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Research Article

Responses of *Jatropha curcas* to Salt and Drought Stresses

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Two greenhouse experiments were conducted to quantify growth responses of *Jatropha curcas* to a range of salt and drought stresses. Typical symptoms of salinity stress such as leaf edge yellowing were observed in all elevated salinity treatments and the degree of the foliar salt damage increased with the salinity of irrigation water. Total dry weight (DW) of Jatropha plants was reduced by 30%, 30%, and 50%, respectively, when irrigated with saline solutions at electrical conductivity of 3.0, 6.0, and 9.0 dS m^{-1} compared to that in the control. Leaf Na^+ concentration was much higher than that observed in most glycophytes. Leaf Cl^- concentrations were also high. In the drought stress experiment, plants were irrigated daily with nutrient solution at 100%, 70%, 50%, or 30% daily water use (DWU). Deficit irrigation reduced plant growth and leaf development. The DW of leaves, roots, and total were reduced in the 70%, 50%, and 30% DWU compared to the 100% DWU control treatment. In summary, salinity stress and deficit irrigation significantly reduced the growth and leaf development of greenhouse-grown Jatropha plants.

1. Introduction

Jatropha (*Jatropha curcas* L.) is a multipurpose shrub (Family Euphorbiaceae) that is native to tropical America but now thrives in many parts of the tropics and subtropics in Africa and Asia [1, 2]. *J. curcas* grows well in lands with low-rainfall harsh climatic conditions and can alleviate soil degradation, desertification, and deforestation [3, 4]. While Jatropha grows well in low-rainfall conditions, requiring only about 200 mm, it can also respond to higher rainfall up to 1200 mm, particularly in hot climatic conditions [5]. Jatropha plants can withstand extremely low humidity in the air and can tolerate long-term drought stress by shielding most of its leaves to reduce transpiration [5]. Due to the above mentioned characteristics, Jatropha has received special attention in many countries and is one of the main crops to be promoted for growing in marginal lands for biodiesel production [5, 6].

To avoid the competition with food production, marginal and wasteland are targeted for producing bioenergy crops. Marginal lands are most likely located in the arid and semiarid regions in many parts of the world where high quality water supply is not available or extremely limited. Marginal lands are characterized with high soil salinity, low fertility, and limited supply of high quality of water. Before exploiting any plant for commercial production in a marginal land, it is imperative to investigate if the selected plants can survive and grow at a seasonably high rate under the stressful environment and quantify the impact of the stresses on plant growth and yield so that potential producers can make the right decisions.

High soil salinity is characterized by the presence of excess levels of soluble salts (saline soil) and/or high amount of sodium (Na^+) in the soil solution. For glycophytes (plant that will only grow healthily in soils with a low content of salts), growth and development are reduced in saline soil mainly due to nutrient imbalance, reduced uptake of nutrients, and specific ion toxicity such as excessive accumulation of Na^+ and Cl^- in the plant tissue [7, 8]. As salinity stress continues to increase, various physiological and chemical processes are damaged and the plant eventually dies. Little information is available on *Jatropha* performance under salt stressed condition.

Contrary to the scarcity of information in salt tolerance, *Jatropha* is reported to be drought resistant and a number of researchers have investigated the performance of *Jatropha*

under dry conditions. In a greenhouse study, drought stress significantly reduced leaf area, biomass, and relative growth rate, but had no effect on specific leaf area, daily range in leaf water potential, leaf water content, and transpiration efficiency [9]. In another greenhouse study, biomass production of *Jatropha* under well-watered condition was 1.49 ± 0.31 g dry mass per day, while under medium water stress (40% plant available water), biomass production was 0.64 ± 0.18 g dry mass per day [10]. In a field experiment, the seed yield was highest when plants were irrigated at 100% potential evapotranspiration (ET_p) and was lowest at 125% and 50% ET_p [5]. Seed yield of *Jatropha* reported in literature ranged from 0.2 t ha^{-1} to 12 t ha^{-1}, depending on production conditions [3]. These studies indicate that the growth and yield of *Jatropha* are affected by drought stress, although differences exist in drought stress imposition in these studies and comparisons across studies are difficult. The objectives of this study were to obtain the baseline information on salt tolerance of *Jatropha* plants and to further quantify the growth responses of *Jatropha* to a range of deficit irrigation.

2. Materials and Methods

2.1. Plant Materials and Culture. Seeds of *J. curcas* (Surinam provenance) were sown (13 Jan, 2011) in 164 mL, 2.5 cm-deep Ray Leach "Cone-tainers" (Stuewe and Sons, Inc., Tangent, OR) filled with a commercial potting mix (Sunshine Mix No. 5, SunGro Hort., Bellevue, WA). Thirty-five days after sowing, seedlings were transplanted into 7.65-L tree pots (TP812, Stuewe and Sons, Inc., OR, USA) filled with a substrate mixture comprising Sunshine Mix No. 4 (SunGro Hort., Bellevue, WA, USA), composted mulch (Western Organics Inc., Tempe, AZ) at 1 : 1 (v/v) amended with 5 kg m^{-3} powdered dolomite limestone (Carl Pool Earth-Safe Organics, Gladewater, TX, USA) and 1 kg m^{-3} Micromax (Scotts, Marysville, OH, USA). Plants were grown in the greenhouse and were well watered with nutrient solution containing 0.5 g L^{-1} of 20 N—8.6 P—16.7 K (Peters 20–20–20, Scotts, Marysville, OH) before the initiation of the treatments. In addition, plants were applied with 28 g per pot of slow released fertilizer (Osmocote 14–14–14; Scotts-Sierra Hort. Products, Marysville, OH). Greenhouse temperature was controlled by a natural gas heating system during winter and a pad and fan system for cooling. The average air temperature in the greenhouse ranged from 20°C to 35°C during the experiment (25 April to 2 June). The average daily relative humidity ranged from 20% to 48% and daily light integral (photosynthetically active radiation) from 13.0 to 20.8 mol m^{-2} d^{-1}. The maximum photosynthetic photon flux (*PPF*) during the experiment ranged from 600 to 980 μmol m^{-2} s^{-1}. The temperature was measured by copper-constantan thermocouples and *PPF* was measured by a quantum sensor (Model QSO-SUN, Apogee Instruments, Inc., Logan, UT, USA) every 10 s and the hourly averages were recorded by a 21X datalogger (Campbell Scientific, Logan, UT).

2.2. Treatments. Salt stress treatments (irrigation with saline solutions) were initiated on 8 April, 85 days after sowing and terminated on 31 May (54 days). Saline solutions were prepared by dissolving calculated amounts of sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), and calcium chloride ($CaCl_2$) at 87 : 8 : 5 (w/w) to the nutrient solution mentioned above. Electrical conductivity (EC) of the saline solutions was 1.6 (control, no addition of salts to the nutrient solution), 3.0, 6.0, and 9.0 dS m^{-1}. A 100-L tank of saline solution was prepared each time for each treatment with confirmed EC. Plants were hand-watered whenever the substrate surface started to dry to prevent water stress and overwatering. Plants were irrigated with saline solutions or nutrient solutions alternatively to prevent excessive root zone salt accumulation by monitoring leachate EC described below. Irrigation interval was determined based on observed plant water use (high EC-treated plants generally used less water than control plants) and weather conditions.

On 25 April (102 days after sowing), drought stress treatments were initiated by irrigating plants daily with 100%, 70%, 50%, or 30% daily water use (DWU), which were 1000 mL, 550 mL, 400 mL, and 300 mL, respectively. These DWUs were determined at the beginning of the experiment: plants were weighed 1 h after thorough irrigation to container capacity and all plants were weighed again in 24 h. The DWU was calculated as the differences between the two weights in 24 h and the average of the weight difference in each treatment was determined as the DWU. Treatments were terminated on 29 May, 34 days after treatment.

2.3. Measurement. For the salt stress experiment, stem length (from substrate surface to growing point) and leaf number were recorded twice a week. Leaf stomatal conductance was measured six times during the experiment using a porometer (SC-1, Decagon Devices, WA) on cloudless days between 11:00 AM to 3:00 PM. Shoots were severed at the substrate surface and leaves and stems were separated at the end of the experiment. Roots were washed free of substrate. Dry weights of leaves, stems, and roots were determined after oven dried at 70°C to constant weight. In addition, EC of leachate was determined according to Wright [11] six times during the course of the experiment. Specifically, 1 hour after well watering the plants with no more drainage, 100 mL reverse osmosis water (EC ≈ 0) was added through the substrate surface and the leachate was collected. The EC of the leachate was measured using an EC meter (Model B-173, Horiba, Ltd., Kyoto, Japan).

To analyze leaf Na^+ and Cl^- concentrations, leaf samples were collected at the end of experiment, washed three times with deionized water, and oven dried at 70°C. Dried leaves were ground to pass a 40-mesh screen with a stainless Wiley mill and the samples were submitted to the Soil, Water, and Air Testing Laboratory of New Mexico State University (Las Cruces, NM) for Na^+ and Cl^- analyses. Na^+ concentrations were determined by EPA method 200.7 [12] and analyzed using an Inductively Coupled Plasma/Atomic Emission Spectrophotometer Trace Analyzer (Thermo Jarrell Ash, Franklin, MA). Chloride was determined by EPA method 300.0 [12] and analyzed using an Ion Chromatograph (Dionex, Sunnyvale, CA).

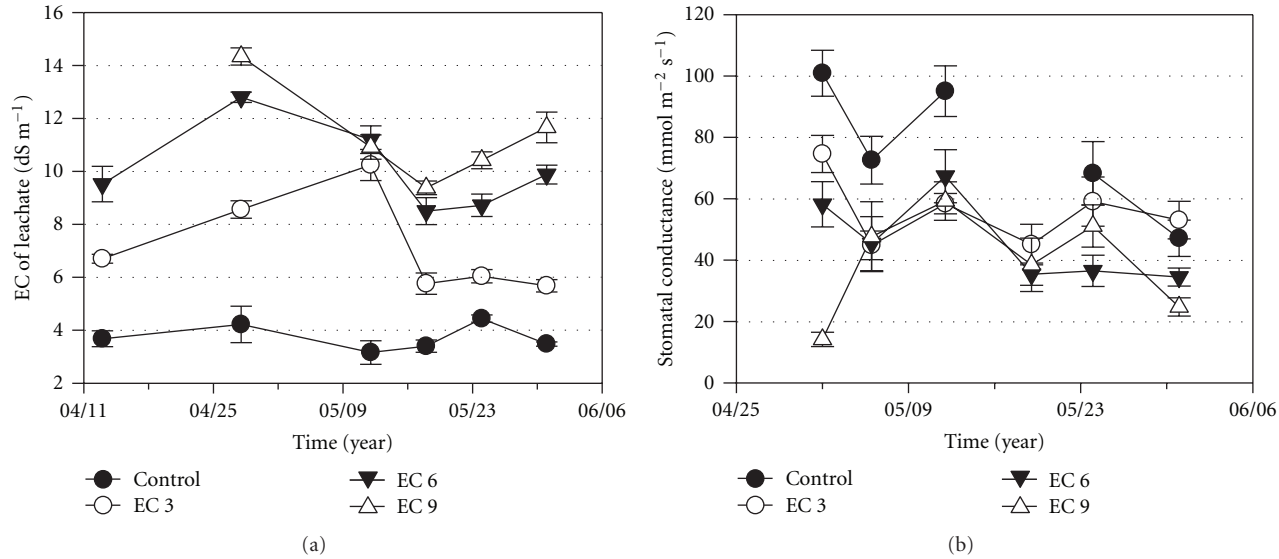

Figure 1: The electrical conductivity (EC) of the substrate leachate (a) and the leaf stomatal conductance (b) of Jatropha plants irrigated with nutrient solution (control) or saline solutions at EC of 3.0, 6.0, or 9.0 dS m^{-1}.

For drought stress experiments, stem length, leaf number, dry weights of leaves, stems, and roots were determined in the same way as in the salt stress experiment. Leaf stomatal conductance was measured 12 times (approximately twice per week) during the experimental period using the same methodology described in the salt experiment. Substrate volumetric moisture contents in the drought experiment were monitored using soil moisture sensors (10HS, Decagon Devices, WA) connected to EM50 datalogger (Decagon Devices). The sensors were calibrated against the same substrate used in this experiment. Actual daily water use was determined gravimetrically: difference in two weights in 24 h plus the amount of irrigation water, that is, Daily water use (mL) = Weight$_1$ − Weight$_2$. No leaching was observed in all irrigation treatments. Five weeks after irrigation treatments, plants were well watered for 4 days before destructive harvest. Leaves, stems, and roots were separated and the dry weights were determined after oven dried at 70°C to constant weight.

For both salt and drought stress treatments, the relative increases in stem length and leaf number were calculated as follows: relative increase (%) = (final height or leaf number − initial height or leaf number)/initial height or leaf number × 100%. The average initial height and number of leaves were 45.5 cm and 33.

2.4. Experimental Design and Statistical Analysis. The experiment was a completely randomized designed with 6 and 5 replications in the salt and drought experiments, respectively. One-way ANOVA was used to determine the effects of salt or drought treatments. Means were separated by Student-Newman-Keuls (SNK) multiple comparisons at $P = 0.05$, when main effect was significant. All statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).

Table 1: Dry weight of leaves, stems, and roots of *Jatropha* plants irrigated with saline solution at electrical conductivity (EC) of 1.6 (control), 3.0, 6.0, or 9.0 dS m^{-1}.

Treatment	Leaves (g)	Stems (g)	Roots (g)	Total (g)	Root/Shoot
Control	101^{az}	70^{a}	42^{a}	213^{a}	0.25^{a}
EC 3	67^{b}	52^{b}	29^{b}	149^{b}	0.25^{a}
EC 6	58^{b}	52^{b}	28^{b}	149^{b}	0.23^{a}
EC 9	49^{c}	38^{c}	19^{c}	106^{c}	0.22^{a}

^{z}means in the same column with same letters are not significantly different tested by Duncan's multiple comparison.

3. Results

In the salt stress experiment, plants had foliar damage exhibiting leaf edge yellowing in older leave. As the salinity of the irrigation water increased, the leaf area and number of leaves with leaf edge yellowing increased. Approximately, one third of the leaves in EC 9 treatment exhibited leaf edge yellowing on older leaves, while it was one fourth in EC 3 and EC 6 treatments. Plants in the control did not have any leaf damage symptoms. Salinity of the substrate leachate for control was below 4 dS m^{-1}, while it was between 6 to 10 dS m^{-1} for EC 3, 8 to 13 dS m^{-1} for EC 6, and 10 to 14 dS m^{-1} for EC 9 (Figure 1).

Dry weights of leaves, stems, and roots were highest in the control, followed by EC 3 and EC 6, and those of EC 9 were smallest (Table 1). As salt stress increased, plant elongated slowly and developed fewer leaves, reflected in leaf number, compared to the plants without receiving saline solution. The relative increase in stem length was higher in the control (65%) compared to other treatments where plants received saline solutions (48% to 55%, Table 2). However, there were

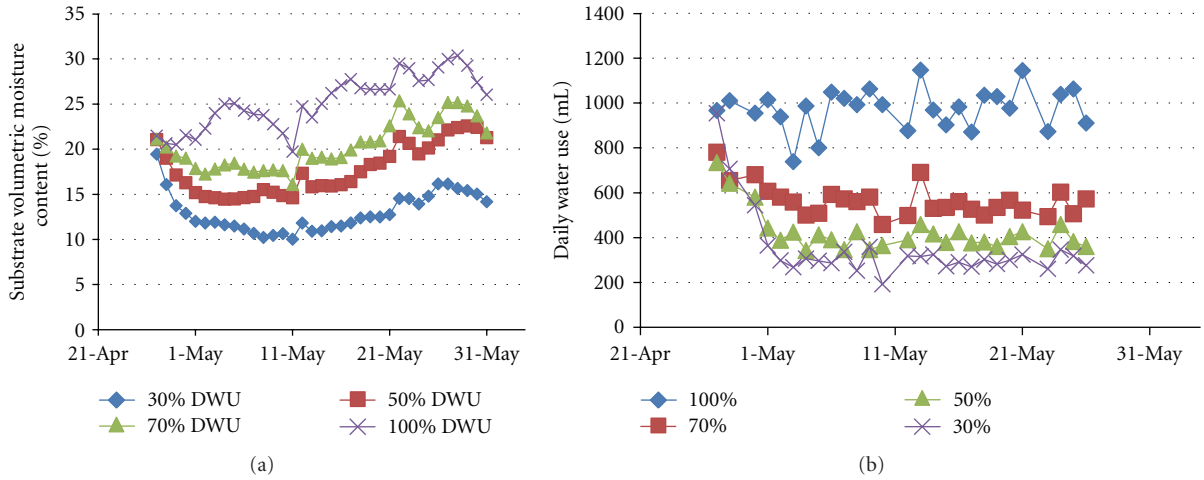

Figure 2: Substrate moisture content (a) and the actual daily water use (b) of *Jatropha* plants irrigated with 100%, 70%, 50%, or 30% daily water use (DWU) during the experiment.

no significant differences among EC 3, EC 6, and EC 9 in the relative increases in stem length. Similarly, there were no statistical differences in relative increases in leaf number among these elevated salinity treatments but were lower compared to the control.

The leaf sodium Na^+ concentration was the highest in plants in EC 9 (34.6 g kg DW^{-1}) and the lowest in the control (10.5 g kg DW^{-1}). No difference was observed in leaf Na^+ concentration between EC 3 and EC 6. Leaf Cl^- concentration was higher in plants in EC 6 and EC 9 and lowest in the control (Table 3).

Salt stress affected leaf stomatal conductance at the beginning of the experiment. The control plants had higher leaf stomatal conductance compared to that of those irrigated with saline solution. However, all plants had low stomatal conductance from the middle part of the experiment (with approximately 68 leaves and 71 cm tall) regardless of treatment (Figure 1).

In the drought experiment, no visual foliar damages were observed in any treatment. Substrate moisture contents started to exhibit differences among the four irrigation treatments two days after the initiation of the irrigation treatments (Figure 2). The substrate moisture contents ranged between 20% to 30%, 15% to 25%, 14% to 23%, and 10 to 17%, for 100% DWU, 70% DWU, 50% SWU, and 30% DWU irrigation treatments, respectively. The actual daily water use of the plants in each treatment was roughly equal to the amount of the irrigation.

The final dry weight (DW) of leaves, stems, and roots was reduced in the 70%, 50%, and 30% DWU compared to 100% DWU (Table 4). Leaf DWs in 70%, 50%, and 30% DWU treatments were similar and were reduced by 28% to 40% compared to that in 100% DWU. No statistical difference was observed in stem DWs between 50% and 30% DWU but was reduced by 20% and 40% compared to 70% and 100% DWU, respectively. There were no differences in root DWs and total DW among 70%, 50%, and 30% DWU treatments. The irrigation treatments did not affect the root to shoot ratio.

Table 2: Relative increases in stem length and leaf number of *Jatropha* plants irrigated with saline solution at electrical conductivity (EC) of 1.6 (control), 3.0, 6.0, or 9.0 dS m^{-1}.

Treatment	Height (%)	Leaf count (%)
Control	65^{az}	138^{a}
EC 3	48^{b}	69^{b}
EC 6	55^{b}	89^{b}
EC 9	49^{b}	90^{b}

[z]means in the same column with same letters are not significantly different tested by Duncan's multiple comparison.

Table 3: Leaf sodium (Na^+) and chloride (Cl^-) uptake of *Jatropha* plants irrigated with saline solution at electrical conductivity (EC) of 1.6 (control), 3.0, 6.0, or 9.0 dS m^{-1}.

Treatment	Na^+ (g kg DW^{-1})	Cl^- (g kg DW^{-1})
Control	10.5^{cz}	11.9^{c}
EC 3	23.6^{b}	17.0^{b}
EC 6	27.1^{b}	21.0^{a}
EC 9	34.6^{a}	20.3^{ab}

[z]means in the same column with same letters are not significantly different tested by Duncan's multiple comparison.

Table 4: Dry weight of leaves, stems, roots, and total of Jatropha plants irrigated with 100%, 70%, 50%, or 30% daily water use (DWU).

Treatment	Leaves (g)	Stems (g)	Roots (g)	Total (g)	Root/shoot
100%	87^{az}	65^{a}	49^{a}	201^{a}	0.32^{a}
70%	63^{b}	47^{b}	32^{b}	142^{b}	0.30^{a}
50%	51^{b}	37^{c}	26^{b}	115^{b}	0.29^{a}
30%	58^{b}	38^{c}	27^{b}	123^{b}	0.28^{a}

[z]means in the same column with same letters are not significantly different tested by Duncan's multiple comparison.

Stem length increased with time in all drought stress treatments but the increments were different (Figure 3).

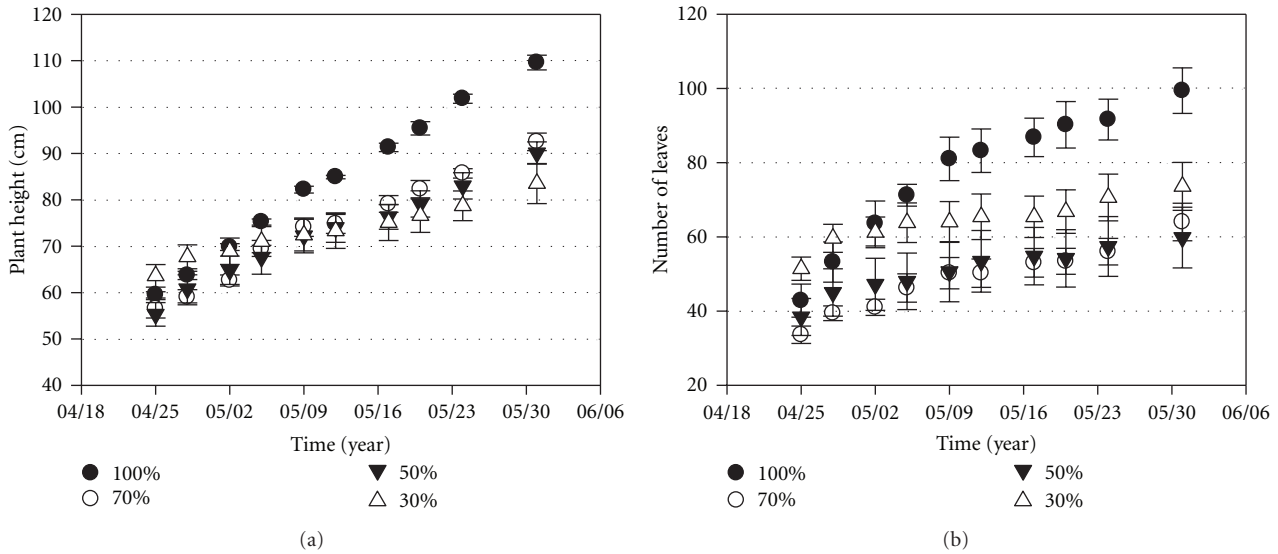

Figure 3: The time course of plant height (stem length, (a)) and number of leaves (b) of *Jatropha* plants irrigated with 100%, 70%, 50%, or 30% daily water use (DWU) during the experiment.

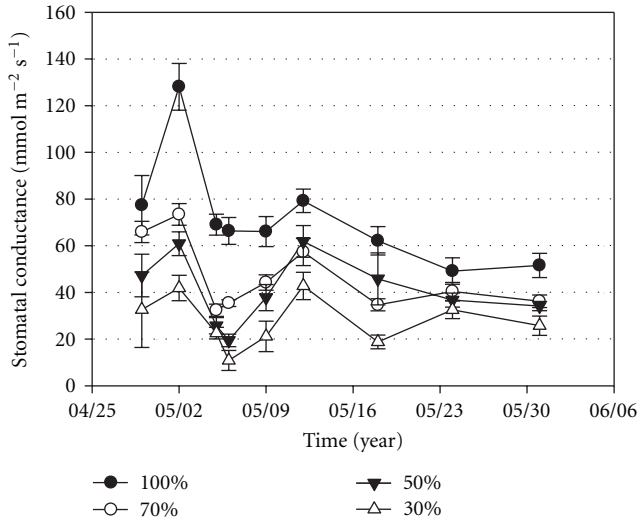

Figure 4: Leaf stomatal conductance of *Jatropha* plants irrigated with 100%, 70%, 50%, or 30% daily water use (DWU) during the experiment.

Stem length exhibited significant difference about 3 weeks after the treatments. Leaf development also showed similar tendency among the treatments. The relative stem length increases during the 38 day experimental period were 84%, 64%, 64%, and 31% in 100% DWU, 70% DWU, 50% DWU, and 30% DWU, respectively. The relative leaf number increases were 136%, 92%, 55%, and 43% in 100% DWU, 70% DWU, 50% DWU, and 30% DWU, respectively. The relative growth in stem length and leaf development was greatest in 100% DWU and smallest in 30% DWU. The average daily shoot elongation rates were 1.42, 1.02, 0.97, and 0.55 cm d^{-1} for 100%, 70%, 50%, and 30% DWU, respectively. The average daily leaf development rates were 1.57, 0.84, 0.67, and 0.45 for 100%, 70%, 50%, and 30% DWU, respectively.

Leaf stomatal conductance was highest in 100% DWU and lowest in 30% DWU for most days (Figure 4). Similar to salt stress experiment, leaf conductance was relatively higher at the beginning of the experiment, but it was low, between 20 to 80 mmol m^{-2} s^{-1}, and decreased generally over time.

4. Discussion

Most crops tolerate salinity up to a threshold level, above which growth and yield decrease as salinity increases [13]. This threshold differs from species to species or from cultivar

to cultivar in some species. Characteristics such as survival, growth, yield, and foliar salt damage are commonly used as criteria for evaluating salt tolerance among different plant genotype [14]. In the current study, leaf salt damage and significant growth reduction were observed in all salt stressed plants. For example, growth of leaves, stems, roots, and total dry weights were reduced by 34%, 26%, 31%, and 30%, respectively, in plants irrigated with saline solution at 3.0 dS m^{-1} compared to those in control. Vegetative growth reduction often leads to reduced yield. Therefore, based on the growth results of this study, *Jatropha* is not tolerant to salinity and its yield would be reduced when grown in a salt affected land, such as marginal land.

The restriction of ion uptake by the roots and the prevention of ion accumulation in the shoots are important mechanisms in salt tolerance of glycophytes [15]. In the current study, leaf Na^+ concentration of *Jatropha* was 10.5 g kg^{-1} in the control and was as high as 34.6 g kg^{-1} in the EC 9 treatment, which was extremely high being treated for 54 days at the this salinity level based on our results in other crops and literature [16–18]. The leaf Cl^- concentrations were 11.9 g kg^{-1} in the control and 20.3 g kg^{-1} in the EC 9, which may be in the average range for most glycophytes. In a salt tolerance study of four rose rootstocks where plants were irrigated with saline solution in a range of salinity up to 8.0 dS m^{-1} for approximately 4 months [16], the leaf Na^+ concentrations were about 0.5 mg g^{-1} in the control (same water source) and 1.0 to 2.6 mg g^{-1} in the EC 8 treatments, which are less than one tenth that in *Jatropha* leaves in the current study. The leaf Cl^- concentrations in the same rose rootstock study ranged from 6.2 to 30 mg g^{-1}, in the control to EC 8, which were comparable to those found in *Jatropha* leaves. In our previous studies with a range of ornamental herbaceous plants and a number of peppers, the concentrations of leaf Na^+ were much lower than those of leaf Cl^- [16, 17, 19–21], while in the current study *Jatropha* leaf Na^+ concentrations were higher than leaf Cl^-.

The relatively high levels of Na^+ accumulated in *Jatropha* leaves may be responsible for the observed salt damage and growth reduction. The most important salt tolerant trait is the ability to limit the concentration of Na^+ that enters the xylem [22]. Wheat genotypes having low Na^+ contents in leaves resulted in higher biomass and better salt tolerance in saline conditions [23].

Excessive accumulation of Cl^- can also cause specific ion injury. Tolerant crops such as barley (*Hordeum vulgare* L.), spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.), and sugar beet (*Beta vulgaris* L.) did not exhibit leaf injury at leaf Cl^- concentrations of 20 to 30 mg g^{-1} [18]. For some sensitive greenhouse rose genotypes, leaf Cl^- at as low as 4.5 mg g^{-1} can cause growth reduction and foliar damage [24]. Average leaf Cl^- concentrations in *Jatropha* plants ranged from 17–20 mg g^{-1} and were probably high enough to cause leaf damage, although it was not possible to separate the toxic effects of Na^+ from those of Cl^-.

Jatropha is reported to thrive in a range of rainfall conditions at as low as 200 mm to as high as 1200 mm [5]. Our study indicated a growth reduction of 29% to 43% in the plants irrigated with 70% to 30% DWU, compared to plants irrigated at 100% DWU. The ability to sustain growth (stem elongation and leaf development) under drought stress conditions is usually considered to be an indicator of drought tolerance. For example, drought tolerant oleander (*Nerium oleander* L.) clones continued to grow, while susceptible clones developed few or no new shoots [25]. In the current study, *Jatropha* plants irrigated with 30% DWU continued to grow without exhibiting any foliar damage or leaf shedding, but the growth was slow. Several research reports have also reported drought-induced reductions in growth and yield of *Jatropha* plants under various growing conditions [3, 5, 10]. Our results are consistent with these previous studies and also suggest that yet-to-be determined mechanisms may allow *Jatropha* to survive moderate to severe drought stress episodes without significantly impacting productivity. Such mechanisms would be beneficial especially on marginal lands with limited soil moisture availability.

In summary, salt stress significantly reduced growth and development of *Jatropha* plants. Leaf edge yellowing was observed in older leaves in all salt stressed treatments and the higher salinity led to more severe leaf damage. Leaf Na^+ concentrations were excessively high compared to most glycophytes. Deficit irrigation decreased the growth and development of *Jatropha* plants but the plants continued to grow even irrigated with as low as 30% daily water use. Growth and yield of *Jatropha* would be reduced at suboptimal moisture conditions and its reduction depends on the degree of drought stress. Further studies are needed to investigate the salt and drought tolerance of *Jatropha* plants at other growth stages and under field conditions.

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