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Polyamine Accumulation and Osmotic Adjustment as Adaptive Responses to Water and Salinity Stress in *Conocarpus lancifolius*

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ABSTRACT

Conocarpus lancifolius is an ornamental plant that flourishes under the semi-arid conditions of Kuwait. The adaptive response of this species to drought, salinity stress and the relationship of free polyamines in leaves, leaf osmotic potential, chlorophyll content and fluorescence were determined. These were done with single shoots (14-15 leaves) in a controlled environmental chamber at 25°C and RH of 45-50%. The leaves of plants treated with ≥ 1.37 M NaCl had a relative water content (RWC) of 65% or lower. Shoot elongation and leaf development ceased just after leaf osmotic potential (ψ_s) of ≥3.32 MPa and the threshold for salt damage in the leaves appeared to be at 1.03-1.37 M NaCl after 10 days. The level of salt tolerance indicated that *C. lancifolius* may be a xerohalophyte. The chlorophyll (chl) content index increased by the formation of green islands and declined with ultrastructural changes of the chloroplasts in 1.37-1.71 M NaCl. The minimal Chl fluorescence (Fo) increased with increase in salinity and drought but the electron transport rate ETR, photochemical quantum yield (Y), photochemical quenching (qP) and variable to maximal fluorescence (Fv/Fm) declined after 10 and 4 DAT with increase in salinity and drought, respectively, an indication of some damage to PSII. Putrescine (Put) was the predominant polyamine during the early stages of drought and salinity stress. Although Put was the most abundant polyamine in 0.17-0.34 M NaCl, at higher concentrations spermidine (Spd) and spermine (Spm) were more abundant. The titer and type of polyamine accumulated in *C. lancifolius* appeared to be related to the nature, intensity and duration of environmental stress. Understanding its response to drought and salinity stress will assist in the management and longevity of this species.

Keywords: arid agriculture, drought, putrescine, salinity, spermidine, spermine, solute potential, xerohalophyte
Abbreviations: CCI, chlorophyll content index; **ETR**, electron transport rate; **Fm**, maximum chlorophyll fluorescence; **Fo**, minimal chlorophyll fluorescence; **Fv**, variable chlorophyll fluorescence; **PA**, polyamine; **PSII**, photosystem II; **qN**, non-photochemical quenching; **qP**, photochemical quenching; **RWC**, relative water content; **Y**, photochemical quantum yield

INTRODUCTION

Conocarpus lancifolius is a tree in the family Combretaceae (a family of trees, shrubs and lianas) which was introduced to Kuwait from one of the Gulf States. It was brought into the Gulf States probably from Yemen, Djibouti or Somalia as an ornamental plant in the 1990's. The plant is propagated vegetatively, produces abundant biomass and copious amount of seeds. It has adapted extremely well to the semi-arid conditions of Kuwait to the extent that it has replaced date palm as the major ornamental plant. The plant also thrives well when irrigated with brackish water (salinity >3500 ppm). The species is able to withstand the hot summer temperatures but appears to be slightly sensitive to frost. Although the optimum growth temperature is unknown the most vigorous growth occurs in summer (temperature range of 40-45°C). It is not an invasive plant, although well adapted to the environment.

High temperatures, drought and salinity are the major environmental factors that limit plant growth and development in Kuwait. These stress factors lower the internal water potential of plants tissues and the cellular water potential of photosynthetic machinery. Most plants adjust osmotically under saline conditions to protect tissues by generating a lower water potential that allows for continuous but slow growth. The effects of water deficit could be tolerated through osmotic adjustment by the accumulation of solutes. Adaptation to water stress can be accomplished through a number of strategies which include maintaining an appropriate turgor by accumulating organic substances or compatible solutes such as sugar alcohols or polyols, proline and polyamines (PAs).

PAs are low molecular weight molecules found in all higher plants. The most common PAs are the diamine (putrescine; Put), triamine (spermidine; Spd) and tetramine (spermine; Spm). They occur in free or conjugated forms as macromolecules that promote cell development and normal growth. Chemically, they are straight and aliphatic hydrocarbon chains with amino and imino groups. Due to their cationic nature PAs are involved in numerous biological and physiological processes (Walden *et al*. 1997). The production and accumulation of PAs in plant cells depends to a large extent on the physiological and biochemical activities of plant species. Higher plant cells synthesize PAs and utilize them as a response to different environmental stress factors (Walden *et al*. 1997; Kuznestov and Shevyakova 2007). The concentration of each polyamine as well as the total PAs in plants under normal growing conditions depends on the plant species, organ, tissue and developmental stage (Kuznetsov *et al*. 2006). The PAs Put, Spd and Spm prevent Chl loss that normally occurs during the senescence of plant tissues. Endogenous synthesis of PAs in plant cells has been associated with maintenance of thylakoid structure integrity (Pal Bais and Ravishankar 2002). Plants treated with exogenous PAs were more effective in retarding Chl loss at higher concentrations or in smaller plant tissues (Tiburcio *et al*. 1994). PAs in some plants under stress, increase several fold and as such could be viewed as "stress markers". Endogenous titers of Put, Spd and Spm in stressed plants tend to be much higher in plants responding to abiotic stress factors thus, enabling plants to adapt and grow under harsh environmental conditions (Nayyar 2005). It appears that plants exposed to different stress conditions accumulate a specific PA for each abiotic stress

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factor. Thus, one type of stress factor could induce high levels of a particular PA than the others or induce the production of a PA at the expense of others.

Salt tolerance is dependent on the accumulation of different PAs (Basu and Ghosh 1991; Pang *et al.* 2007). Tang and Newton 2005 found Put controlled salt damage in Virginia pine and in barley seedlings (Zhao and Qin 2004). In rice, salt tolerance was associated with increased levels of Spd and Spm (Krishnamurthy and Bhagwat 1989; Santa-Cruz *et al*. 1997). In salt-sensitive cultivars however, the accumulation of Put and not Spd or Spm was involved in the protection of the plants (Erdei *et al*. 1996). The role of PAs in drought tolerance has also been reported (Yamaguchi *et al*. 2007; Yang *et al.* 2007).

We tested the hypothesis that the accumulation of PAs may be related to drought and salinity as stress factors in *C. lancifolius* as an evergreen plant, with prolific growth under drought and saline conditions.

The objective was achieved by determining the effect of drought and salinity on the relative water content (RWC), leaf solute potential (ψs), Chl content index (CCI), Chl fluorescence and correlating drought and salinity to the accumulation of the most common free PAs in *C. lancifolius*.

MATERIALS AND METHODS

Plant material

C. lancifolius clonal plants were grown from cuttings in plastic pots (14 cm diameter × 14 cm height) containing a mixture of local sandy loam soil: peat moss (3: 1). The plants were maintained in a growth chamber at 25°C, relative humidity of 45-50%, 13 h photoperiod and photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1} at the top of the canopy. The experimental design was complete randomized design and unless otherwise stated each study was conducted twice with three replicates per treatment. The plants were irrigated with deionized water to field capacity prior to each experiment. Single shoot plants with 14-15 leaves were selected for each test. Plants were subjected to drought stress for 3 and 4 days by withholding water while control plants were maintained at field capacity. The plants for salinity experiment were watered three times with 50 mL of different concentrations of NaCl solutions at two-day intervals. Control plants were maintained at field capacity with deionized water. The leaves of the treated plants were used to determine the RWC, ψs, CCI, Chl fluorescence and the concentrations of free PAs, 1, 3, 7 and 10 days after treatment. The number of new leaves that developed and the increase in shoot length were recorded for salt stressed plants after 10 days.

Determination of relative water content

The relative water content (RWC) of plants under different salinity treatments was determined using 10 mm diameter leaf discs. Avoiding the mid-vein, leaf discs were excised and the fresh weight (FW) of 10 leaf discs/treatment were determined. The discs were floated on sterile, distilled and deionized water for 4 h at 4°C and the saturated fresh weight (SFW) determined. The dry weight (DW) was obtained by keeping leaf discs at 70°C until a constant weight was attained. The relative water content was calculated as follows: RWC = FW - DW / SFW - DW x 100.

Determination of leaf solute potential (ψs)

The mature leaves were taken from plants subjected to NaCl treatments and frozen at -80°C. Leaf sap was extracted using a garlic press, centrifuged at 10,000 rpm for 15 min at 4°C and filtered through a 0.22 µm Millipore filter. An aliquot (0.1 mL) of the undiluted sap was used to determine the ψs using a Wescor Vapor Pressure Osmometer, 5520 (Wescor Inc. Logan, Utah, USA) at 22 ± 1°C. Three replicates of each treatment were used for the measurement. The osmolalities were converted to MegaPascals (MPa) using van't Hoff's equation: MPa = RTc, where RT = 2.454 at 22°C and c = osmolality in mol/kg.

Determination of chlorophyll content index (CCI)

Chl index of plants subjected to salinity treatments was measured using a portable Chl content meter (CCM-200, OPTI-SCIENCES, Tyngsboro, MA, USA), which is a reliable measurement of leaf Chl (Richardson *et al.* 2002). Measurements were taken by inserting a portion of an attached leaf into the chamber of the Chl meter. Measurements were taken from 3 replicates of the same mature leaves that were used for leaf fluorescence measurement.

Histological study

Leaf samples of plants subjected to salt treatments used to measure the Chl content index were taken for light and electron microscopy. Leaf sections were taken from control plants, stressed plants with "green islands" and yellowing leaves. The sections were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2 for 3-4 h at room temperature, postfixed in 2% osmium tetraoxide for 2 h and then dehydrated through a graded ethanol series. The tissues were embedded in epon resin, cut into semi-thin sections (1 µm thick) and stained with 1% toluidine blue in 1% borax and examined with a light microscope. Ultrathin sections (50 nm) were then cut and stained with 2% uranyl acetate followed by 0.2% lead citrate and examined with a JOEL's JEM-1200 EX II at kV 80.

Determination of leaf fluorescence

The leaf fluorescence was measured with a modulated Chlorophyll Fluorometer OS5-FL (OPTI-SCIENCES, Hudson, NH, USA) with photodiode detector, at 23 ± 1°C using the kinetic test mode. Plants were dark adapted for 45-60 min prior to fluorescence measurements. The leaves remained attached through out the determination of fluorescence. They were placed in the 60 degree angle clip with external illuminator at 1.0 cm to give an acceptable illumination level. The fluorescence transients recorded were: the minimal fluorescence (Fo), the maximal fluorescence (Fm), variable fluorescence (Fv), the ratio of the variable to maximal fluorescence (Fv/Fm), the overall photochemical quantum yield (Y), photochemical fluorescence quenching (qP), non-photochemical quenching (qN) and the apparent photosynthetic electron transport rate (ETR). The data were collected at 200 ms interval for about 3 s. These parameters representing the activity of photosystem (PSII) were measured to assess the functional damage by salinity and drought to the plants.

Effect of drought and salinity on PA accumulation

(a) Plants at 14-15-leaf stage in growth chambers at 25°C were watered with deionized water to field capacity and allowed to acclimate form 7 days. The plants were then subjected to drought treatment for 3 and 4 days by withholding water. Control plants were maintained at field capacity. Leaf samples were collected as described earlier to determine the accumulation free PAs.

(b) Plants grown and maintained as described earlier were watered with 0.0-1.71 M solutions of NaCl. They were watered with 50 mL of saline solutions 3 times every other day and control plants were given the equivalent amount of deionized water. Treatments were replicated 4 times and matured leaves of plants were sampled to determine accumulated free polyamines. Data for total PAs were fitted to a non-linear regression model while linear regression was used to determine the relationship between Put, Spd and Spm and salinity.

Determination of polyamines

Extraction, dansylation and HPLC quantification of PAs in leaf tissues were done as described by Marcé *et al.* (1995). Leaf tissues were powdered in liquid nitrogen using a pre-cooled mortar and pestle. The PAs were extracted in 5% percholic acid (PCA) using 300 mg fresh weight tissue per ml of PCA. The homogenates were incubated in centrifuge tubes in ice for 30 min and centrifuged at 27,000 × *g* for 20 min at 4°C. The supernatant fractions were collected for dansylation.

Dansylation of polyamines was carried out in dim light by adding 400 µL of dansyl chloride (5 mg/mL in acetone) to 200 µL

of supernatant, 200 µL of saturated Na_2CO_3 solution and 40 µL of 1,7 diaminoheptane (0.05 mM) as an internal detector. The mixture was incubated in the dark overnight at room temperature and the reaction was stopped by adding 100 µL proline solution (100 mg/mL in H_2O) to remove the excess of dansyl chloride. Extraction of the dansylated PAs was done by adding 500 µL of toluene. Dansylated PAs were dried using nitrogen gas, and re-dissolved in 800 µL of acetonitrile. The mixture was passed through a 0.22 µm Millipore syringe filter and analyzed using HPLC.

The PAs were separated using HPLC (Waters Inc, Milford, MA, USA), equipped with Brownlee reverse-phase ODS Spheri-5 (C18 5 µm spherical, 80 A° pore size 0.220 × 4.6 I.D.) and a fluorescence detector. The PA extract in acetonitrile (20 µL) was injected into the HPLC system with a mobile phase of filtered acetonitrile and water. The initial mobile phase mixture was pumped for 4 min with 70% acetonitrile and 30% water at a flow rate of 1.5 mL per min. The concentration of acetonitrile was raised to 100% for 4 min and finally back to 70% acetonitrile and 30% water. The quantification of PAs (Put, Spd and Spm) was performed by comparing each sample with a known internal standard (Sigma, St. Louis, MO, USA) using a photodiode detector. Data were stored and analyzed on PC equipped with Waters Empower 600 software.

Statistical analysis

Experiments were set up in a complete randomized design and unless otherwise stated were done at least twice with three replications per treatment. Data on PAs from drought tress plants were subjected to analysis of variance (ANOVA) at P = 0.05 level and means were separated with Fisher's least significant difference (LSD) test using Minitab software version 13, (Minitab Inc., State College, PA.) Regression was also used to describe the relationship between salinity and explanatory variables: RWC, leaf ψs, CCI, Fv/Fm, Y, qP and the PAs.

RESULTS

Leaf relative water content and solute potential

The leaves of plants subjected to different NaCl treatments showed significant differences in their relative water content (RWC). Control plants had the highest RWC of 91.4%. A day after NaCl treatment, there was no significant difference between the RWC of 0, 0.17 and 0.34 M NaCl treatments. After day 3, there was still no significant difference between 1.03-1.71 M NaCl treatments (data not shown). However, at 10 DAT there was a significant difference between treatments (**Fig. 1A**). The difference between the RWC of control and 1.71 M salt treatment was 29.4%. Leaves with RWC below 72% appeared slightly flaccid.

The solute potential of the leaves decreased as the concentration of NaCl in solution increased (**Fig. 1B**). The leaves in NaCl treatments 0 to 1.37 M appeared normal with a range of solute potential of 1.28 to 1.54 MPa after day 1 but those from 1.71 M NaCl with solute potential of 1.84 MPa were flaccid. The leaf solute potential remained relatively unchanged and appeared normal 3 DAT in treatments 0 to 0.68 M NaCl. However, 10 DAT, only leaves from the 0, 0.17 and 0.34 M NaCl treatments still appeared normal or healthy and the leaf ψs remained relatively unchanged. The incremental shoot growth was 25, 23 and 13 mm for 0, 0.17 and 0.34 M NaCl treatments, respectively (**Fig. 1C**). Shoot elongation was completely inhibited in 1.37-1.71 M NaCl and cessation of new leaf development was in 1.03-1.71 M NaCl.

Determination of chlorophyll content index

The CCI for mature leaves was about the same for 0 to 1.03 M NaCl treatments and thereafter a gradual increase 3 DAT (**Fig. 2**). After 7 days, CCI increased with increase in NaCl concentration up to 2 times in 1.71 M NaCl treatment. Generally these leaves developed dark "green island" symptoms. However, after 10 days there was a rapid decline in the CCI in leaves treated with 1.03-1.71 M NaCl. The regression line forms for CCI of mature leaves after 3, 7 and 10 days showed r^2 values of 0.94, 0.97 and 0.90, respectively. In the drought stressed plants after day 4, the CCI regression line was similar to that of salinity after day 10.

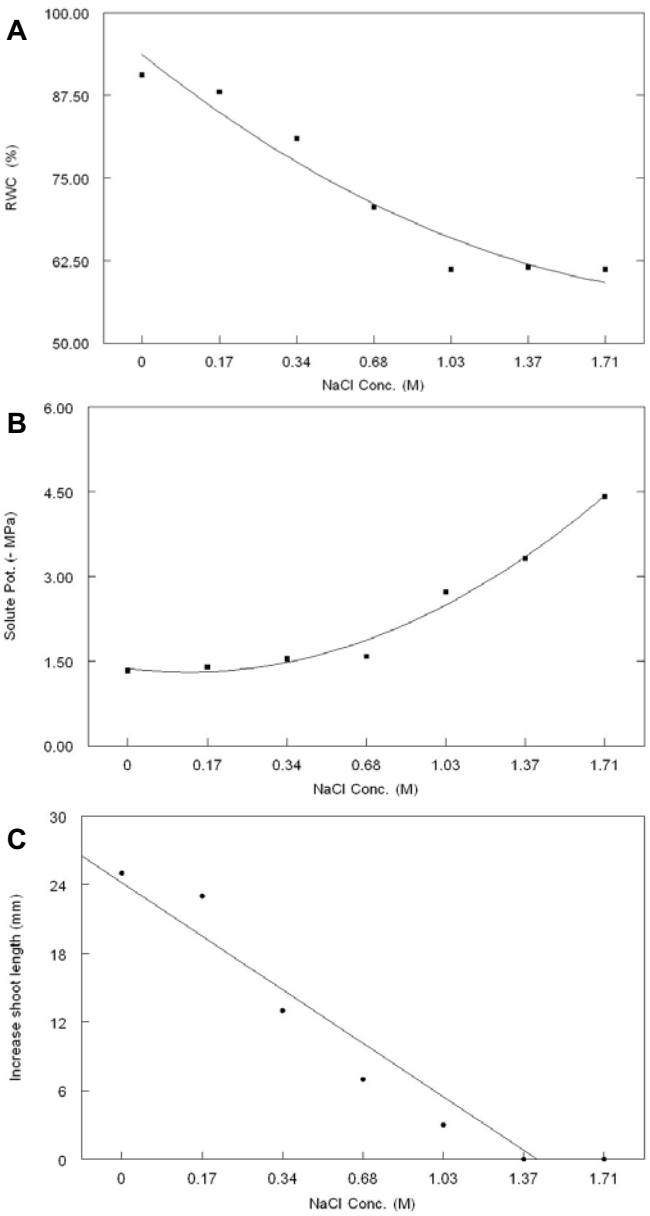

Fig. 1 (**A**) The relationship between leaf RWC and NaCl treatments of *C. lancifolius*, 10 days after treatment (DAT). (**B**) The relationship between leaf solute potential and NaCl treatments applied to *C. lancifolius*, 10 DAT. (**C**) The relationship between shoot length of *C. lancifolius* and NaCl treatments, 10 DAT. The values of all parameters represent means of three measurements taken from replicates. The regression lines are of the forms: $y = 0.6x^2 - 10.5x + 103.6$, $r^2 = 0.97$ for RWC, $y = 0.1x^2 - 0.4x + 1.7$, $r^2 = 0.99$ for solute potential and $y = -4.7x^2 + 28\ 9$, $r^2 = 0.96$, where y is either RWC, solute potential or shoot length and x is the NaCl concentration.

Histology

The leaf samples from control plants had normal but fewer intact chloroplasts compared to NaCl treated plant leaves (data not shown). The chloroplasts appeared normal with well defined grana and stroma lamellae or frets. The chloroplasts were lined-up against the cell wall and generally had a single large starch grain or inclusion and some plastoglobuli (**Figs. 3A, 3B**). Leaf samples with "green islands" had many more chloroplasts, with relatively no starch grains (**Fig. 3C**). The grana and stroma lamellae appeared to form a series of long parallel thylakoid membranes that

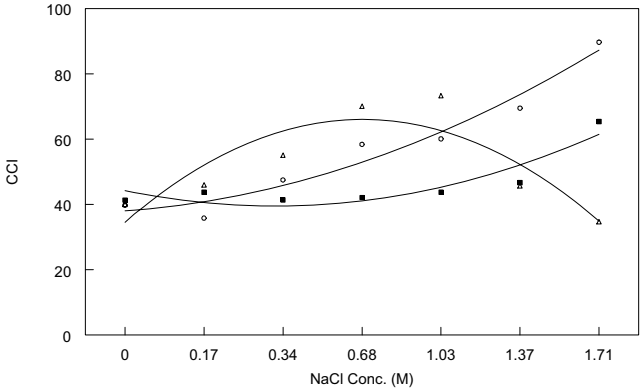

Fig. 2 The relationship between NaCl concentration and leaf chlorophyll content index, 3, 7 and 10 DAT. The CCI values represent means of three measurements; ■ 3 DAT, ○ 7 DAT and Δ 10 DAT. The regression lines are of the form: $y = 0.50x^2 - 3.93x + 47.80$, $r^2 = 0.94$ for 3 days, $y = 0.20x^2 + 2.52x + 36.30$, $r^2 = 0.97$ for 7 days and $y = -1.59x^2\ 18.59x + 18.30$, $r^2 = 0.90$ for 10 days after treatment; where y is the chlorophyll content index and x is the NaCl concentration.

filled the entire stroma of the chloroplasts. Yellowish or mottled leaf tissue had cells with damaged cell walls, distorted or damaged chloroplasts, most of them were curved, "C" or spherical in shape (**Figs. 3D, 3E**). These damaged chloroplasts had distorted grana, some plastoglobuli and the tonoplasts were either partially destroyed or not discernable.

The effect of drought and salinity on chlorophyll fluorescence

The leaves of *C. lancifolius* showed typical Chl fluorescence transients of Fo, Fm, Fs and Fms in control as well as drought or salinity stressed plants. The fluorescence transient graphs for salinity are shown in **Figs. 4A, 4B**. Similar transient graphs with slightly lower values were observed for drought stressed plants. Both salinity and drought stressed plants after 10 and 4 days, respectively showed a decline in Y, qP, ETR and Fv/Fm. Compared to the control, there was a 70% increase in Fo for 1.71 M NaCl and that of drought increased by 53.4%. However, the Fv/Fm, Y, qPand ETR decreased by 71.0, 65.0, 71.2, and 10.2% respectively, after 10 days for salinity. The regression lines for Y, qPand Fv/Fm were similar for salinity and drought, those for salinity are shown in **Fig. 5**. The r^2 values were 0.96, 0.85, 0.85 for Y, qP and Fv/Fm, respectively.

The effect of drought on PA accumulation

Put increased significantly with increase in the drought stress period. The level of putrescine after 3 and 4 days drought stress was about 2 times the level detected in the control plants (**Fig. 6**). Spm accumulation was relatively the same in the control and drought stressed plants. Put was the predominant polyamine throughout the stress period. The total PAs accumulated increased with increase in the duration of drought.

The effect of salinity on PA accumulation

Put, Spd and Spm accumulated in all treatments. After 24 h, the concentrations of Put and Spm increased by 14.3 and 44.4%, respectively in plants treated with 1.71 M NaCl. Ten days after treatment, Put increased in the 0.34 M NaCl treated plants and thereafter declined with increase in NaCl (**Fig. 7**). Spm accumulation was relatively the same in 0 to 1.03 M NaCl but increased thereafter. In 1.71 M NaCl, Spd and Spm were the predominant PAs and the titers were approximately the same. The regression line forms between NaCl and PA titers are shown in (**Fig. 7**). The total free PAs appeared to be accumulated in three different NaCl range of concentrations or groups of concentrations. The first concentration group (0.0-0.34 M) showed an increase of total

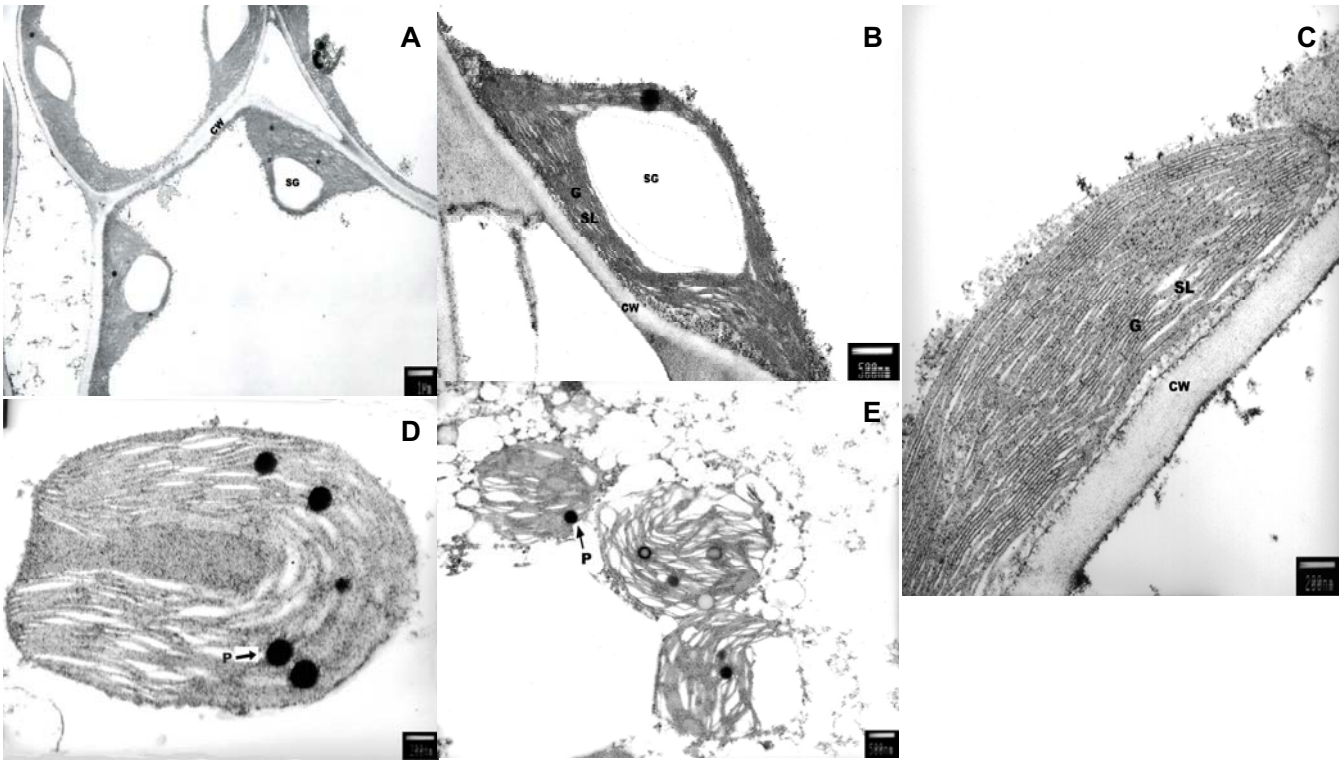

Fig. 3 (**A**) Transmission electron micrograph showing a portion of leaf cells from control plants with normal chloroplasts containing a single starch grain (SG) aligned against the cell wall (CW). Scale bar = 1 µm. (**B**) Transmission electron micrograph of chloroplast with starch grain (SG), grana (G), stroma lamella (SL) and cell wall (CW) from a control plant. Scale bar = 500 nm. (**C**) Transmission electron micrograph showing a chloroplast of salt treated plant leaf, with the grana (G) and stroma lamella (SL) as parallel thylakoid membranes in the chloroplast aligned against the cell wall (CW). Scale bar = 200 nm. (**D**) Transmission electron micrograph showing damaged chloroplasts of plant leaf treated with 1.71M NaCl. Distorted "C" shaped chloroplast with plastoglobuli (P). Scale bar = 200 nm. (**E**) Damaged spherical chloroplasts with plastoglobuli (P). Scale bar = 500 nm.

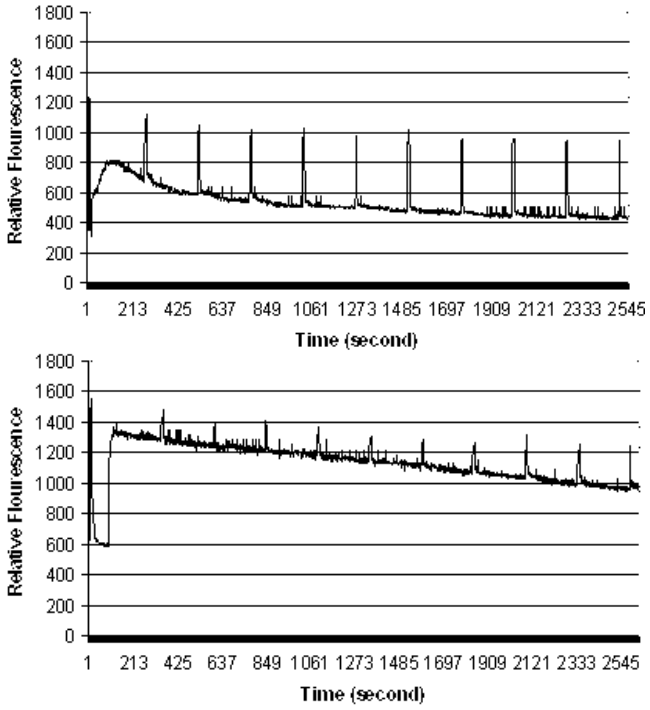

Fig. 4 (**A**) The kinetics of chlorophyll fluorescence induction in control of *C. lancifolius* plants at 25°C after illumination of dark-adapted plant leaves. (**B**) A typical kinetics of chlorophyll fluorescence induction in 1.71 M NaCl, 10 DAT of *C. lancifolius* at 25°C after illumination of dark-adapted plant leaves.

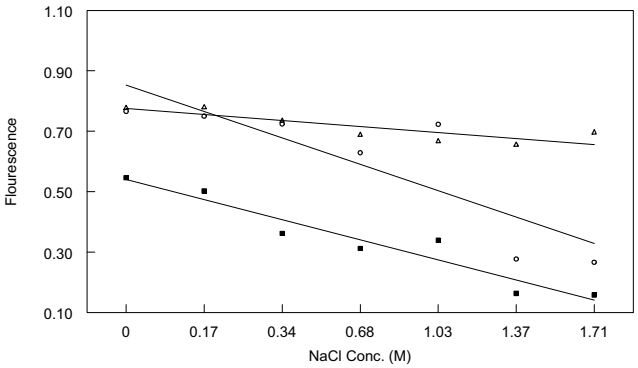

Fig. 5 The relationship between fluorescence parameters; quantum yield, Y (■), photochemical quenching, qP, (○) ratio of variable and maximum fluorescence, Fv/Fm ratio (Δ) and NaCl concentration, 10 DAT.

PAs. The plants in this group showed no visible physiological damage and still continued to grow and to develop new leaves. The second group (0.34-1.03 M) showed a steady decline in total PAs. The plants in these treatments showed no visible damage but growth was minimum. The third group (1.37-1.71 M) had a slight increase above the second group in total PAs. The lower leaves of the plants began to wilt and the middle leaves showed "green island" symptoms. The r^2 values were 0.62, 0.54, 0.59 and 0.79 for Put, Spd, Spm and total PAs, respectively.

DISCUSSION

The relative water content (RWC) in plants is a useful indicator of the amount of water required to reach saturation in plants since it is related to the water potential in plant tissues. Generally, RWC below 80% implies a water potential of 1.5 MPa or less which could result in metabolic changes including cessation of photosynthesis, increase in respiration and accumulation of proline and ABA in plants (Gonzalez and Gonzalez-Vilar 2001). *C. lancifolius* leaves began to show symptoms of water stress after 24 h in plants subjected to 1.71 M NaCl. These leaves had RWC of 65% or solute potential of 1.84 MPa showed the range of NaCl concentration at which leaf ψs was equal to leaf RWC was 1.03-1.37 M. Apparently, at this threshold little or no shoot elongation and no new leaf development were observed. Plants that grow and survive in seawater (3% NaCl) are classified as halophytes. In this study *C. lancifolius* could tolerate up to 6% or 1.03 M NaCl, as such could be classified as a xerohalophyte plant capable of growing in a habitat where soil is always saline however, the soil may dry to the extent that water availability becomes limited for the plant. Examination of the leaves of *C. lancifolius* showed they have secretory glands or gland dots that may have a role in the secretion of salts. The salt regulating capacity of *C. lancifolius* was probably manifested by the abscission of lower mature leaves at NaCl concentrations greater than 1.03 M. Thus, it is feasible that *C. lancifolius* maintained low internal water potential by multiple mechanisms of osmotic adjustment including salt exclusion. The mechansims of salt secretion and osmotic adjustment by *C. lancifolius* would require further investigations.

There was an increase in the chlorophyll content index (CCI) from day one to ten and this may be associated with the development of "green islands", areas of green tissue with a substantial chloroplasts in their cells. These chloroplasts were probably regenerated in cells with many proplastids or other plastids. Aldesuquy *et al*. (2000) showed an increase in the number of chloroplasts which developed

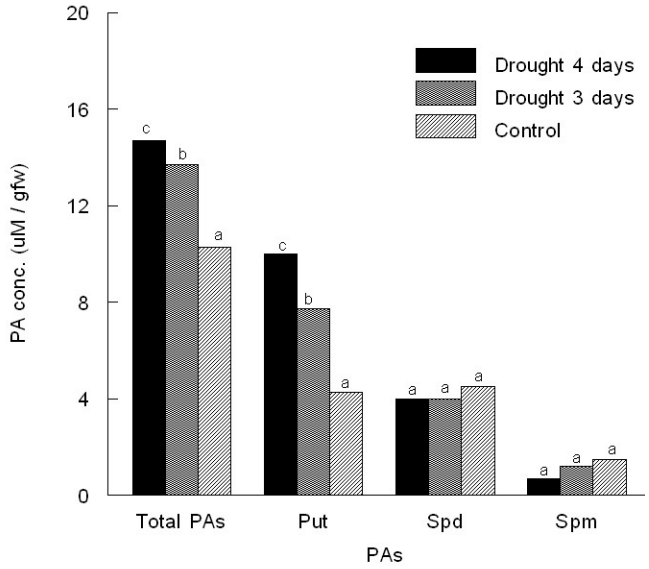

Fig. 6 The accumulation of putrescine, spermidine, spermine and total PAs in the leaves of drought stressed and control *C. lancifolius* plants. Means followed by the same letter are not significantly different at P≤0.05 according to Fisher's protected least significant difference (LSD).

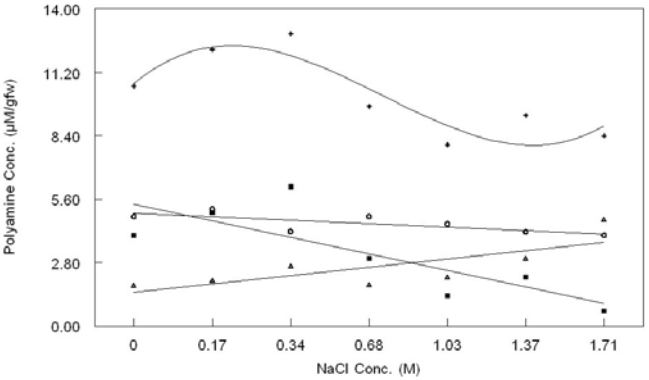

Fig. 7 The relationship between the accumulation of putrescine (■), spermidine (○), spermine (Δ) and total PAs (+) in the leaves of *C. lancifolius* plants and NaCl treatments, 3 DAT.

from proplastids in wheat within 7 days. Retaining green tissue delayed senescence which was probably due to the accumulation of PAs This is in agreement with the observations of Cheng and Kao 1983; Walters 2003. The mode of action for antisenescence of these polyamines could be due to free radical scavenging (Drolet *et al.* 1986).

Salt treatments in this study increased Fo 10 DAT, particularly with 1.37-1.71 M NaCl which may be due to salt damage to PS II. This was reported by Park *et al*. (1995) who correlated an increase Fo to a reduction in the numbers of functional reaction centers in PS II. A reduction in the functional reaction centers could also lead to increase fluorescence in the photosynthetic antennae (Osmond *et al*. 1999). The decrease in electron transport (ETR), quantum yield (Y) and Fv/Fm coupled with an increase in Fo in this study indicated a decrease in the efficiency of photoprotection (Weiss and Reigosa Roger 2001). It appeared that increased salinity affected photosynthesis directly by low cellular water potential of the photosynthetic machinery which was manifested by the reduction in ETR and Fv/Fm.

The species grows vigorously in hot, dry summers and does not appear to be under environmental stress. *C. lancifolius* has the ability to produce the PAs: Put, Spd and Spm. Put was the predominat PA at the lower NaCl treatments and at higher levels of NaCl Spm became dominant. Some plants under stress probably respond to increase photorespiration by increasing the production of ammonium (NH_4^+) that could stimulate the synthesis of glutamate. Glutamate is converted to arginine and ornithine, which ultimately are used in the synthesis of Put (Slocum and Weinstein 1990). Under semi-arid conditions this could be a pathway for the production of PAs in *C. lancifolius.*

Spd and Spm are known as antisenescent compounds (Besford *et al*. 1993) and have been implicated in various plant growth and developmental processes (Bouchereau *et al*. 1999; Kakkar *et al*. 2000; Liu *et al*. 2006). The semi-arid conditions coupled with the use of brackish water for irrigation in Kuwait tend to increase the salinity of the soil through evapotranspiration. Spd and Spm were reported to accumulate in plant tissues as a result of salinization (Krishnamurthy and Bhagwat 1989; Santa-Cruz *et al*. 1997; Maiale *et al*. 2004; Groppa and Benavides 2008). Our observations appeared to be agreement. Polyamine accumulation under stress has been reported in a number of plant species (Sánchez *et al*. 2005; Groppa and Benavides 2008). Although the precise role(s) of PAs in plants is/are still under investigation, there is evidence that they ameliorate salt stress by accumulating proline and Put (Aziz *et al*. 1998). The data in this study showed the accumulation of Put as the predominant PA as an early response to salinity stress and drought stress. The decline in Put with increased intensity of stress (salinity) could be due to its utilization, contribution to proline production and/or its conversion to the triamine Spd and then to the tetramine Spm that could ameliorate osmotic stress. The accumulation of PAs in this study correlated significantly with the duration of drought and increase in salinity. Drought and salinity appeared to increase the accumulation of the three most common PAs in different proportions in *C. lancifolius*. However, it appeared the accumulation of Put, Spd and Spm in this study was probably dependent on the nature, intensity and duration of the environmental stress for *C. lancifolius* which in agreement with the observations of Ashraf and Harris (2004) and Kasinathan and Wingler (2004). Finally, when we imposed drought and salinity on *C. lancifolius* at levels that would not favor osmotic adjustment ETR, Y, Fv/Fm, CCI declined after 10 days in intact and functional chloroplasts.

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