DESERT 2021, 26(1): 16-27
DOI:10.22059/jdesert.2020.284473.1006735

RESEARCH PAPER

Mitigation of salt stress by mycorrhizal inoculation on *Nitraria schoberi* as a native landscape plant in the arid regions

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Received: 30 June 2019, Revised: 14 November 2020, Accepted: 5 April 2021

Abstract
Increasing the salinity in the water and soil can negatively affect plant growth and development. Mycorrhizal fungi application is one of the ways to reduce the undesirable effect of salt stress on plants. An experiment was conducted in 2017 to assess the effect of salt stress on *Nitraria schuberi*, as a native Iranian plant in arid regions, inoculated with mycorrhizal fungi. Seedlings of this plant were treated under three different levels of NaCl in three stages. The stages including low salt concentrations (0, 20, 60, and 100 mM NaCl), medium salt concentrations (0, 40, 120, and 200 mM NaCl) and high salt concentrations (0, 80, 240, and 400 mM NaCl). Mycorrhizal treatment including two levels: non-inoculated (control) and mycorrhizal inoculated. Experimental designs were factorials (4×2 treatments) based on the completely randomized design with four replications. In this study, content of chlorophyll, carotenoide, sugar, proline and Na, Mg, K, Fe and Ca were measured. The results indicated that with increasing salinity levels from the first (low) to third (high) stage, chlorophyll content was decreased while carotenoid, proline and sugar were increased. The application of NaCl salinity led to a reduction in Fe and enhancement in Na. In the mycorrhizal plants, sugar content decreased but magnesium, calcium and potassium levels increased. Based on these findings it seems that *Nitraria schuberi* is a salt tolerant plant and mycorrhizal fungi can mitigate salinity stress in this plant. Therefore this plant could be applied in the urban landscape of arid and semi-arid regions.

Keywords: Arid regions, Elements, Proline, Salinity, Urban landscape.

Introduction

Climate change is a global phenomenon that can affect soil and water resources. Increase of temperature and decrease of precipitation as results of climate change lead to soil and water salinity (Asseng *et al.*, 2007). The largest water-use sector in the world, allocate to plant irrigation that accounting for 70 percent of all water withdrawn (Billib *et al.*, 2009).

Although the salinity is expected to affect the world more vigorously and extensively in coming years (MacFarlane and Williamson, 2002) but due to population growth and urban expansion, the use of plants in the urban environment and development of green areas in the cities is essential. Trees, grasses, plants and associated hardscape components, due to provide environmental, human health, psycho-social and economic benefits are a vital component of the urban areas (Frank, 2003). Salt in the water and soil is a limiting factor for growth and the productivity of plants (Shrivastava and Kumar, 2015). Salinity can adversely affect plant growth, flowering, germination and yield due to changes in nutritional balance, water relationship, photosynthesis, oxidative stress and combinations of these factors (Negrão *et al.*, 2017), (Munns and Tester, 2008), (Acosta-Motos *et al.*, 2017), (García-Caparrós and Teresa Lao, 2018).

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Many researches indicate that among of the various plants, most of the ornamentals and the plants using in the urban landscaping are sensitive to salinity (García-Caparrós and Teresa Lao, 2018), (Valdés *et al.,* 2015), (García-Caparrós *et al.,* 2016), (Niu and Cabrera, 2010), (Cassaniti *et al.,* 2013), (Acosta-Motos *et al.,* 2017). From the general responses of ornamental plants to salinity is reducing of aesthetic values due to reduction of the flower quantity and quality (Kucukahmetler, 2002), decrease the leaf growth and plant height (Acosta-Motos *et al.* 2017), leaf thickening (Ibrahim *et al.,* 1991), leaf necrosis (Cassaniti, 2008), defoliation (Fox *et al.,* 2005), leaf loss, discoloration and wilting (Niu *et al.,* 2007), (Aroca *et al.,* 2012).

One of the biological methods to overcome the negative effects of salt stress in plants is the application of arbuscular mycorrhizal fungi that colonize with the plant roots. This fungi can alleviate the toxicity induced by salt stress (Hameed *et al.,* 2013). Arbuscular mycorrhizal fungi improves the uptake, mobilization and supply of essential elements by modifying root (Al-Karaki *et al.* 2001). The enhanced ability of some ornamental plants to cope with salt stress by arbuscular mycorrhizal fungi have reported in some researches (García-Caparrós and Teresa Lao, 2018), (García-Caparrós *et al.,* 2016).

Recently using of native plants in the landscapes and gardens of arid and desert areas is considered as a smart option. Native plants due to exposure to scarce and saline water resources are mostly drought and salinity resistant and therefore generally require less watering and fertilizing than non-natives (Simmons *et al.,* 2011). *Nitraria schoberi* L. is a shrub species belongs to family Nitrariaceae that is widely distributed all over Middle East. It also grows in the arid and semi-arid areas of Iran. Some local studies indicate that this plant is a drought (Karimian *et al.,* 2017) and salinity resistant plant (Naseri, 2014). Regarding this plant can grow and survive in the saline and dry regions such as the central desert of Iran and Kashan, it seems to be a resistance shrub to a high level of salinity (Karimian et al., 2017).

Despite the enormous researches of plant responses to salt stress and arbuscular mycorrhizal fungi, the responses of native salinity resistant plants and this fungi under salt stress has not been properly studied. Therefore, the present study was conducted to evaluate changes in the salinity resistant of *Nitraria schoberi* as a native plant in Middle East under different NaCl concentrations. Content of some elements, proline level, sugar and chlorophyll content were measured to evaluate the salinity resistant changes in this plants.

Materials and Methods

Plant materials and Mycorrhizal inoculums

In the early of autumn 2017, the wild seeds of *Nitraria schoberi*, were collected from the natural environments in the central parts of Iran, Meyghan desert of Arak city. Before sowing, the soaked seeds were placed in the dark room with temperature of 4 ^{o}C for 4 weeks. After stratification, seeds were planted in the planting trays and seedlings about 45 days old (5-7 cm height), were transplanted to 15 L pots. All pot contained 12 kg washed and sterilized sandy soil, were put in the greenhouse of Research Center for Plant Sciences, Ferdowsi University of Mashhad. Plants were allowed to grow at day/ night temperature of 25/ 15°C, 60 % relative humidity and 12 hours photoperiod.

Arbuscular mycorrhizal fungi was ordered from an Iranian company with brand name of Mycoroot (a secret mixture of some species of *Glomus*). According to the company's instruction, before transplanting, the mycorrhizal inoculum was added to soil, near the seedling roots.

Treatments

To prevent of plants and fungi shock by high NaCl concentrations, salinity stress were commenced ten days after transplanting by applying the saline irrigation water progressively. The five salt treatments in three stages were prepared. NaCl concentrations after each stage was double. Stages including 1. Low salt concentrations (0, 20, 60, and 100 mM NaCl), 2. Medium salt concentrations (0, 40, 120, and 200 mM NaCl) and 3. High salt concentrations (0, 80, 240, and 400 mM NaCl). Mycorrhizal inoculation consisted of two levels: 1. non-inoculated (control) and mycorrhized inoculated. Salinity treatment was planned using field capacity (FC) method and plants were irrigated with saline water to field capacity.

Measurements

Leaf nutrient analysis

At the end of experimental period, physiologically mature leaves were randomly harvested per treatments and dried in an oven at 70°C to constant weight for mineral determination. Na, Ca, Fe, K and Mg were determined by atomic absorption spectrophotometry and multi-element method in the central labratoare of Ferdowsi university of Mashhad.

Determination of biochemical compounds

Chlorophyll (Chl) was extracted with 80% acetone from dry leaves. Supernatant was quantified with a spectrophotometer at 645 and 663 nm. Chlorophyll was calculated as described by Arnon (1949). Total chlorophyll content was reported as mg/g fresh weight.

The free proline was measured by the method of Bates *et al.* (1973). 500 mg of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid, and then heated in a water bath at 85°C for 60 min. By a spectrophotometer the absorbance was measured at 528 nm. Two ml of filtrate was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid for 1 hour at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm.

The research was done according to a factorial experiment (4×2) based on completely randomized design with four replications. Data were analyzed using a simple regression analysis comparing regression equations and elevations.

Results

As can be seen in the figure 1, in the first stage, only content of carotenoid significantly ($p\leq 0.05$) affected by the interaction of salinity levels and mycorrhiza. The lowest carotenoid content was observed in the control and treatment MSA0 (above 45 mg/g) that were significantly different than other treatments. Also treatments MSA100 and MSA20 indicated a significant difference to each other whereas other treatments had no a significant difference. In the second stage, salinity levels and also its interaction and mycorrhiza could significantly ($p\leq 0.05$) affect chlorophyll b. Among of different salinity levels, only SA120 (highest chlorophyll b) were significantly different compared with control (lowest chlorophyll b) (Figure 2). The lowest chlorophyll b content (nearly 0.5 mg/g) was obtained in the control and also M0SA40 that were significantly different from MSA120 but no significant differences was found among other treatments (figure 3).

Table 1. Analysis variance (mean squares) of some traits in *Nitraria schoberi* under salinity and mycorrhiza treatments

Sources of variance	Df.	Ch. a	Ch. b	Carot.	Sug.	Prol.	Na	Mg	K	Ca	Fe
Mycorrhiza	1	0.014 ^{ns}	0.006 ^{ns}	8.355 ^{ns}	806.99 ^{ns}	112.44 ^{ns}	208923032^{ns}	13859476*	40017068*	29550615*	15221 ^{ns}
Salinity	3	0.004 ^{ns}	0.017*	60.159 ^{ns}	105.34 ^{ns}	63.22 ^{ns}	236716241*	4375566 ^{ns}	11639356 ^{ns}	10725496 ^{ns}	26462*
Sa×My	3	0.012 ^{ns}	0.048*	70.811*	418.64 ^{ns}	30.25 ^{ns}	226899192^{ns}	2809371 ^{ns}	7882248 ^{ns}	12012841 ^{ns}	15618 ^{ns}
Error	24	0.021	0.011	63.015	191.3	30.88	81241860	2938609	9848317	4304070	3825
CV%		10.86	11.11	12.13	11.6	18.48	8.25	10.67	11.06	11.56	19.68

Ns: not significant. *: Significant at the 95% level, My: Mycorrhiza treatment, SA: Salinity treatment, Ch.a: Chlorophyll a, Ch.b: Chlorophyll b, Carot.: Carotenoide, Sug.: Sugar, Prol.: Proline

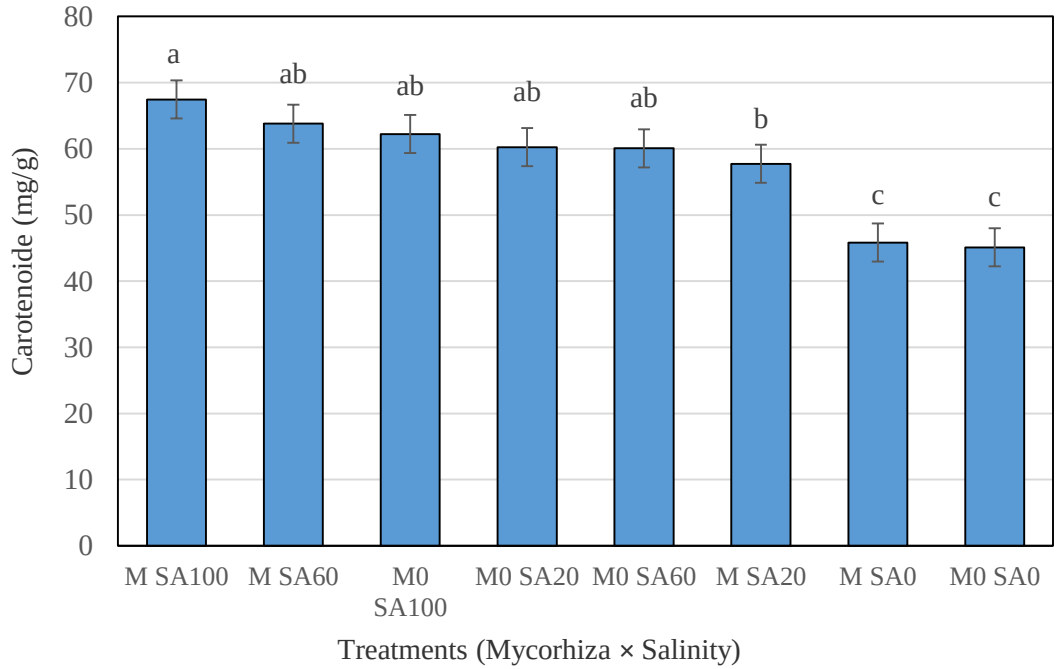

Figure 1. The effects of salinity and mycorrhiza interactions on the carotenoid during first stage
M: Mycorrhizal treatment, M0: Non mycorrhizal treatment, SA0, 20, 60 and 100: Salinity concentrations (mM)

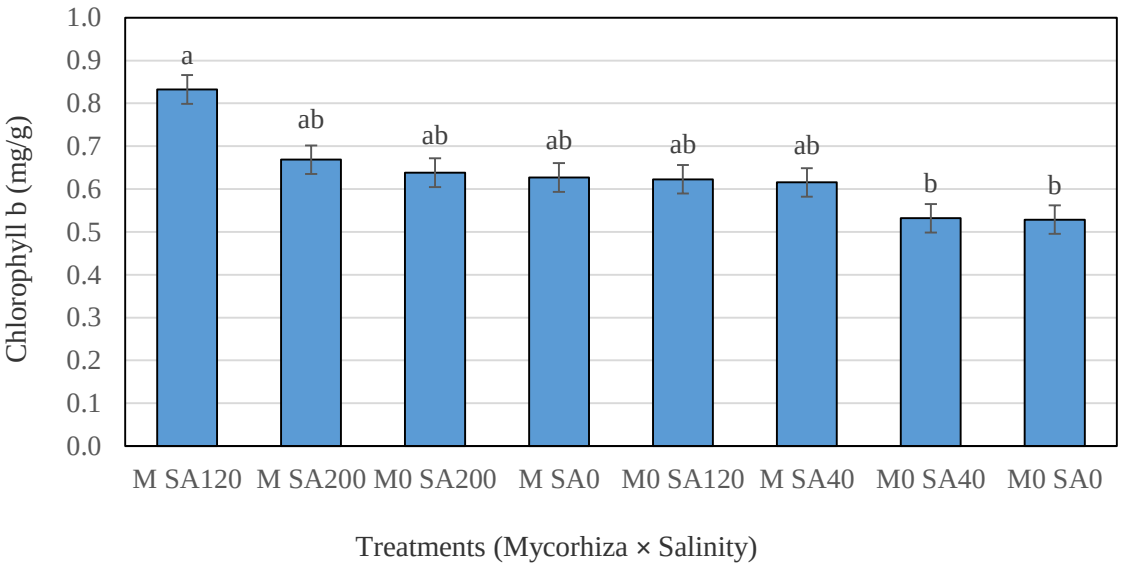

Figure 2. The effects of salinity on the chlorophyll b during second stage
M: Mycorrhizal treatment, M0: Non mycorrhizal treatment, SA0, 20, 60 and 100: Salinity concentrations (mM)

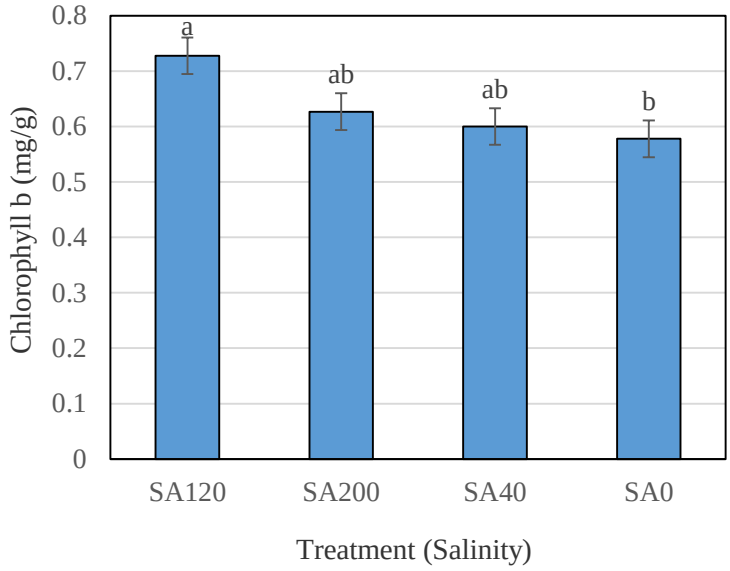

Figure 3. The effects of salinity and mycorrhiza interactions on the chlorophyll b during first stage
M: Mycorrhizal treatment, M0: Non mycorrhizal treatment, SA0, 20, 60 and 100: Salinity concentrations (mM)

Changing trend of chlorophyll a, b and carotenoid under treatments of salinity during the three stages has been illustrated in figures 4. According to figure 4, with increasing of salinity level from stage 1 to stage 3, content of chlorophyll a and b decreased. The most changes of chlorophyll a and b respectively was obtained in the medium and high NaCl concentrations. As can be seen in the figure 4, with increasing salinity levels from stage one to three, carotenoid content also increased. The most changes was observed in high concentration of NaCl.

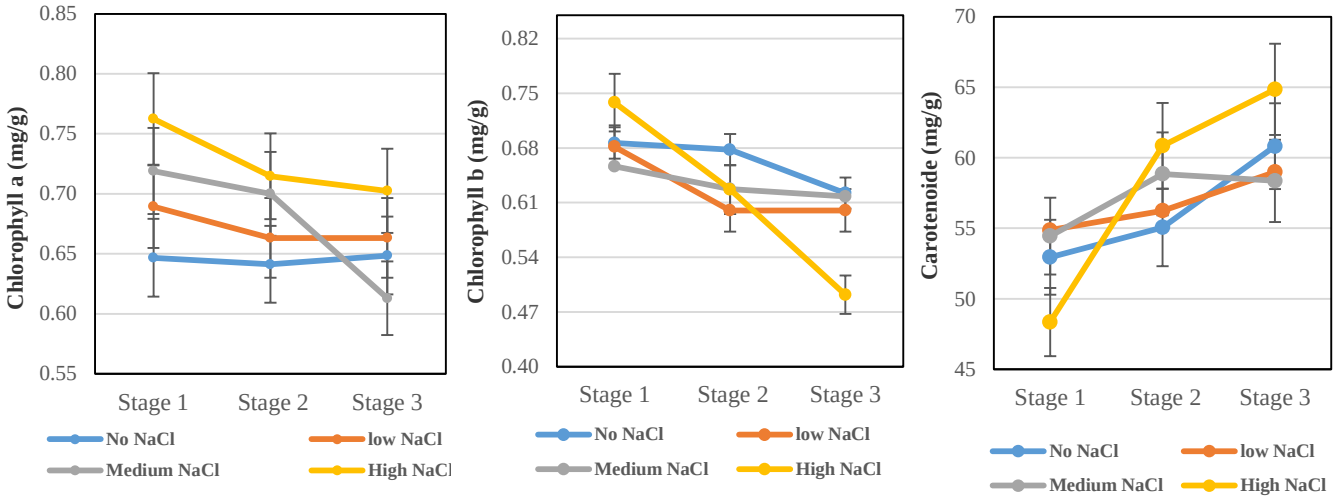

Figure 4. Changing trend of the chlorophyll a, b and carotenoid under salinity treatment during three stages

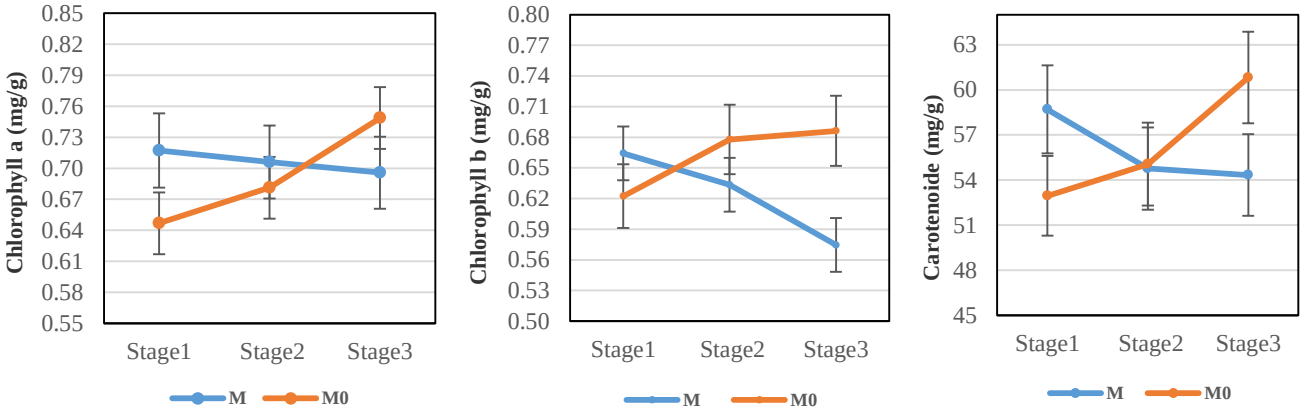

Figure 5. Changing trend of the chlorophyll a, b and carotenoid under mycorrhizal inoculation during three stages

Changings of chlorophyll a, b and carotenoid Inoculated with mycorrhiza indicated a decreasing trend, so that most changing slop was observed in the chlorophyll b content (figure 5). According to the figure 6 (A), proline changes in the plants treated with mycorrhiza and also in the control plants increased during three stages. In the first stage proline concentration in the control plants was significantly higher in comparison with the plants treated with mycorrhiza (Figure 6). Changing proline in the plants treated with different concentrations of NaCl indicated an increasing trend during three stages. Although proline content had also an increasing trend in the control plants, but it was significantly lower than other plants that treated with NaCl during three stages (Figure 6 (B)).

Sugar changings in the Inoculated plants with mycorrhiza and also in the control plants showed an increasing trend. As can be seen in the figure 7 (B), sugar content of control plants in the third stage was significantly higher in comparison with the treated plants with mycorrhiza. Sugar concentration of treated plants with different salinity levels and also in control plants indicated an increasing trend during three stages. In the third stage, high concentration of NaCl, was significantly lower than other treatments (Figure 7 (A)).

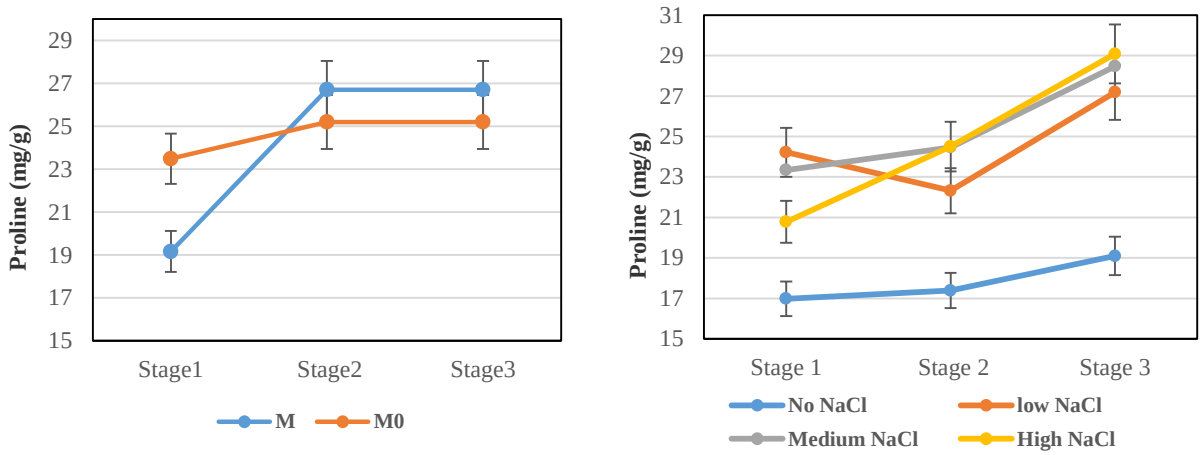

Figure 6. Changing trend of the prolin under salinity (B) and mycorrhiza treatment (A) during three stages

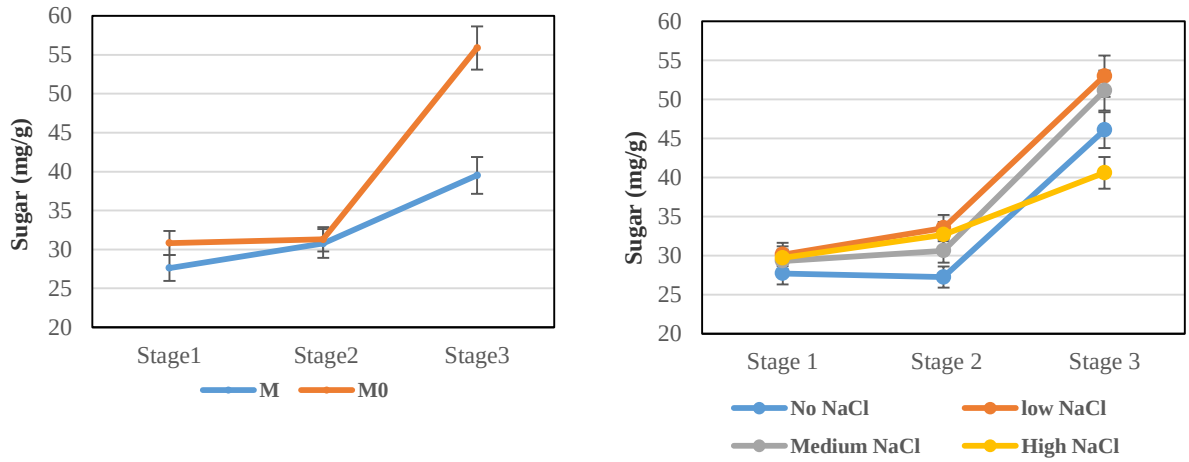

Figure 7. Changing trend of the sugar under salinity (B) and mycorrhiza treatment (A) during three stage

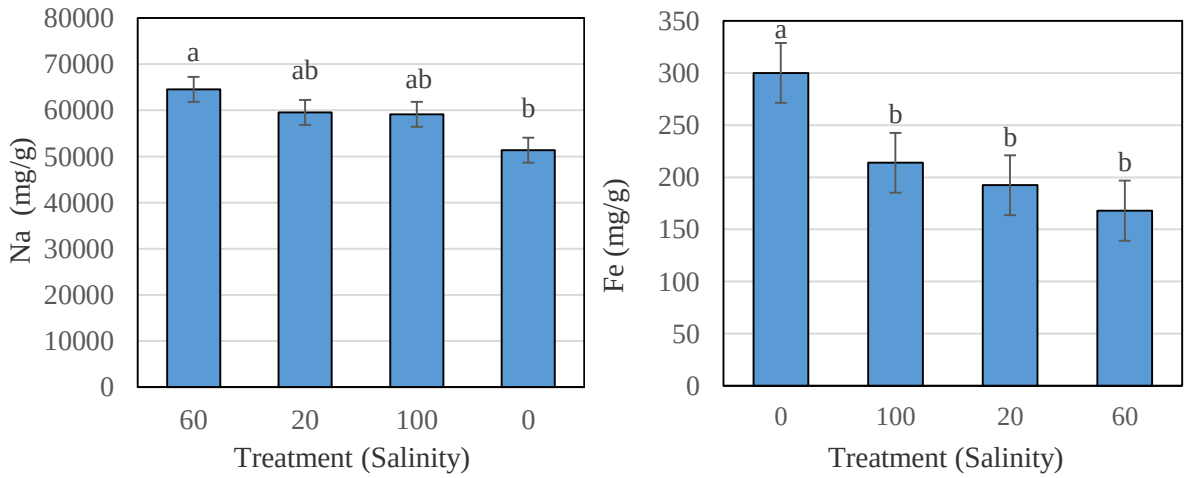

Figure 8. The effect of salinity treatment on the content of Na and Fe in the end of experiment

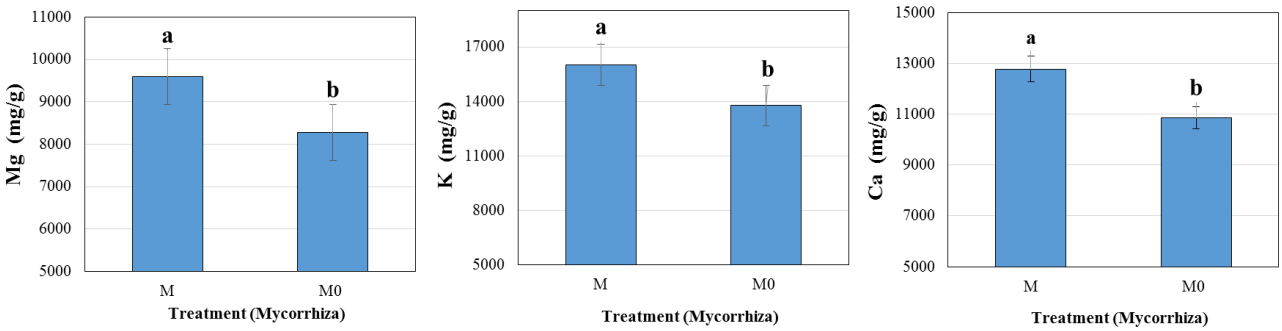

Figure 9. The effect of mycorrhiza treatment on the content of Mg, K and Ca in the end of experiment

According to figure 8, among of different salinity levels, only plants treated with SA 60 (highest Na content) were significantly different than control plants (lowest Na content). The highest Fe content (300 mg/g) content was observed in the control plants that was significantly different compared with plants treated with s alinity (20, 60 and 100 mM NaCl) (Figure 8).

As can be seen in figure 9, the contents of Mg, K and Ca were significantly higher in the plants Inoculated with mycorrhiza than control plants.

Results indicated that contents of Mg, K and Ca were not affected by salinity levels. Mycorrhizal treatment also did not show any significantly change in the Fe and Na contents.

Discussion

In the current study, experiments showed that the application of NaCl salinity in *Nitraria schoberi* increased carotenoid, proline, sugar and Na content whereas the concentration of chlorophyll and also Fe decreased. The results also indicated that with increasing of salinity levels from the first (low) to third (high) stage, chlorophyll content decreased whereas carotenoid, proline and sugar increased. Studies indicated that salinity resistance in the plants depends on different factors including salinity level, plant species and growth stage (Ebrahim and Saleem, 2017), (Ebrahim, 2005). Generally, high salinity levels, have inhibitory and suppressing effects in germination, growth, elements uptake (Ebrahim and Saleem, 2017).

Salinity stress due to reducing of photosynthetic rate, can decrease chlorophylls. Reduction in chlorophyll content and increase of carotenoid content as a typical symptom of salt stress have been recorded in some researches. Our findings are also in line with results reported by García-Caparrós and Teresa Lao, 2018, García-Caparrós *et al.*, 2016, Acosta-Motos *et al.*, 2017, Bres et al., 2016, Bahadoran and Salehi, 2015. Chlorophyll content and photosynthetic efficiency can affect salinity stress in two ways. The first way comprises the regulation of the enzyme activity and expression levels involving in chlorophyll biosynthesis and photosynthesis. The Second way is regulating antioxidant enzyme systems (Yang et al., 2020). In the current study with increasing of salinity levels content of chlorophyll a and b decreased whereas carotenoid content increased. Studies show that high concentrations of salt cause to decrease in photosynthetic pigments (Aghaleh et al., 2009), (, García-Caparrós *et al.*, 2016), (Chaves *et al.*, 2009).

Accumulation of proline in the plants under salinity stress is considered as an adaptive mechanism and can improve salt tolerance in the plants (Kaur and Asthir, 2015). Due to osmosis adjustment, osmoprotection and carbon storage, plants accumulate sugar in their tissues under salt stress (Sami *et al.*, 2016). Proline accumulation in plants help to water absorption, decreases the osmotic potential and contributes to scavenging reactive oxygen species (Pottosin et al. 2014). Sugar and proline accumulation is an adaptive mechanism

against salt stress in the plants (García-Caparrós and Teresa Lao, 2018) that it was confirmed by the present study. In the current study, the content of proline and sugar indicated an increasing trend during three stages. Our findings are in line with results reported by Talei et al. (2012), Karimi et al. (2009), García-Caparrós and Teresa Lao, (2018).

NaCl accumulation in the soil due to increasing of alkalinity level causes to decrease of the Fe absorption and Iron shortage in the plants. Fe deficiency interrupts chlorophyll synthesis and plant growth. Although Iron shortage affects plant growth negatively however, Yasmeen *et al.* (2016) in their research on a rice cultivar proved that there was a positive correlation between Iron deficiency and salt tolerance. Therefore, in the current study, reducing of Fe under salinity treatment may be as a tolerance mechanism in *Nitraria schoberi*. Although scientific and logical deduction about this subject need to molecular and more detailed studies.

According to our results in the mycorrhizal plants, sugar content decreased but contents of Mg, K and Ca increased. Salinity stress due to decrease of nutrient uptake and transport of them to shoot can change the nutritional status of plants (Munns and Tester 2008). Mg, Ca and K are considered as macronutrients which play a key role in the plant activity and growth. Plants under salinity stress exhibited a decrease of some elements such as Mg, K and Ca concentration in leaves. Maintenance of Ca/Na can improve plant colonization by arbuscular mycorrhiza fungi and under salt stress mycorrhizal plants have appropriate Ca/Na by improving Ca uptake (Evelin et al., 2012). Many studies indicated that mycorrhizal fungi can modify root architecture and therefore, improve plant growth by increase of essential elements uptake and mobilization (Wu *et al.,* 2010), (Ahanger *et al.*, 2014), (Hameed *et al.*, 2014), (Hodge and Storer, 2015). In this research results showed that mycorrhizal fungi could enhance the concentration of these three elements in the salt treated plants. This finding is in line with some reports (Ebrahim and Saleem, 2017), (Garcia-Sancheza *et al.*, 2014).

Our finding show that despite decreasing the chlorophyll and increasing the carotenoid contents in some salinity levels, due to increase of proline and sugar during the three stages, *Nitraria schuberi* can probably be considered as a salt tolerant plant. In some researchers reported that the accumulation of proline and also sugar under salinity stress are adaptive mechanisms which can improve salt tolerance in the plants (Kaur and Asthir, 2015), (García-Caparrós and Teresa Lao, 2018).

The presence of mycorrhizal fungi in natural saline environments has been proved. Studies indicate that mycorrhizal fungi can improve salinity stress tolerance in plants (Hashem et al., 2015), (Rabie, 2005), (Hodge and Storer, 2015). According to many researches, mycorrhizal fungi can improve plant growth, nutrient uptake and tolerance to some stresses (Munns and Tester, 2008), (Wu *et al*., 2010), (Ahanger *et al.,* 2014), (Hameed *et al.* 2014), (Hodge and Storer, 2015). Although in the present study the effects of mycorrhizal fungi on chlorophyll, sugar and proline content in comparison with control were not statistically significant, however increase of Mg, Ca^{2+} and K contents in the mycorrhizal plants can be a reason to improve of these nutrients uptake. According to some studies, mycorrhizal fungi increase the accumulation of K and Mg (Kaur and Asthir, 2015), (García-Caparrós and Teresa Lao, 2018). Kopittke (2012) reported that K^+ and Ca^{2+} can mitigate the toxic effects of Na^+ and improved plant growth, respectively in the saline condition.

Conclusion

Due to proline and sugar accumulation in *Nitraria schuberi* we may conclude that this plant can be considered as a salt tolerant plant. Mycorrhizal fungi mitigate salinity stress in the studied plant by enhancing of some nutrient uptake. Applying of *Nitraria schuberi* as a native

plant by mycorrhiza fungi in urban landscape of Middle East countries can be an appropriate option for regions with the relatively saline soils and waters.

Acknowledgment

This work was supported by Ferdowsi University of Mashhad, Iran, research plant code and date, are 45790 and 4/10/2017, respectively.

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