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Responses of *Conocarpus lancifolius* to environmental stress: a case study in the semi-arid land of Kuwait

Respuestas de *Conocarpus lancifolius* al estrés ambiental: estudio de un caso en la zona semiárida de Kuwait

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Abstract. *Cococarpus lancifolius* Engl. grows wild in Djibouti, Somalia and East Africa, and South Asia. The species was introduced into Kuwait, where it is exposed to several stresses: oil pollution, salinity, high temperature and harsh climatic conditions. These conditions are prevalent in its semi-arid environment. In this study, the impact of salinity (2, 6 and 10%), temperature (20, 30 and 40 °C), polyethylene glycol (10, 30 and 60%) and drought has been investigated on *C. lancifolius*. Proline (Pro) accumulation (5.5 ± 0.03 µg/g dry weight; p≤0.05-0.005), protein (16.47 ± 0.052 mg/g; p≤0.001), free amino acids (10.92 ± 0.082 mg/g; p≤0.006) and lipids (116.05 ± 2.17 µg/g; p≤0.05) were measured in plants irrigated with 60% polyethylene glycol (PEG) for 6 days. Similar increases in the accumulation of osmolytes were observed under water deficit, NaCl, and temperature-stressed plants. Accumulation of osmolytes was correlated with photosynthetic capacity and electron transport rate. Fifteen different phospholipids were detected in leaf extracts by TLC and 6 major fatty acids were identified by GC/MS analyses. On day 4, the omega-3 fatty acid, linolenic acid was observed the predominant fatty acid present at 61.32% in the 10% PEG-treated plants. The high concentration of this omega-3 fatty acid may help *C. lancifolius* to adapt to semi-arid conditions in the State of Kuwait. Implications of this study may highlight sustainability of *C. lancifolius* in the Middle East, African peninsula and other semi-arid regions of the world.

Keywords: *Conocarpus lancifolius*; Drought; Electron transport rate; Environmental stress; Salinity; Temperature; Photosynthesis.

Resumen. *Conocarpus lancifolius* Engl. crece naturalmente en Djibouti, Somalía y Este de África, y Sur de Asia. La especie fue introducida en Kuwait, donde está expuesta a varios estreses: contaminación ambiental, salinidad, alta temperatura y condiciones climáticas adversas. Estas condiciones prevalecen en este ambiente semiárido. En este estudio, se investigaron los efectos de la salinidad (2, 6 y 10%), temperatura (20, 30 y 40 °C), polietilenglicol (10, 30 y 60%) y sequía en *C. lancifolius*. La acumulación de proline (Pro) (5,5 ± 0,03 µg/g de peso seco; p≤0,05-0,005), proteína (16,47 ± 0,052 mg/g; p≤0,001), aminoácidos libres (10,92 ± 0,082 mg/g; p≤0,006) y lípidos (116,05 ± 2,17 µg/g; p≤0,05) se midieron en plantas regadas con 60% de polietilenglicol (PEG) por 6 días. Incrementos similares en la acumulación de osmolitos se observaron en plantas expuestas a estreses por deficiencia hídrica, NaCl, y temperatura. La acumulación de osmolitos se correlacionó con la actividad fotosintética y la tasa de transporte de electrones. Se detectaron quince fosfolípidos diferentes en extractos vegetales por TLC, y se identificaron seis ácidos grasos principales por análisis GC/MS. Al 4º día, el ácido graso omega-3, ácido linoleico, fue el ácido graso predominante (61,32%) en las plantas tratadas con PEG al 10%. La alta concentración de este ácido graso omega-3 podría ayudar a *C. lancifolius* a adaptarse a las condiciones semiáridas en el Estado de Kuwait. Las implicancias de este estudio podrían destacar la sustentabilidad de *C. lancifolius*

Palabras clave: *Conocarpus lancifolius*; Sequía; Tasa de transporte de electrones; Estrés ambiental; Salinidad; Temperatura; Fotosíntesis.

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INTRODUCTION

In Kuwait, efforts are being made to introduce plants with the unique ability to tolerate local environmental pressures. In this endeavor, *Conocarpus lancifolius* Eng. has been introduced from Djibouti for greenery projects in the State of Kuwait. Frequent sand storms, high temperatures, drought, salinity, oil pollution and excessive sunlight are key stress parameters for plants growing in the semi-arid environment of Kuwait. Desiccating environmental conditions along with strong solar radiation that suppresses CO_2 fixation contribute to the oxidative injury to plant tissues. Abiotic stress alters membrane permeability, the nature of membrane lipids, hormones and osmotic parameters. Furthermore, variation in soil water results in an increase in osmotic pressure and the elongation of plant cells (Mayabaf et al., 2001). However, the variation in the effects of abiotic stress is based on how well a plant responds to the quality and quantity of stress. Thus, drought-stressed plants with compromised photosynthetic proton circuit (Kohzuma et al., 2009) diminish their osmotic potential by accumulating amino acids, proline, ions and soluble polyols, such as mannitol (Shen et al., 1997; Cicek & Cakirlar, 2002). In addition to an important role as an osmoprotectant, accumulated N-containing compounds, such as proline and other amines such as citrulline, play major roles as physiological regulators and potent hydroxyl radical scavengers (Akashi et al., 2004). Notably, the protective role of proline in the solubilization of proteins, nitrogen conservation and energy production has been recognized (Aloni & Rosenshtein, 1984). Other roles of proline include the prevention of heat denaturation of enzymes and the accumulation of ArgE-related proteins, which lead to an increase in the amount of free amino acids, such as arginine (Kawasakil et al., 2000).

Salinity is also a major factor that affects plant physiology. Salinity affects plants through a variety of mechanisms that alter osmotic effects, membrane permeability, ion specific transport, transpiration rates, photosynthesis and growth (Stoeva & Kaymakanova, 2000; Renault et al., 2001). Importantly, the vital and delicate balance of Na^+/K^+ ions is affected by salinity and has serious implications on plant growth. As a result, the measurements of Na^+/K^+ are used as markers for evaluating plant stress (Palfi & Juhasz, 1971). Many other osmolytes such as glutathione, cysteine, ascorbate and the late embryogenesis abundant (LEA) group of proteins accumulate in response to salinity (Mansour, 2000; Sahu et al., 2010). Changes in osmolytes occur when salt accumulates in the root zone of the plant (Zhu, 2001). An increase in lipid peroxidation in response to salinity and sodium sulfate has been indicated in bean plants by a consequential increase in malondialdehyde (MDA), a marker of lipid peroxidation (Kayamakanova et al., 2008).

Almost all abiotic and biotic stress conditions promote oxidative stress (Haberer et al., 2008) in the chloroplast and the mitochondria (Navrot et al., 2007). Heath & Packer (1968) reported that photo-oxidation affects the stoichiometry of fatty acids. Moreover, antioxidant defenses, such as glutathione and α-tocopherol (Tausz, 2001; Munne-Bosch, 2005), are depleted due to reactive oxygen species (ROS) generated by drought, salinity and other types of stresses (Gechev et al., 2006). The levels of natural antioxidant enzymes are also altered due to stress induced by high concentrations of sodium chloride (Kalir & Poljakoff-Mayber, 1981). As a result, lipid peroxidation metabolites and related molecules act as cell signaling molecules influencing the plant cell cycle and its physiology (Kalir & Poljakoff-Mayber, 1981).

Drought can induce ROS through photo-oxidative stress, altering a plant's redox system (Sircelj et al., 2005). Changes in membrane structure and permeability in response to cytotoxic ROS generated by drought and salinity have been previously reported by several research groups (Wang et al., 2002; Sun et al., 2003). Notably, gradient vapor due to drought stress has been observed to stimulate the synthesis of phospholipids and their metabolites, and affects energy metabolism and signal transduction, thereby altering metabolic processes. Lipids, which are important membrane components, undergo an irreversible change that affects membrane transport and the physiological responses of the plant cell. Both enzymatic and non-enzymatic antioxidant defenses operate through a complex mechanism that coordinates these defenses to protect plants from the damaging effects of ROS (Sahu et al., 2010). Additionally, other biological systems or apparatuses that are affected by ROS are proteins and nucleic acid synthesis (Mittler, 2002). In the present study, we report the effects of stressors such as salinity, temperature, polyethylene glycol, and water deficit on proteins, lipids and free amino acids in *C. lancifolius.*

MATERIALS AND METHODS

Plant material. Shoots of vegetatively propagated *C. lancifolius* plants were used and maintained in the greenhouse at Kuwait University. Plantlets of uniform height and various growth stages of leaves (13-15) were randomly selected and transferred to plastic pots (19.0 cm diameter and 16.3 cm deep) containing local sandy soil and peat moss (3:1 v/v). Pots were then transferred to walk-in growth rooms where they remained for one week to acclimate prior to the experimental treatments at 22 °C. All plants were divided into five groups (A-E) and subgroups. The subgroups, each containing six plants, were exposed to a different stressor for six days. Plants in group A were subdivided into three subgroups (a1, a2, a3), and each subgroup of plants was watered daily with 10, 30 or 60% polyethylene glycol (PEG 6000). In group B, plants were subdivided into three subgroups (b1 – b3), and each was watered daily with varying amounts of NaCl (2, 6, or 10%). Plants in group C were divided into three subgroups

(c1, c2, c3), and each subgroup of plants was exposed to different temperatures (20, 30 or 40 °C) while being irrigated daily. In group D, plants were subjected to drought conditions by withholding water for six days at 22 °C, and plants in group E served as controls.

Photosynthesis and electron transport rate. In order to associate changes in the accumulation of osmolytes, with the photosynthetic capacity of the stressed plants, both photosynthesis and electron transport rates were measured using an OS5-FL chlorophyll fluorometer (OPTI-SCIENCE, Hudson, NH, USA) with a photodiode detector at 25 ± 1 °C, as described by Hortensteiner (2006). The rate of photosynthesis was measured using an LCi portal photosynthesis meter (ADC BioScientific Ltd., Hoddesdon UK).

Lipid analysis. Lipids were extracted from fresh plant material via homogenization in ice-cold chloroform:methanol (2:1, v/v) as described by Folch et al. (1957). The total amount of lipids was determined after the complete evaporation of solvent under a gentle stream of N_2 gas. These samples were also used for two-dimensional TLC analyses of phospholipids using chloroform-methanol-water (75:25:2.5, v/v) and chloroform-methanol-acetic acid-water (80:9:12:2, v/v) as described by Touchstone (1995). In this double development, 15 phospholipids were resolved on a glass-coated HPTLC plate stained with a 2% ethanolic solution of phosphomolybedic acid (Fig. 1). The lipid extract (2 mg) was then dissolved in a methanolic solution of boron trifluoride (500 µL) and incubated at 40 °C for 20 min. Two milliliters of ice-cold water was added to chilled reaction vials, and the fatty acid methyl esters (FAME) were extracted with redistilled hexane (1 mL × 3). The pooled extract was dried with anhydrous sodium sulfate and then filtered, and the solvent was evaporated under a gentle stream of N_2 gas. The FAME mixture was reconstituted in hexane and injected onto an OV1 GC capillary column (30 m × 0.25 mm ID) for GC-MS analysis using a 6890 series GC system and an Agilent model 5973 Network Mass selective detector. Helium gas pressure was maintained at 19.0 psi with a flow rate of 2 mL/min. The oven temperature program used was set to 80 °C for 4 min, followed by a gradual increase of 3 °C/min to 250 °C, and then the temperature remained at 250 °C for 5 min. The total running time was 45.67 min. The MSD Chemstation data analysis with the Agilent library was used for identification of each FAME.

Protein and free amino acid analyses. Protein analysis was performed on plants exposed to differential stress treatments on days 2-6. Whole plants were washed with distilled water to remove soil from the roots. Plants were first dried with papers towels to remove extra water and then dried in an oven at 40 °C for 18 h. Dried plants were powdered in a milling machine and the resulting powder was used to quantify the amounts of proline, protein and free amino acid. Plant powder (2 g) was stirred in 10 mL of 3% sulfosalicylic acid for 30 min, centrifuged and passed through a 0.45 µm membrane filter. The amount of protein in the resulting colorless extract was quantified using ninhydrin (Bates et al., 1973). The absorbance of pure protein bovine serum albumin (BSA) at 570 nm was used to make a standard curve.

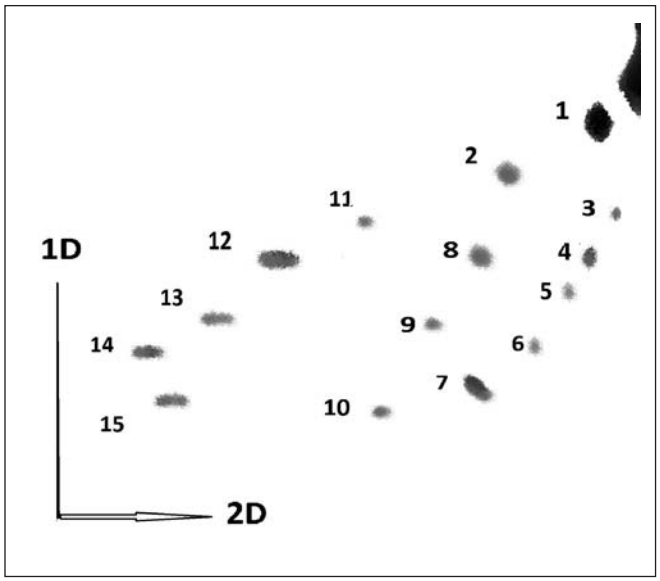


Fig. 1. TLC Separation of phospholipids on two dimensional thin layer chromatography. Spots identified were as follows: 1. Phosphatidic acid; 2. Phosphatidyl inositol; 4. phosphatidyl ethanolamine; 5. phosphatidyl choline; 15. Phosphatidyl serine.
Fig. 1. Separación TLC de fosfolípidos por cromatografía de capa fina bidimensional: Los grupos se identificaron como sigue: 1. Ácido fosfatídico; 2. Fosfatidil inositol; 4. fosfatidil etanolamina; 5. fosfatidil colina; 15. fosfatidil serina.

Protein and free amino acids were extracted using 100 mM phosphate buffer pH 7.8, containing 1 mM EDTA, 1% triton X-100 and 10% glycerol, and concentrations were determined according to the method described by Wang et al. (2006). Free amino acids (FAAs) from the dried plant material were extracted into methanol:chloroform:water (MCW 12:5:3 v/v) as described by Marur et al. (1994). Concentration of free amino acids was determined photometrically by reacting a known volume of the extract with ninhydrin at 90 °C. Alanine was used to generate a standard curve.

Statistical analyses. All measurements were made in triplicate with mean centered and analyzed using one-way-analysis of variance (ANOVA), using GraphPad Prism 5 software (San Diego, CA, USA). Mean differences were determined using Tukey's multiple comparison test for p value at 95% confidence ($p \leq 0.05$). GraphPad Prism 5 software was used for graphics.

RESULTS

Conocarpus lancifolius thrives well under semi-arid conditions by adjusting its physiological and biochemical processes. Our results demonstrate that *C. lancifolius* is affected by differential environmental stresses, and the effect is dependent on the exposure time and the type of the stress.

Effect of drought and PEG on the accumulation of lipids. Plants exposed to non-permeant 30 and 60% PEG accumulated significant ($p \leq 0.05$) amount of lipids on day 4 and 6 (Fig. 2a). Lipids showed a two-fold increase from 55.5 ± 2.35 mg/g (control) to 83.46 ± 2.48 and 116.2 ± 3.58 mg/g after exposure to 30 and 60% PEG, respectively (Fig. 2a). Water deficit plants showed lipid accumulation of 84.23 ± 3.2 mg/g after six days

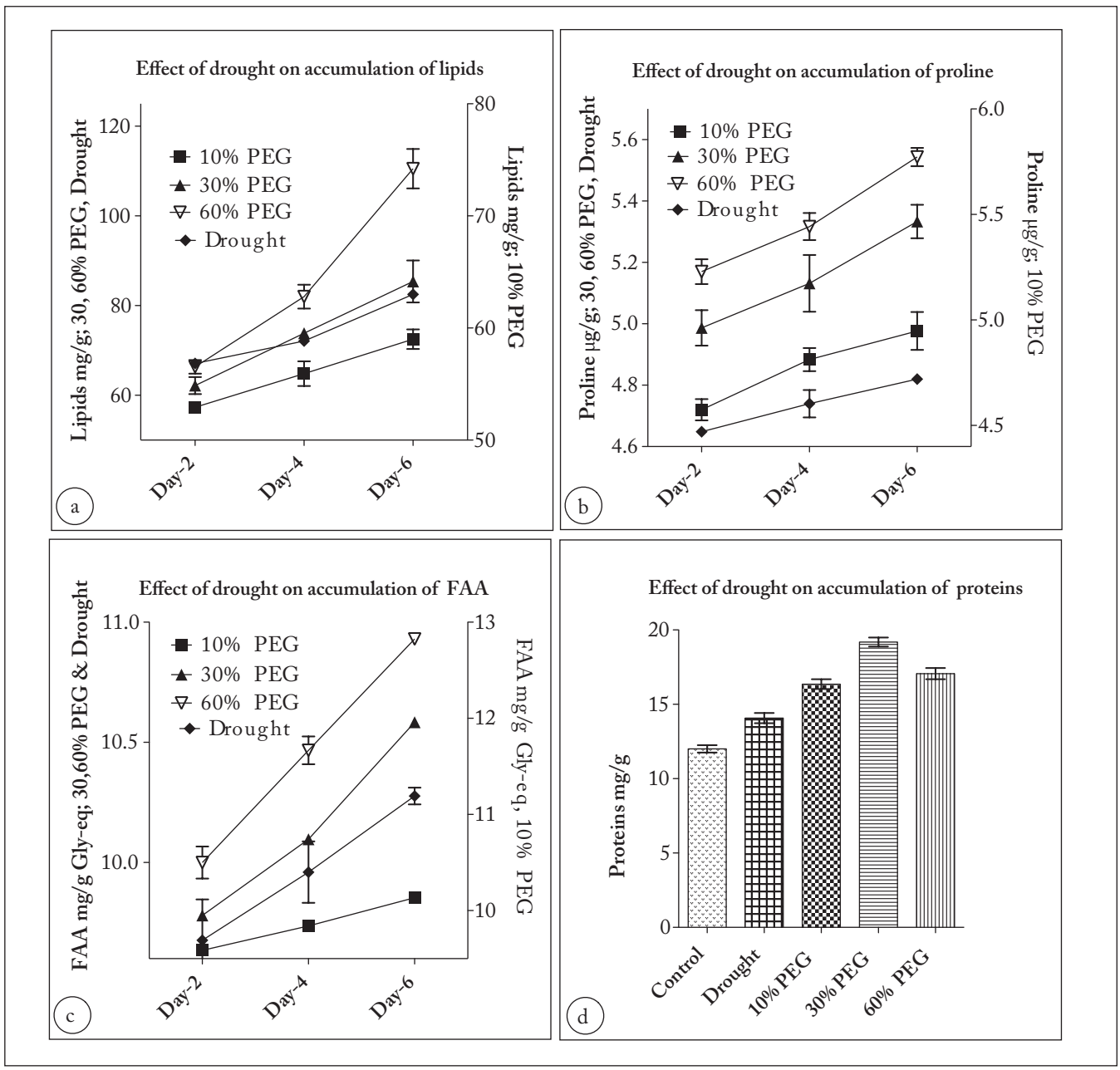


Fig. 2. Effect of PEG and drought: 2a. Effect of PEG and drought on accumulation of lipids; 2b. Effect of PEG and drought on the accumulation of proline; 2c. Effect of PEG and drought on the accumulation of free amino acids (FAA); 2d. Effect of drought on the accumulation of proteins.

Fig. 2. Efecto de PEG y sequía: 2a. Efecto de PEG y sequía en la acumulación de lípidos; 2b. Efecto de PEG y sequía en la acumulación de prolina; 2c. Efecto de PEG y sequía en la acumulación de aminoácidos libres (FAA); 2d. Efecto de sequía en la acumulación de proteínas.

of exposure to drought. A not significant (ns) increase in lipids was observed when plants were exposed to 10% PEG for 2-6 days. Accumulation of competitive solutes such as proline also took place after exposure of the plants to drought and PEG. The accumulation of proline was ns when plants were exposed to water deficit or 10% PEG after exposure for six days. However, proline accumulation was significantly higher [4.37 ± 0.01 μg/g (control)] when plants were exposed to 30% (5.32 ± 0.07 μg/g) or 60% (5.52 ± 0.03 μg/g) PEG for a period of six days (Fig. 2b). Accumulation of proline in drought exposed plants was 4.85 ± 0.045 μg/g. Free amino acids (FAA) also accumulated in response to PEG and drought. 10% PEG and drought had ns effect on the accumulation of FAA. However, exposure of plants to 30 and 60% PEG resulted in a significant accumulation of FAA. Thus FAA increased from 9.55 ± 0.04 mg/g (control) to 10.25 ± 0.015 and 10.93 ± 0.017 mg/g after six days of exposure to 30 and 60% PEG, respectively (Fig. 2c). Accumulation of proteins in drought and 10% PEG exposed plants was ns. However, exposure to 30 and 60% PEG resulted in a significant accumulation of proteins after six days of exposure, and exposure to 30% PEG resulted in higher (19.34 ± 0.18 mg/g) accumulation of proteins than exposure to 60% PEG (16.54 ± 0.17 mg/g) compared with the control (12.32 ± 1.29 mg/g) (Fig. 2d).

Effect of temperature on the accumulation of lipids. Exposure of the plants at 20 and 30 ˚C for six days showed ns accumulation of lipids. However, plants exposed to 40 ˚C for 6 days exhibited a significant ($p \leq 0.05$) increase in lipids in a time-dependent manner. An increase of 92.8% in lipids was observed after 6 days of exposure to 40 ˚C (Fig. 3a). Thus, exposure to higher temperature (40 ˚C) resulted in lipid accumulation [from 55.5 ± 2.35 (control) to 107.45 ± 4.55 mg/g]. Accumulation of competitive solutes such as proline was also observed at higher temperature when it increased from (Fig. 3b) 4.37 ± 0.01 (control) to 4.4 ± 0.02 μg/g in plants exposed to 20 ˚C (cooling effect), while exposure to higher temperatures (30 and 40 ˚C) resulted in a significant accumulation of proline: 4.58 ± 0.01 μg/g (30 ˚C) and 4.81 ± 0.02 μg/g (40 ˚C) (Fig. 3b). Lower temperatures (i.e., 20 ˚C) did not show accumulation of FAA. However, increased temperature to 30 and 40 ˚C showed a significant increase in the accumulation of FAA. Thus, FAA accumulation changed from 9.55 ± 0.04 (control) to 11.13 ± 0.03 (30 ˚C) and 12.83 ± 0.03 mg/g (40 ˚C), glycine equivalent (Fig. 3c). A ns accumulation of proteins resulted in plants exposed to variable temperatures (Fig. 3d). Relationships of proline, FAA and proteins with ETR, and photosynthesis with variable temperature are shown in Fig 5a-c.

Effect of NaCl on the accumulation of lipids. Exposure of the plants to 2% NaCl showed ns effect on the accumulation of lipids (55.50 ± 2.35 - 59.34 ± 0.46 mg/g). However, exposure to 6 and 10% NaCl showed a significant increase in lipids to 85.35 ± 3.55 and 92.42 ± 5.23 mg/g, respectively, after 6 days of salinity exposure (Fig. 4a). Plants exposed to 2% NaCl accumulated proline in a ns manner (Fig 4a). However, exposure of the plants to higher concentration of NaCl (6 and 10%) resulted in a significant accumulation of proline. Thus, an increase in proline was observed from 4.37 ± 0.01 μg/g (control) to 5.14 ± 0.02 and 5.32 ± 0.05 μg/g after 6 days of plant exposure to 6 and 10% NaCl, respectively (Fig. 4b). Protein accumulation was ns at all temperatures (results not shown in figure). Accumulation of FAA in response to 2% NaCl was ns but exposure of plants to higher salinity (6 and 10% NaCl) resulted in a significant accumulation of FAA. Thus, accumulation of FAA was noted from 9.55 ± 0.04 mg/g (control) to 10.45 ± 0.04 and 10.85 ± 0.02 mg/g in plants exposed to 6 and 10% NaCl, respectively (Fig. 4c).

An analysis of the fatty acid profile of the total lipids revealed that the unsaturation index (UI, calculated as linolenic (18:3)/stearic acid (18:0); L/S) of the plants increased under all stress conditions. At 20 ˚C, a cooling effect was observed among plants in which the UI increased from 6.33 ± 0.04 on day 2 to 6.63 ± 0.037 on day 6; thus, the cell wall integrity and fluidity at low temperatures were maintained. The UI trend decreased in plants that were exposed to increasing temperatures (30 and 40 ˚C) for 2-6 days, indicating the synthesis of more saturated fatty acids in response to higher temperatures. A similar trend in decreasing UI during 2-6 days was observed in plants exposed to increasing salinity (2-10%), PEG (10-60%) and drought (Table 1). The accumulation of ω3 linolenic acid at 14.62% of the total fatty acids on day 4 and 13.68% on day 6 in plants exposed to 40 ˚C makes the *Conocarpus* plant attractive for its use as a nutritional source of ω-3 fatty acid. Our results are supported by previous studies reporting an increase in unsaturated fatty acids in plants in response to drought and low temperature (Kasamo et al., 1992; Torres-Franklin et al., 2008).

DISCUSSION

The decrease in water potential with differential permeating stressors, such as NaCl and the nonpermeating PEG 6000 solute, results in osmotic stress that induces membrane lipid modifications, ionic imbalance, photosynthesis, competitive solute accumulation and variation in enzyme activities (Koyro, 2006). Salt tolerance in plants and the effect of salt on plant physiology have been reviewed (Hamdia & Shaddad, 1997; El-Samad et al., 2010).

In response to salinity, membrane accumulation of polyunsaturated fatty acids (PUFAs) in the form of phospholipids, is known to protect photosystem II in plants (Koyro, 2006; Sui et al., 2010), In response to increasing salinity, PEG, drought and temperature, an increase in the amount of total lipids may be a protective mechanism against stress and accumulated osmolytes, which can disturb the ionic balance within the plant tissue. Salinity stress may also modify the Na/K-ATPase

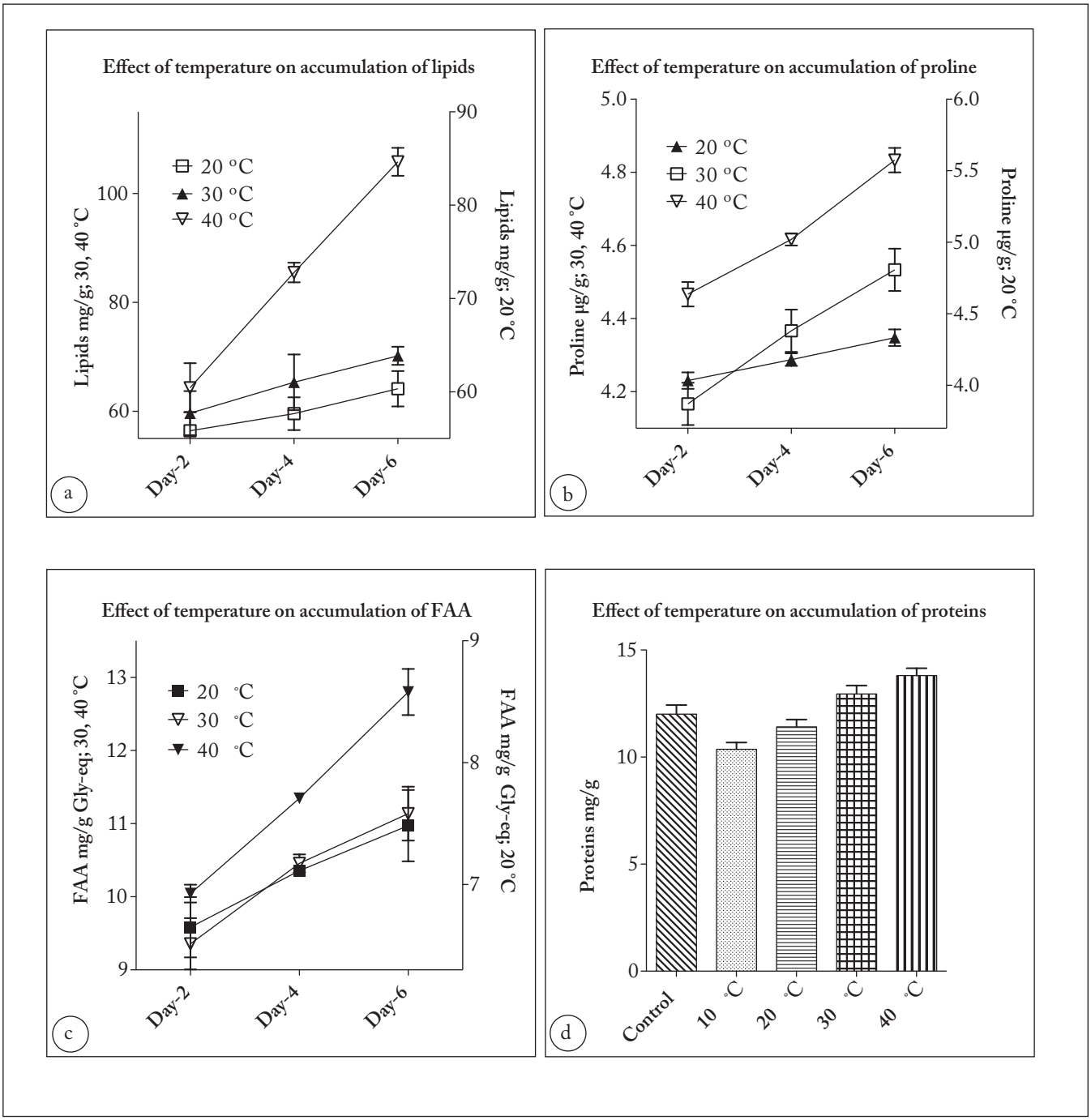
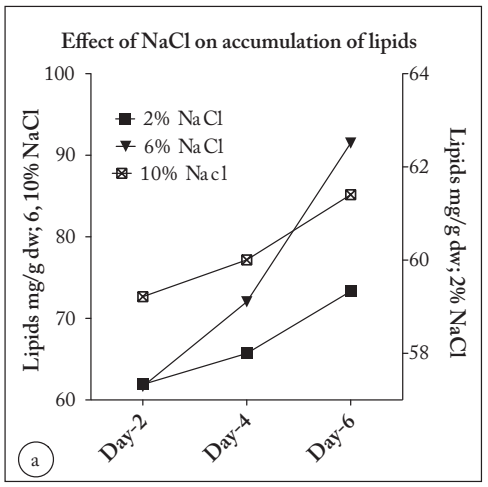
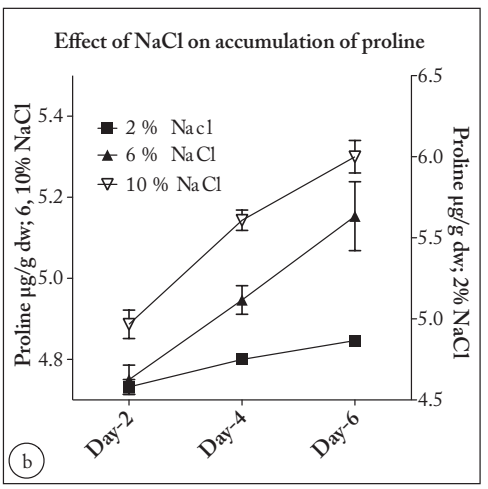


Fig. 3. Effect of Temperature: 3a. Effect of temperature on the accumulation of lipids; 3b. Effect of temperature on the accumulation of proline; 3c. Effect of temperature on the accumulation of free amino acids; 3d. Effect of temperature on the accumulation of proteins.

Fig. 3. Efecto de Temperatura: 3a. Efecto de temperatura en la acumulación de lípidos; 3b. Efecto de temperatura en la acumulación de prolina; 3c. Efecto de temperatura en la acumulación de aminoácidos libres; 3d. Efecto de temperatura en la acumulación de proteínas.





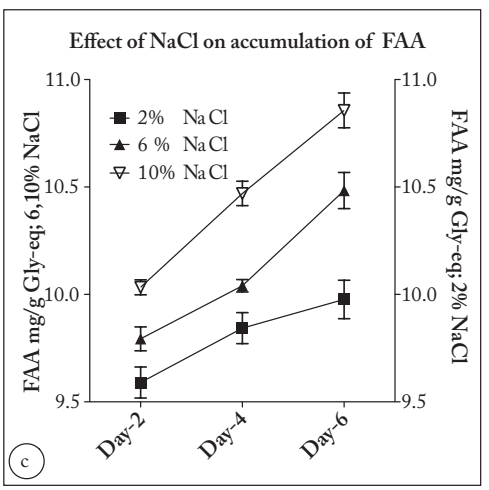
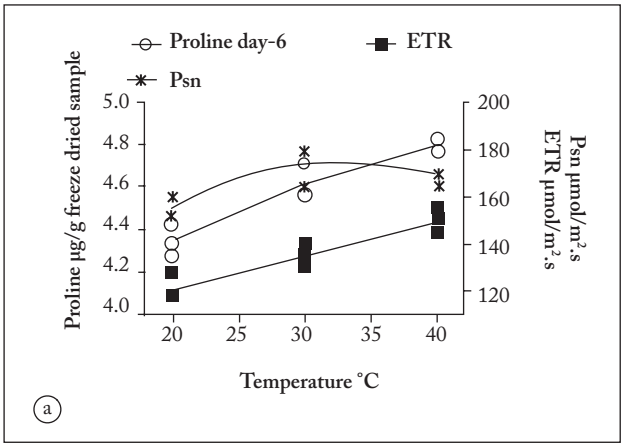
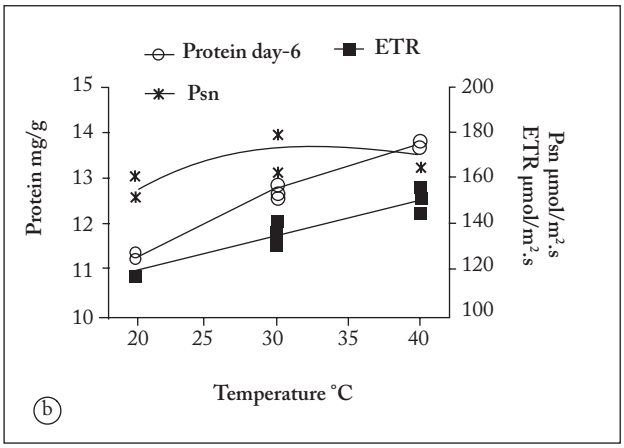


Fig. 4. Effect of salinity: 4a. Effect of NaCl on the accumulation of lipids; 4b. Effect of salinity on the accumulation of proline; 4c. Effect of NaCl on the accumulation of free amino acids.

Fig. 4. Efecto de la salinidad: 4a. Efecto del NaCl en la acumulación de lípidos; 4b. Efecto de la salinidad en la acumulación de prolina; 4c. Efecto del NaCl en la acumulación de aminoácidos libres.





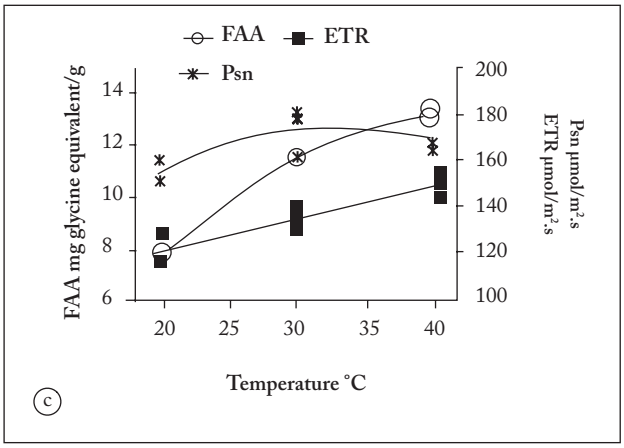


Fig. 5. 5a. Relationship between proline and Psn, ETR at different temperatures; 5b. Relationship between protein and Psn, ETR at different temperatures; 5c. Relationship between FAAs and Psn, ETR at different temperatures. ETR = Electron transport rate; Psn = Photosynthesis rate.

Fig. 5. 5a. Relación entre la prolina y Psn, ETR a diferentes temperaturas; 5b. Relación entre proteína y Psn, ETR a diferentes temperaturas; 5c. Relación entre FAAs y Psn, ETR a diferentes temperaturas. ETR =tasa de transporte de electrones; Psn = tasa fotosintética.

Table 1. Linolenic and stearic acid levels and their ratio in multiple treatments of *C. lancifolius* samples.
Tabla 1. Niveles de los ácidos linoleico y esteárico y su relación en tratamientos múltiples de muestras de *C. lancifolius*.

Stressor	Stress %	Days	Linolenic Acid %	Stearic Acid %	Ratio L/S
PEG[a]	10	2	26.936	45.185	0.596
		4	61.321	5.758	10.649
		6	29.374	42.383	0.693
	30	2	29.500	40.259	0.733
		4	30.767	40.157	0.766
		6	30.202	40.955	0.737
	60	2	26.10	44.619	0.584
		4	26.808	43.175	0.620
		6	26.437	42.802	0.617
Salinity[a]					
	2	2	18.341	53.159	0.345
		4	32.163	38.173	0.842
		6			
	4	2	17.400	54.255	0.327
		4	35.761	36.967	0.967
		6			
	6	2	17.500	54.255	0.313
		4	36.158	39.303	0.919
		6			
	8	2	17.630	53.660	0.328
		4	33.663	41.327	0.814
		6			
	10	2	17.392	55.338	0.314
		4	38.440	38.384	1.001
Temp.*					
	20 °C	2	51.980	8.220	6.322
		4	41.480	24.760	1.680
		6	44.630	6.740	6.620
	30 °C	2	16.210	39.650	0.408
		4	14.060	46.360	0.301
		6	12.240	42.770	0.290
	40 °C	2	10.990	49.010	0.224
		4	14.620	42.980	0.340
		6	13.680	46.200	0.296
Drought					
		2	36.137	36.310	0.995
		4	27.855	42.143	0.660
		6	35.800	39.161	0.914

pump with changes in membrane lipid composition (Palacios et al., 2004). In the present study, high percentages of salinity, especially 6 and 10% NaCl, had a profound effect on both lipid accumulation and the UI of plants, whereas low percentages of salinity, PEG and temperature had a nonsignificant (ns) effect on total lipids and osmoregulators.

The decrease in linolenic acid content with increasing stress may have resulted from the oxidative damage caused by ROS generated in response to salinity and drought. With the exception of temperature treatment, all other types of stress resulted in decreasing UI. These data corroborate a previous report that a decrease in PUFA occurs in response to water stress in higher plants (Sueldo et al., 1996).

A cooling effect was observed in plants exposed to 10-20 °C; these plants accumulated unsaturated fatty acids probably to maintain membrane integrity and fluidity at these temperatures. Previous studies (Kasamo et al., 1992) have reported the synthesis of unsaturated fatty acids at low temperatures in various organisms. Enzymes such as 1,2–diacylglycerolcholine phosphotransferase (CPT) and acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT), responsible for the synthesis of PUFA are activated at low temperatures, increasing the PUFA content (Cheesbrough, 1989). Catalase is known to play a role in linoleate desaturation in the biosynthesis of linolenic acid (Browse & Slack, 1981).

One of the most common responses of plants under stress is the accumulation of osmolytes. Low molecular-weight osmolytic substances play an important role in protecting the plant from osmotic pressure, oxidative damage, and decreased membrane integrity and enzyme instability. The accumulation of proline has been universally observed in plants under various types of stress (Nedjimi et al., 2006). Additionally, proline is known to protect subcellular structures, such as proteins, from oxidative damage generated by ROS in stressed plants (Koyro, 2006). Accumulation of proline in response to NaCl or other types of osmotic stress is also known to induce antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and it helps to mitigate the stress by intensifying photosynthetic performance (Khan et al., 2007; Tan et al., 2008; Ahmed et al., 2010). Accumulation of organic and FAA in response to salt stress has been reported by Gavaghan et al. (2011). Thus, proline and FAA accumulation result from a reduced level of oxidation and/or an increased breakdown of proteins under stress conditions. The synthesis and transport of proline and FAA to sink tissues under stress conditions, protecting photosystem II has been reported (Marur et al., 1994). In the present study, PEG-treated plants accumulated the highest amount of proline (from 4.42 to 5.54 μg/g). Our results show that PEG-induced drought is much stronger that the drought created by water withdrawal. For this reason, accumulation of lipids, proline, protein and FAA was higher in PEG treated plants (Fig 2a-d; Fig. 3a-d; Fig. 4a-c). Under PEG, NaCl or temperature stress, increase in these osmolytes may increase the

antioxidant level with a simultaneous increase in photosynthetic capacity of *C. lancifolius*. FAA, proline and proteins are antioxidants that increase photosynthesis in stressed plants (Barry, 1993; Almajano et al., 2007; Tan et al., 2008). PEG-induced stress also resulted in a significant accumulation of proteins in plants compared to plants exposed to other types of stresses applied in this study, and protein accumulation was higher at 30% PEG than at 60% PEG (Fig. 2d). Proteins and proline metabolism play an important role in water-stressed plants, and PEG-induced oxidative stress has a critical function in enzyme modification and protein accumulation (Xu & Huang, 2010).

In the present study, a consistent increase in FAA accumulation was observed with increasing temperature, PEG, drought and NaCl (Fig 2c; 3c; 4c). Thus, the highest FAA accumulation [from 9.55 ± 0.04 mg/g (control) to 12.85 ± 0.045 mg/g at 40 ˚C] was observed when plant growth rate was optimal (Fig. 4c). The accumulation of proline, protein and other soluble solutes in response to PEG and NaCl may be due to osmotic adjustment (Mohammad Khani & Heidari, 2008). NaCl and drought stress resulted in a ns accumulation of FAAs in plants with the exception of plants exposed to 10% NaCl on days 4 and 6 ($p \leq 0.05$). On day 6, plants exposed to 10% NaCl were physically stressed. Under these conditions, the increased accumulation of FAA including proline in *Conocarpus* may be due to increased protein synthesis. However, the increases in proline, proteins, FAA, and lipids with differential stress and time dependency clearly reflect the contribution of each stress type, and not the metabolic turnover rate of plants. Accumulation of amino acids in response to salt stress has been recently reported in maize plants (Gavaghan et al., 2011). An increase in the accumulation of proline, proteins and FAA in *Conocarpus* not only mitigate the oxidative stress, but also benefit photosynthesis and electron transport (Fig. 5a-c). Our results show that *Conocarpus* experiences differential environmental stresses and has the capacity to persist within the Kuwait environment.

CONCLUSION

C. lancifolius is sensitive to environmental stresses including salinity, extreme drought. However, the plant thrives well at elevated temperatures. Plants of *C. lancifolius* alter its physiology by accumulating soluble osmolytes, such as proline, proteins and FAA. The results of this study demonstrated that an elevated growth temperature, prevalent in Kuwait, helps the plant to accumulate polyunsaturated fatty acids, which may play a protective role in the survival of this plant in the Kuwait environment.

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