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MORPHOPHYSIOLOGICAL RESPONSE AND RECLAMATION POTENTIAL OF TWO AGROFORESTRY TREE SPECIES (*Syzygium cumini* AND *Vachellia nilotica*) AGAINST SALINITY

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Problem of salinity has been recognized as core issue across the globe. *Syzygium cumini* (L.) and *Vachellia nilotica* (L.) are multipurpose agroforestry tree species of subcontinent where poor management practices result in soil salinity. Therefore, two studies were designed to test out the reclamation potential and growth of these tree species against salinity. The results of the field study that was conducted in Satiana, a town of District Faisalabad, Pakistan indicated that the soil EC was lower at different depths (0-15 cm and 15-30 cm) under the tree canopies of both species as compared to open field. Consequently, second study was designed to assess the growth of these important agroforestry tree species under different salinity levels (i.e. 10 dSm^{-1}, 20 dSm^{-1}, 30 dSm^{-1}, 40 dSm^{-1} including control) at seedling stage. Morphophysiological parameters (Shoot and root length, shoot and root fresh and dry weights, stem diameter and leaf area, transpiration rate, photosynthetic rate and intrinsic CO_2 concentration etc.) and ionic concentrations (Na^+ and K^+) were studied. Salinity negatively affected the growth of *V. nilotica* but with more prominent effects on *S. cumini*. Good survival rate of *V. nilotica* was observed at higher salinity level (40 dSm^{-1}) but *S. cumini* did not survive at the same level. 84.1% decrease in shoot and 70% in root fresh weight of *V. nilotica* was seen at higher salinity level. Drastic changes were observed in case of shoot and root fresh weights of *S. cumini* at 30 dSm^{-1} i.e. 72.8% and 70.2%, respectively. Increase in Na^+ and decrease in K^+ ion concentration was recorded in different organs of both species. Stomatal conductance and photosynthetic rate of both species were decreased at higher salinity levels. It was concluded that *V. nilotica* is more tolerant to salt stress but *S. cumini* can be cultivated in moderate saline stress condition.
Keywords: Salt tolerance, biomass, tree survival and growth, gas exchange, ion accumulation.

INTRODUCTION

Vachellia nilotica (Gum Arabic tree) is a multipurpose tree, it provides timber, fuelwood, dye, gum and can also be used as live fence. It also impacts the environment through soil reclamation and soil enrichment (Saqib *el al*.,2006). *Syzygium cumini* (Black plum) is also an important tree species present in subcontinent with great economic importance (Rafiullah, 2006). Among different abiotic stresses, salinity is thought to be a key factor which inhibits growth of plants (Saqib *et al*.,2006; Yousaf *et al*.,2018). Reduction in food supply, abandonment of land, environmental contamination, water efficacy and crop yield reductions are some adverse effects of salinity (FAO, 2005). Globally, lands affected by salinity cover more than 397 million hectares. Most of such lands have Magnesium Sulfate (MgSO4), Calcium Chloride (CaCl2), Sodium Chloride (NaCl) and Sodium Sulfate (Na_2SO4) as major salts contributing in salinization (Ghosh *et al*.,2012). Severe salinity problems exist in different countries of various continents including Australia, Pakistan, China, Egypt, Iraq, India, Syria, Mexico, United States and Russia. Out of total land area in Asia-pacific zone almost 6.1 % has been affected by salts (Wicke *et al*.,2011). Assessments revealed that globally about 45 million hectares of irrigated lands have been affected by salinity (World Bank, 2006; Hasanuzzaman *et al*., 2014). Pakistan ranks 8th in the world with respect to land affected by salinity (FAO, 2005; Corbishley and Pearce, 2007).

Application of brackish irrigation water and overuse of fertilizes in most parts of Pakistan are considered as major reasons of saintly. Due to such practices 13 % of irrigated agricultural land of the country is classified as strongly saline (WAD, 2018). Nearly salinity has affected about six million hectare land in Pakistan and it falls under Indus Basin, most of which is located in Sindh and Punjab provinces (Qureshi *et al*., 2008). Plant growth and crop production is greatly restricted by salt stress (Munns, 2002; Abdul-Jaleel *et al*., 2007). Numerous enzymatic activities and photosynthetic rates are reported to be affected by salt stress. In addition, it also restrains development of seedling and germination process, which is the crucial phase for any plant (Alaoui-Sosse *et al*., 1998; Sairam and Srivastava, 2002; Misra and Dwivedi, 2004; Yang *et al*., 2009; Zushi *et al*., 2009; Bacchetta *et al*., 2010; He *et al*., 2011; Li *et al*., 2011; Abid *et al*., 2011). Salts affect key biological processes of plants like photosynthetic and respiration rates etc. (Parida and Das, 2005). Reduction in biomass of the plants in response to high salinity levels has also been reported in salt tolerant plants (Wang *et al*., 2009; Parida *et al*., 2016). In crops different types of problems are caused by salinity such as oxidative, osmotic, secondary and ionic stresses (Sha *et al*., 2019). Salinization can have serious impacts on the root growth but studies on growth of roots related to salt stress are scarce. Sodicity disturbs the physicochemical properties of soil which ultimately affects root penetration of trees. Higher leaf fall and disturbance in root biomass was observed in highly saline patches (Hossain and Nuruddin, 2016). Salinity reduces the plant growth and limits various physiological processes (Bhaskar and Bingru, 2014). Photosynthetic rate of seedlings was significantly reduced by increasing the salt stress levels. High doses of NaCl decreased the photosynthesis and transpiration rates of seedlings which ultimately increased the photosynthetic water use efficiency (Negrao *et al*., 2017). Higher salt accumulation in young leaves reduced the leaf area and ultimately the photosynthetic rate (Munns *et al*., 2002; Karimi and Kuhbanani, 2014). Poor stomatal conductance in tree species under salt stress reduced the carbon dioxide which significantly affected the photosynthesis. A relatively low level of intracellular CO_2 was observed in *P. australis* due to salt stress (Choi *et al*., 2005). Heavy accumulation of Na^+ in different parts was a major reason for osmotic disturbance, nutrient imbalance and specific ion toxicity (Junbo Xiong *et al*., 2017). There are various techniques to reclaim saline soils like engineering, chemical and biological approaches. Engineering and chemical approaches are costly and require recurring budgets but biological approach is cheaper and also provides socio-economic benefits to the farmers. In biological approach, salt tolerant tree species are used to reclaim the problematic soil (Hasanuzzaman *et al*., 2014).

Syzygium cumini and *Vachellia nilotica* are multipurpose tree species commonly grown in social forestry. Therefore, this study explored the potential of *S. cumini* and *V. nilotica* against salt stress at seedling stage in term of morphophysiological growth and ionic response.

MATERIALS AND METHODS

Description of Study Sites: In current research program first phase of study was conducted in Satiana, a small town situated 27 km from Faisalabad. It has an area of 5856 km^2 and lies at 31°12'06.47"N latitude and at 73° 10'12.17"E longitude, located at the height of 184 m above sea level. About 3-4 decades ago salinity and waterlogging problems were reported as serious issues of Faisalabad division, especially in Satiana.

Surveying and Collection of Soil Samples*:* To assess the current condition of these issues a survey was carried out in Faisalabad (Satiana) by using a pre tested questionnaire with the help of Agriculture Extension Officers. About 46 farmers were interviewed in person and the collected data were analyzed by using statistical software SPSS. Soil samples were collected from selected sites of Satiana. Sampling was carried out with the help of auger from the site where the farmers were already using different tree species for the reclamation of saline soil. Sampling was done from under and away the canopies of the selected tree species (V. nilotica, S. cumini) in the affected fields. Samples were taken from 2 depths i.e. (0-15 cm, 15-30 cm), from four directions i.e. east, west, north and south around the tree and were mixed to attain a composite sample for each depth during the study. Samples were analyzed from Saline Agriculture Laboratory, University of Agriculture, Faisalabad and Ayub Agricultural Research Institute, Faisalabad.

Based upon the results of field study an experiment was conducted under controlled conditions in Department of Forestry and Range Management, University of Agriculture, Faisalabad, it lies at 31°25'7.37"N latitude and at 73° 4'44.79"E longitude. To study the influence of salts on *V. nilotica* and *S. cumini,* a pot trial was performed under control conditions. Soil samples were analyzed for EC, pH, saturation percentage (SP), soil organic matter % (OM) and textural class (Table 1). Physicochemical properties were measured according to USDA handbook No 60 (Richards, 1954).

Table 1. Physicochemical properties of soil.

Characteristic	Values
Texture	loam
Sand (%)	35.44±0.45
Silt (%)	41.71±0.92
Clay (%)	21.60±0.82
SP (%)	29.00±1.72
OM (%)	0.78±0.13
pH	7.51±0.04
EC dSm^{-1}	2.56±0.31
SAR	8.54±0.45

Planting material and experimental design**:** Before developing salinity levels soil was air-dried, 12 kg of soil was added to each pot. Except control, four salinity levels were developed by the addition of calculated volume of NaCl i.e. 10, 20, 30 and 40 dSm^{-1}, respectively, with five replications and were organized according to CRD in factorial arrangement. After developing the desired salinity levels, soil was tested from each treatment. Uniform sized seedlings of *V. nilotica* and *S. cumini*, raised in the nursery of Department of Forestry were used for the said study. After shifting, seedlings were allowed to acclimatize in normal conditions before the application of treatments. Weekly data were recorded for each plant with respect to its survival at different salinity levels. Time period of trial was six months and after that following parameters were recorded.

***Plants harvesting and measurement of biomass*:** At the termination of experiment plants were harvested. Plant height, root and shoot length were measured with measuring tape. Stem diameter was recorded by using Vernier Caliper. Leaf area of the plants was measured with leaf area meter (YAXIN-1241/CI-20-CID). After harvest, data regarding fresh and dry shoot and root weights were recorded with the help of weighing balance (Extech SC600 electronic counting scale USA). For measuring roots dry weight and shoots dry weight plants were separately oven dried (DHG 9202 series, Thermostatic Drying Oven) at 75^0C for 48 hours.

Measurement of gas exchanges parameters:

Before harvesting different physiological parameters i.e. transpiration rate, stomatal conductance, photosynthetic rate, intrinsic CO_2 concentration and photosynthetic water use efficiency (Bashir *et al*., 2018) were measured with the help of portable Infra-Red Gas Analyzer (IRGA) between 10:00 and 12:00 am with maximum day light intensity.

***Measurement of ionic concentration*:** 50 mg of dried sample was digested by using 2 ml of HNO_3. K^+ and Na^+ ion concentration was determined by using flame photometer (Sherwood-410).

***Statistical analysis*:** Means were compared by using Tukey's test. Data were statistically analyzed by using SPSS software.

RESULTS

***Tree services and soil reclamation*:** Results of first phase of study revealed that maximum number of respondents had much information regarding services of trees such as soil reclamation, shelter etc. The results showed that problematic soil was increasing with the passage of time due to mismanagement and overuse of brackish underground water. Satiana had the respondents 65 %, 30 % and 22 % who thought that they have land facing the problems of salinity, waterlogging + salinity and only waterlogging, respectively (Figure1a). It was observed that tree planting trend at selected site was increasing gradually. The 60% of the respondents believed that trees planting trend was increasing whereas 35% of the respondents were of the view that it was decreasing (Figure 1b). Satiana site had 70.1% respondents, who thought that trees reclaimed the soil, 20.2 % respondents were agreed that trees acted as windbreaker and 10.2 % respondents thought that trees provide shade, shelter, habitat for wildlife etc (Figure 1c). The results revealed that after three years slight reduction in EC under the tree canopies was recorded as compared to control site i.e. away to canopy (Figure 2). The EC values were higher at 0-15 cm as compared to 15-30 cm soil depths. In Satiana soil EC was higher during 1st year at 0-15 cm soil depth (12.2, and 12.1 dSm^{-1}) as compared to 3rd year (11.5, and 11.9 dSm^{-1}) for both tree species, respectively (Figure 2). The results indicated that the soil EC was lower at both depths (0-15 cm and 15-30 cm) under tree canopy as compared to open field (Figure 2).

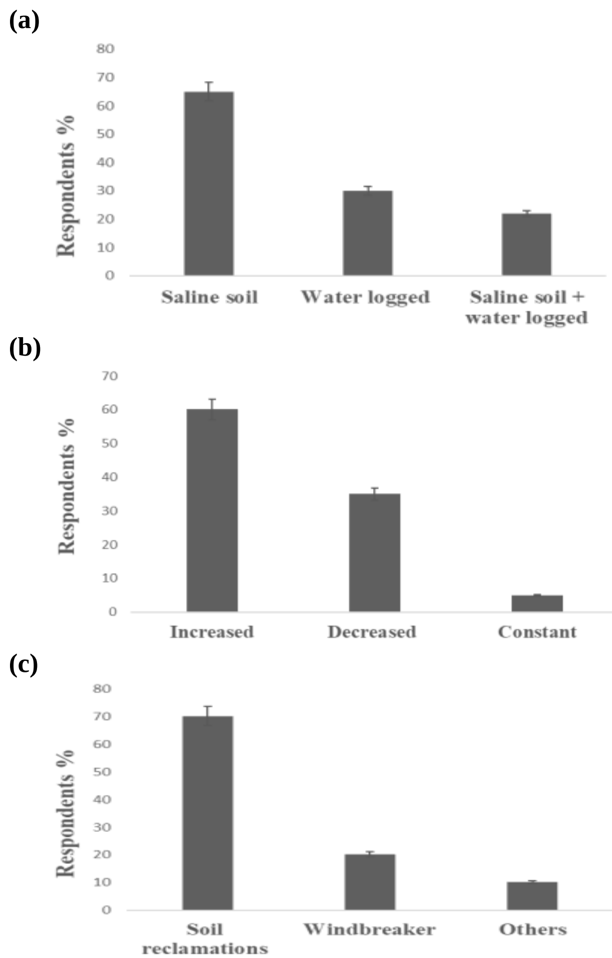

Figure 1. Distribution of respondents according to a) problematic soil (b) trend of panting during last five years and (c) tree services at Satiana.

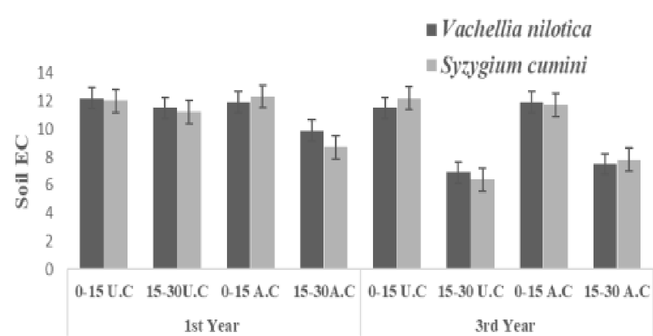

Figure 2. Effect of time duration on soil EC (dSm^{-1}) under the tree canopy (U.C) and away to tree canopy (A.C) of *V. nilotica* and *S. cumini* at two depths (0-15 and 15-30 cm) at Satiana site

Morphological response: All plants of *V. nilotica* survived against higher salt stress i.e. 40 dSm^{-1} but *S. cumini* did not survive at same level. Reduction in biomass production in both tree species was recorded at higher salinity levels as shown in Fig. 3. Comparison of means of recorded morphological attributes revealed significant decline with the increase in salinity levels for both tree species ($p<0.05$). Moderate and high salinity adversely affected the root length of *S. cumini* and *V. nilotica* up to 65 % (Figure 3a), shoot length also behaved likewise. The shoot fresh weight significantly decreased at higher salt stress (Figure 3e) i.e. 72.85% and 84.1%, respectively, dry weights upto 70 % in both species (Figure 3c, 3d). A significant decrease in plant leaf area was recorded between 30 to 40 dS dSm^{-1} i.e. 21.42 % and 56.31 %, respectively (Figure 3g, 3h). A drastic decrease in stem diameter was recorded at high salinity for both species (Fig. 3b).

***Physiological response*:** Comparison of means of recorded physiological attributes revealed significant decline with the increase in salinity levels for both tree species ($p<0.05$). At higher salinity level the photosynthetic water use efficiency increased to 33.7 % in *S. cumini* and 30.39 % in *V. nilotica* (Figure 4a), transpiration rate was reduced to 75.9 % and 67.9 % (Fig. 4e), intrinsic CO_2 concentration was reduced i.e. 47.85% and 38.4% (Figure 4b), respectively, stomatal conductance was decreased to 38.54 % and 30.56 % (Figure 4d), photosynthetic rate was reduced to 74.07% and 78.6% in both species, respectively (Fig. 4c).

***Potassium and sodium concentration*:** Na^+ and K^+ ion concentration was significantly different in all treatments ($p<0.05$). Significant reduction in K^+ ion concentration was recorded in the shoots, leaves and roots at higher salinity level (Figure 5). Na^+ ion concentration in roots and leaves increased up to 70.1 % and 80.2 %, respectively (Fig. 5d, 5f). K^+ ion concentration in shoots was reduced as compared to control conditions (Figure 5c). Na^+ ion concentration in shoot increased to 70.8 % in *S. cumini* and 73.5 % in *V. nilotica*,

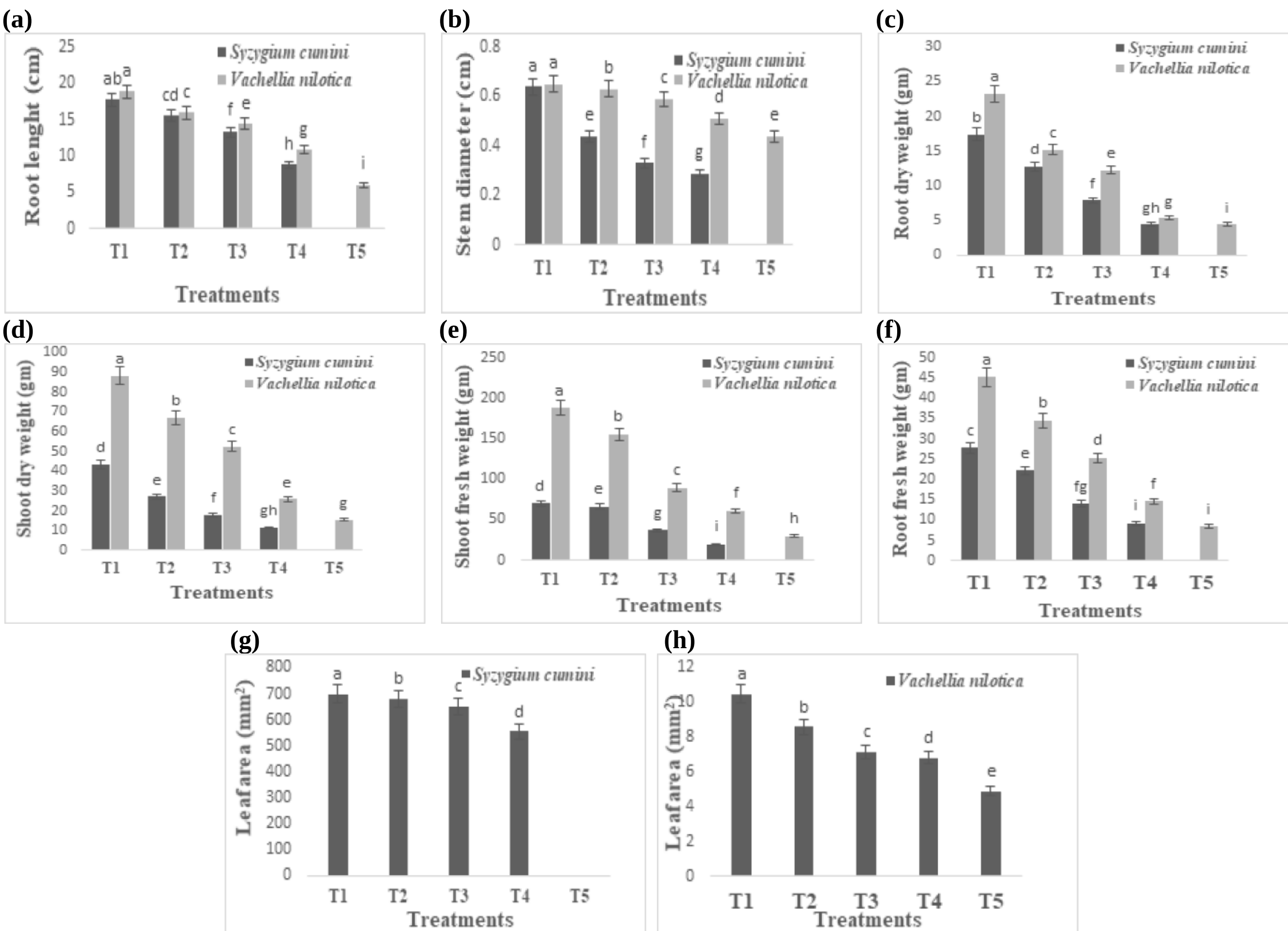

Figure 3. Effect of NaCl salinity on (a) root length (b) stem diameter (c) root dry weight (d) shoot dry weight (e) shoot fresh weight (f) root fresh weight (g) leaf area of *Syzygium cumini* (h) leaf area of *Vachellia nilotica*. Values are means with letters reported significant differences (*p*< 0.05).

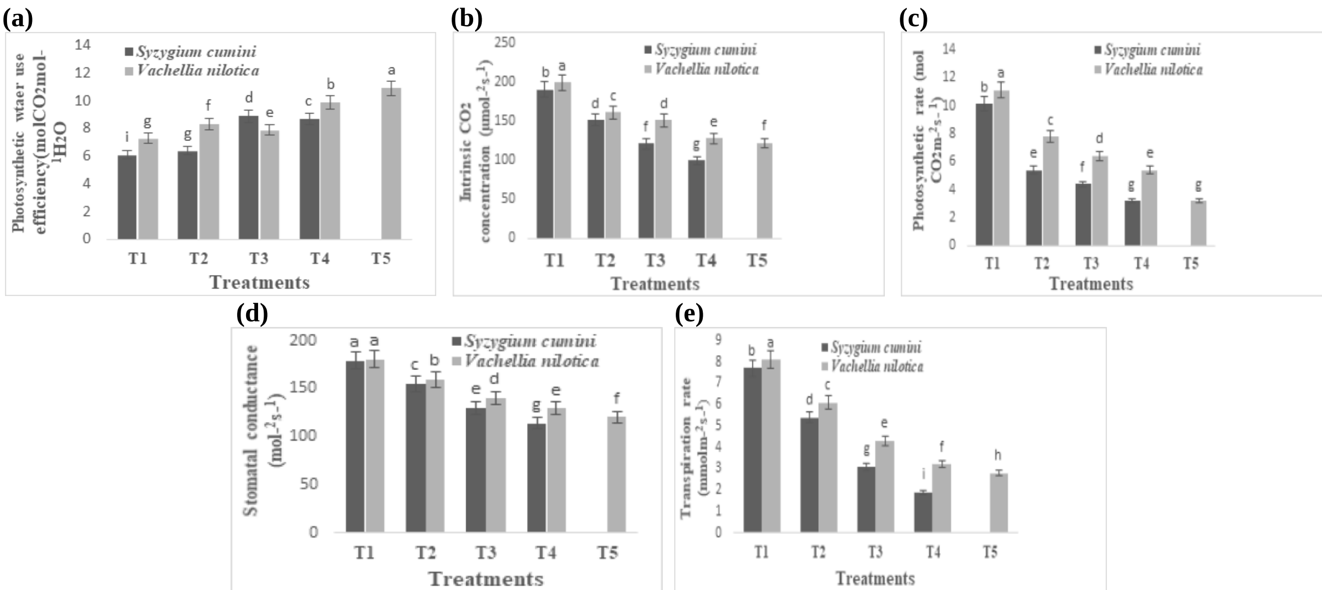

Figure 4. **Effect of NaCl salinity on (a) photosynthetic water use efficiency (b) intrinsic CO_2 Con. (c) photosynthetic rate (d) stomatal conductance (e) transpiration rate. Values are means with letters reported significant differences (*p*< 0.05).**

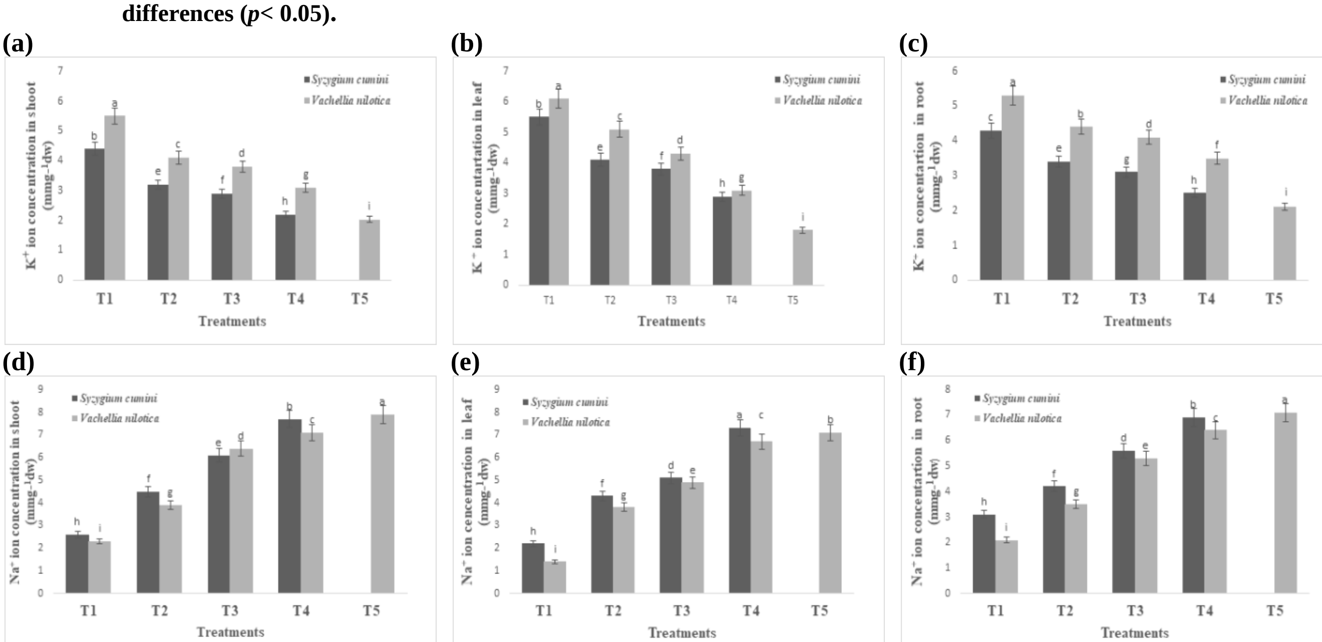

Figure 5. **Effect of NaCl salinity on (a) K^+ ion concentration in shoot (b) K^+ ion concentration in leaf (c) K^+ ion concentration in root (d) Na^+ ion concentration in shoot (e) Na^+ ion concentration in leaf (f) Na^+ ion concentration in root. Values are means with letters reported significant differences (p< 0.05).**

respectively. However, decrease in K^+ ion concentration in *V. nilotica* shoot was recorded i.e. 49 % under 40 dSm^{-1} (Fig. 5c). This increase was more prominent in the shoot for *V. nilotica* than *S. cumini*.

DISCUSSION

In field study higher EC levels (12-13 dSm^{-1}) was observed in top soil as compared to subsoil which is in line to those of Noureen *et al*. (2008) who observed more EC in top soil. Moreover, the researcher observed that leaf litter of *Acacia jacquemontii* in Cholistan desert slightly reduced the EC of soil. Increasing trend was observed for the promotion of agroforestry and major tree species planted were *Populus deltoides* (W. Bartram) *Dalbergia sissoo* (Roxb.), *Vachellia.*

nilotica and *Eucalyptus camaldulensis* (Dehnh.) during last decade (Yousaf *et al*., 2018). Current study concluded that trees improve soil fertility which is exactly in line with the findings of Safdar (2012), who described that trees improve physicochemical properties of soil. In terms of plant biomass and growth in saline conditions, *V. nilotica* responded much better as compared to *S. cumini*. This suggested that the *V. nilotica* is suitable species for cultivation in saline soils. In this investigation although a decreasing trend was observed in above (i.e. plant height, leaf area, stem diameter, shoot fresh and dry weights) and underground (i.e. root fresh and dry weights) biomass allocation but survival of seedling was not affected and results are in agreement with various pervious findings (Wang *et al*., 2011; Nawaz *et al*., 2016). The results demonstrated that *S. cumini* and *V. nilotica* biomass was significantly reduced at higher salinity levels. Salinity reduces plant biomass in halophytic tree species due to toxic and osmotic effects (Koyro *et al*., 2006; Turan *et al*., 2010; Wang *et al*., 2011; Khalil *et al*., 2012; Teakle *et al*., 2013). Variation in growth and biomass production of halophytic tree species in saline soil was also observed due to the salinity (Munns, 2002). Growth rate of seedling was affected under salt stress conditions (Maas and Hoffman, 1977; Qureshi *et al.*, 2008; Munns and Tester, 2008; Yue, 2012; Abbas *et al.*, 2013; Harter, 2014). The reduction of root and stem diameter of *S. cumini* and *V. nilotica* with increased NaCl levels are similar to the results of Beritognolo *et al*., 2007, who reported reduced growth of *Hibiscus cannabinus* under salt stress. When salt stress was increased, plants leaf area was reduced significantly because salt stress inhibited plant growth. The same finding was also observed by many other researchers as well (Kramer, 1983; Curtis and Lauchli, 1986; Brungnoli and Lauteri, 1991; Alberico and Cramer, 1993; Mahmood *et al*., 2009; Hameed *et al*., 2010; Jiang *et al*., 2014; Behera *et al*., 2015; Jose *et al*., 2017). Decrease in shoot and root length was observed because of high NaCl concentrations as compared to normal soil. Increase in salinity resulted in the reduction of the length, similar results were also observed in other woody plants by Nabil and Coudret (1995). According to Rahimi *et al*. (2012) salinization is main reason for reductions of biomass. Decrease in shoot and root dry weight was observed only at higher salinity levels. Similar findings were reported for *Atropa belladonna* by Bilski *et al*. (1988).

In current study, significant decrease was observed in leaf gas exchange parameters with increasing salinity levels. The finding of this study indicated that photosynthetic rate of seedlings in *S. cumini* and *V. nilotica* was significantly reduced by increasing the levels of salinity. This supported the results of previous studies (Brugnoli and Lauteri, 1991; Chaves *et al*., 2009; Bhaskar and Bingru, 2014; Geissler *et al*., 2015; Hassan and Narges, 2017), who found that photosynthetic rate in the seedlings of halophytes decreases with increasing salinity levels. In current study stomatal conductance drastically decreased at higher salinity level but it remained unchanged at moderate salinity level which confers the findings of Parida *et al*. (2004). Similar results were reported earlier on *Avicenna marina* (Eller *et al*., 2014; Hoa *et al*.,2015). Decreased stomatal conductance is followed by low transpiration rate and less assimilation of CO_2 (Marler and Zozor, 1996; Romero-Aranda *et al*., 2001; Ashraf, 2001; James *et al*., 2002; Martínez-Alcántara *et al*., 2015) which is in close relation with the findings of this study where increase in salinity decreased transpiration rate remarkably. Similar results were observed in *Avicenna marina* (Eller *et al*., 2014). The reduction in transpiration rate was likely due to the reduced photosynthetic activity. Poor stomatal conductance resulted in less transpiration rate and closing of stomata (Parida *et al*., 2003; Negrao *et al*., 2017). A comparatively low level of intracellular CO_2 concentration was recorded in *V. nilotica* at 40dSm-^{1} and similar results have been reported by Choi *et al*., 2005. Photosynthetic water use efficiency (PWUE) significantly increased when the salinity level was increased. Increase in PWUE of *V. nilotica* at high salinity level resulted in rapid decline of transpiration rate relatively than assimilation of CO_2 (Tan *et al*., 2019) which is similar to the finding of this study at higher salinity level. Na^+ contents of the shoots, leaves and roots were increased with increasing concentration of NaCl and K^+ contents were significantly reduced in all parts of plant. Similar results have been reported by Renault *et al*. (2011) in seedlings of *Gleditsia tricanthos*. Heavy NaCl accumulation has also been reported for many tree species when exposed to salinity (Nabil and Coudret, 1995). In current study, Na^+ ion accumulation was higher in the shoots as compared to leaves and roots. Na^+ ion concentration was higher in shoots of *V. nilotica* and *S. cumini* seedling, due to higher Na^+ ion concentration in soil. Usually, in cultivars which are sensitive to salts, sodium contents are increased by increasing salinity (Alaoui-Sasse *et al*., 1998; Sairam *et al*., 2002; Poustini and Siosemardeh, 2004; Zhu *et al*., 2016) while concentration of potassium ion (K^+) reduced in all plant parts when salt concentration is increased. This strange relation between concentrations of sodium ion (Na^+) and potassium ion (K^+) is thought to be a significant physiological mechanism in plants and it plays a vital role in various plant species (Willadino and Câmara, 2005).

Conclusion: On the basis of present investigations, it is concluded that *V. nilotica* can be planted in highly saline soils having EC (40dSm^{-1}) and *S. cumini* can be used for moderate soils (15-20dSm^{-1}) as biological reclaiming agent.

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