Indian Journal of Agricultural Sciences **92** (12): 1480–1483, December 2022/Article
https://doi.org/10.56093/ijas.v92i12.102244

Physiological adaptability of *Salvadora oleoides* to sodicity and salinity stress

ASHWANI KUMAR[1]*, ANITA MANN[1], CHARU LATA[1] and ARVIND KUMAR[1]

ICAR-Central Soil Salinity Research Institute, Karnal, Haryana 132 001, India

Received: 10 July 2020; Accepted: 1 November 2022

ABSTRACT

An experiment was conducted in microplots at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana during 2013–16 to study the adaptive response of *Salvadora oleoides*, a facultative halophyte under saline, sodic and mixed saline-sodic conditions. *S. oleoides* was not able to survive at higher sodicity (*p*H ~ 10.0) and salinity (ECe ~ 35 dS/m) as well as on the mixed sodicity and salinity levels (*p*H ~ 9.0 along with ECe ~ 10, 15, 20 dS/m). Stress either salinity or sodicity brought reduction in plant height, chlorophyll content and gas exchange attributes, but *S. oleoides* maintained gaseous exchange at moderate stress level. *S. oleoides* accumulated 4 fold higher proline under sodic stress and 6 fold under salinity stress. Other osmolytes, TSS and soluble protein decreased with stress intensification to maintain osmotic balance. In comparison to control, sodicity enhanced Na^+ and Cl^- by 54.57% and 20.33%, while under salinity, Na^+ was enhanced by 141.52–256.09% and Cl^- by 47.83–115.58% at ECe ~ 15 and 25 dS/m, respectively. Inspite of such higher increase in Na^+ and Cl^-, *S. oleoides* retained good amount of mean K^+ (1.22%) in leaf tissue. *S. oleoides* also maintained leaf Na^+/K^+ below 1.0 under stress condition of *p*H ~ 9.5 and ECe ~ 15 dS/m. Based on the studied physio-biochemical analysis, *Salvadora oleoides* exhibited good adaptive potential under moderate salinity and sodicity stress and could be used as a promising salt-tolerant plant species for plantation in salt affected areas.

Keywords: Na^+/K^+, Osmolytes, Photosynthetic traits, Salinity, *Salvadora oleoides*, Sodicity

The arid and semi-arid regions of the world witnessed the most unstable and vast tracts of barren land because of high erratic rainfall, evapotranspiration, solar radiation and low atmospheric humidity. Nearly one billion hectares of arid and semi-arid areas of the world are salt-affected and remain barren. Salt-induced land degradation is a major ecological impediment in India which is predicted to increase from the current 6.73 million hectares (Mha) to 16.2 Mha by 2050. Salt tolerance is a multifarious phenomenon, as salinized plants experience physiological stress initially from the osmotic stress and subsequently from the specific ion effects (Kumar *et al*. 2016, Mangalassery *et al*. 2017, Kumar *et al*. 2018a). Salt tolerance is a complex trait, involving multiple alterations and integration in the transport and compartmentalization of K^+, Na^+ and $Cl^−$ ions, compatible solute synthesis, and morphological adaptation (Kumar *et al*. 2018b, 2019a). Rehabilitation of salt affected soils with increasing soil salinization and shortage of fresh water availability needs a major shift to evolve innovative technologies and domesticate halophytes of high economic value.

Salvadora oleoides Decne. (Salvadoraceae) locally known as *mithijal* is an ecologically, economically and socially important plant species that is grown as multipurpose tree in arid regions of western India and Pakistan and is one of the dominant tree species in the vast area of Kutch (northwest saline desert) and in the semi-arid central area of the Saurashtra region, south to the Kutch, Gujarat, India (Vaghela *et al*. 2009). *S. oleoides* is a typical facultative halophytic tree of great importance because of its pharmaceutical, fodder, fuel, and timber values (Ramoliya and Pandey 2002). Its seeds are also a rich source of non-edible oil (45–50%). Leaves of *S. oleoides* are excellent source of fodder and stem and branches are used for fuel. Owing to adaptability of *S. oleoides* to saline habitats and its economic use, this species is selected and characterized physiologically and biochemically under alkaline and saline conditions to know its tolerance limit.

MATERIALS AND METHODS

Root slips of *Salvadora oleoides* were collected from the Great Rann of Kutch in the Northwest India (Bhuj, Gujarat). The plants were established through cuttings in micro-plots (2.5 m × 1.5 m × 0.5 m) filled with sandy loam soil (ECe ~ 043; *p*H ~ 8.1) in a screen house under natural environment during 2013–2016. After establishment, different stress conditions i.e. control, two levels of sodicity

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[1] ICAR-Central Soil Salinity Research Institute, Karnal, Haryana. *Corresponding author email: Ashwani.Kumar1@icar.gov.in

stress (*p*H ~ 9.5 and 10.0), three levels of salinity stress (ECe ~ 15, 25 and 35 dS/m) and mixed saline sodic (*p*H ~ 9.0 along with ECe ~ 10, 15 and 20 dS/m) were imposed under natural environment. Stress treatments were given through saline/sodic irrigation water supplemented with 3:1 of Cl$^-$ dominated salts for salinity and NaHCO$_3$ for sodicity. Regularly, ECe and *p*H were checked and desired stress levels were maintained. After 6 months of growth, plant height was measured with the help of meter scale rod from the ground surface to the tip. Fully expanded leaves were sampled to quantify the chlorophyll content (Hiscox and Israelstam 1979). Gas exchange attributes were measured with an infrared open gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA) between 10:00 AM to 12:00 PM. Chlorophyll fluorescence (Fv/Fm) was determined using a portable pulse modulated fluorescence measurer (Junior PAM Chlorophyll Fluorometer, Germany) after adapting the leaves to dark for 25 min via special leaf clips. Estimation of osmolytes were done using the methods of Yemm and Willis (1954) for soluble sugars, Bates *et al*. (1973) for proline content and Bradford (1976) for soluble protein content. Na$^+$ and K$^+$ contents were measured in di-acid digested plant material with the flame photometer (PFP7, Jenway, Bibby Scientific, UK). The data were analyzed statistically using randomized block design (Version 9.3, SAS Institute Inc., Cary, NC, USA) and DMRT was applied at 5% probability level to compare the mean differences.

RESULTS AND DISCUSSION

Results obtained from the present experiment showed that *Salvadora oleoides* could not survive at higher sodicity (*p*H ~ 10.0) and salinity (ECe – 35 dS/m) as well as on the mixed sodicity and salinity levels (*p*H ~ 9.0 along with ECe – 10, 15, 20 dS/m). Salt stress generally hastens plant physiology and metabolism aspects, which is attributed to the water and nutritional imbalance and toxic ion accumulation (Kumar *et al*. 2019a, 2021). Among phonological traits, reduction in plant height is a viable indicator of salt tolerance, or sensitivity. *S. oleoides* had plant height of 160.98 cm under control conditions which was decreased by 53.7% under sodic stress (*p*H ~ 9.5), while salinity stress caused reduction of 32.3% and 47.1% at ECe ~ 15 and 25 dS/m, respectively (Table 1). This reduction in plant height might be due to reduced osmotic potential, alterations in cell wall extensibility and turgor pressure, inhibition of cell division and expansion, and nutritional imbalance caused by higher uptake of Na$^+$ and Cl$^-$ (Tavakkoli *et al*. 2011, Pooja *et al*. 2019).

In earlier studies, it was very well documented that salinity caused reduction in the rate of photosynthesis in terms of CO$_2$ exchange, either by restricting CO$_2$ diffusion into the leaves or by the reduced efficiency of ribulose-1, 5-bisphosphate carboxylase/oxygenase for carbon fixation and photophosphorylation (Sudhir and Murthy 2004). Total chlorophyll content was 2.24 mg/g in *S. oleoides* under control condition, which was reduced by 6.7% under sodic stress (*p*H ~ 9.5) and 9.82% and 20.54% under salinity stress (ECe ~ 15 and 25 dS/m) in comparison to control (Table 1). This decrease in chlorophyll contents might be attributed towards reduced chlorophyll biosynthesis due to reduction in activity of ALA synthase and by nutrient deficiency particularly magnesium, required for the biosynthesis of chlorophyll pigments or increased chlorophyllase enzyme activity and ROS formation due to photo-inhibition (Garg and Singla 2004). Present results showed that net photosynthesis (Pn), stomatal conductance (gS), transpiration rate (E) and chlorophyll fluorescence (Fv/Fm) decreased significantly with stress intensification in *S. oleoides* (Table 1). Photosynthesis is the backbone for producing biomass by means of source activity and found that *S. oleoides* showed net photosynthetic rate of 28.73 μmol CO$_2$/m^2/s under control condition. Sodicity stress caused 26.38% reduction in Pn while salinity stress led to 29.52% and 35.89% reduction at ECe ~ 15 and 25 dS/m, respectively (Table 1). Stomatal conductance (gS) regulates the entry of CO$_2$ and exit of H$_2$O in the form of water vapour (Chaves *et al*. 2002) and found significant reduction under stress condition. Both these stresses led to reduced gS by 21.0–28.0% but salinity of ECe ~ 25 dS/m caused higher reduction. Transpiration mainly reflects the potential of plant water consumption which was closely associated with stomatal conductance and it was also found in literature that abiotic stresses particularly salinity caused an imbalance in leaf water status and dehydration at the cellular level

Table 1 Effect of salt stress (sodicity and salinity) on physiological traits of *S. oleoides*

Treatment/Trait	Plant height (cm)	Chlorophyll content (mg/g FW)	Pn (μmol/m^2/s)	gS (mmol/m^2/s)	E (mmol/m^2/s)	Chlorophyll fluorescence (Fv/Fm)
Control	160.98 ± 3.82^{a}	2.24 ± 0.04^{a}	28.73 ± 0.696^{a}	0.567 ± 0.015^{a}	7.08 ± 0.169^{a}	0.749 ± 0.006^{a}
*p*H ~ 9.5	74.48 ± 1.58^{d}	2.09 ± 0.015^{b}	21.15 ± 0.212^{b}	0.447 ± 0.009^{b}	5.51 ± 0.091^{b}	0.710 ± 0.004^{b}
ECe ~ 15 dS/m	108.98 ± 3.82^{b}	2.02 ± 0.027^{c}	20.25 ± 0.584^{b}	0.417 ± 0.013^{c}	5.79 ± 0.239^{b}	0.707 ± 0.004^{b}
ECe ~ 25 dS/m	85.19 ± 2.18^{c}	1.78 ± 0.01^{d}	18.42 ± 0.259^{c}	0.409 ± 0.005^{c}	4.29 ± 0.159^{c}	0.670 ± 0.006^{c}
General mean	107.4	2.03	22.13	0.46	6.44	0.71
CD (P=0.05)	8.61	0.071	1.285	0.014	0.592	0.015
CV (%)	4.94	2.17	3.58	1.901	6.437	1.343

Means with at least one letter common are not statistically significant ($P<0.05$) using DUNCAN's Multiple Range Test. Pn, net photosynthesis; gs, stomatal conductance; E, transpiration rate.

(Kumar *et al.* 2019b). In *S. oleoides*, transpiration rate (E) was 7.08 mmol $H_2O/m^2/s$ under control environment and decreased by 22.18% at *p*H ~ 9.5, while salinity stress of 15 and 25 dS/m reduced E by 18.22 and 39.41% (Table 1). It was noted from the results that *S. oleoides* showed lesser reduction or maintained E under lower salinity level. Such decrease in leaf gas exchange attributes might be attributed to decrease in CO_2 fixation due to stomatal closure (Dhansu *et al.* 2022) or due to reduction in carboxylase activity and enhanced oxygenase activity of Rubisco enzyme (Sudhir and Murthy 2004). Fluorescence of leaf chlorophyll was a good indicator of photosynthesis activity which also provides an insight to how efficiently a leaf is performing photosynthesis. Maximal efficiency of PSII photochemistry (Fv/Fm) was 0.749 under control conditions. Results revealed that sodicity caused 5.21% reduction whereas Fv/Fm reduced by 5.61% at ECe ~ 15 dS/m and 10.55% at ECe ~ 25 dS/m (Table 1). This decrease in Fv/Fm under salt stress is mainly due to damage or inactivation of PSII reaction centres, a phenomenon commonly observed in plants under stress (Baker and Rosenqvist 2004).

To mitigate the adverse effects created by salt stress, *S. oleoides* accumulates organic solutes, such as proline, soluble sugar and soluble proteins that could adjust cellular osmotic potential for better uptake of water (Kumar *et al.* 2018b). Under control condition, *S. oleoides* accumulated 0.674 mg/g proline which increased to 2.34 mg/g at *p*H ~ 9.5, 2.04 mg/g and 3.78 mg/g at ECe ~ 15 and 25 dS/m (Table 2). Proline accumulation under stress condition helps in stabilization of proteins, membranes and sub-cellular structures and protecting cellular functions by scavenging reactive oxygen species (Mann *et al.* 2019). It was also noted from the results that *S. oleoides* accumulated 5.81 mg/g TSS, which decreased to 4.83 mg/g at *p*H ~ 9.5, 5.23 mg/g and 4.38 mg/g at ECe ~ 15 and 25 dS/m. Stress caused reduction in TSS might serve as a protective molecule and also provide source of energy and carbon (Lata *et al.* 2019) or enhance the activity of hydrolytic enzymes that lead to decreased TSS content (Pooja *et al.* 2019).

Soluble proteins involved in energy metabolism and cellular maintenance, also played a central role in stress tolerance (Kosová *et al.* 2013). The maximum proteins content was recorded at control, i.e. 10.95 mg/g which decreased with the increased stress (Table 2). Sodicity and salinity stress caused 9.5–16.0% reduction in soluble protein content and this decrease in protein content might be due to increased protease activity that could act as a source of stored nitrogen that could be re-mobilized during stress period (Kumar *et al.* 2015, Pooja *et al.* 2017).

In addition to accumulation of organic osmolytes, *S. oleoides* also accumulated inorganic solutes in the form of Na^+, K^+ and Cl^- but salt stress led disturbance in availability, absorption and transportation of nutrient contents (Lata *et al.* 2022). These ions could also serve as inorganic osmolytes upto certain limits but higher accumulation resulted in ion toxicity. Present results revealed that salt stress led to enhanced Na^+ and Cl^- of leaves by 54.57% and 20.33% under sodic stress of *p*H ~ 9.5 (Table 2). Whereas, much higher increase was noted under salinity stress, i.e. 141.52% and 256.09% Na^+ and 47.83% and 115.58% Cl^- at ECe ~ 15 and 25 dS/m, respectively in comparison to their respective control (Table 2). It is interesting to note that inspite of such higher increase in Na^+ and Cl^-, *S. oleoides* retained good amount of mean K^+ (1.22%) in leaf tissue, i.e. sodic stress led to substantial decrease in K^+ by 20.2%, whereas salinity of ECe ~ 15 and 25 dS/m reduced K^+ by 12.28% and 18.34% (Table 2). It is believed that good supply of K^+ could minimize the harmful effects of high Na^+ under stress condition and such diminution of K^+ in leaf tissue might also be due to direct competition between K^+ and Na^+ at plasma membrane (Kumar *et al.* 2016).

Excessive accumulation of Na^+ and Cl^- in the leaf tissue was always considered highly harmful for normal metabolism, but maintaining Na^+/K^+ homeostasis was vital and positively related with higher salt tolerance (Kumar *et al.* 2016). *S. oleoides* showed Na^+/K^+ of 0.328 under control condition which increased to 0.636 at *p*H ~ 9.5, 0.903 at ECe ~ 15 dS/m and 1.43 at ECe ~ 25 dS/m. Na^+/K^+ ratio was regarded as one of the most significant measures to see the plant response to salt stress (Kumar *et al.* 2018c) and it was noted from the results that *S. oleoides* maintained their Na^+/K^+ below 1.0 under salt stress condition of *p*H ~ 9.5 and ECe ~ 15 dS/m. From the results, it was concluded that *Salvadora oleoides* exhibited good adaptive potential under moderate salinity and sodicity stress in terms of photosynthetic carbon assimilation, accumulation of osmo-protectants and by maintaining low Na^+/K^+. *Salvadora oleoides* could be used as a promising salt-tolerant plant

Table 2 Effect of salt stress (sodicity and salinity) on osmolytes accumulation and ionic analysis of *S. oleoides*

Treatment/Trait	Proline content (mg/g FW)	Soluble sugar (mg/g FW)	Soluble protein (mg/g FW)	Na^+ content (% DW)	K^+ content (% DW)	Cl^- content (% DW)
Control	0.674 ± 0.022^d	5.81 ± 0.082^a	10.95 ± 0.355^a	0.460 ± 0.017^d	1.401 ± 0.01^a	0.738 ± 0.043^c
*p*H ~ 9.5	2.335 ± 0.115^b	4.83 ± 0.034^c	9.89 ± 0.09^b	0.711 ± 0.013^c	1.118 ± 0.014^c	0.888 ± 0.043^c
ECe ~ 15 dS/m	2.038 ± 0.049^c	5.23 ± 0.039^b	9.91 ± 0.109^b	1.111 ± 0.058^b	1.229 ± 0.017^b	1.091 ± 0.03^b
ECe ~ 25 dS/m	3.775 ± 0.138^a	4.38 ± 0.077^d	9.19 ± 0.08^c	1.638 ± 0.073^a	1.144 ± 0.019^c	1.591 ± 0.059^a
General Mean	2.21	5.06	9.97	0.98	1.22	1.08
CD (P=0.05)	0.296	0.221	0.678	0.134	0.043	0.16
CV (%)	8.285	2.697	4.196	8.44	2.185	9.175

Means with at least one letter common are not statistically significant (p <0.05) using DUNCAN's Multiple Range Test.

species for plantation in salt affected areas that also provides fodder and shelter for the livestock.

ACKNOWLEDGMENT

The authors sincerely acknowledge Indian Council of Agricultural Research through National Agriculture Science Fund (NASF), New Delhi for funding this work.

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