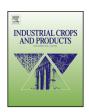
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Seedling emergence, growth, and leaf mineral nutrition of *Ricinus communis* L. cultivars irrigated with saline solution



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ABSTRACT

Two greenhouse experiments were conducted to evaluate the salt tolerance of five cultivars ('Brigham', 'Energia', 'Hale', 'Memphis', and 'Ultra dwarf') and one hybrid ('HCastor') of castor (Ricinus communis L.) by germinating seeds in saline substrate and irrigating plants with saline solutions. Seeds were sown in substrate that was moistened with reverse osmosis water [Electrical conductivity (EC) = 0.02 dS m⁻¹, control), wastewater (EC = 1.9 dS m⁻¹), or saline solution (EC = 10.3 or 17.5 dS m⁻¹). Both wastewater at EC of 1.9 dS m⁻¹ and saline solution at EC of 10.3 dS m⁻¹ increased the seedling emergence of 'Brigham', 'Hale', and 'Memphis', but decreased that of 'HCastor' and 'Ultra dwarf'. Saline solution at EC of $17.5~\mathrm{dS}~\mathrm{m}^{-1}$ significantly inhibited seedling emergence of all cultivars, except 'Memphis'. The results indicated that 'Memphis' was the most tolerant cultivar, and 'Brigham', 'Hale', 'HCastor', 'Energia', and 'Ultra dwarf' were less tolerant to salinity stress. In the 2nd experiment, plants were irrigated with nutrient solution (no addition of salts, control) or saline solution at EC of 10 dS m⁻¹ for 69 days. All plants irrigated with saline solution were shorter than those irrigated with nutrient solution. The reduction in height was 40%, 39%, 44%, 38%, 51%, and 50%, respectively, for 'Memphis', 'Brigham', 'HCastor', 'Ultra dwarf', 'Energia', and 'Hale', compared to their respective controls. The fruit dry weight (DW) of 'Brigham', 'HCastor', 'Ultra dwarf', and 'Hale' irrigated with saline solution was significantly reduced by 65%, 66%, 60%, and 78%, respectively. Although a reduction of 57% and 55% in the fruit DW was recorded for 'Energia' and 'Memphis', respectively, there was no significance between saline solution and nutrient solution. Total DW of the plants irrigated with saline solution decreased by 60% for 'Memphis' and 70-78% for other cultivars as compared with those irrigated with nutrient solution. Salt treatment significantly increased leaf Na⁺, Ca²⁺, and Cl⁻ concentrations, while decreased K⁺ concentration. However, leaf Na⁺ and Cl⁻ concentrations were relatively low compared to other salt sensitive crops. Based on the results from both experiments, 'Memphis' was more tolerant to salinity than other cultivars.

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1. Introduction

Salt salinization and fossil fuel pollution are two major global issues. They become an ever-increasing problem as the global population grows dramatically. Recent estimates of FAO-AGL indicate that 397 million hectares of land throughout the world are affected by salinity and 434 million hectares of land by the associated condition of sodicity (FAO, 2005). Most of them are marginal lands which have low inherent productivity and have been abandoned or degraded (Cai et al., 2011). Marginal lands are majorly located in arid and semi-arid regions where soil salinity is too high for most

common economic crops and groundwater with high salinity is the primary water source. Therefore, identifying salt tolerant crops and improving salt tolerance for salt affected lands are critically important tasks.

Biodiesel as an alternative to petrodiesel has drawn considerable attention due to diminishing availability of discoverable fossil fuel reserves and environmental consequences of exhaust gases from fossil fuel. A total of 23.4 billion liters of biodiesel was produced worldwide in 2011 (U.S. Energy Information Administration, 2011). Biodiesel, alkyl esters of fatty acids, is made from renewable resources including animal fats and vegetable oils. The edible fatty oils derived from rapeseed, soybean, palm, sunflower, coconut, linseed, etc. have been exploited commercially (Korbitz, 1999). In 2010, 2.32 billion liters of biodiesel were produced from soybean oil, tallow, and cottonseed oil (MME, 2010). As a consequence,

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biodiesel feedstock production may compete with food supply and/or with food crops for arable land in the long run. Growing bioenergy crops on marginal lands would be an alternative way to conserve water, reduce fossil fuel pollution, and secure food safety.

Ricinus communis L. (Castor), a member of Euphorbiaceae, is widespread throughout subtropical and tropical regions (Weiss, 2000). A total of 1.54 million hectares of castor is harvested, and 1.76 million tons seeds are produced in 2010 in the world (FAO, 2010). The seed contains 40-60% oil that is rich in triglycerides (Severino et al., 2012a). Castor oil production accounts for 0.15% of vegetable oils produced in the world (Scholz and Silva, 2008). Castor is an ideal plant for industrial oil production and bioenergy usage because it has high yield with unique fatty acid composition and is non-food crop with wide adaptation to marginal lands and tremendous genetic potential (Severino et al., 2012a). Many varieties have been selected for oil production and bioenergy usage. For instance, 'Brigham' is a variety with 10-fold reduction in ricin content adapted for Texas (Auld et al., 2001). 'Hale' is a dwarf variety with multiple racemes (Brigham, 1970). Salt tolerance of castor has been documented (Janmohammadi et al., 2011; Kumar et al., 1989; Pinheiro et al., 2008; Raghavaiah et al., 2006). However, a considerable variation in salt tolerance exists within genotypes or cultivars of castor (Kumar et al., 1989; Raghavaiah et al., 2002, 2006). To provide further information on such variation, salt tolerance of six castor cultivars was evaluated by examining the responses of growth, gas exchange rates, and leaf ion accumulation of the six castor cultivars when irrigated with nutrient solution or saline solution

2. Materials and methods

2.1. Plant materials and treatments

2.1.1. Seedling emergence

Castor seeds of five cultivars ('Brigham', 'Energia', 'Hale', 'Memphis', and 'Ultra dwarf') and one hybrid ('HCastor') were sown on November 2, 2011 in germination cells (Dillen® 6-pack insert, $5.7 \, \text{cm} \times 6.4 \, \text{cm} \times 5.7 \, \text{cm}$) filled with a potting mix (Sunshine Mix No. 4, SunGro Hort., Bellevue, WA). 'Brigham' and 'Hale' were Lubbock, TX; 'Energia' was from Brazil; 'Memphis' and 'Ultra dwarf' were from Mississippi; and 'HCastor' was from USDA National Plant Germplasm System. The potting mix was saturated with same amount of saline solution at electrical conductivity (EC) of 10.3 or $17.5 \,\mathrm{dS}\,\mathrm{m}^{-1}$, wastewater (EC = $1.9 \,\mathrm{dS}\,\mathrm{m}^{-1}$), or reverse osmosis water (RO, EC = $0.02 \, dS \, m^{-1}$) (four treatment solutions). Saline solution was prepared by dissolving the calculated amounts of NaCl and CaCl2 salts at 2:1 molar ratio into RO water. Wastewater is the treated municipal effluents and its major ions were Na⁺, Ca²⁺, Mg²⁺, K⁺, Cl⁻, NO₃⁻, and SO₄²⁻ at 272.0, 55.0, 13.7, 25.8, 349.5, 17.5 and 165.6 mg L⁻¹, respectively. The experiment followed a completely randomized design with three replications, 12 seeds per replication per treatment per cultivar. Pots were misted with tap water whenever substrate surface started to dry to prevent water stress. Emergence was counted daily as soon as the first emergence appeared and lasted for 35 days. A seedling was considered emerged when the hypocotyls hook was visible above the surface. The average day temperature was at 20.6 °C, night temperature at 15.5 °C, and average daily relative humidity at 29.5%. Germination flats were placed on bottom heating pad with a root zone set temperature of 20 °C.

Emergence percentage (EP) was calculated using the following formula:

$$EP~(\%) = \frac{Number~of~emerged~seedlings}{Total~number~of~seeds} \times 100$$

A relative EP was calculated for each cultivar in the salt treatment as:

Relative EP (%) =
$$\frac{\text{EP in salt treatment}}{\text{Averaged EP in control}} \times 100$$

In addition to emergence percentage, emergence index (EI) was calculated as: EI = $\sum_{i=1}^{n} (\text{EP}_i/T_i)$; where EP_i is the emergence percentages on day i, and T_i is the number of days after sowing seeds. Similar to relative EP, relative EI was calculated as:

Relative EI (%) =
$$\frac{\text{EI in salt treatment}}{\text{Averaged EI in control}} \times 100$$

One-way analysis of variance (ANOVA) was used to analyze relative EP and relative EI. When significant difference among cultivars existed, means were separated by Tukey's Honestly Significant Difference (HSD) multiple comparison at P < 0.05.

2.2. Seedling growth

Castor seeds mentioned above were sown in 2.3-L poly-tainer container #1S (16.5 cm \times 16.5 cm) filled with Sunshine Mix #4 (SunGro Hort., Bellevue, WA) on November 2, 2011. Seedlings were watered with nutrient solution until treatments were initiated. The nutrient solution at EC of 1.5 dS m $^{-1}$ was prepared by adding 0.72 g L $^{-1}$ of 15 N-2.2 P-12.5 K (Peters 15-5-15; Scotts) to tap water. The major ions in the tap water were Na $^+$, Ca $^{2+}$, Mg $^{2+}$, Cl $^-$, and SO $_4$ 2 – at 184, 52.0, 7.5, 223.6, and 105.6 mg L $^{-1}$, respectively.

On 22nd December, treatments were initiated by irrigating plants with nutrient solution (control) or saline solution with 10–20% leaching fraction. Plants were then irrigated with nutrient or saline solutions whenever substrate surface started to dry. Saline solution at EC of $10.0\,\mathrm{dS\,m^{-1}}$ was prepared by adding calculated amount of sodium chloride (NaCl) and calcium chloride (CaCl₂) at 2:1 (molar ratio) to the nutrient solution. Solutions were prepared in 100-L tanks with confirmed EC of 1.40 ± 0.14 and $10.78\pm0.28\,\mathrm{dS\,m^{-1}}$ (mean and standard deviation) for nutrient and saline solutions, respectively.

Seedlings were transplanted to 9.6-L poly-tainer container #3 (27 cm \times 22 cm) with fresh Sunshine Mix #4 on 17 January 2012. Marathon® 1% G (1% lmidacloprid; OHP, Inc., Mainland, PA), 12 g per container, was applied to control thrips. Leachate was collected periodically, and the EC of the leachate was measured using an EC meter (Model B-173, Horiba, Ltd., Japan). To reduce the salt accumulation, plants were flushed with tap water to lower the salinity in the root zone. The substrate final EC, as determined using saturated paste extract (Gavlak et al., 1994; USDA Staff, 1954), was $3.97\pm1.70~\rm dS\,m^{-1}$ and $12.45\pm4.86~\rm dS\,m^{-1}$ for control and salinity treatment, respectively. The temperatures in the greenhouse were maintained at $23.0\pm5.4~\rm ^{\circ}C$ (mean \pm standard deviation) during the day and $19.2\pm2.4~\rm ^{\circ}C$ at night. The daily light integral (photosynthetically active radiation) was $11.1\pm4.7~\rm mol\,m^{-2}\,d^{-1}$.

2.3. Growth data

Plant height from the pot rim to the top of the inflorescence and number of leaves were recorded biweekly. Number of nodes, number of inflorescence, total length of all inflorescences, and stem diameter right above the 3rd node were recorded at the end of experiment (i.e. 1st March). Upon termination of the experiment, shoots were severed at the substrate surface, and dry weight (DW) of fruit, leaf, and stem was determined after oven-dried at 65 °C to constant weight. All growth parameters were converted to relative values of the control using the same equation as for relative EP and relative EI.

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