Journal of Arid Environments 119 (2015) 56–60

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Is 5-aminolevulinic acid concentration in plants related to soil salinity? A test with 17 native species of Bahrain

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ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form
24 March 2015
Accepted 1 April 2015
Available online 6 April 2015

Keywords:
Arid environment
Compatible solutes
Drought stress
Osmoprotection
Salt stress

ABSTRACT

The objective of this study was to determine the effect of soil salinity on the concentration of ALA in 17 native plants species collected from different locations across the Kingdom of Bahrain. ALA was extracted from the various plant species and measured by flourometric analysis. ALA concentrations ranged between 21.53 ± 2.05 μg g^{-1} and 66.03 ± 4.42 μg g^{-1}. The highest ALA concentration was found to be in *Aizoon canariense* whereas the lowest concentration was recorded in *Avicennia marina*. Significant difference ($P < 0.05$) in ALA concentration among all plant species was found between 108 out of 136 possible combinations (79.4%). Furthermore, ALA concentrations of desert and mangrove plants only were found to be significantly different at $P < 0.05$. However, no correlation was observed between soil salinity and ALA concentrations in any of the plant species studied. Therefore, the results of this study suggest that the main role of internal ALA in plants' is to maintain plant specific chlorophyll content and photosynthetic parameters regardless of soil salinity.

1. Introduction

The Arabian Peninsula is an extremely arid environment with elevated temperatures and scarce precipitation. Agricultural activities are therefore limited and mainly dependent on ground water which has been over used in the past two decades resulting in the salinization of water and therefore soil (Watanabe et al., 2004; Youssef and Awad, 2008). Salt stress can adversely affect various plant processes such as reduction in the seed germination, yield, and the photosynthetic rate (Wang et al., 2001; Perveen et al., 2010). In recent years, the salinization of soil has become a more serious challenge facing countries such as those of the Arabian Peninsula leading to the gradual increase of desertification which hinders the efforts of increasing the green area (UNEP, 1991; Youssef and Awad, 2008).

ALA, an important amino acid in plants, is the initial precursor of porphyrins such as chlorophyll and heme. ALA production is directly associated with chlorophyll content, photosynthetic rate and parameters of photosynthesis such as gas exchange, water potential and oxidative stress. Several studies were published regarding the promotive effects of exogenous ALA application on such parameters (Hotta et al., 1997a; Memon et al., 2009; Naeem et al., 2010). However, limited information is available on the role of ALA external treatment under saline and dry conditions in maintaining water balance, water use efficiency, and Na uptake in plants. A small number of studies have been conducted on some plants such as spinach (Nishihara et al., 2003), and sunflower (Akram et al., 2012), to determine the effect of foliar ALA treatment on improving salt tolerance. Nishihara et al. (2003) stated that ALA treatment could protect plants grown under different NaCl concentrations against the oxidative damage of activated oxygen species (AOS). However the proper amount of exogenous ALA that has to be administered is still unknown due to the fact that plants differ among themselves as well as in their environmental conditions. Akram et al. (2012) have proven that foliar ALA application on sunflower plants had no significant effect on alleviating adverse effects of salt stress in regard to most physiological process associated with salt tolerance.

On the other hand, several studies have shown very effective role of ALA exogenous application in minimizing salt-induced adverse effects on some crop plants such as cotton seedlings (Watanabe et al., 2000), spinach (*Spinacia oleracea*) (Nishihara et al., 2003), date palm (*Phoenix dactylifera*) (Youssef and Awad, 2008), oilseed rape (*Brassica rapa*) (Naeem et al., 2010), potato (*Solanum tuberosum*) (Zhang et al., 2006), and pakchoi (*Brassica campestris*)

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http://dx.doi.org/10.1016/j.jaridenv.2015.04.002
0140-1963/

(Wang et al., 2005). In addition, foliar application of ALA at 50–100 mg/l on wheat grown under Saudi Arabia's dry conditions had a substantial enhancement on water use efficiency of crops and significant promotive effects (Al-Thabet, 2006). Similar results were also reported by Zsembeli et al. (2008) showing a significant effect of ALA treatment blended in a nitrogen liquid fertilizer PENTAKEEP-V (PV) on the water use efficiency in 3 sorghum hybrids under dry circumstances. The effect of ALA application on the dominant fruit tree in the Arabian Peninsula (date palm), a plant that displays true halophytic adaptation to salt stress (FAO, 1982; Ramoliya and Pandey, 2003), was investigated by Al-Qureshi and Awad (2011). They have reported promotive effects of ALA application on the fruit quality of two date palm cultivars under hot arid climate.

Most studies available today are about the importance of external application of ALA in reducing the salt-induced effects in many plants. However, to our knowledge, the internal concentration of ALA in plant extracts and its relationship with soil salinity has never been investigated. Therefore, the present study is a first attempt to provide information about the ALA concentrations in native plants collected from different habitats across the Kingdom of Bahrain in relation to soil salinity.

2. Materials and methods

2.1. Plant materials and sampling

The plants analyzed in this study were collected in February 2012. Plant samples representing 17 native species were collected from different sites (*Sesuvium verrosum* Raf. Aizoaceae, *Suaeda vermiculata* Forssk. ex J.F.Gmel. Amaranthaceae, *Zygophyllum qatarense* Hadidi Zygophyllaceae., *Heliotropium crispum* Desf. Boraginaceae, *Halocnemum strobilaceum* (Pall.) M. Bieb. Amaranthaceae, *Avicennia marina* (Forssk.) Vierh. Verbenaceae, *Suaeda maritima* (L.) Dumort. Chenopodiaceae, *Salicornia herbacea* auct. non (L.) L. Chenopodiaceae, *Arthrocnemum macrostachyum* (Moric.) K.Koch Amaranthaceae, *Anabasis setifera* Moq. Amaranthaceae, *Helianthemum lippii* (L.) Dum.Cours. Cistaceae, *Salsola baryosma* (Schult.) Dandy Chenopodiaceae, *Atriplex leucoclada* Boiss. Chenopodiaceae, *Malva parviflora* L. Malvaceae, *Asphodelus tenuifolius* Cav. Asphodelaceae, *Emex spinosa* (L.) Campd. Polygonaceae and *Aizoon canariense* L. Aizoaceae).

Plants were collected from four different habitats: coastal plain, inland sabkha, desert, and mangrove. Sabkha (plural: sabkhas) is an Arabic name for a habitat that is characterized by soil that has high concentrations of salts. Sabkhas originate in the coastal and in land saline mud flat plains due to capillary suction and intense evaporation as well as deposition of silt, clay, and sand in shallow, sometimes extensive depressions (Khan et al., 2006).

Plants collected for analysis were distributed among 3 main soil types, the solonchak, regosols, and raw mineral soils. The solonchak soil has two different groups, cultivated and natural. The cultivated solonchak can be further sub-grouped as loamy and sandy based on their texture, whereas the natural solonchak can also be sub-grouped into gypsiferous and the sabkhas. In both cases the ground water is at less than 1 m depth. Regosols soil type can be divided into two groups based on their parent material as aeolian stable sand and recent beach deposits. Raw mineral soil type can also be divided into three sub-subgroups, soils of interior basins, soils of detrital fans, and soil with stone pavements based on their landscape (Doornkamp et al., 1980).

The collected plants were kept in plastic bags and transported to the laboratory where they were immediately cleaned from debris and washed with de-ionized water to remove any remaining soil. The samples were then dried to a constant weight (48–72 h) in an oven at 60 °C. Finally, samples were mechanically ground to fine powder and kept in tightly closed dark glass bottles and stored in a dry, dark and cool place. A minimum of 3–10 (30) individuals from each species were used to form a composite plant specimen from each site.

2.2. Soil sampling and analysis

The top 1–2 cm of the soil surface was first scraped and then approximately 0.5–1 kg of soil from the upper 20–30 cm was collected in a plastic bag and transported to the laboratory. Soil samples were air dried and sieved through a 2 mm mesh. Electrical conductivity (salinity) was determined using PWA1 water analyzer and expressed as μs cm^{-1}. Soil types of each of the 97 collection sites were identified according to Doornkamp et al. (1980).

2.3. ALA extraction and analysis

A total of four replicate from each composite specimens were used for the ALA analysis. ALA extraction was carried out following the procedure of Kolossov and Rebeiz (2005) with some modifications. One gram of the powdered plant leaves was homogenized with 10 ml 10%TCA using Waring commercial blender (Waring Product Division, Dynamic Corporation of America, New Hartford, Connecticut 0605, USA). The homogenate was centrifuged at 10,000 rpm for 30 min and the supernatant was collected and used for the spectrofluorometric analysis of ALA.

Fluorometric measurement of ALA was carried out following the procedure of Lee et al. (2004) with some modifications. The fluorescent derivatization of ALA was prepared by mixing 0.55 ml of the extracted samples or standard solutions with 3.5 ml acetylacetone reagent and 0.45 ml of 10% formaldehyde. A reagent blank was prepared by mixing 0.55 ml of 10% TCA with 3.5 ml acetylacetone reagent and 0.45 ml of 10% formaldehyde. A control sample with known amount of ALA (62.5 μg/ml) was also prepared and analyzed in every run. The mixtures were heated for 10 min at 100 °C and then cooled in an ice bath. The fluorescence emission spectrum was scanned from 400 to 700 nm with an excitation wavelength at 370 nm using Turner Designs filter fluorometer TD-700, Turner Designs Instrument, Turner Designs, Sunnyvale, CA, USA.

2.4. Data analysis

The statistical analysis was performed using the statistical package from Excel 2007 (Microsoft Corporation) for calculation of means and standard deviation (SD). Results are presented as mean values of 3 replicates and ±SD. Correlation tests among ALA concentration of the plant species individually, collectively, and within habitats with salinity were conducted according to Pearson correlation coefficient. Comparison of means for ALA concentration among all species was carried out according to t-test. Differences with P value ≤ 0.05 were considered statistically significant.

3. Results

The 17 plant species studied were collected from 97 different sites representing 4 different habitats. Most of the plant species studied were mainly distributed in two habitats, deserts (9 species) and mangroves (4 species), whereas the remaining 4 species were distributed among all 4 habitats.

The soil salinity concentration values obtained in this study fall within 4 salinity types; non-saline (NS) (0–0.45 μs cm^{-1}), slightly saline (SS) (4.5–9 μs cm^{-1}), medium saline (MS) (9–18 μs cm^{-1}), and high saline (HS) (>18 μs cm^{-1}); based on the categories as described by FAO (1990).

ALA concentrations of plant species studied along with the soil