# Tuber and Inulin Production of Jerusalem Artichoke (Helianthus tuberosus, L.) Under Salinity Stress





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## 1. INTRODUCTION

Jerusalem artichoke (Asteraceae) is a North American crop that was consumed by Native Americans before European settlers arrived. Its tubers are a rich source of inulin (50% DW), a fructan (polymer of fructose molecules) valued as a non-caloric probiotic fiber and source of sugars for biofuels. The whole plant can provide inulin, protein (6-12%), and aminoacids as feed and food. Although reported as tolerant to drought and salinity, salinity effects on the production of inulin, inulin's degree of polymerization, and tuber antioxidant capacity have not been reported. Its high adaptability to diverse edaphoclimatic conditions, pests and diseases, and ability to re-sprout from overwintered tubers make it a potential biofuel, food, and feed crop adaptable to areas unsuitable for conventional agricultural crops. Our objective was to evaluate crop growth, tuber yield, inulin tuber concentration and degree of polymerization, tuber antioxidant capacity, and concentration of free sugars under saline irrigation.

# 2. MATERIAL & METHODS

The experiment was conducted with the cultivar 'Stampede' at the US Salinity Lab in Riverside, CA. The average temperatures during growing season (April 29 to July 18, 2014) were 30°C (day) and 15°C (night). Day lengths ranged from 13.5 h (April 29, planting) to 14.15h (July 18, shoot harvest), then to 12.75h (September 4, tuber harvest). Tubers were planted in large (3.0 m L x 1.5 m W x 2.0 m D) sand tanks (Figs. 1 & 2). This system enables complete recycling of the irrigation waters while maintaining stable root-zone salinity. Riverside tap water, pH=7.5-8.0 of average electric conductivity (ECw) =  $0.7 \text{ dS m}^{-1}$  was mixed with high salinity water, or HSW, (EC, = 12.0 dS m<sup>-1</sup>) enriched with macro and micronutrients, Na<sup>+</sup>, and Cl<sup>-</sup> (Dias et al. 2016). EC of irrigation waters (ECw) were 1.2 (tap water plus nutrients and low NaCl), 3.9, 6.6, 9.3, and 12 dS/m (equivalent to 1/4 seawater).



Figure 1. Helianthus tuberosus cv. Stampede under salinity. Planting: April 29, 2014 Left: June 27 (45 DAP, left), Middle: July 8 (79 DAP), Right: September 4 (harvest, 128 DAP). Tubers shown on right are from plants irriaated with ECw = 6.6 dS/m (83 tons tuber ha-1).



Figure 2. Outdoor lysimeter system with 24 sand tanks (left). A pump system irrigates sand tanks (top right) with waters from underground 3,600L reservoirs (bottom right) containing the desired treatments and maintaining a stable root-zone salinity.

## 3. RESULTS & DISCUSSION

'Stampede' tubers generated multi-stemmed plants that started flowering on June 20<sup>th</sup> (53 DAP), anthesis on July 8<sup>th</sup>(Fig. 1). Half the plants were harvested for shoot biomass on July 18 (80 DAP), after 26 blooming days. Plants irrigated with LSW (1.2 dS m<sup>-1</sup>) were ≥1.5m high, while plants irrigated with HSW (12 dS m<sup>-1</sup>) were 1.2 m high. 'Stampede' is an intermediate-flowering cultivar with inductive photoperiod estimated to be 14h:20min (day length 17 days before flowering). 'Stampede' completed its cycle in 128 days in California.

#### 3.1 Salt accumulation and biomass accumulation:

Statistically-significant decreases in leaf, stem, and total shoot (leaf+stem) biomass occurred only between LWS (ECw=1.2 dS m<sup>-1</sup>) and HSW (ECw=12 dS m<sup>-1</sup>) with shoot biomass being similar at ECw ranging from 3.9 to 9.3 dS m<sup>-1</sup>. Pearson's correlation coefficient showed that salinity had a significant and negative impact on shoot biomass (Fig. 3A). While Cl<sup>-</sup> increased with salinity in all organs. Na<sup>+</sup> only increased in roots and tubers (Fig. 3B). Thus, Cl<sup>-</sup>, not Na<sup>+</sup>, was responsible for decreased shoot and tuber biomass (Fig. 3B). Shoot biomass decreased 67% from LSW to HSW (Fig. 3A, Table 1).



Figure 3. A) Relation between leaf ( A, Series 3), stem ( I, Series 2) and total shoot dry matter ( •, Series 3) of 'Stampede' and salinity levels of blended waters. \*\*Significance of Pearson's correl. coef. for 22 degrees of freedom. B) Inverse response of stem and leaf dry weight to chloride tissue accumulation. Concentration of chloride (Cl<sup>-</sup>) and sodium (Na<sup>+</sup>) in stems and leaves (based on DW) of plants irrigated with saline waters.

#### 3.2 Tuber yield, total soluble solids, and sugars:

Tuber yield was reduced by salinity, similar to shoot biomass. Although reduction in tuber yield plant<sup>-1</sup> was not significant, there was a tendency for tuber yield to decrease with salinity, and in 47% from LSW to HSW.

Treatment (EC <sub>w</sub> in dS m <sup>-1</sup> )		Means	Table 1. Effect of water
	g plant <sup>-1</sup>	Mg ha-1	salinity on tuber yield
1.2	$1663\pm224$	$92\pm12$	per plant and per
3.6	$1486 \pm \ 126$	$83\pm7$	hectare. Blended waters
6.6	$1488\pm311$	$83\pm17$	ECw ranged from 1.2 to $12 \text{ dS m}^{-1}$
9.3	$1048\pm139$	$58\pm 8$	12 05 117 .
12	$885 \pm 187$	$49\pm10$	

Total soluble solids (TSS, in °Brix) increased with salinity up to 6.6 dS m<sup>-1</sup> (31.4 °Brix) (Fig. 4), suggesting that soluble sugars, such as inulin, may be involved in osmoregulation to counteract Na<sup>+</sup> accumulation in tubers up to a certain salinity level. A similar increase in glucose from control to 7.5 dS m<sup>-1</sup>, then decreasing at 12.5 dS m<sup>-1</sup>, was reported for leaves of Olea europaea (Petridis et al., 2012, in Dias et al. 2016).



Inulin-type fructans and glucose tended to increase with salinity from 50 to 60% DW (inulin) and from 6 to 12 mg g<sup>-1</sup> DW (glucose), while sucrose and total sugars decreased significantly and linearly as salinity increased (Fig. 5). Results indicate that sucrose (5-10% of tuber DW) might have been used to maintain cell osmoticum or decreased due to reduced sucrose biosynthesis in response to salinity.

Figure 4. Second order regression for the <sup>o</sup>Brix of tubers in response to water salinity  $(EC_w)$  levels of mixed low-salinity water and high-salinity water. \*\* Significant at p<0.01.



Figure 6 (Right). Oxygen radical absorbance capacity (ORAC) (A), total phenolics (TP) (B). 3 plants from each tank were combined into one replicate tank<sup>-1</sup>, n=3).

## 3.3. Total antioxidant capacity:

There was no salinity effect on total antioxidant capacity of tubers, by either ORAC or TP (Fig. 6). 1. Salinity affected shoot more than ORAC correlated highly with TP (r<sup>2</sup>=0.69\*\*) indicating tuber TP function as antioxidants. The increase in both Na<sup>+</sup> and Cl<sup>-</sup> in tubers seemed to have triggered biochemical responses that increased 2. Plants produced 83 tons of tubers tuber antioxidants in cell vacuoles, but decreased sugars in the cytosol (e.g., sucrose, Fig. 5E). Interestingly, the correlation between TP vs. Na<sup>+</sup>  $(r^2=0.83^{***})$  and  $Cl^ (r^2=0.71^{***})$ , and between ORAC vs. Na<sup>+</sup> ( $r^2=0.69^{***}$ ) and Cl<sup>-</sup> ( $r^2=0.54^{*}$ ) in tubers was significant at the salinity range 3.9 - 9.3 dS m<sup>-1</sup>, where both plant growth and shoot Cl<sup>-</sup> 3. Inulin degree of polymerization remained stable (Fig. 3B). These results suggest that at this salinity range, the mechanism by which plants sustained growth and biomass accumulation involved non-enzymatic antioxidants in addition to 4. Tuber nutritional value (not osmotic balance provided by sugars.

Source: Dias et al. 2016. Jerusalem artichoke (Helianthus tuberosus. L.) maintains high inulin, tuber vield, and antioxidant capacity under moderately-saline irrigation 5. Chloride, not sodium, was the waters. Industrial Crops and Products 94:10009-1024.



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Figure 5 (Left). Tuber concentrations of fructans (A), degree of polymerization (DP) (B), free fructose (C), free glucose (D), free sucrose (E), and total sugars (F) from plants irrigated with waters of different electrical conductivities (EC<sub>w</sub>). ns = not significant.\*\*,\*significant at  $p \le 0.01$ , and at  $p \le 0.05$ , respectively.



- tuber vield (67% vs. 47%) at ECw = 12 dS/m, but reduced tuber yield
- in only 11% at ECw=6.6 dS/m.
- ha<sup>-1</sup> (42 tons of inulin ha<sup>-1</sup>) at ECw=6.6 dS m<sup>-1</sup> (moderate salinity), and 92 tons of tubers ha-<sup>1</sup> (46 tons inulin ha<sup>-1</sup>) at control salinity (ECw=1.2 dS m<sup>-1</sup>). Tubers had 5-10% sucrose, 50-60% inulin.
- (DP) of 'Stampede' was low and unchanged by salinity, and is suitable for biofuel production.
- presented) and antioxidant capacity was maintained at all salinity levels.
- most toxic ion for the crop.
- 6. 'Stampede' can be irrigated with waters of low-moderate salinity, completing its cycle in 4 months.