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EFFECT OF SEA SALT IRRIGATION ON PLANT GROWTH, YIELD POTENTIAL AND SOME BIOCHEMICAL ATTRIBUTES OF *CARISSA CARANDAS*.

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Abstract

Carissa carandas (varn. Karonda) is an edible and medicinal plant having ability to grow in saline and water deficit conditions, however, little is known about its salinity tolerance. Therefore, the effect of salinity on vegetative (height and volume), reproductive (number of flowers and number, size and weight of fruits) and some biochemical parameters (leaf pigments, ions, soluble sugars, proteins, and phenols) of *C. carandas* were studied. Plants were grown in drum pot culture and irrigated with non-saline or saline water of 0.6% and 0.8% sea salt concentrations, for a period of 30 months. Results showed that, plant height, and canopy volume decreased with increasing salinity. The chlorophyll contents and chlorophyll a/b ratio followed the similar trend as for growth, however, carotenoids increased at 0.6% sea salt and subsequently decreased in higher salinity. Unchanged soluble sugar and protein content at 0.6% sea salt, as compared to control, could be attributed to leaf osmotic adjustments which decreased with further increase in salinity. Linear increase in soluble phenols and carotenoid/chlorophyll ratio indicating a protective strategy of *C. carandas* to minimize photo-damage. Besides increasing Na^+ and decreasing K^+ contents, plant seemed to maintain K^+/Na^+ ratio (above 1), especially at 0.6 sea salt, which disturbed at higher salinity. Salinity adversely affected reproductive growth of *C. carandas* where, production of flowers, and fruits were significantly reduced. In addition, fresh and dry weights of fruits decreased with increasing salinity, but salinity did not affect fruit length and diameter. Present study provides basic information related to plant growth, fruit yield and some biochemical attributes, which suggest that *C. carandas* is moderately salt tolerant plant. This plant showed potential to grow on saline marginal lands using brackish water irrigation and provide biomass for edible and medicinal purposes. However, in-depth analysis of field and greenhouse experiments could be helpful to understand the detailed eco-physiological responses and mechanisms of salinity tolerance of this species.

Key words: Marginal lands, Salt tolerance, Non-conventional plants, Fruit crops.

Introduction

Rapid increase in population growth and competition between urban expansion and agricultural land utilization are major factors creating huge gap between demand and supply of food crops. In addition, soil salinity and shortage of good quality irrigation water are threatening the conventional agricultural productivity by limiting plant growth and survival (Sobhanian *et al.,* 2010). Agricultural production has already decreased around 35% and it is expected to decrease more with alarming pace (Anon., 2008). Poor irrigation practices and drainage miss-managements are normally the main causes of arable land losses. These malpractices upsurge underground water table and due to high evapotranspiration, salt deposition increased at the soil surface, leading to soil degradation (Munns &Tester, 2008; Tayyab *et al.,* 2015). In such soils, growing conventional crops and getting economically feasible yields is difficult. These threats are creating food crises which are directly related to human survival, especially in third world countries. Utilization of non-conventional plants, which are adapted to counter harmful effects of soil salinity, as alternate sources of food, fodder, medicine and other industrial raw material, is one of the well-established solutions (Qasim *et al.,* 2010, 2014; Abideen *et al.,* 2012; Abideen *et al.,* 2015b). For instance, cultivation of salt resistant plants on marginal lands with saline water irrigation is attracting the attention of progressive farmers. These plants include salt bushes (Atriplex species), Kallargrass (*Leptochloa fusca*), forage wheatgrass (*Agropyron cristatum*), Barley (*Hordium vulgare*), Oat (*Avena sativa*), and Janter (*Sesbania sesban*) among other fodder or cereal crops. Additionally, some fruit plants including Falsa (*Grewia asiatica*), Chicko (*Manilkara zapota*), Jujube (*Ziziphus mauritania*) and Karonda (*Carissa carandas*) could also be cultivated using saline resources (Qureshi & Barrett-Lennard, 1998; Maas & Grattan, 1999).

Soil salinity disrupts osmotic, ionic and nutritional balance of plants, eventually leading to death of sensitive species, due to insufficient tolerance mechanisms (Munns &Tester, 2008). Salinity adversely affects growth and development of these plants at various life cycle events, from seed germination to yield production, which ultimately challenge their survival in saline substrates (Munns, 2002). Individual and synergistic effects of limited water uptake and ion imbalance, hindered vegetative (height, cover, biomass) and reproductive (number and weight of flowers and fruits) performance of plant (Gupta & Huang, 2014). In saline substrates, plant accumulates inorganic ions (Na^+ and K^+) and organic osmolytes (sugars, proteins, and proline), for maintaining osmotic balance. To achieve this, discrimination between the uptake of Na^+ and K^+ is essential for plant survival (Benlloch *et al.,* 1994) because Na^+ is toxic for most of the sensitive plants. However, some tolerant species effectively use Na^+ in osmotic adjustments (Bell & Leary, 2003) while majority accumulates high amount of compatible solutes. Many studies clearly indicated that ion toxicity inhibit photosynthesis by altering chlorophyll and carotenoid contents (Azeem & Ahmad, 2011). Salinity also brings about changes in other biochemical processes related to flower, fruit and seed production and supress overall yield (Taffouo *et al.,* 2010).

Carissa carandas L. (Syn. *Carissa congesta* Wight) commonly known as Karonda or 'Christ thorn' is a large, evergreen shrub belongs to family Apocynaceae. It's a dichotomously branched, short stem plant, bearing fruits. It is widely distribution in South East Asia. Fruits of *C. carandas* contained high amount of pectin, vitamin A, C and anthocyanins, hence used in prickle, jelly, jam, squash, syrup and in sauces production (Lindsey *et al.,* 2000). These fruits are also the source of essential minerals i.e. iron, calcium, and phosphorous (Singh & Uppal, 2015). The plant is used in traditional medicines to treat diarrhea, dysentery, skin infections, parasitic worms, inflammation of joints/muscles, and brain and heart disease. Several biological activities (anti-inflammatory, antipyretic, analgesic, hepatoprotective and antiviral) are also reported from different parts of *C. carandas* (Singh & Uppal, 2015). Chemical analysis showed the presence of sesquiterpene glucoside, triterpenoid, tannins, isomers of urosolic acid, carissic acid, lupeol and β-sitosterol (Tyagi *et al.,* 1999).

Besides its high nutritional and medicinal value, *C. carandas* has the ability to grow in saline and water deficit conditions and could provide possible commercial benefits from theoretically unproductive soils. Such properties making this plant a good candidate to bring vast area of degraded/marginal lands under cultivation, particularly in arid/semiarid region. Literature is available on several medicinal, pharmacological and chemical properties, however, reports on salinity tolerance and yield potential of this species under saline conditions are scanty. Therefore, present work was carried out to study the effect of sea salt on vegetative and reproductive growth, ion content, and some biochemical parameters of *C. carandas* using drum pot culture.

Materials and methods

Experimental setup: About six month's old *C. carandas* saplings of almost equal height and volume were purchased from local market and transplanted in drum pots/lysimeters (Ahmad & Abdullah, 1982). Pots filled with sandy loam (300 kg) containing cow dung manure (9:1 soil: manure) were capable of retaining around 45 litre water (at field capacity). Each drum pot was irrigated (weekly in summer and fortnightly in winter) with 20 litre either tap water (EC_{iw}= 0.6 $dS.m^{-1}$) or saline water of 0.6% (10 $dS.m^{-1}$) and 0.8% sea salt (13 $dS.m^{-1}$). The plants were established for two weeks and later salinity treatment started gradually by dissolving sea salt in irrigation water till final concentration reached. Three replicates were maintained for each treatment. Urea, DAP and KNO3 (3:1:2) along with Osmocot granules (50g; Scotts-Sierra Horticulture Products) and Mericle-Gro (50g; Scotts Miracle-Gro Products, Inc.) were mixed in irrigation water as nutrient source at six monthly intervals. Experiment was conducted for a period of 30 months (June, 2009 to December, 2011) during which environmental data (Fig. 1) including average humidity (%), high and low temperatures (^{o}C), wind velocity (kmph) and rainfall (cm) was recorded (Pakistan Metrological Department, Karachi).

Vegetative and reproductive growth: Vegetative growth in terms of plant height and canopy volume was recorded initially (prior to salinity treatment) and then after every six month interval till 30 months. Reproductive growth was observed on the basis of number of flowers and number and weight (fresh and dry) of fruits per plant. Length and diameter of ten randomly selected fruits were also recorded.

Biochemical analysis: Biochemical parameters including chlorophylls (Arnon, 1949), carotenoids (Duxbury & Yentsch, 1956), soluble and insoluble sugars (Yemm & Willis, 1954), soluble proteins (Bradford, 1976), and total phenols (Singleton & Rossi, 1965) were determined on leave samples collected at grand period of growth (onset of flowers).

Mineral composition: Oven dried ground leaves (1g) were ashed at 550ºC for 6 hours and dissolved in 5 ml of 2N HCl (Chapman & Pratt, 1961). Diluted and filtered extracts were used for Na^+ and K^+ estimation using flame photometer (Petracourt PFP I). Concentration of these ions was calculated against the respective standard curves using following equations.

Na^+ (ppm) = 0.016135.x1.879824
K^+ (ppm) = 0.244346.x1.314603

Statistical analysis: Values are expressed as means (± standard error) of minimum 5 replicates using completely randomised block design. Analyses of variance (ANOVA) and post-hoc LSD test were used to compare individual means. SPSS (version 16) and Sigma Plot (version 11) were used for all statistical analyses and graph preparation, respectively.

Results

The vegetative growth (height and canopy volume) of *C. carandas*, growing under varying sea salt concentrations, for a period of 30 months, is presented in figure 2. A significant increase ($p<0.001$) in plant height and canopy volume was observed with time, which decreased significantly ($p<0.001$) with saline treatments.

Leaf carotenoid content and a/b ratio were decreased significantly ($p<0.001$) with increasing salinity (Fig. 3). However, at 0.6% sea salt, chlorophyll 'b' content remain unchanged which showed that the decrease in total chlorophyll was due to reduction in chlorophyll 'a'. Carotenoid content increased at 0.6% sea salt, while it decreased ($p<0.01$) with further increase in salinity. Carotenoid/chlorophyll ratio was increased throughout the experiment and it was highest at 0.8% salinity (Fig. 3).

Soluble proteins and sugars (soluble and in-soluble) of *C. carandas* leaves followed a similar trend as of leaf pigments, which were unaffected at 0.6% sea salt and decreased at high salinity, as compare to control (Fig. 4). On contrary, leaf phenolic content showed a gradual increase with increasing salinity (Fig. 4).

Sodium content of *C. carandas* increased significantly ($p<0.001$) whereas, potassium content and K^+/Na^+ ratio decreased ($p<0.001$) with increasing salinity (Fig. 5).

Reproductive growth in terms of number of flowers, flower shedding percentage and length, diameter and weight (fresh and dry) of *C. carandas* fruits, under varying salinities, is presented in figure 6. Salinity adversely affected ($p<0.001$) the number of flowers and fruits production in *C. carandas*, although, no significant difference was observed in initiation of flowers at control and salt treated plants. However, flower shedding increased proportionally with increasing salinity. Interestingly, salinity did not affect fruit length and diameter, but, their fresh and dry weights were decreased ($p<0.01$) with increasing salinity.

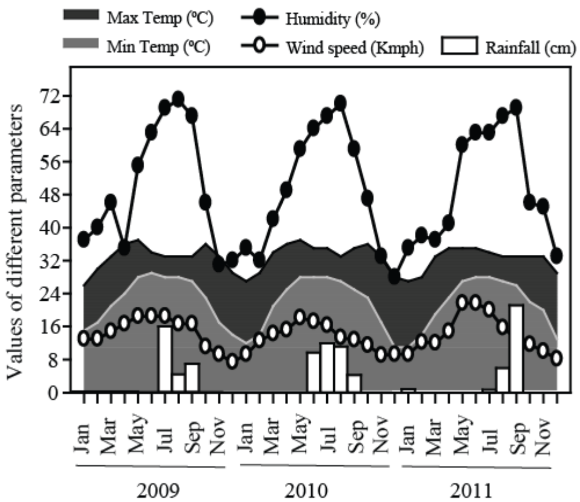

Fig. 1. Environmental data of the study area during experimental period (Pakistan meteorological department).

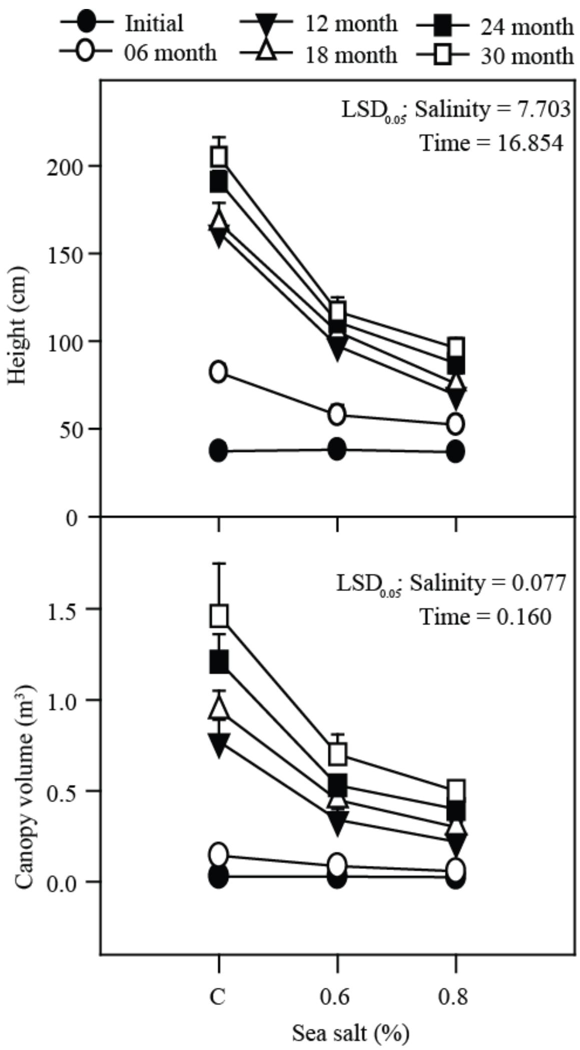

Fig. 2. Vegetative growth in terms of height and canopy volume of *C. carandas* growing under different saline treatments for 30 months (June, 2009 to December, 2011).

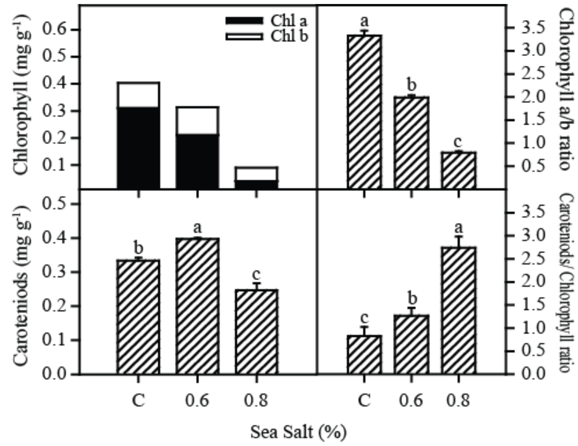

Fig. 3. Chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a / b ratio , carotenoids contents and chlorophyll / carotenoids ratio of *C. carandas* growing under salinities created by irrigation of different dilutions of sea salt. Bars represent means ± standard error of each treatment and significance among the treatments was recorded at $p<0.05$.

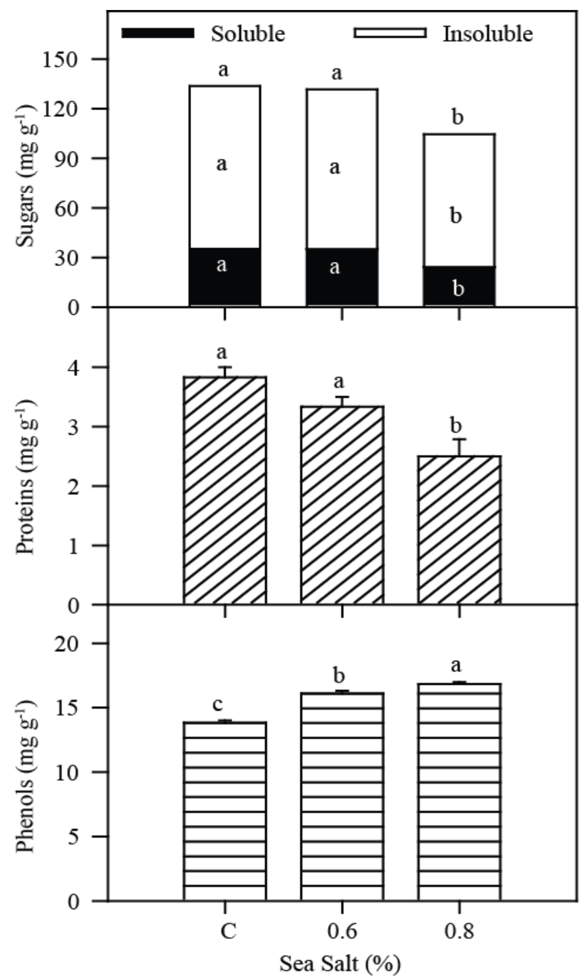

Fig. 4. Total protein, sugars and phenolic contents of *C. carandas* growing under salinities created by irrigation of different dilutions of sea salt. Bars represent means ± standard error of each treatment and significance among the treatments was recorded at $p<0.05$.

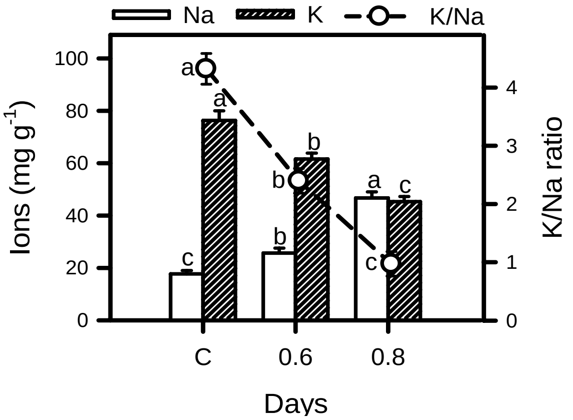

Fig. 5. Mineral analysis including Na and K ions was done on leaves of *C. carandas* growing under salinities created by irrigation of different dilutions of sea salt. Bars represent means ± standard error of each treatment and significance among the treatments was recorded at p<0.05).

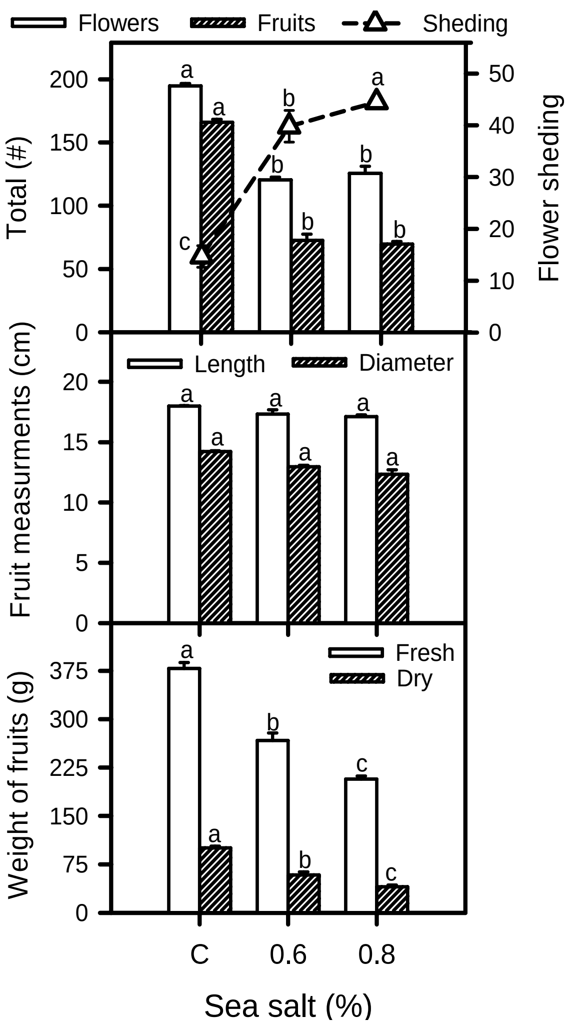

Fig. 6. Reproductive growth in terms of flowers and fruits numbers, flower shedding, length, diameter, fresh and dry weight of fruits of *C. carandas* growing under different salinity treatments. Bars represent means ± standard error of each treatment and significance among the treatments was recorded at p<0.05).

Discussion

This study explains the salt resistance ability of *Carissa carandas* and provides baseline information regarding its growth and yield potential, under different salinity regimes. Studies explain that growth and yield production of crop plants is decreased under salinity (Tayyab *et al.,* 2015), likewise, in this study, vegetative and reproductive growth and some biochemical parameters of *C. carandas* were decreased under salinity. The salt induced growth reduction is mainly accounted for combined effect of osmotic imbalance and ionic toxicities (Silvera *et al.,* 2001). Excessive salt in growth medium hindered the absorption of other essential minerals resulting ion imbalance and due to increase uptake of toxic ions (Na^+ and Cl^-), physiological, biochemical and other metabolic plant processes are severely compromised, which leads to overall growth reduction (Del Amor *et al.,* 2001). High concentration of cytosolic ions (Na^+ and Cl^-) disrupts the compartmentalization capacity of vacuole, which hampered other cellular processes related to cell division, cell elongation, and multiplication (Munns *et al.,* 2006). Our results are parallel with some other studies in which significant growth inhibition of peas, chickpea, and faba beans was reported against salt stress (Delgado *et al.,* 1994). Simpson *et al.* (2014) observed that height, growth rate and leaf area of grafted and un-grafted citrus trees decreased with increasing salinity. Felker *et al.* (1986) reported adverse effect of salinity on stem elongation and biomass production of six species of Prosopis. Chartzoulakis *et al.* (2002) grew six olive cultivars on different NaCl concentrations and found linear reduction in shoot length, leaf area and dry biomass with increasing salinity. Similar results are also reported in Sesbania (Mahmood *et al.,* 2008), Canola (Humaira & Ahmad, 2004), Tomato (Azeem & Ahmad, 2011) and Cotton (Meloni *et al.,* 2003).

In this study, total chlorophyll and chlorophyll *a*/*b* ratio of *C. carandas* decreased with salinity and this reduction was more pronounced at 0.8% sea salt. Growth of *C. carandas* also corresponded to chlorophyll content. Adverse effects of salinity on leaf pigments was presumably due to the destruction of enzymes responsible for chlorophyll synthesis (Ashraf & Harris, 2013) and/or increased chlorophyllase activity (Sudhakar *et al.,* 1997). Thus, insipid leaf is considered a visible indicator of chlorophyll damage, which is correlated with quantified values, as reported in alfalfa (Al-Khanjari *et al.,* 2002). It was observed that, chlorophyll *a* of *C. carandas* was more sensitive than chlorophyll *b*, especially under low salinity. At this level, it can be assumed that chlorophyll *b* may be converted into chlorophyll *a* and the decrease in total chlorophyll was mainly attributed to chlorophyll *a* destruction (Eckardt, 2009). Decline in chlorophyll contents across different salinity regimes was also reported in cotton (Ahmed & Abdullah, 1979), pea (Hernandez *et al.,* 1999), *Vicia faba* (Gadallah, 1999), mulberry genotype (Agastian *et al.,* 2000), chickpea (Garg, 2004) and in mangrove (*Bruguiera parviflora*; Parida *et al.,* 2004). It is also noteworthy that plants growing in saline mediums are under high risk of chlorophyll mediated reactive oxygen species (ROS) production and the proteins of photosystem are highly susceptible to oxidative damage. Hence, reducing the amount of chlorophyll and improving the photo-protective measures (carotenoids, carotenoids/ chlorophyll ratio and phenolic compounds), would be a strategy to minimize the chance of ROS mediated photo-damage (Koyro *et al.,* 2013). On the other hand, it limits the carbon assimilation capacity, which in turn reduces overall plant growth, as reported earlier (Moradi & Ismail, 2007; Koyro *et al.,* 2013). Increased carotenoids content of *C. carandas* leaves at 0.6% sea salt and a linear increase in carotenoids/chlorophyll ratio in both salinities indicate the role of carotenoids to protect the over-reduction of photosynthetic reaction centres. It decreases the light absorption and help to dissipate excessive energy of leaves that otherwise, use to generate ROS (Christian, 2005). In this context, detailed study related to gas exchange, chlorophyll florescence and antioxidative parameters of *C. Carandas* would be required to elaborate photosynthetic responses of this plant.

Leaf sugars and protein contents of *C. carandas* were unchanged at 0.6% sea salt but decreased at higher salinity. These results are parallel with other reports where synthesis of sugars and availability of soluble protein was linked with ionic burden (Azeem & Ahmad, 2011). The synthesis of organic osmolytes requires high amount of energy, therefore, some plants rather reduce the transport of photosynthetic product than investing in osmolyte synthesis (Ashraf & Foolad, 2007). Their major function includes osmotic adjustment, osmoprotection, radical scavenging, and carbon storage (Parida & Das, 2005). Changes in soluble sugars content and translocation of sucrose, and other simpler forms of sugars has been reported in a number of salt stress plants (Kerepesi & Galiba, 2000). In *Vicia faba* salinity decreased soluble and hydrolyzable sugars (Gadallah, 1999). Sugar content increased in some genotypes of rice but also decreased in some genotypes (Alamgir & Ali, 1999). Besides their role in osmotic adjustments, sugars may also work as signalling molecules and feedback inhibitors of several metabolic process, related to plant's salinity tolerance (Kerepesi & Galiba, 2000). On the other hand, salt induced protein reduction is mainly attributed to inhibition of protein synthesis and/or protein hydrolysis (Ahmad *et al.,* 2009). Deleterious effects of salinity on protein content were also reported in ginger (Ahmad *et al.,* 2009), sorghum (Ali *et al.,* 2013a, 2013b; Sahito *et al*., 2013), and tomato (Azeem & Ahmad, 2011).

A linear increase in phenolic content of *C. carandas*, with increasing salinity was also demonstrated previously in other species like *Achillea collina* (Giorgi *et al*., 2009), *Lactuca sativa* (Kim *et al.*, 2008) and *Bruguiera parviflora* (Parida *et al.,* 2004). Synthesis and accumulation of plant phenolics is generally enhanced under biotic/abiotic stresses (Qasim *et al.,* 2010). These compounds take part in the neutralization of harmful free radicals, which are excessively produced when photosynthetic metabolism is impaired by salt stress (Giorgi *et al.*, 2009). Phenolic compounds act as free radical scavengers, reducing agents, and elicitors of the anti-oxidative defence through hydrogen donation and/or electrons delocalization abilities, thus preventing oxidative damages (Zhou & Yu, 2004; Abideen *et al*., 2015a). In addition, higher accumulation of polyphenols in leaves also suggest their fundamental role to protect photosynthetic machinery from heat dissipation, providing shield against UV or high irradiance and stimulates antioxidant enzymes (Tattini *et al.,* 2005). These compounds are also involved in signalling of several metabolic processes and their role become more crucial when plant under goes salt stress (Parida & Das, 2005).

One of the major causes of growth reduction in salt stressed plants is the specific ion toxicity (mainly Na^+ and Cl^-). The linear increase in Na^+ content and decreased values of K^+ and K^+/Na^+ correlated well with decreased growth of *C. carandas* under saline conditions. Plants, either glycophytes or halophytes, cannot tolerate high accumulation and mobility of toxic ions. In order to survive, toxic ions must be compartmentalized in the vacuole and/or transported to older tissues that are eventually sacrificed (Cheeseman, 1988). High amount of Na^+ hindered the vital metabolic functions by interfering the absorption and transport of essential minerals (like K^+), leading to nutrient deficiency (Guo *et al.,* 2012). Therefore, maintaining high concentrations of K^+ and low concentrations of Na^+ is crucial to avoid ion toxicity (Shabala, 2009). The K^+/Na^+ ratio considered as an important parameter in salt stress studies and maintaining K^+/Na^+ value greater than 1, is needed for cellular integrity to prevent salt induced plant death (Silva *et al.,* 2012). In this experiment, *C. carandas* seemed to maintain this ratio (above 1), which was disturbed at higher salinity. This disturbance would be attributed to growth inhibition as K^+ is involved in alleviating the damaging effects of salinity to plant metabolism. Similar relationship between Na^+ and K^+ concentration was also reported in guava (Ferreira *et al.,* (2001), *Vicia taba* (Gadallah, 1999) and *Casurina equsetifolia* (Dutt *et al.,* 1991).

The reproductive growth of *C. carandas* in terms of number of flowers and fruits was decreased and percent flower shedding was increased with increasing salinity. The weight of fruits (fresh and dry) and total fruits per plant were also decreased with increasing salinity, however, length and diameter per fruit were unchanged. Garcia-Sanchez *et al.* (2003) also reported the decrease in fruit number in citrus trees, without significant change in individual fruit weight, under salinity. Salinity adversely affect different reproductive phases of plants i.e. flowering, fertilization, fruit setting, yield and seeds quality (Shannon *et al.,* 1994). Garcia-Sanchez *et al.* (2003) suggest that, salinity induced impairment of protein, and carbohydrate metabolism along with nutrient imbalance could lead to biochemical disturbances which may impair yield and/or fruit quality. Howie and Lloyd (1989) found decreased flowering intensity, fruit setting and number of fruits of *Citrus senensis,* with medium salinity.

Conclusions

It appears that *Carissa carandas* is able to tolerate moderate sea salt salinity, however, its growth and yield is reduced. This inhibition was due to lower photosynthetic ability in terms of decreased chlorophyll content which could be attributed to increasing Na^+ toxicity. In addition, high Na^+ also disturbed the availability of K^+ which also adversely affects soluble sugars and proteins contents, particularly at higher salinity. On the other hand, plant increased soluble phenols and carotenoid/chlorophyll ratio to minimize photo-damage. Production of flowers and fruits were also declined under salinity. Yield decline was due to reduction in number of fruits rather fruit length and diameter. *Carissa carandas* showed potential to grow on vast degraded lands with sea water of permissible concentration to produce edible fruits and active biomass of medicinal value at local and commercial scale. However, further research is needed to convert scientific knowledge into practically feasible outcome, which would help to improve socio-economic condition of poor farmers from theoretically unproductive soils.

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