benefits of using Si is very detailed investigated in wheat, cucumber and rice (Ashraf *et al.*, 2008; Zhu *et al.*, 2014; Mahdieh *et al.*, 2015; Wang *et al.*, 2015).Considering oilseed rape as an important agricultural crop (Grassano *et al.*, 2011), mostly known by its role in livestock nutrition and oil production it is necessary to investigate the possibilities of mitigating damage due to increased concentration of NaCl in substrate. Although there are some studies of the application of high concentration of NaCl in later stage of rapeseed growth, the influence of silicon has been studied only in terms of prevention (Hashemi *et al.*, 2010). The corrosive effect of silicon in oilseed rape that is being affected with different

NaCl concentrations is not known. In the present study, growth, physiological traits, concentration of N, K and Na, as well as activity of some ROS scavenging enzymes were examined in oilseed rape exposed to excessive salts. The aim was to investigate potential of Si to alleviate harmful effects of NaCl.

MATERIALS AND METHODS

The experiment was conducted in a glasshouse, in semi-controlled conditions. Seeds of spring oilseed rape (*Brassica napus* L., cv Jovana) were germinated in the presence of treatments (Table 1) (in the previously sterilized sand in which it was added;0, 50 or 100 mM NaCl with or without addition of 5 mM Si), in an incubator at the temperature of 26 °C. Seedlings were transferred to half-*strength Hoagland* nutrient solution (1/2 Hoagland) (Hoagland and Arnon, 1950) where the plants were grown in pots of 750 mL capacity. The source of Si was fertilizer Siliplant Univerzalni (Eko Plant d.o.o. Beograd) in amounts recommended by the producer**,**5 mM Si. Experiment was done in 5 replications, with 8 plants per replication. One month after planting, analyses were performed.

Table 1. The composition and labels of ½ Hoagland nutrient solutions used in the experiment

Nutrient solution (Treatment)	NaCl concentration (mM)	Siliplant concentration (mM)	Label
1	-	-	Control
2	-	5	Si
3	50	-	50
4	50	5	50 Si
5	100	-	100
6	100	5	100 Si

Fresh (FW) and dry weight (DW) (drying to a constant mass at 70 °C) of roots, stems and leaves, as well as number of leaves per plant were recorded. Leaf area was assessed using Ci-203 Laser area meter (CID Bio-Science). Transpiration intensity (It) was measured gravimetrically during 3 consecutive days. Concentration of photosynthetic pigments was assayed in acetone extracts (Holm, 1954; Von Wettstein, 1957).

The intensity of lipid peroxidation was measured on the basis of malondialdehyde concentration (MDA) in leaves and roots of plants (Devasagayam *et al.,* 2003). Free proline concentration was assessed as described by Bates (1973).

Activity of catalase (CAT) was determined by method of Aebi (1984) and guaiacol peroxidase (GPx) by method of Simon *et al.* (1974).

Concentration of mineral elements was expressed in dry weight (DW). Nitrogen concentration of oilseed rape leaves and roots was determined by Kjeldahl method (Kjeldahl, 1883). Sodium and potassium concentrations were measured by flame atomic absorption spectrophotometry (AAS SHIMADZU AA6300).

Data analyses**:** Statistical analyses of data were carried out using Statistica 13 software. All data were subjected to ANOVA and means comparison was done using Fisher's least significant difference test (LSD).

Principal component analysis (PCA) was programed and analyzed using R software packages (R Core Team, 2016), in order to characterize and visualize patterns of measured morphophysiological parameters by the first two PCA dimensions. Prior to analysis, dataset was scaled (normalized) making the distributions of variables normalized, with stabilized variance and making tested model multilative on the raw scale.

RESULTS

Oilseed rape relatively tolerates lower salt concentrations and it is very sensitive to higher concentrations. In the presence of 50 and 100 mM NaCl seeds germinated, while at the concentration of 200 mM NaCl seeds did not germinate.

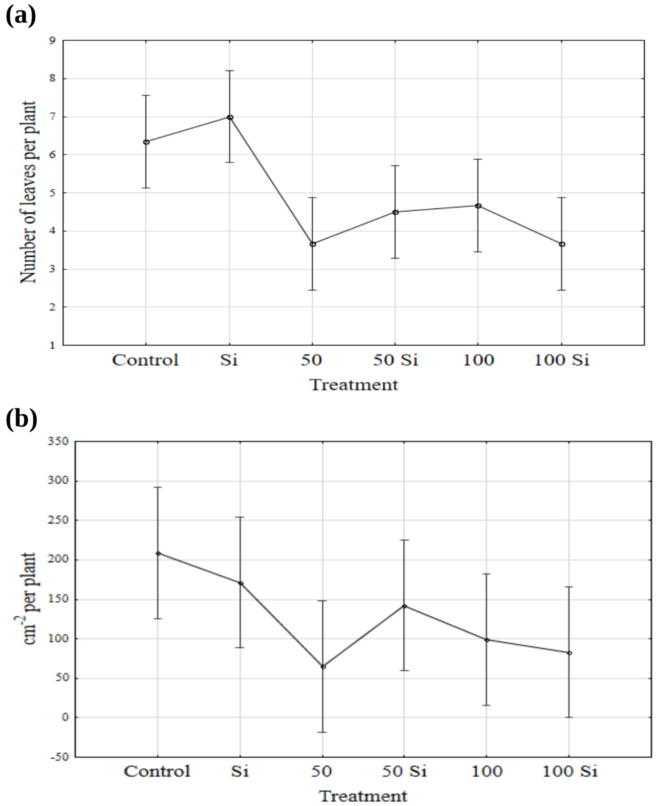


Figure 1. Number of leaves per plant (a) and total leaf area (b) grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.

The presence of Si at lower salt concentrations (50 mM NaCl) reduced the effect of salt, as it did not affect the metabolism to a great extent, but it affected productivity. Because NaCl is potentially present, research is being done to reduce the toxicity by using Si that is not in excess and can help in production.

Addition of Si to 50 mM NaCl -containing solution significantly increased number of leaves per plant (by 19%) with respect to the 50 mM NaCl without Si oilseed rape (Figure 1a). Leaf area in presence of 50 Si showed increase of 54%, relative to the same level of salinity in the absence of Si (Figure 1b). Similar was recorded in fresh weight (FW) of root, leaf and stem tissues (Figure 2). FW of roots, stems but also leaves per plant increased up to 56% in presence of 50 Si, relative to 50 treatment.

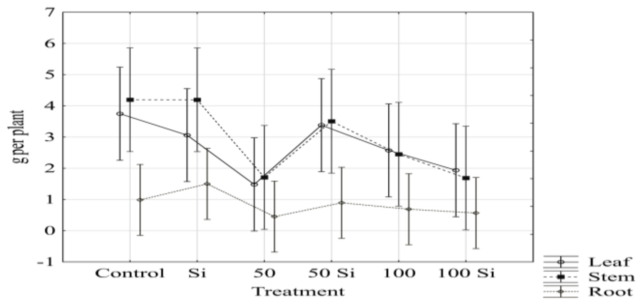


Figure 2. Fresh weight of leaves per plant, stem and root grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.

Silicon increased intensity of transpiration. In the presence of 50 Si transpiration intensity increased by 52% and by 30% in presence of 100 Si (Figure 3).

In the presence of 50 mM NaCl, a significant decrease in the concentration of photosynthetic pigments was recorded. Analyses of chloroplast pigments pointed out positive effect of addition of Si to 50 mM NaClin case of all pigments (Chl a, Chl b, Chla+b and Car), although the stimulation was significant in carotenoids (22%) (Fig. 4).

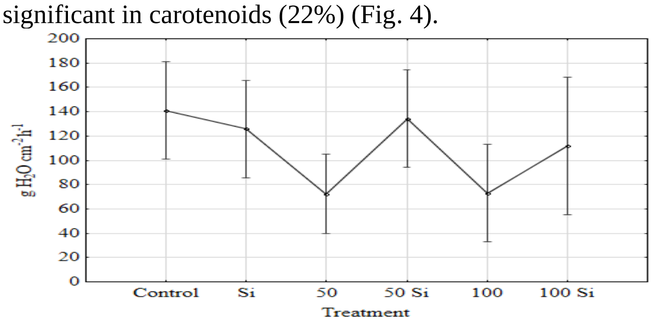


Figure 3. Transpiration intensity (It) of plants grown with and without additional Si in NaCl- containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.

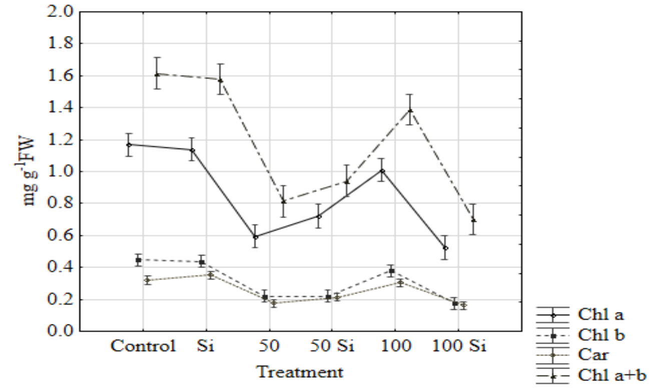
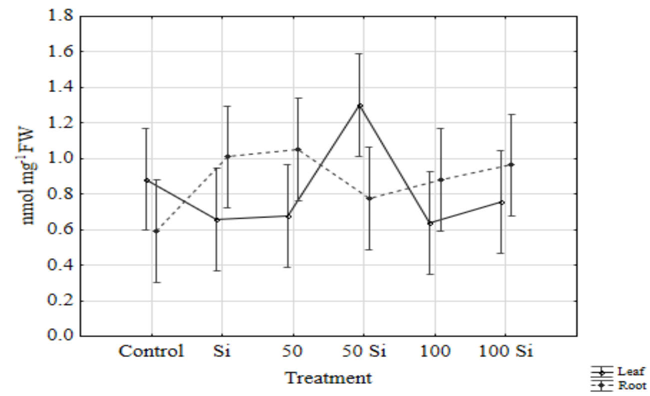


Figure 4. Photosynthetic pigment concentration in leaves of oilseed rape grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.

(a)



(b)

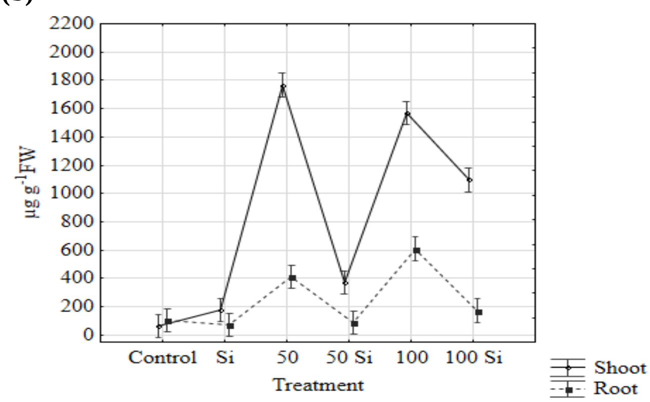


Figure 5a and 5b. Concentration of malondialdehyde (MDA) in leaves and roots (a) and free proline content (b) in shoot and root of oilseed rape grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.

MDA content increased with Si addition to 50 and 100 mM NaCl, in leaves and roots relative to Si-absent treatments (Figure 5a). However, statistically significant decrease of 27% in MDA content was found in roots of plants exposed to 50 Si, when compared to 50. Both shoot and root free proline content significantly declined (up to 4.5-fold) when Si was added to 50 mM NaCl (Figure 5b). Free proline content in plant tissues at higher salt concentrations (100 Si) decreased relative to 100. It was up to 72% in shoot.

CAT and GPx activity increased by 70% in presence of 50 Si in leaves (Figure 6). The same was observed with higher salt concentration in the nutrient solution. On the contrary, Si markedly decreased CAT and GPx activities in roots when applied both to 50 and 100 mM NaCl (approximately 7.5-fold).

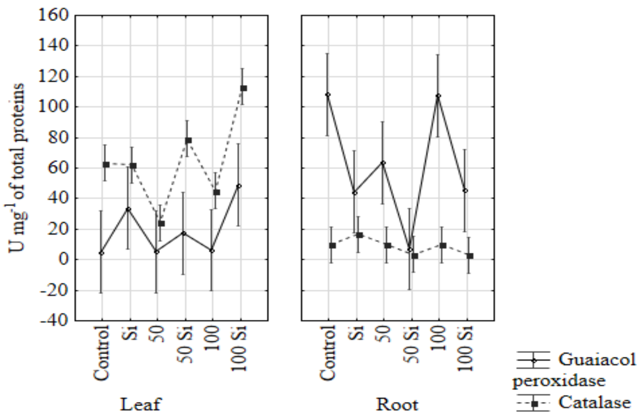


Figure 6. **Catalase (CAT) and guaiacol peroxidase (GPx) concentration in leaves and roots of oilseed rape grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.**

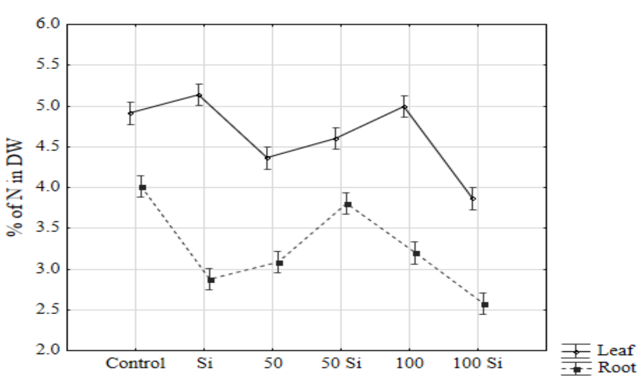


Figure 7. **Nitrogen content in leaves and roots of plants grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.**

Lower concentration of NaCl in the solution, led to significant decrease in nitrogen and potassium concentration in DW, and increase concentration of Na (Figure 7 and Figure 8). In the presence of 50 Si, N concentration in DW of leaves and roots increased by 8% and 36%, respectively (Figure 7). Na concentration in leaves, stems and roots in the presence of Si decreased, relative to plants grown in presence of 50 and 100 mM of salt (up to 33% in leaves). Adding Si to 50 mM NaCl solution decreased K concentration in leaf and root of oilseed rape, up to 4 times (Figure 8).

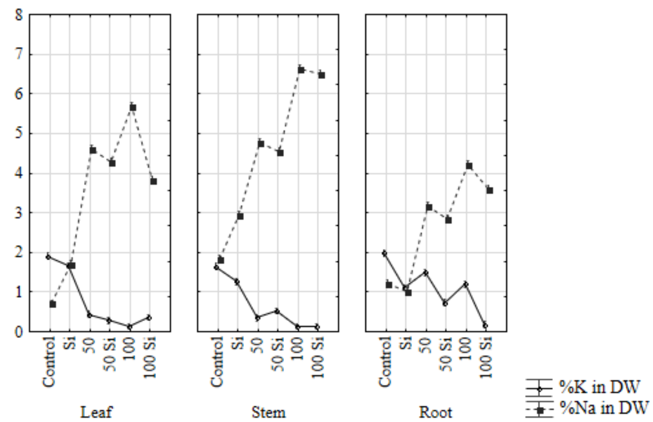


Figure 8. **Na and K percentage in leaves, stems and roots of plants grown with and without additional Si in NaCl-containing solution. Vertical bars denote 0.95 confidence intervals. Labels of treatments are explained in Table 1.**

DISCUSSION

Excessive concentrations of salts such as NaCl in the soil and nutrient solution may affect plant metabolism in many ways. In order to promote their growth and production, attention nowadays is focused on improvement of their salt tolerance. In that sense, next to its role in plant nutrition, Si is an element that might alleviate different stress influences in plants. Thus, Si supplementation, during crop cultivation is generally considered as favorable. Analyses of growth and physiological parameters in the current study proved Si application efficiency mostly when applied to 50 mM NaCl solution (number of leaves per plant, leaf area per plant, FW per plant). The positive effect of Si addition to nutrient solution which contained 50 mM NaCl could be assigned to the effect of Si on photosynthetic rate (Adatia and Besford, 1986). Furthermore, both salinity levels (50 and 100) significantly reduced chloroplast pigments concentration in leaves, with respect to control. Decreased pigment concentrations together with reduction in carotenoid content in saline medium may be the result of changes in antioxidant activities (Mittler, 2002). In addition, Si application to 50 mM NaCl solution, stimulated accumulation of Chl a, b and particularly Car (22%). According to Gunes *et al.* (2007), carotenoids encounter as natural non-enzymatic antioxidants which may prevent oxidative damage in plant cells. Similar to present results, Sacala (2017) recorded enhanced chlorophyll content in maize in presence of Si in substrate which contained NaCl. The increase in CAT activity may contribute to prevention of chlorophyll degradation due to excess salts. Both CAT and GPx activities in leaves of oilseed rape were stimulated by Si presence by nearly 90%. Our results are consistent to those observed by Abdolzadeh and Sadeghipour (2010), when oilseed rape showed 3.4-fold greater CAT activity in the presence of Si in saline medium. The higher activities of antioxidant enzymes following Si addition were reported in barley, indicating mitigation of salt toxicity symptoms (Zhu *et al.*, 2004). Presence of salt in the nutrient solution decreased transpiration intensity of oilseed rape, because NaCl caused changes in water regime. Presence of Si under both salinity levels (50 and 100) increased transpiration intensity almost to the levels observed in plants cultivated under non saline substrates. Differences were not statistically significant, but increasing trend was observed. Obtained results are similar to those recorded by Farshidi *et al.* (2012) where transpiration increased in hydroponically grown oilseed rape with the addition of Si to 100 mM NaCl-containing solution. Increased lipid peroxidation under salt treatment was reported in many plants (Vaidyanathan *et al.*, 2003) and reflects damages of membranes. Si supplementation in the present study was efficient in declining level of lipid peroxidation in roots subjected to 50 Si. Similarly, Abdolzadeh and Sadeghipour (2010) reported that only roots of *B. napus* grown for 25 days in 150 mM NaCl -containing solution with additional Si, showed decreased MDA content. Significant reduction in lipid peroxidation, observed in the roots of 50 Si oilseed rape, following Si supplementation, may be related to the increase in the activity of antioxidative enzymes (CAT and GPx) which helped to protect cell membrane integrity.

Proline is one of the compatible solutes which keeps osmotic potential in plants in balance (Motomura *et al.*, 2002). Si application was very efficient in decreasing proline concentration to a great extent, by more than 5 times. These results are in consistence with those of Nezami and Bybordi (2011) obtained in canola exposed to three levels of salinity and Si.

Although Si is regarded as non-essential nutrient, present results show strong beneficial influence of Si in Na accumulation in leaf and root tissue. Al-aghabary *et al.* (2005) pointed out that the reduction in Na accumulation improves the ROS scavenging. The same was observed for K concentration. Despite this, several investigators reported increase in K content following Si application (Ashraf *et al.*, 2010). The increase of N concentration in presence of 50 Si and the difference in the growth can be explained by changes in the intensity of transpiration.

***Multivariate interactions of measured parameters*:** Principal component analyzes has been proven as a very useful tool in data analyses. Utilization of PCA method may help in distinguishing the variations in large datasets through simultaneous consideration of multiple parameters (Polutchko *et al.*, 2018).Since it can condense a large number of original variables into a new compact set of principal components with a minimal loss of information (McGarigal *et al.*, 2000), it has been utilized to assess the plant's response to experimental queries.

According to the analysis of multi-variant functional traits (Figure9), experimental groups had been clearly separated in two-dimensional system. The first two principal components (Dim 1 and 2) accounted for 72.9% of overall variation, which is pointing to the importance of variables which are closely positively correlated with them. Experimental groups were separated across the two-dimensional plot without any overlapping of individual measurements. An interesting fact is that control and Si groups were separated across the first dimension from NaCl treatments with or without Si applied. Such observation might point to the fact that applied Si did not affect plant metabolism in an adverse manner as NaCl treatments did. Likewise, Si application on NaCl treated plants had led to differential response of measured parameters in comparison to non-Si treated NaCl groups. It is obvious that biomass production and leaves elemental composition were highly influenced by applied treatments. On the other hand, CAT and MDA have mostly contributed to variability of the second principal component. Such distribution of variables did not diminish the importance of examined biochemical traits in elucidation of salt stress effects.

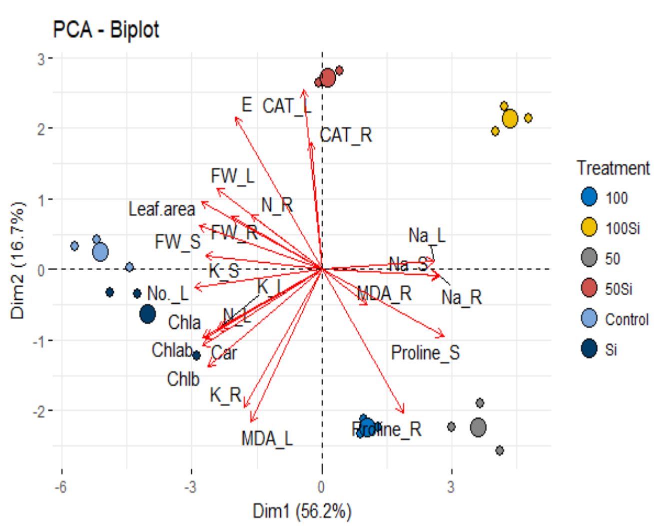


Figure 9. **Principal component analysis (PCA) of investigated morpho-physiological traits. Biplot is presented with first and second principal components (Dim1 and Dim2). * L - *leaves*; S - *shoots*; R - *roots*.**

***Conclusions*:** According to the results it can be concluded that addition of Si may reduce the adverse effects of lower concentration of NaCl in nutrient solution. It is efficient in alleviation of adverse effects caused by moderately increased salt concentrations (50 mM NaCl), but lower efficiency was recorded in case of high salt concentrations (100 mM NaCl). NaCl significantly influenced changes in young plants and parameters such as number of leaves per plant, photosynthetic pigments, concentration of free proline, catalase in leaves, concentration of N, K and Na. Addition of Si may help to reduce stress responses induced by NaCl which was evidenced in all tested parameters exposed to 50 mM NaCl, but only number of leaves per plant, concentration of free proline in shoot and root, catalase in leaves, concentration of N in root, statistically differed significantly.

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