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Gas exchange and photochemical efficiency of transplanted seedlings of *Jatropha curcas*. L: accessions under salinity

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Abstract

Jatropha (*Jatropha curcas* L.) has been used as a biofuel source for a long time. However, Jatropha photosynthesis and photochemical reaction investigations have gotten a lot less attention, especially regarding salinity. As a result of the injury caused to the leaf photosynthetic machinery, salt stress is a severe environmental constraint for its establishment. The study is aimed at evaluating the impact of salinity (100 mM and 15 mM NaCl) on gas exchange parameters such as net photosynthetic rate (Pn), transpiration rate (E) and, stomatal conductance (Gs), and maximal photochemical efficiency of PSII (Fv/Fm) and quantum efficiency of PSII (ϕ_{PSII}) of Jatropha. Six accessions *viz.*, A6002, A6004, A6007, A6008, A6011, and A6014 of Jatropha transplanted seedlings were subjected to 100 mM and 15 mM NaCl for 360 days. A6002, A6004, A6007, and A6008 accessions were sensitive to salinity as growth (in terms of height, stem girth, leaf area, and dry weight of shoot and root) gas exchange measurements, SPAD chlorophyll, Fv/Fm and ϕ_{PSII} were significantly decreased at 150 mM NaCl at 360 DAT. However, when exposed to 150 mM NaCl at 360 DAT, the accessions A6011 and A6014 showed no significant decline in Pn, Gs, E, Fv/Fm, and ϕPSII, indicating that PSII function was preserved by suppressing photo-oxidative damage and thereby main taining carboxylation efficiency. As a result, we can conclude that A6011 and A6014 accessions were more resistant to NaCl stress, whereas A6002, A6004, A6007, and A6008 accessions were more sensitive.

Keywords: stress study, salinity, jatropha, gas exchange, photochemical

Introduction

Jatropha curcas L. is a perennial shrub native to tropical America that belongs to the Euphorbiaceae family. Jatropha seeds contain between 28% and 38% oil (Fukuzawa *et al*., 2012) [5], which can be converted into high-quality biodiesel by esterification with methanol; as a result, there is growing interest in Jatropha seeds for biodiesel production [1]. Jatropha genus has a remarkable combination of valuable traits with high adaptability to various environmental conditions (semi-arid climates) and soils (marginal soils). The capacity for heavy metal bioremediation, ease of propagation by grafting or seed, widespread natural ranges, and expansive growth make it a unique plant. Jatropha has recently attracted attention for their alternative source of sustainable energy, i.e., biodiesel production [2, 3]. It is now being implemented successfully in some tropical and subtropical areas of Africa and Asia [4]. Jatropha originals from Mexico had already been planted on an estimated 900,000 ha worldwide by 2008 [5].

Significant progress has been made for Jatropha in bioremediation or phytoremediation and bioenergy applications [6, 7]. The performance of Jatropha species under different abiotic stresses such as nutrient deficiency flooding, drought, and chilling [8-16] little is known of its growth and photosynthetic responses under salinity. Soil salinity is defined as an excess of dissolved inorganic salts or total soluble salts in soil that prevents adequate plant growth [17]. Most crops do not grow well in saline soils. Salinity is a major abiotic stress that limits crop productivity on millions of hectares of land worldwide, costing US$11 billion per year [18]. Furthermore, the salt-affected soil grows each year due to natural salinization and increased irrigation, particularly in arid and semi-arid regions [19]. Beyond the threshold levels, sodium chloride (NaCl) in the soil causes ionic imbalance and disrupts ion homeostasis in plant cells. Subsequently, it creates specific ionic toxicity, affects the distribution and supply of essential mineral nutrients such as K^+, $CA6002^{+,}$ and Mn^{2+}, and lowers root osmotic potential, all of which affect the normal physiology of plant cells and hamper plant growth [20, 21]. High concentrations of Na^+ and Cl^- ions in leaves affect the stomatal and non-stomatal components and decrease the assimilation of photosynthetic CO_2 [22-24]. This work investigates the effect of different concentrations of NaCl on photosynthetic responses (photosynthesis, transpiration, leaf conductance), photochemical efficiency, and growth of Jatropha.

Material and Methods

Plant material

The six *J. curcas* accessions *viz*., A6002, A6004, A6008, A6007 A6011 and A6014 were selected randomly from ICRISAT Jatropha collections (ICJC) and National Bureau of Plant Genetic Resources (NBPGR) germ-plasm collections. Three months before the start of the experiment, healthy, large, and uniform seeds of *J. curcas* accessions were selected. One seed of each accession was sown at 2-4 cm depth in polybags filled with free-draining growing media containing organic matter (1:1:1 sand-soil-manure).

The seeds were well watered till it attained the height of 30-40 cm (within 6-15 days)

Growth, experimental design, and salt treatments

Seedlings of *J. curcas* accessions were transplanted at the three-leaf stage into pots filled with 15 kg of soil mixture consisting of a 1:2 ratio of sand and red soil (18 cm height ×16 cm diameter). These pots were then placed in a shaded net for initial management and were watered as needed. Thinning was done during the third month after sowing, and only one healthy plant was kept. Plants were subjected to different levels of salinity treatment after three months of acclimation. NaCl induced salinity stress and salt concentrations were stepped up in 25 mM per day increments until final concentrations (100 mM and 150 mM NaCl) were achieved to avoid sudden osmatic shock. All plant was irrigated daily, according to the treatment, with 800 mL of saline solution (enough volume to occur leaching), in the early hours of the morning (06:00-07:00 h). The pots were sealed at the bottom to prevent leaching and to minimize soil evaporation covered with cartons wrapped with aluminum folio. A few pots were weighed periodically to note the moisture loss, and soft water was added to make up for the deficiency. During the experiment, daily water loss was measured at 9 a.m. by recording the weight of pots and tap water was supplied to 80% of water holding capacity. Care was taken not to water excessively to prevent waterlogging. Weeds were periodically hand removed, and occasionally, 2gm L^{-1}Bordeaux mixture was sprayed after spotting powdery mildew on leaves and stems. This study was carried out in the glasshouse of the Botany Department, Osmania University, Hyderabad, with a day/night temperature of 28/24 ℃ and relative humidity between 70%-80% and 800 to 1000 μmol m^{-2} s^{-1} the maximum photosynthetic photon flux. The experimental design was a randomized complete block in a split-plot layout with three replications. The experiments were repeated three times with similar results each time.

Data collection

Non-destructive growth measurements were taken after 6 (180 DAT) and 12 (360 DAT) months of treatment. Plant height, stem diameter, shoot dry weight leaf area, and SPAD chlorophyll measurements were taken for each pot. At the end of the experiment, plants were harvested and separated into shoots and roots. Shoot and root dry weights were measured.

Gas exchange measurements

In both experiments, leaf gas exchange parameters were measured on young fully expanded leaves from the upper crown of plants. Gas exchange and chlorophyll fluorescence were measured in the same leaf. Gas exchange parameters such as net photosynthetic rate (Pn), transpiration rate (E), and stomatal conductance (Gs) were measured with an LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE, USA). These measurements were carried out on the middle part of the youngest (fully opened second leaf), which avoided the leaf vein. The measurements were conducted from 8:30 to 10 a.m., during this time greenhouse curtain was shut down to prevent the effects of different light conditions. The saturating photosynthetic photon flux density was between 1000 and 1500 µmol m^{-2} s^{-1} in the leaf chamber during the measurement periods. The temperature, CO_2 concentration, and relative humidity inside the leaf cuvette were always close to ambient air values.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were determined using a PAM-2500 chlorophyll fluorescence analyzer (WALZ, Germany) between 8:30 and 10 a.m. After a 20 min dark adaptation period, the maximal photochemical efficiency of PSII (Fv/Fm) and quantum efficiency of PSII (ϕ_{PSII}) were determined. The cuvette of the gas exchange system was modified to accept the fiber optic of the fluorimeter at a 60° angle without significantly interfering with PPFD distribution at the leaf surface. Minimal fluorescence (F_0) was measured under a weak pulse of modulating light over a 0.8 s period, and a saturating pulse of light-induced maximal fluorescence (Fm) (5000 µmol $m^{-2}s^{-1}$) applied over 0.8 s. The maximal quantum efficiency of PSII was determined as F_v/F_m, where F_v is the difference between F_0 and F_m. An actinic light source (600 µmol m^{-2} s^{-1}) was then applied to achieve steady-state photosynthesis and to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.7 s to obtain F'_m (light-adapted maximum fluorescence). FMS-2 calculated the fluorescence parameters based on the dark-adapted and light-adapted fluorescence measurements. The quantum efficiency of PSII (ϕ_{PSII}) was calculated as $(F'_m–F_s)/F'_m$.

Chlorophyll content

Chlorophyll content was estimated by SPAD (Soil Plant Analysis Development). Chlorophyll meter (SPAD-502, Minolta) reading is a unitless value that calculates the amount of chlorophyll in the leaves by measuring the intensity of green color. It corresponds to the relative amount of chlorophyll content in the leaves by estimating the variation between light disintegration at 430 nm. Chlorophyll a and b have a peak wavelength at 750 nm near Infrared without transmittance. SPAD chlorophyll reading was taken on the fully expanded secondary leaf from the intermodal position on the main stem.

Growth attributes

Stem girth at 5 cm from soil: This was carried out using a 30 cm ruler and a string.

Plant height: Observations were recorded using a ruler; if the plant was more than 30 cm, it was measured by marking the plant at the end of 30 cm.

Leaf area: Leaf area was recorded with an LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA).

Shoot and root dry weight: After harvesting, shoots and roots were placed in an oven at 60 ℃ for 72 hours to a constant weight, and weights were recorded.

Statistical analysis

The results presented are the mean values of 5 replicates. Pots were arranged in a completely randomized block design with five replicates (pot) for each treatment. The data analyses were carried out using one-way analysis of variance (ANOVA) followed by Post Hoc Test (Multiple Comparisons) using SPSS (SPSS Inc., Chicago, IL, USA). The differences were considered significant if p was ≤ 0.05. The mean values were compared, and lower case letters were used in figures/tables to highlight the significant differences between the treatments.

Results

Gas exchange parameters

Net photosynthesis (Pn)

When exposed to 100 mM and 150 mM NaCl, all J. curcas accessions propagated by transplanted seedlings showed decreased Pn. At 180 DAT, the average Pn rate in A6002, A6007, and A6008 accessions declined significantly with increasing salt treatments compared to their respective controls. At 360 DAT, the stress was greater than at 180 DAT. By 180 DAT, the A6011 and A6014 accessions

showed no significant decline compared to their controls with salt treatments, but at 360 DAT, the drop was considerable. The Pn rate was found to differ considerably between accessions. The negative effect of salinity on Pn at 150 mM was more negligible in A6011 (%) and A6014 (%) accessions, whereas substantial (P<0.05) in A6002, A6007, and A6008 accessions. But the A6004 accession was marginally affected (Fig.1 A&B).

Stomatal conductance (Gs)

The average Gs decreased with increasing salinity concentrations in all accessions. At 360 DAT, the Gs of A6002, A6007, A6008, and A6004 accessions decreased significantly (p<0.05) by 57, 49, 45, and 27%, respectively, when compared to their corresponding controls at the highest salinity treatment. Up to 15 mM NaCl treatment, no significant reduction in Gs was observed in the accessions. Following the 180 and 360 DAT, the accessions A6002, A6007, A6008, and A6004 had the most significant (p<0.05) reduction in Gs at 100- and 150-mM salinity. At 150 mM NaCl, the Gs of A6011 and A6014 accessions were relatively higher than the remaining accessions until 360 DAT (Fig.1 C&D).

Transpiration rate (E)

All accessions showed an increased transpiration rate (E) with increased salt concentrations. At the highest salinity treatment, the average E was 122, 132,119, 121, 91, 68 % (p <0.05) in A6002, A6007, A6008, A6004, A6011, and A6014 accessions, respectively. Surprisingly, salinity caused the greatest transpiration rate at 180 DAT rather than 360 DAT in all accessions. With increasing salinity treatments, there was a high degree of accession variation in the E rate of Jatropha. Under the highest salinity treatment, accessions A6002, A6007, A6004, and A6008 had significantly (p <0.05) higher E rates. The accessions A6004, A6011 and A6014 were not significantly suffered from enhanced E rate when challenged with 150 mM NaCl concentration (Fig.1 E&F).

SPAD chlorophyll

The chlorophyll content of all tested J. curcas accessions decreased as salt concentration increased. At 180 DAT, the average chlorophyll content was reduced by 18-44% at 100 mM salinity and 26-59% at 150 mM NaCl in A6002, A6004, A6007, and A6008 accessions. At 150mM NaCl treatment, the reduction was 35-69% (p<0.05) on average at 360 DAT. At 180 DAT, J. curcas accessions A6011 and A6014 had no statistically significant reduction in chlorophyll content up to 150 mM NaCl. At the highest salinity treatment, there was a significant increase in chlorophyll content by 26% (p=0.0178) and 36% (p=0.0281) under prolonged conditions, i.e., at 360 DAT in A6011 A6014 accessions at highest salinity treatment compared to their respective controls. Under salinity conditions, chlorophyll content was relatively higher (p<0.05) in A6011 and A6014 accessions. J. curcas accessions A6002, A6004, A6007, and A6008 had the greatest reduction in chlorophyll content when exposed to 150 mM NaCl DAT (Fig.1 G&H).

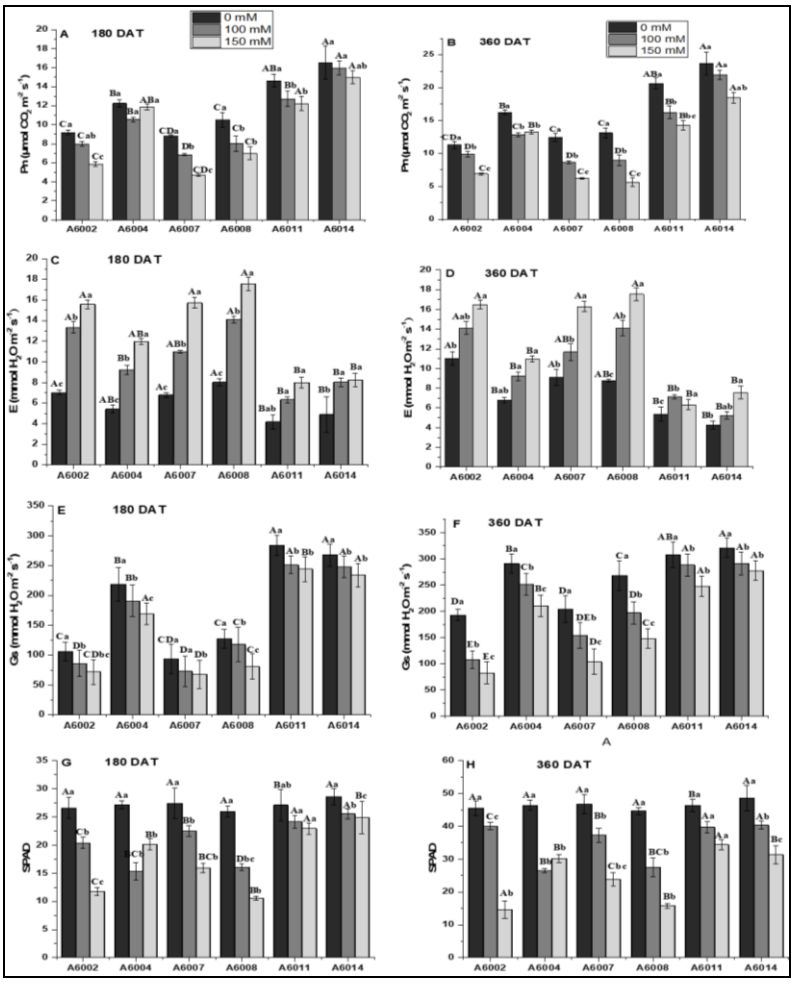

Fig 1: Net photosynthesis (Pn; A and B), stomatal conductance (gs; C and D) and transpiration (E; E and F) and SPAD chlorophyll (G and H) of six accession of transplanted seedlings of *Jatropha curcas* under 0, 100 and 150 mM NaCl at 180 DAT and 360 DAT. Vertical bars represents means ±SE (n = 5); Different letters on the top of bars denotes significant differences. The small letters and capital letters denote significant difference of salt treatments of within each accession and among accessions, respectively at p≤0.05 according to post hoc test.

Chlorophyll fluorescence

Maximal photochemical efficiency of photosystem II (Fv/Fm)

In the current study, at the highest salinity treatment, when compared to their corresponding controls, Fv/Fm of A6002, A6007, and A6008 accessions decreased significantly ($p<0.05$) at 180 DAT (25, 27, and 26%), and 360 DAT (23, 28, and 20%). However, even at 360 DAT, the accessions A6004, A6011, and A6014 had the most minor ($p> 0.05$) reduction in Fv/Fm under 150 mM NaCl treatment. Under control and salt treatments, the average value of Fv/Fm of Jatropha accessions differs significantly ($p<0.05$) (Fig.2 A&B). Accessions A6004, A6011, and A6014 were more resistant to Fv/Fm reduction with an average decrease of 3-11%.

Quantum efficiency of PSII (ΦPSII)

At 150 mM salinity treatment, accessions A6002, A6007, and A6008 had significantly reduced the quantum efficiency of PSII (ΦPSII) by 37, 24 and 33 % respectively over their controls at 180 DAT.

However, the salinity effect was relatively low at 360 DAT than at 180 DAT. The average of ΦPSII of Jatropha accessions significantly ($p <0.05$) differs under control and salt treatments. Accessions A6004, A6011 and A6014 were more resistant to ΦPSII reduction than A6002, A6007, and A6008, having an average decrease of 2-18% compared to that of A6002, A6007, and A6008, which displayed an average 20-37% reduction at highest salinity treatment (Fig.2 C&D).

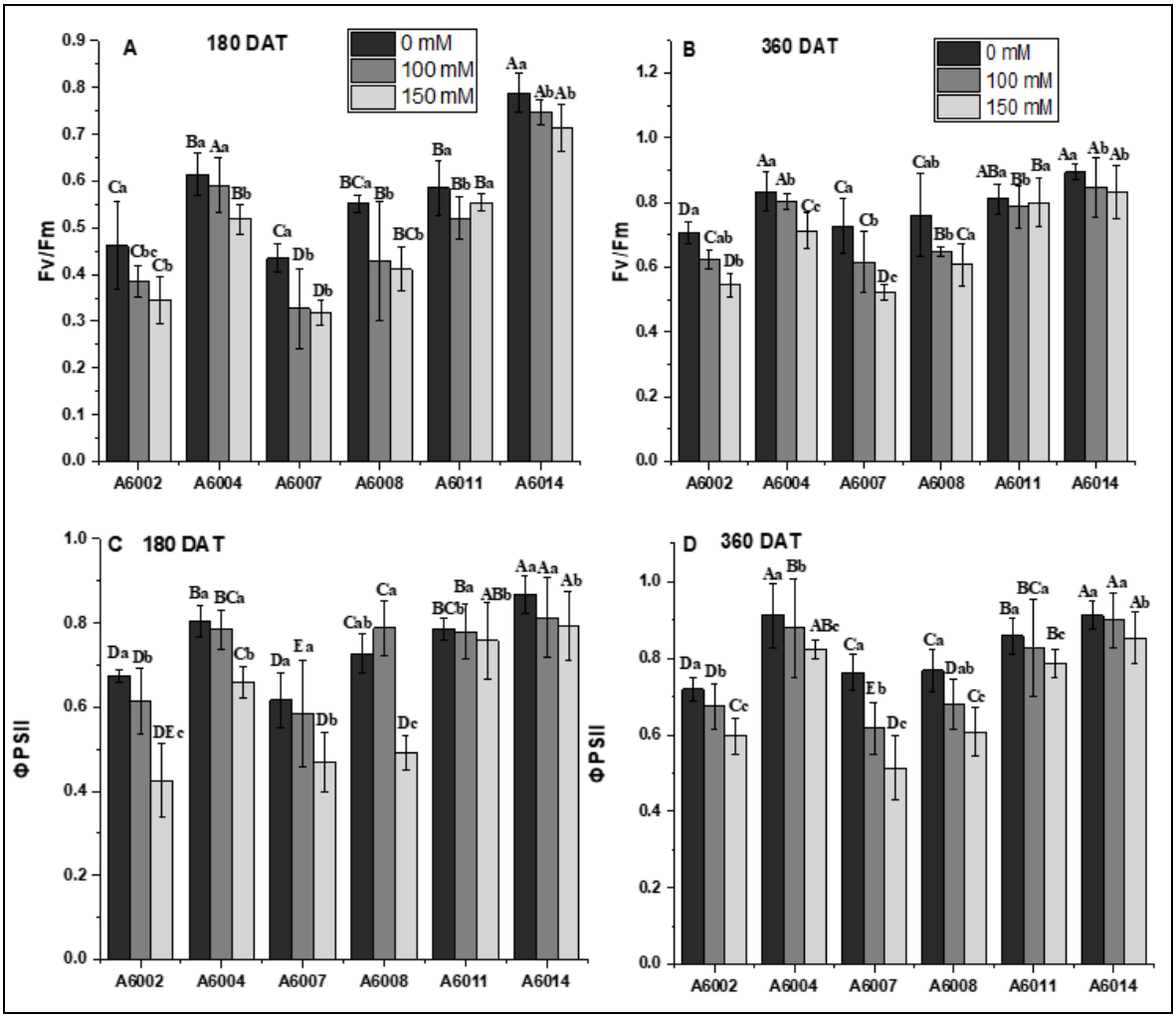

Fig 2: Maximal photochemical efficiency of PSII (Fv/Fm, A and B) and quantum efficiency of PSII (C and D, ΦPSII) of six accession of transplanted seedlings of *Jatropha curcas* under 0, 100 and 150 mM NaCl at 180 DAT and 360 DAT. Vertical bars represents means ±SE (n = 5); Different letters on the top of bars denotes significant differences. The small letters and capital letters denote significant difference of salt treatments of within each accession and among accessions, respectively at $p\leq0.05$ according to post hoc test.

Growth attributes

Plant height

Jatropha accessions A6011 and A6014 grew, on average, taller with greater stem diameters in all treatments over the 360-days growth trial than A6002, A6004, A6007, and A6008 accessions grown under similar conditions. Although A6011 and A6014 accessions did not significantly reduce height growth at 100 mM NaCl compared to control treatments, they appear to experience significant growth retardation at 150 mM NaCl at 180 DAT ($p < 0.05$). At 360 DAT, they didn't experience considerable growth retardation at 150 mM NaCl. The plant height of A6002, A6004, A6007, and A6008 accessions was significantly diminished considerably ($p<0.05$) by at 180 DAT (by 26, 12, 19, and 24 % respectively) and 360 DAT (by 21, 13, 27, and 18% respectively) at highest salinity treatment. A considerable degree of variation was observed in *J. curcas* accessions for plant height at the control and salinity treatments. The plant height of A6002, A6004, A6007, and

A6008 accessions was more susceptible to salinity stress than A6011 and A6014 accessions (Fig.3 A & B).

Stem girth

When compared to the control, 150 mM NaCl treatment made a significant ($p<0.05$) decrease in stem girth at 180 and 360 DAT (Fig.3 C&D) for all tested accessions. When exposed to 100 mM NaCl, A6002 accessions reduced stem girth by 18% at 180 DAT and A6002, A6004, A6007, and A6008 accessions reduced by 20, 25, 27, and 15%, respectively, at 360 DAT, when compared to the corresponding controls. In both periods, stem girth was less affected ($p<0.05$) by salt stress in A6011 and A6014 accessions than other *J. curcas* accessions A6002, A6004, A6007, and A6008. *J. curcas* accessions suffered significant reductions in both average plant height and stem girth at all salt treatments, but response varied in accessions.

Leaf area

All accessions accounted for the marginal loss in leaf area at 100 mM NaCl treatment at 180 DAT, except A6008 (by 20%; P=0.0410). At 150 mM treatment, A6002, A6004, A6007, and A6008 accessions showed substantial reductions in Leaf area at 180 DAT (by 49, 35, 28, and 33 percent, respectively) and 360 DAT (by 49, 35, 28, and 33 percent, respectively) (by 63, 38, 64, 39 percent respectively).

At 180 DAT, the A6011 and A6014 accessions leaf area remained unaffected, but at 360 DAT, it was reduced by 23% (p=0.0370) and 20% (p=0.0267), respectively, at the highest salinity treatment. Under the highest salinity treatment and control conditions, J. curcas A6011 and A6014 accessions had more leaf area than other accessions (Fig.3 E&F).

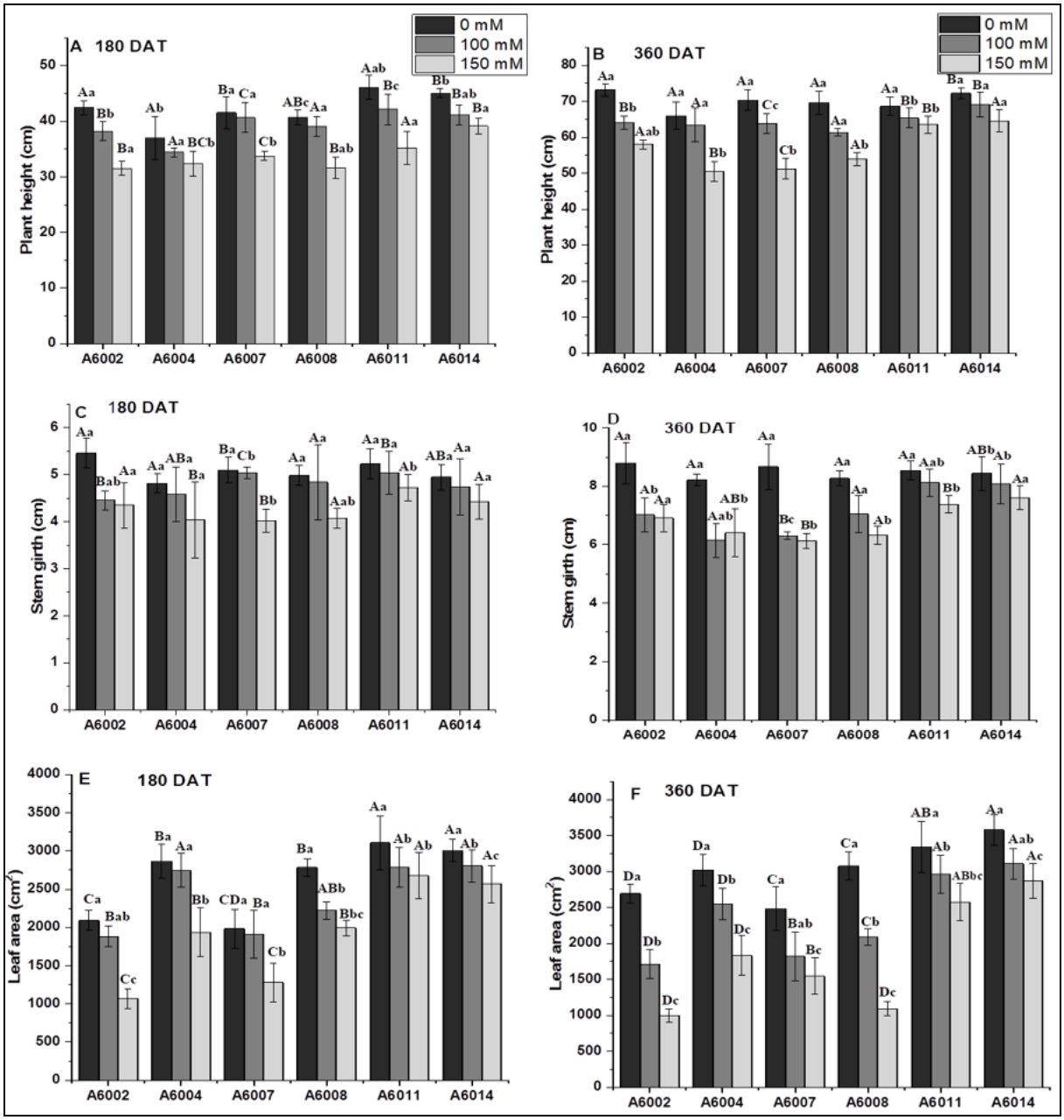

Fig 3: Plant height (A and B), stem girth (C and D) and Leaf area (E and F, LA) of six accession of transplanted seedlings of *Jatropha curcas* under 0, 100 and 150 mM NaCl at 180 DAT and 360 DAT. Vertical bars represents means ±SE (n = 5); Different letters on the top of bars denotes significant differences. The small letters and capital letters denote significant difference of salt treatments of within each accession and among accessions, respectively at $p\leq0.05$ according to post hoc test.

Shoot dry weight and root dry weight

With increasing salt treatments in all *J. curcas* accessions, dry weights of shoot and root diminished significantly, except A6014 (Fig.4 A&B). Average shoot dry weight was significantly ($p<0.05$) reduced by 19-56% at 100 mM salinity treatment and reached up to 33-67% at 150 mM NaCl application for A6002, A6004, A6007, A6008 and A6011 accessions (Fig.4 A). Similarly, A6002, A6004, A6007, A6008 and A6011 accessions root dry weight displayed an average significant reduction of 16-40 % ($p<0.05$) at 100 mM NaCl treatment and of 26 -51 % ($p<0.05$) at 150 mM NaCl treatment respectively in comparison with control. The dry mass of shoot and root *of* A6002, A6004, A6007, A6008, and A6011 accessions was more susceptible to salinity stress. Under control conditions, A6004, A6011, and A6014 are significantly ($p<0.05$) higher dry mass than others. *J. curcas* accessions suffered significant reductions in average dry mass of shoot and root at all salt treatments, but response varied in accessions.

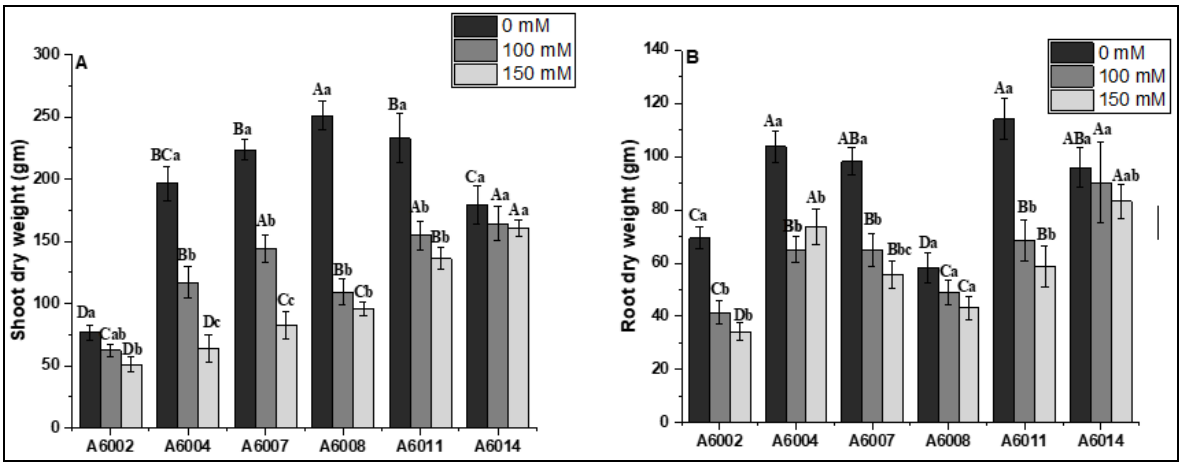

Fig 4: Shoot dry weight (A) and root dry weight (B) of six accession of transplanted seedlings of *Jatropha curcas* under 0, 100 and 150 mM NaCl at 360 DAT. Vertical bars represents means ±SE (n = 5); Different letters on the top of bars denotes significant differences. The small letters and capital letters denote significant difference of salt treatments of within each accession and among accessions, respectively at $p\leq0.05$ according to post hoc test.

Discussion

Photosynthesis is the most crucial process by which green plants convert solar energy to chemical energy in organic compounds synthesized by the fixation of atmospheric carbon dioxide. Our results showed that the gas exchange parameters decreased in *J. curcas* accessions propagated *via* stem cuttings subjected to 100 and 150 mM NaCl concentrations at both tested periods (Fig.1 A-F). Examining individual accessions, A6002, A6008, and A6007 are sensitive to salinity as they experienced the significant reduction of Pn, Gs, and E following the 360 DAT. While the A6004, A6011, and A6014 accessions are relatively tolerant to salt stress by displaying no considerable reduction of Pn, Gs, and E (Fig.1 A-F). In line with our results, Regni *et al* [25] found that *Olea europaea* subsp. europaea var. europaea cultivars exhibited the declined values of Pn, E, Ci and Gs treated with 100 and 200 mM NaCl, at 210 and 240 DAT. Similar reduction in gas exchange parameters were reported in cotton [26] (Soares *et al*., 2018), pongamia [27] (Marriboina *et al*., 2017) and Jatropa [28]. Recently, Zheng *et al* [29] observed the significant reduction of Pn, E, Ci, and Gs in Castor bean genotypes subjected to 100 and 200 mM NaCl treatment for 112 DAT. Freo and Zibo cultivars were more resistant than Wanneroo, Forrestdale, and Wycombe cultivars. The drop of photosynthesis rate (Pn) could be due to stomatal (gs) or other non-stomatal limitations. The stomatal limitation is attributed to the osmatic stress due to the accumulation of Na ions, consequently limiting the water absorption and reducing the intercellular CO_2 concentrations [21, 30]. Non-stomatal factors such as declined Rubisco, stromal fructose bis-phosphatase enzyme activities, ribulose-1,5-bisphosphate (RuBP) regeneration [18, 31-32], disturbance of the photosynthetic electron transport chain of PSII and PSI which are no longer able to accept excess excitation energy from light-harvesting chlorophyll protein complexes (LHCP). It leads to ROS generation which may also significantly lower the rate of photosynthesis in *J. curcas* accessions under salinity stress [21, 22, 33].

Chlorophyll fluorescence is a non-invasive technique used to study the photosynthetic performance of plants by measuring absorption, transmission, dissipation, and distribution of light energy of the photosynthetic reaction in photosystem II [34]. Our results showed a significant difference in leaf photochemistry of investigated Jatropha accessions under different salinity regimes at 180 DAT and 360 DAT. The Fv/Fm and ΦPSII values were significantly reduced in A6002, A6007, and A6008 accessions under 100 and 150Mm NaCl concentrations (Fig.2 A-D). It indicates photoinhibition of PSII, reaction centres of PSII were damaged or photochemically inactive, resulted in decreased excitation energy reaching PSII reaction centres under salinity stress in jatropa leaves [33]. This could be due to PSII's reduced ability to re-oxidized QA or transport electrons when exposed to salt stress [19, 34, 35]. It is also evident that excess Cl^- disrupts the oxygen-evolving complex of PSII and affects PSII performance under salt stress [36]. The Fv/Fm and ΦPSII values of accessions A6004, A6011 and A6014 were reduced at 150 mM NaCl condition but not significant, reflecting that are relatively resistant to accessions against salinity (Fig.2 A-D). These results indicated that the photo-oxidative damage was inhibited, and the function of PSII was maintained in A6004, A6011, and A6014 accessions under salinity stress. This protection can be associated with, at least in part, the excess energy dissipation at the PSII level. These results are

congruent with Magalhes *et al* who found that salt stress reduced the maximal quantum efficiency of photosystem II, as mirrored by the drop in Fm/Fv values and gas exchange Pn, gs, E, Ci, and A/Ci in *J. curcas* plants. Saddiq *et al* [37] observed the significant reduction of *gs*, chlorophyll content index, and light-adapted leaf chlorophyll fluorescence, i.e., Fv/Fm and instantaneous chlorophyll fluorescence (Ft) of wheat (*Triticum aestivum* L.) spring and winter genotypes under salt stress. Similarly, the varied response of chlorophyll fluorescence and gas exchange parameters were observed in cultivars of varieties of citrus rootstocks (common Sunki mandarin, Florida Rough lemon, Santa Cruz Rangpur lime, and Volkamer lemon). The 'Sunki' mandarin and the 'Florida Rough' lemon genotypes are more sensitive to salinity levels (0.3 and 4.0 dSm^{-1}) as they exhibited the significant reduction of chlorophyll fluorescence characteristics (F0, Fm, Fv, and Fv/Fm) and gas exchange parameters (Pn, E, Ci, and Gs) (Brito *et al*., 2016). Salt stress down regulated the genes encoding photosystem I, photosystem II i.e. LHC (light-harvesting complex) proteins in *J. curcas* L. leaves [38] (Cartagena *et al*., 2015). The Soil Plant Analysis Development (SPAD)-chlorophyll meters provide a simple, quick, and non-destructive method of determining relative chlorophyll content values per unit leaf area based on measurement of leaf transmittance (at 650 and 940 nm) [39, 40]. In the present study, the application of NaCl at both 100 and 150 mM concentrations significantly reduced the SPAD chlorophyll readings in all tested Jatropha accessions. Evaluation of individual accessions it was noticed that at 180 DAT, A6002, A6008, and A6007 accessions were suffered from higher chlorophyll degradation, whereas A6004, A6011, and A6014 accessions were relatively tolerant to salt stress. However, prolongation of salinity stress till 360 days all the tested accessions exhibited markedly lower levels of SPAD chlorophyll (Fig.1 G&H). Our results run parallel with the findings of Saleh [41], who observed the decrease of SPAD chlorophyll readings in cotton varieties (*Gossypium hirsutum* L.) but varieties showed some differences in their response to salt treatment. Abrar *et al* [42] also observed that drought and salinity significantly decreased the SPAD chlorophyll readings in *J. curcas.* Similarly, *Ricinus communis* seedlings showed reduced chlorophyll and carotenoid content with increasing salt (40, 80, and 120 mM NaCl) and alkali ($NaHCO_3$) stress [43]. It may be because excess NaCl (accumulated ions -Na^+ and Cl^-) hamper the chlorophyll biosynthesis by inhibiting δ-aminolevulinic (ALA) dehydrogenase, ALA synthase, and protochlorophyllide reductase enzyme activities, or displacement of central Mg^+ ion associated with the tetrapyrrole ring of chlorophyll molecules or activation of chlorophyll degradation by increased chlorophyllase activity [44].

Our results showed that NaCl at 100 and 150 mM concentrations significantly reduced the plant height, shoot dry weight, and root dry weight of transplanted seedling accessions, however, the responsiveness of the decline was varied. The accessions A6002, A6004, A6007, and A6008 were more sensitive to salinity than the A6011 and A6014 accessions (Fig.3 A-F and Fig. 4 A&B). In addition, NaCl treatment arrested the stem girth, and leaf area expansion was significantly arrested in all Jatropa accessions. Reduction of stem girth enlargement and LA expansion was more pronounced in A6002, A6008, and A6007 accessions over A6011 and A6014 accessions at 100 and 150 mM NaCl for 360 days (Fig.3 C-F) results are incoherent with the findings of Elhag and Gafar [45] who observed that 0.4 and 0.8% of NaCl significantly reduced the shoot length, fresh weight and, dry weight of shoot and root, and several leaves of Jatropha transplants. A study conducted by Sapeta *et al* [13] observed that the reduction of stem diameter elongation, leaf emergence, and total leaf area of two *J. curcas* accessions from different geographical and climatic origins, one from wet tropical climate) and the other from Cape Verde islands (semi-arid climate) under severe drought stress for 28 days. A similar response was found by Raoufia *et al* [46] in the rootstock of six pistachio genotypes. In this study, salinity at 12 and 18 dSm^{-1} significantly reduced the stem diameter, leaf area number of leaves, fresh weight, and dry weight of root, shoot, and leaves of 'Badami', 'Ghazvini', and 'Italyayi' genotypes. They suggested that these genotypes were less tolerant to salinity compared to 'Akbari', 'Ahmad-Aghai'and 'UCB-1'. Under salinity stress, a reduction in leaf area might result in decreased carbohydrate synthesis, resulting in diminished plant growth and development, as evidenced in the present study. According to the current study, plant height, shoot diameter, and leaf area were found to be negatively affected by salinity due to osmotic potential, nutritional constraint and high concentration of Na^+ and Cl^- ions in the cell wall disturbed metabolism while diminishing the cell wall's flexibility [47, 48]. Xyloglucan endotransglucosylase (XTHs) and expansin enzymes remodel the cell wall and cell expansion by modulating the interactions between the xyloglucans (hemicellulosic polymers of dicotyledonous plants) and cellulose fibrils there by conferring greater cell wall extensibility of tissues (leaf and root), and better plant adaptation to abiotic stress [48, 49]. Our data suggest that the negative effect of salt stress on the wall remodeling and cell expansion accounted for poor growth of A6002, A6004, A6007, and A6008 accessions. Whereas, lower reduction of growth A6011 and A6014 accessions could be due to positive or no effect of salt on Xyloglucan end otransglucosylase (XTHs) and expansion enzymes conferring greater cell wall extensibility and better plant adaptation to NaCl stress. Similarly, RNA-Seq transcriptome investigation of *Jatropha curcas* L. accessions revealed that the XTH enzyme is involved in wall remodeling and cell expansion. Exposure to 150 mM NaCl resulted in a minor reduction in plant growth [50].

Conclusion

Based on the results, Jatropha accessions exposed to 100 mM and 150 mM NaCl concentration for 360 days harmed all screening growth attributes, but their response varied among the accessions. Among the accessions, A6002, A6004, A6007, and A6008 were sensitive to salinity and exhibited significant growth reduction. Jatropha accessions 6011 and 6014 maintained the carboxylation efficiency when exposed to 150 mM NaCl at 360 DAT as there was no significant decline in Pn, Gs, or E levels. Chlorophyll fluorescence measurements also indicated that photo-oxidative damage was inhibited under salinity stress, and PSII function was preserved in A6004, A6011, and A6014 accessions. Thus, we conclude that the A6011 and A6014 accessions can be considered promising accessions for future studies of genetic improvement that involve the search for elite genotypes tolerant to salinity. These

accessions may be used for cultivating Jatropha in saline soils. However, more research is needed to explain variations across propagation and, methods and accessions and the causes responsible for variations

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