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Eco-physiological responses of *Aeluropus lagopoides* (grass halophyte) and *Suaeda nudiflora* (non-grass halophyte) under individual and interactive sodic and salt stress

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ABSTRACT

Aeluropus lagopoides (grass halophyte) and *Suaeda nudiflora* (non-grass halophyte) collected from extreme saline sodic Kachchh plains, Bhuj (Gujrat), were established in micro-plot research facility of CSSRI, Karnal, to evaluate their eco-physiological responses under different salt stresses. The experiment was designed in split plot design (two factorial randomized complete block design) having 2 halophytes and 9 different treatments of salinity/sodicity, i.e., control (pH_2 7.1; ECe: 0.56), sodic (pH_2 9.5 and 10.0), saline (ECe: 15, 25, 35 dS m^{-1}), and saline sodic (pH_2 9.0 with ECe: 10, 15, 20 dS m^{-1}). Eco-physiological responses were measured in terms of gas exchange attributes, chlorophyll fluorescence, ionic relations (Na^+, K^+, and Cl^- content) and biochemicals (total soluble sugars, total soluble protein, proline content, epicuticular wax load, and peroxidase activity). Both these halophytes showed maximum gas exchange properties under unstressed control conditions. The highest photosynthetic rate (34.5 and 33.5 μmol CO_2 m^{-2} s^{-1}) was recorded in control treatment, which was decreased with the intensified stress and reduced to minimum under stress condition of pH_2 9.0 + ECe 20 dS m^{-1} (18.1 and 16.9 μmol CO_2 m^{-2} s^{-1}) in *A. lagopoides* and *S. nudiflora*, respectively. Reductions were also noticed in the rates of stomatal conductance and transpiration rate under different saline/sodic levels. As the stress conditions prevailed, these grass and non-grass halophytes accumulated higher amount of Na^+ and Cl^- in their leaves. *Aeluropus* accumulated 10.23% Na^+ at ECe 35 dS m^{-1}, which was approximately 6 times higher than control (1.65%) and 2 times than pH_2 9.0 + ECe 20 dS m^{-1} (5.8%) stress level, whereas *S. nudiflora* accumulated 2.75% Na^+ in control, which increased to 17.33% at ECe 35 dS m^{-1} and 22.25% at pH_2 9.0 + ECe 20 dS m^{-1} treatment. Chloride content showed similar trend of increase. Increased accumulation of K^+, i.e., 103.3% and 39.5%, was observed at ECe 35 dS m^{-1} in *A. lagopoides* and *S. nudiflora*, respectively, with respect to control treatment. TSS content was decreased under sodic environment, while increasing pattern was observed under salinity and combined stress. Maximum protein accumulation of 23.24 mg/g F.W. was observed at ECe 35 dS m^{-1} in *A. lagopoides* while in *S. nudiflora* (19.18 mg/g F.W.) at pH_2 9.0 + ECe 20 dS m^{-1}. Approximately 10 times higher proline accumulation was observed in both halophytes with increasing stress conditions, which showed higher osmotic adaptations. This study confers the eco-physiological potential of salt tolerance in both the halophytes and these could be used as good material for forages under salt-affected environments.

1. Introduction

Natural saline habitats vary in their salinity levels spatially and temporally due to differences in topography, soil properties, and micro-climate (Bazihizina et al., 2009). Globally, more than 900 million hectares of land, approximately 20% of the total agricultural land (FAO, 2007), are affected by salt, accounting for more than 6% of the world's total land area. In India, SAS (salt-affected soils) occupy an area of about 6.73 million ha of which saline and sodic soils constitute roughly 40% and 60%, respectively (Singh et al., 2010). Salt-affected soils (SAS) are widespread in irrigated arid and semiarid regions of the world where irrigation is essential to increase agricultural production to satisfy food requirements (Abrol et al., 1997). Rann of Kachchh in North West India is a unique saline marshy desert located in Thar Desert. It is described as “a desolate area of unrelieved, sun-baked saline clay desert, shimmering with the images of a perpetual mirage” (Mountfort et al., 1991) and is regarded as the largest salt desert in the world (GTD, 2008). In the Indian part, it stretches in 7505.22 km^2 known as Great Rann and 4953 km^2 known as Little Rann. Owing to the extreme salinity

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characteristics associated with these soils, salt-tolerant halophytes form predominant vegetation in the region. This ecosystem support many flowering plants, shrubs, climbers, herbs, trees, and grasses as reported by Ishnava et al. (2011) and supply fuel, fodder, and timber for local people and livestock. Saline lands are not suitable for growth of traditional crops because of extreme salinity and other adverse factors. If plant salt tolerance cannot be improved, then vast amount of soils may be left uncultivated. This will severely threaten the national food security and biomass energy production. Salinity is one of the rising problems causing tremendous yield losses in many regions of the world especially in arid and semiarid regions. To maximize the land productivity, these areas might be brought under utilization where there are options for removing salinity or using the salt-tolerant crops. The use of salt-tolerant crops has their own threshold limitations, whereas halophytes have the capacity to accumulate and exclude the salt in an effective way. Recently, attention has been given to the possibility of growing halophytes as irrigated crops on a large scale (Al-Hadrami et al., 2004; Al-Dakheel et al., 2006). Many halophyte grasses and non-grasses having potential to be used as fodder grow in the region that exhibit inherent potential to survive under high salt concentration even greater than seawater (Goswami et al., 2014). They survive saline environments by developing mechanisms to overcome water deficit in the root zone arising from low water potential, ion toxicity, and nutrient imbalances (Arndt et al., 2004).

Aeluropus lagopoides is a perennial species that produces flowers throughout the year. *Aeluropus* is a genus of Eurasian and African plants in the grass family, found primarily in desert regions (Watson and Dallwitz, 2008). It propagates vegetatively by underground rhizomes after monsoon rains, and through sexual reproduction with numerous seeds and flowers produced between April and October. *A. lagopoides* has a large habitat range, which spans through Southeast Europe, North Africa from Morocco to Somalia, the Middle East and the Arabian Peninsula, and central Asia, including India and Pakistan. Mostly, it inhabits damp, saline soil on the fringes of salt marshes and sulfurous springs, as well as on wasteland. *A. lagopoides* survives at high-salinity habitats that are uninhabitable to many other plant species due to structural adaptations and modifications. The plant itself has a very low salt content (Gulzar and Khan, 2001), and it is able to expel the salt it gains from the highly saline soil through glands on the leaves. The small waxy leaves and strong root network also help this species to survive in stressful salty environments, especially throughout the summer months when there is a three-fold increase in soil salinity (Mohsenzadeh et al., 2006).

On the other hand, *Suaeda nudiflora* (Chenopodiaceae), a bush species, grows abundantly in the low-lying area of Kutch (northwest saline desert) of Gujarat State of India. *Suaeda is* a halophytic species that grows wild in highly saline, dry, and extreme high tidal belt along the sea cost. It is a prostrate herb having erect branches, immature green twigs, but it becomes radish after maturity. Tap roots are well developed, and deep sunken roots are exposed during land erosion. Internods are not rigid but solid, woody, rough, terete, and green. Leaves are simple, alternate exstipulate sessile, 3 cm long with 0.3 cm width, oblong lanceolate, and green but becomes red to reddish after maturity. Flowers are bisexual, and pistillate intermixed staminate flowers are also sometimes present; perianth actinomorphic, zygomorphic, or irregular; perianth segments persistent and enclosing fruit 5, distance, or proximally to almost completely connate. As the seeds contain 30–35% oil, plant has the potential as a future oilseed crop and is highly suitable for producing for high protein biomass in saline soils due to C_4 photosynthesis. Leafy shoots of this plant are highly palatable to camels and wood is used as fuel.

Keeping the above view in focus, two major halophytes of extreme saline sodic Kachchh region, namely, *A. lagopoides* and *S. nudiflora*, were selected for the eco-physiological investigation under sodic, saline and saline sodic stresses.

2. Materials and methods

2.1. Experimental details (plant material and growth conditions)

The present study was conducted to evaluate the physiological responses of halophytic grass and non-grass on salt-affected soils (alkaline/saline). Seeds as well as root slips of *A. lagopoides* (Linn.) Trin. Ex Thw. (grass; Monocot) and *S. nudiflora* (Wild.) Moq. (non-grass; Dicot) were collected from extreme saline sodic Kachchh plains, Bhuj, Gujrat, and established in pots under controlled conditions. After establishment, these grasses were transferred to micro-plot (2.5 m × 1.5 m × 0.5 m) facilities of Crop Improvement Division, Central Soil Salinity Research Institute (CSSRI), Karnal (29°43`N, 76°58`E, and 245 m above the mean sea level), Haryana, India, with plant to plant spacing of 30 cm and row to row spacing of 60 cm. Annual rainfall of the site is between 700 and 800 mm. The experiment was conducted in split plot design (factorial randomized complete block design) having different treatments of alkalinity/salinity were imposed in these sandy loam filled micro-plots separately (pH_2 9.5 and 10.0, and ECe: 15, 25, and 35 dS m^{-1}) and in combination (pH_2 9.0 with ECe: 10, 15, 20 dS m^{-1}) with 3 replications having control (pH_2 7.1 and ECe: 0.63). The micro-plot area was suitably covered with a high quality polythene sheet to avoid the entry of rainwater and maintain the desired salt stress in the micro-plots as per treatments.

2.2. Gas exchange attributes and chlorophyll fluorescence

Physiological parameters were recorded thrice (one month interval) after 30 days of the imposition of stress treatments. Gas exchange parameters viz. photosynthetic rate (Pn), transpiration (E), and stomatal conductance (gs) were measured with an infrared open gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA). The photochemical efficiency of plants was obtained from the fluorescent analysis of the chlorophyll. The measurements were made on the same leaves that were evaluated for gas exchange. The maximum photochemical efficiency (Fv/Fm) and the quantum photochemical yield [Y(II)] of photosystem II were determined using a portable pulse modulated fluorescence measurer (Junior PAM Chlorophyll Fluorometer, Germany) after adapting the leaves to the dark for 5 min via special leaf clips. The readings were measured after saturating 1 s light pulses to promote the closing of the photosystem II reaction center.

2.3. Osmoprotectants and peroxidase enzyme

Freshly harvested leaves were weighed and analyzed for total soluble sugars (Yemn and Willis, 1954), protein content (Bradford, 1976), proline content (Bates et al., 1973), epicuticular wax load (Ebercon et al., 1977), and peroxidase enzyme activity (Siegel and Siegel, 1969).

2.4. Ion concentrations

For Na^+ and K^+ content, 100 mg of oven dried and well ground plant material was digested with 10 ml of HNO_3: $HClO_4$ (3:1) di-acid mixture and readings were taken with flame photometer (PFP7, Jenway, Bibby Scientific, UK) using standard NaCl and KCl. Chloride content of leaves was determined volumetrically by modified method of Chhabra (1973).

2.5. Forage quality

About 1 kg of representative sample was collected for forage quality analysis. Samples taken from each plot were dried in shade followed by overnight drying in hot air oven at 80 °C for DM estimation. The proximate analysis of nutrients was carried out as per the AOAC (1995) and cell wall constituents as per Goering and Van Soest (1970).