Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Jerusalem artichoke (Helianthus tuberosus, L.) maintains high inulin, tuber yield, and antioxidant capacity under moderately-saline irrigation waters

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ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form 2 September 2016
Accepted 12 September 2016

Keywords:
Sequential management strategy
ORAC
Total phenolic content
Fructan
Irrigation water use
Biofuel crop

ABSTRACT

Information on management strategies and alternative crops adaptable to saline waters is scarce. We investigated the effects of high-salinity water (HSW) blended or sequentially applied with low-salinity water (LSW) on growth, mineral nutrients, and tuber biochemistry of Jerusalem artichoke (*Helianthus tuberosus*, L. cv. 'Stampede'). Plants were irrigated with blended waters of electrical conductivity (EC_w) ranging from 1.2 dS m^{-1} (LSW control) to 12 dS m^{-1} (highest HSW treatment) or with LSW followed by hsw at set intervals (sequential management). Both growth and tuber yield were significantly reduced between LSW control (ECw = 1.2 dS m^{-1}) and 12 dS m^{-1}. An increase in salinity from 1.2 to 6.6 dS m^{-1} reduced shoot biomass by 37%, but tuber yield only by 11% showing that the plant can tolerate soil-water salinity (EC_{sw}) = 6.6 dS m^{-1}, while increasing salinity to 12 dS m^{-1} caused a 67% decrease in shoot and 47% decrease in tuber yield. Shoot biomass was similar for blended and sequential treatments of equivalent salinity. Tuber yield of sequential treatments was similar to control salinity if 75% of irrigations used LSW. 'Stampede' accumulated sodium in roots, but not in shoots. Chloride increased in all organs, mainly in leaves and roots, and high chloride, not sodium, accounted for decreased shoot and tuber biomass. In general, salinity had no effect on the concentrations of minerals, inulin-type fructans and their degree of polymerization, or on tuber antioxidants, but decreased tuber sucrose significantly. Tubers had 50–60% inulin-type fructans and less than 0.02% starch. 'Stampede' is an early cultivar adapted to moderate salinity (EC_w = 6.6 dS m^{-1}) producing, at 5.6 plants m^{-2}, 83 Mg ha^{-1} of tubers (41.5 Mg of inulin ha^{-1}) and, at control salinity, 92 Mg ha^{-1} (46 Mg of inulin ha^{-1}). The crop is a rich source of feedstock for biofuels or for non-caloric, prebiotic, soluble fiber for the food industry.

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1. Introduction

Helianthus tuberosus, L. (Asteraceae), also known as Jerusalem artichoke or sunchoke, is originally from North American being one of the few crops taken from the New World to Europe and Asia. Its tubers were an important source of food for Native Americans. Although the whole plant can be used as animal feed (Seiler and Campbell, 2004), and the tubers as food and feed, it has been mainly studied in the past 10 years as a biomass crop for ethanol production (Gunnarsson et al., 2014; Johansson et al., 2015) yielding 7 to 14 t ha^{-1} of carbohydrates (Denoroy, 1996). Its tubers accumulate inulin-type fructans (polymers of fructose molecules) to at least 50% DW (Danilcenko et al., 2008), 6–12% protein (Cieślik et al., 2011; Seiler, 1990), and amino acids (Danilcenko et al., 2013). Fructans with a short chain length are known as fructooligosaccharides – FOS. Inulin is a natural storage fructan carbohydrate present in over 36,000 plant species, including wheat, onion, bananas, garlic, asparagus, Jerusalem artichoke, and chicory. Because inulin is soluble in water, it is osmotically active. Plants can change the osmotic potential of cells by changing the degree of polymerization of inulin molecules through hydrolysis, without changing the total amount of carbohydrates. This osmotic regulation role helps plants tolerate cold and drought during winter periods (Boeckner et al., 2001). Inulins with both high and low degree of polymerization (DP), the latter referred to as FOS, can be used for biofuel production,

Abbreviations: lsw, low-salinity water; hsw, high-salinity water; EC_w, electrical conductivity of irrigation water; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.indcrop.2016.09.029
0926-6690/Published by Elsevier B.V.

nutritional purposes such as low caloric soluble dietary fiber (Al-Sheraji et al., 2013), and as prebiotics that stimulate the growth of probiotic gut bacteria, mediate sugar and lipid metabolism (Wada et al., 2005), and stimulate the immune system (Judprasong et al., 2011). Most of the inulin-type FOS commercially available today are extracted from chicory roots, which contain only 20% inulin (Baert and Van Bockstaele, 1993), or are synthesized from sucrose (Niness, 1999; Wada et al., 2005).

The scarcity of fresh water in semiarid regions is the main factor limiting the increase in irrigated cultivated area needed to feed a growing world population. This limited water supply forces many farmers to use non-conventional, or recycled, irrigation water that lowers the yield of agricultural crops and, in the long run, increases salinization of irrigated areas. Examples of recycled waters are brackish groundwater, saline drainage waters, municipal wastewater, and brine resulting from water desalination plants. Currently, feasible replacement of fresh water with saline water requires the selection of salt-tolerant crops with economically-viable yields according to the expected root-zone salinity, adequate drainage, and the application of a suitable irrigation water management strategy that can save fresh water. Salt tolerance is generally defined by the ratio of yield under saline conditions divided by the yield under non-saline conditions (Maas and Hoffman, 1977). Salt-tolerant crops are often lower yielding under non-saline conditions so crop selection must also consider absolute yield under the specific growing conditions. When saline water is abundant, and fresh water limited, the following management strategies can be used to reduce the effects of salinity on plants: saline waters used alone, blended with fresh water, or in sequence with fresh water (Malash et al., 2008; Medeiros et al., 2014; Nangare et al., 2013). The advantages of sequential use over blending has been established for crop rotations where one crop is salt sensitive and the other crop is more tolerant (Murtaza et al., 2006; Rhoades et al., 1989), but has not been tested in Jerusalem artichoke. Due to its reduced costs, saline water blended with fresh water can be a viable alternative to save fresh water while maintaining crop yield. Although this blended-water management strategy can enable crop irrigation at lower costs, it may increase the total salts retained by soils with poor drainage and result in soil and shallow groundwater salinization.

Salt tolerance differs among species and among cultivars of the same species. Also, in the same cultivar, tolerance may vary according to the plant phenological stage (del Amor et al., 1999). Jerusalem artichoke (*Helianthus tuberosus*, L.) is reported to be both a salt and drought-tolerant species that can adapt and grow in soils that are both saline and alkaline (Newton et al., 1991) and unsuitable for staple agricultural crops. The crop grows well in the pH range of 5.8–7.0, but growth is favored by slightly alkaline pH (Cosgrove et al., 2016). During two years of field trials in Spain, plants of the cultivar Nahodka that had 50% and 75% of their optimal water supply restricted during the first stage of growth, decreased their tuber yield by only 0–13% (Conde et al., 1991). However, most salinity research on Jerusalem artichoke has focused on short-term salt stress and most cultivars used were neither well characterized nor available commercially (Chen et al., 2011). Newton et al. (1991) assessed the salt tolerance of irrigated Jerusalem artichoke for its shoot and tuber biomass in both greenhouse and field trials. Salinity of irrigation water ranged from 0.7 to 12 dS m^{-1} in the greenhouse trial and from 0.2 to 10 dS m^{-1} in the field trial. Based on dry matter yield of tubers of greenhouse-grown plants and of shoots of greenhouse-grown and field-grown plants, their unidentified cultivar was classified as 'moderately sensitive' to salinity, according to Maas and Hoffman (Maas and Hoffman, 1977).

In the past decade, accumulated evidence suggests that abiotic stresses, including salinity, are associated with plant built-up of reactive oxygen species (ROS) that can induce stressed plants to increase enzymatic and non-enzymatic metabolites in an attempt to counteract excessive ROS and adapt to stress (Di Baccio et al., 2004). These authors showed that Jerusalem artichoke irrigated with 10% seawater (EC_w = 6.4 dS m^{-1}) significantly increased levels of ascorbate and glutathione in roots, and glutathione reductase in leaves and roots, while the levels of ascorbate reductase remained unchanged by salinity in both leaves and roots. Xue et al. (2008) reported that salt-stressed (150 $mmol_c$ L-1 NaCl – about 15 dS m^{-1}) reduced the activity of superoxide dismutase, peroxidase, and catalase. Malondialdehyde has also been described as an indicator of membrane damage caused by lipid peroxidation and used to differentiate salt-tolerant from salt-sensitive species (Chen et al., 2011). However, there is no published information on the effects of salinity stress on the concentration of sucrose and inulins, accumulation/decrease of non-enzymatic antioxidants (e.g. flavonoids and phenolics), and on the complete mineral nutrient status of Jerusalem artichoke shoots and tubers.

Our first objective was to relate salinity of irrigation waters to plant growth, shoot and tuber biomass accumulation, tuber inulin concentration, degree of inulin polymerization, fructose, sucrose, and glucose as sources of carbon for biofuels. Second, we aimed to identify salinity effect on the uptake of Na^+ and Cl^-, macro and micronutrients, and tuber antioxidant capacity. The third objective was to evaluate the use of high-salinity water (hsw) blended with low-salinity (tap) water (lsw) *vs*. sequential use of lsw followed by hsw.

2. Material and methods

The experiment was conducted at the U.S. Salinity Laboratory (USDA-ARS), Riverside, CA (Lat. 33.9°58′24″ N, Long. 117°19′12″ E and Alt. 311 m). The average maximum and minimum temperatures during the growing season (April 29 to July 18, 2014) were 29.8 and 15 °C, respectively. There was no rainfall during the period over which the experiment was conducted. Day lengths for the growing season changed from 13 h 29 min (April 29, planting) to 14 h 9 min (July 18, shoot biomass harvest), then to 12 h 46 min (September 4, tuber harvest) according to http://www.calendar-365.com/calendar/2014/April.html

2.1. Plant material and growth conditions

Whole tubers of Jerusalem artichoke (*Helianthus tuberosus,* L., cv. 'Stampede'), obtained from a commercial farm (Potato Garden, Austin, CO, USA), were homogenized for size and planted on April 29, 2014 in large (3.0 m long × 1.5 m wide × 2.0 m deep) outdoor tanks, part of an outdoor lysimeter system, filled with loamy sand and equipped with an automated irrigation system. This system enables complete recycling of the irrigation waters while maintaining stable root-zone salinity (Fig. 1). The system is contained to prevent environmental contamination while maintaining thermal and drainage properties similar to the ones found in sandy soils (Wang, 2002). The irrigated experimental plot had 24 tanks, each connected to irrigation reservoirs (3605 L) through ½ HP pumps that circulate the water inside reservoirs and irrigate the tanks in the corresponding outdoor sand tank (Fig. 1). Irrigation was done twice a week with treatment waters (nutrient/salt solutions) completely saturating and leaching the sand culture medium. In each tank, the nutrient/salt solution returned to the reservoir, after irrigation, through a subsurface drainage system at the bottom of the tanks, thus maintaining an relatively uniform and constant salinity in the root zone.

In each tank, tubers were planted 5-in. deep in two rows spaced 0.6 m apart, and 0.3 m apart within the rows. The loamy sand in the tanks was mixed with 10% peat moss (v/v) resulting in an average bulk density of 1380 kg m^{-3} and an average volumetric water + air

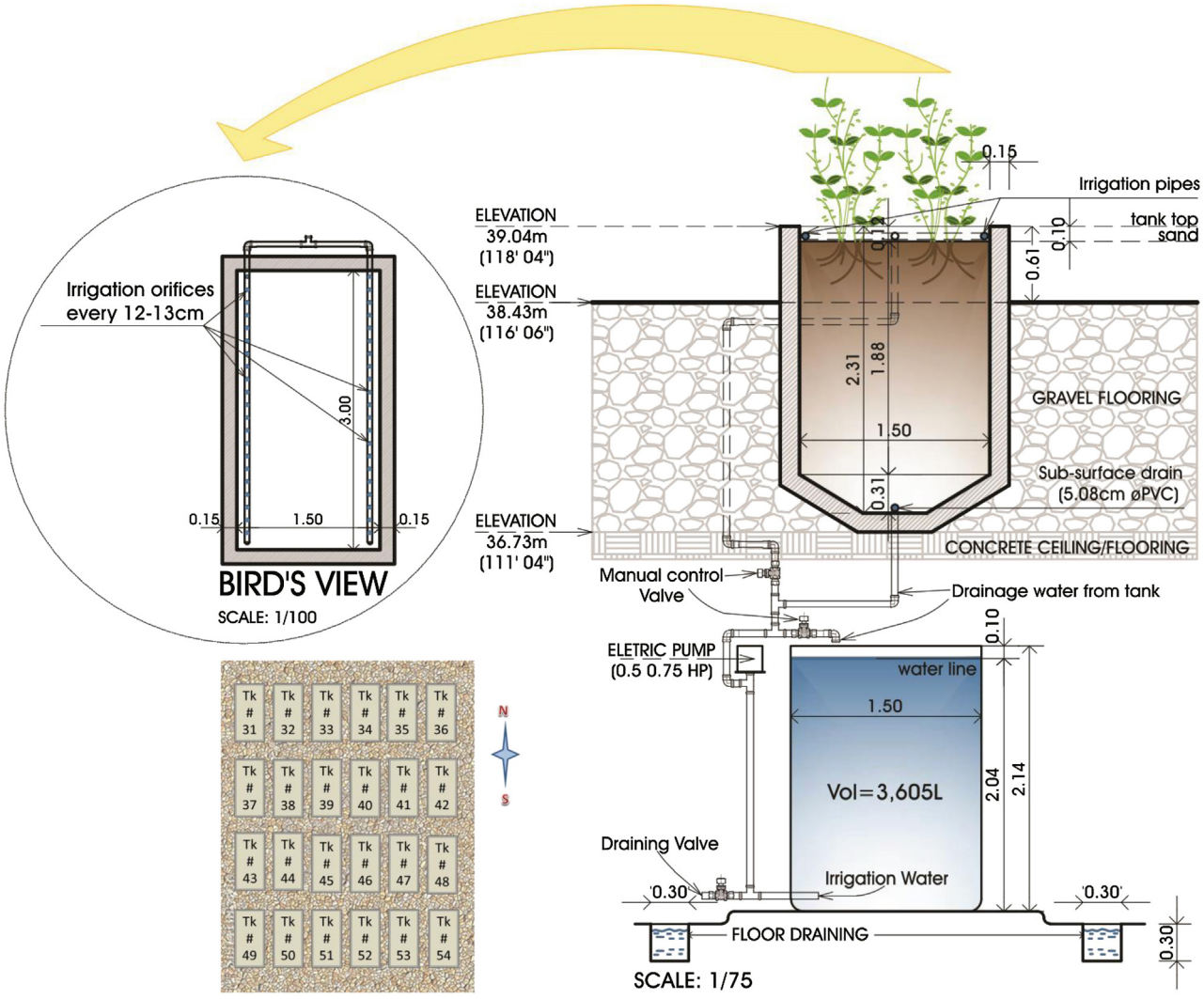

Fig. 1. Schematic drawing of the outdoor lysimeter system with 24 outdoor tanks connected through PVC pipes and an electric pumping system with 24 underground water reservoirs. The aerial view insert (lower left) shows tank area oriented N/S with four rows of six tanks each in a caged area of 418 m^2. Vol = filled volume of reservoir.

content of 0.30 $m^3\ m^{-3}$, determined by packing dry soil, weighing, saturating with water and reweighing. Before the treatments were applied, all plants received low-salinity water (lsw) consisting of Riverside municipal tap water with an electrical conductivity (EC_w) of 0.7 $dS\ m^{-1}$. After nutrients were added to this lsw, the final EC_w was 1.2 $dS\ m^{-1}$ (control salinity). Treatments with high-salinity water (hsw) were initiated 30 days after planting (DAP) on May 29, 2014.

2.2. Evaluation of growth and biomass accumulation

Parameters recorded at the vegetative cycle were leaf, stem, and total plant dry matter, plant height at 45, 55, 65, and 95 DAP, and leaf area at 60, 70, 80, and 110 DAP. Leaf area was calculated according to an equation created with previous measurements in a leaf area meter, correlated to length and width, and multiplied by a correction factor based on leaf shape. Water applications are specified in Table 1. To evaluate salinity effects on shoot biomass accumulation, three plants of each treatment and each replicate (or tank) were harvested 80DAP (July 18, 2014), at blooming. At the end of crop cycle (September 4, 2014, 128DAP), to determine salinity effect on tuber yield, three other plants per tank were harvested and measured for tuber yield per plant, per area, and percent of tuber soluble solids (°Brix). For shoot biomass measurements, each plant was divided into leaves, stems (main and secondary stems) roots and tubers. Shoot samples (stems + leaves) were dried at 72 °C to a constant weight (dry matter).

2.3. Saline water and management strategies

The tap water came from the Riverside, CA, municipal system (average EC_w = 0.7 $dS\ m^{-1}$ and pH = 7.5–8.0), while the hsw (EC_w = 12.0 $dS\ m^{-1}$) was achieved by adding Ca^{2+}, Mg^{2+}, Na^+, SO_4^{2-} and Cl^- to nutrient- enriched irrigation water at 29.2, 14.5, 85.8, 62.3 and 63.1 $mmol_c\ L^{-1}$, respectively. This nutrient-enriched water contained, on average, NO_3^- (5 $mmol_c\ L^{-1}$), K^+ (5 $mmol_c\ L^{-1}$), and P (0.2 $mmol_c\ L^{-1}$). At the highest salinity treatment, salt concentrations were equivalent to 1/4 seawater or to the reject saline water from small-scale water desalination system. Nutrient analyses and EC measurements (ORION conductivity meter, Model 120, Thermo Scientific Orion, Chelmsford, MA) were performed monthly and salinity was corrected with the pertinent salt if concentrations and EC_w were lower than intended for the treatment. Reduced EC_w resulted from nutrient consumption by the crop or from water addition to complete tank volume.

Salt and nutrient translocation to leaves was assessed by determining the concentrations of Na^+, Cl^-, the macronutrients N, P, K^+, Ca^{2+}, Mg^{2+}, and S, and the micronutrients Fe, Cu, Mn, and Zn in fully

Table 1
Treatment waters (tap water with added nutrients designated as low-salinity water, lsw), high-salinity water (hsw) alone or blended with lsw at specified ratios), management strategies (T1–T5, blended and T6–T8, sequential) compared to produce shoot[1] and tuber biomass, application of treatments, and electrical conductivity (EC_w) of treatment waters. The EC_w provided for sequential treatments is a calculated seasonal value.

Treatment	Treatment waters and management strategy	Application (started at)	EC_w (dS m^{-1})
T_1	lsw applied for all 20 irrigations (throughout)	30DAP	1.2
T_2	Mixture of 75% lsw + 25% hsw applied throughout	30 DAP	3.9
T_3	Mixture of 50% lsw + 50% hsw applied throughout	30 DAP	6.6
T_4	Mixture of 25% lsw + 75% hsw applied throughout	30 DAP	9.3
T_5	hsw applied throughout	30 DAP	12.0
T_6	[1]Sequential irrigation using 25% (5 irrigations) lsw, followed by 75% (15 irrigations) hsw[a]	53 DAP	9.3
T_7	[1]Sequential irrigation using 50% (10 irrigations) lsw, followed by 50% (10 irrigations) hsw[b]	71 DAP	6.6
T_8	[1]Sequential irrigation using 75% (15 irrigations) lsw followed by 25% (5 irrigations) hsw[c]	87 DAP	3.9

[1] Sequential irrigations as they applied to tuber yield (September 4 harvest, 128 DAP). However, for shoot biomass (July 18, 80 DAP), consider sequential management as follows:
[a] 38% (5 irrigations) with lsw followed by 62% (8 irrigations) with hsw (seasonal EC_w = 7.9 dS m^{-1}.
[b] 77% (10 irrigations) with lsw followed by 23% (3 irrigations) with hsw (seasonal EC_w = 3.7 dS m^{-1}.
[c] 100% (13 irrigations) with lsw followed by 0% (no irrigation) with hsw (seasonal EC_w = 1.2 dS m^{-1}). Blooming started 53 DAP and ended 99 DAP. DAP = days after planting.

developed leaves collected 80DAP, 50 days after treatment initiation. Leaves were collected from the upper third of the plants, dried at 60 °C for 48 h. After weighing, samples were finely ground. One subsample was analyzed for total N by combustion (Pyro-Cube, Elementar Corp). Subsamples were reacted with 0.1 N HNO_3 in 10% acetic acid and analyzed for chloride by amperometric titration in a digital chloridometer (Model 4425000, Labconco, Kansas City, USA). The concentrations of Na^+, P, K^+, Mg^{2+}, Ca^{2+}, total S, and micronutrients (Fe, Cu, Mn, Zn, and Mo) were determined from concentrated nitric acid digestions of the ground plant material (Ippolito and Barbarick, 2000). Sodium, macro and micronutrients were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES, 3300DV, Perkin-Elmer Corp., Waltham, MA, USA).

Two water management strategies were adopted: low-salinity water (LSW) blended with high-salinity water (hsw) at different ratios or LSW followed by HSW, sequentially, after 25%, 50%, or 75% of the irrigation events (20 irrigations total) were done with LSW. Blended LSW and HSW, or the sequential treatments, were used to irrigate plants after adding a basic nutrient solution at pH 7.5 and with EC_w = 1.2 dSm^{-1} of the following macronutrient composition (mg L^{-1}): monoammonium phosphate (MAP) = 75, calcium nitrate = 375, magnesium sulfate = 400, potassium nitrate = 250. Micronutrient concentrations were (mg L^{-1}): copper sulfate = 0.15, zinc sulfate = 0.2, manganese sulfate = 1.50, boric acid = 1.5, sodium molybdate (Na_2MoO_4. $2H_2O$) = 0.15, ammonium molybdate = 0.15 and Fe EDDHMA-6% Fe = 30. Because shoot nutrients were expected to drop drastically after August, once plants have completed most vegetative growth (Swanton and Cavers, 1989), half of the plants in the tank were collected on July 18 (80 DAP) to evaluate the effects of salinity on mineral nutrient and shoot biomass accumulation. As these plants were harvested right after irrigation event 13 (out of 20), the sequential water treatments had 0%, 23% and 62% of the irrigations with hsw rather than the 25%, 50%, and 75% for plants harvested for tuber yield, which received all 20 irrigation events. We measured crop water consumption for each tank by measuring the water volume to be replaced in each reservoir every 10–15 days, and approximately 14 h after the last irrigation. For the evaluation of water use related to shoot biomass, the percentages of the sequential treatment were calculated based on the number of irrigations and water use up to the time of shoot biomass harvest.

Irrigation waters containing nutrients/salts were reduced from pH 8.5 to 7.5 by addition of nitric acid after target EC_w was reached. The percentage of separate irrigations with lsw and hsw shown in Table 1 are only for the alternative management strategy (considering 20 irrigations applied from 30 DAP to 120 DAP) while for blended water management strategy, lsw and hsw were blended at the specified ratios to reach target ECs and applied throughout the experiment (20 irrigations). Plants were irrigated twice a week during experiment. However, after irrigation event 20 (120 DAP), plants received three small irrigations once a week in order to prevent any possible water stress up to the time of tuber harvest (128 and 129 DAP). These three irrigations were also considered for our water use calculations.

2.4. Tuber extraction for ORAC and total phenolics analyses

Tubers were harvested in the mornings of Sept. 4 and Sept. 5, 2014. Two to three tubers having 14–30 g in fresh weight were taken from each of three plants per tank and combined as one replicate and three tanks were sampled per treatment (n = 3). Tubers were immediately surface-cleaned using damp Kim wipes, sliced and placed in a deep-freezer at −80 °C. Frozen samples were freeze-dried at −52 to −55 °C in a Freeze Dry System (FreeZone 6, Labconco, Kansas City, MO) for 72 h, and ground in a Wiley mill to pass a 40-mesh (0.635 mm) screen.

Ground tubers (0.5 g) were mixed with 5 g of sand, and extracted by pressurized liquid extraction (ASE 350, Dionex Corp., Bannockburn, IL). The ASE 350 was set for static extraction: 5 min; flush: 100%; purge: 60-s cycle: 2; temperature: 80 °C; and 1500 psi (10,342 KPa). Samples were extracted with aqueous acetone (acetone:water:acetic acid = 70:29.5:0.5) for the hydrophilic fraction. The aqueous acetone extract of each sample was used for both oxygen radical absorbance capacity (ORAC) and total phenolic analysis.

The ORAC assay is based upon the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as [2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH)] (Prior et al., 2003). Samples were analyzed for their antioxidant capacity (ORAC) in triplicate in a 96 well plate using a FLUOstar OPTIMA (BMG LABTECH, Offenburg, Germany).

Total phenolics were assayed according to the Folin-Ciocalteu method (Singleton and Rossi, 1965; Slinkard and Singleton, 1977). The absorbance was read at 765 nm using a microplate spectrophotometer (xMark, BIO-RAD, Hercules, CA). Samples were analyzed in triplicate and their total phenol concentration was quantified against a gallic acid (Cat No. 398225, Sigma-Aldrich, St. Louis MO) standard curve.

2.5. Sugars and starch analyses

Freeze dried ground tubers (0.5 g) were weighed and extracted for soluble sugars in 4 mL of 80% (v/v) ethanol in a 80 °C water bath with shaking for 30 min. The extracts were agitated for 5 s using a vortex mixer and centrifuged for 7 min at ∼1000*g* using a bench centrifuge (HN-SII, IEC, Needham Heights, MA). The ethanol supernatant was decanted, and the samples were re-extracted three

more times as above. The pooled supernatant was brought to a fixed volume and used for the common sugar assays based on procedures described by Hendrix (1993) but modified to use glucose hexokinase reagent (TR15421, Thermo Scientific, Fisher Sci., Middletown, VA) to test glucose.

Glucose was assayed in triplicates in a 96-well plate, 20 μL (the volume was adjusted when dilution was needed) of ethanol supernatant was added into each well. The plate was incubated at 55 °C for about 10 min to evaporate ethanol; then, each well received 20 μL of high-purity (resistivity ~1 MΩ cm^{-1}) deionized water (dH_2O), covered with a sealing sticky film, and allowed to dissolve sugars for one hour. Glucose hexokinase reagent was added (150 μL) to each well. Every sample had its own blank prepared in triplicate with 150 μL of ddH_2O. The plate was covered with film and incubated at 37 °C for 5 min. Glucose concentration was quantified by comparison to a known glucose standard curve obtained from the same glucose hexokinase reaction. After the glucose assay, we performed a fructose assay by adding 20 μL of phosphoglucose isomerase (PGI) solution (0.5 EU for each well of sample) to each well (P5381, Sigma-Aldrich, St. Louis MO) prepared in 0.2 M HEPES buffer, pH 7.8, covered by a plastic film and incubated for 15 min at 37 °C to allow PGI to convert fructose into glucose. For sucrose assay, 20 μL of invertase (I4504, Sigma-Aldrich, St. Louis MO) solution (about 60 EU for each well of sample) prepared in 0.1 M citrate buffer at pH 6.0 was added into each well after glucose and fructose analyses. The plate was covered with a plastic film and incubated for 15 min at 37 °C for invertase to hydrolyze sucrose into fructose and glucose. For all sugar analyses, after incubation, plates were read at absorbance of 340 nm using a microplate spectrophotometer (xMark, BIO-RAD, Hercules, CA). The differences in absorbance before and after the additions of PGI solution and invertase solutions, respectively, were used for the calculation of fructose and sucrose concentrations based on ODs of mixed glucose, fructose and sucrose standards in a series of concentrations. Tuber total soluble solids (TSS) content (°Brix, %) was determined with a digital refractometer (MA871, Milwaukee Instruments, Rocky Mount, NC) from a composite sample of two tubers from each of three plants per treatment, and from each replicate (n = 9).

Starch analysis was done as previously described (Hendrix, 1993; Liu et al., 1999). The residues from sugar extraction were oven dried at 55 °C, resuspended in 3.0 mL of 2N KOH, and incubated in boiling water bath for one hour to gelatinize the starch. After cooling, 3.0 mL of 2N acetic acid was added to each sample to neutralize the KOH. Starch was then hydrolyzed to glucose using an amyloglucosidase (10115, Sigma-Aldrich) solution prepared in 50 mM of sodium acetate buffer at pH 4.5 (about 100 EU per sample). Starch hydrolysis was carried out at 55 °C in a water bath with overnight shaking for 17 h. The reaction was completed by boiling the sample tubes in a water bath for 3 min. The glucose released from the hydrolysis was used to determine starch concentration according to the above procedures for glucose assay.

For inulin analysis, freeze dried tubers (0.4 g) were ground and mixed with 5 g of sand, then extracted with hot water, under 1500 psi of compressed nitrogen in an accelerated solvent extraction system (ASE 350—Dionex-Thermo Fisher). The water extract was analyzed separately for free glucose, fructose, and sucrose according to the sugar analysis procedures described previously. Also, 0.25 mL of extract was mixed with 4.75 mL of 0.2 M HCl in test tubes and placed in a hot water bath at 97 °C for 60 min to hydrolyze inulin into fructose and glucose (Pekić et al., 1985; Saengkanuk et al., 2011). The solution was then neutralized by adding 5 mL of 0.2 M NaOH, and analyzed again for fructose, glucose and sucrose. Inulin (I) was calculated as I = k (F + G − f − g − 1.05s) as in previous reports (Baert, 1997; Monti et al., 2005; Saengkanuk et al., 2011), where k = 0.995 (correction factor) is recommended for unknown inulin DP (degree of polymerization); F + G, and total fructose + glucose content after acid hydrolysis; f, g, and s are free fructose, glucose, and sucrose contents. The average DP was calculated as DP = 1+ number of net fructose units per number of net glucose units (Baert, 1997; Saengkanuk et al., 2011).

2.6. Statistical analysis

The experiment was conducted in a randomized complete block design with eight treatments and three replicates (8 × 3 = 24 tanks) with three plants per replicate and six plants per tank. From six plants from each tank, three were harvested for shoot biomass evaluation 79DAP and three were harvested 128 DAP for tuber yield evaluation. Significant differences at $p \leq 0.05$ were determined for macro and micronutrients across salinity treatments including blended and sequential treatments at $P \leq 0.05$ using the Bonferroni multi-comparison method in GLM procedure of SAS (version 9.3, SAS Institute, Cary, N.C.). Correlations between parameters and their respective equations were done using the graph and statistics functions of Excel, the statistical package in Sigmaplot v11.0 or Pearson's correlation coeficient. Significance differences for ORAC, total phenolics, sugars, fructan and the related parameters were also analyzed only among the five blended salinities at $p \leq 0.05$ using the Bonferroni multi-comparison method in GLM procedure of SAS (version 9.3, SAS Institute, Cary, N.C.).

3. Results and discussion

3.1. Phenological aspects of 'Stampede' as related to salinity and tuber yield

'Stampede' tubers planted on April 29 generated multi-stemmed plants that flowered on June 20th (53 DAP), with anthesis around July 8th, blooming for 46 days (August 5, 99 DAP). When half of the plants were harvested to evaluate shoot biomass on July 18 (80 DAP), they had been blooming for 26 days. Plants irrigated with lsw (1.2 dS m^{-1}) were 1.5 m in height or taller, while plants irrigated with hsw (12 dS m^{-1}) were around 1.2 m in height. As plants bloomed 53 DAP, 'Stampede' could be classified either as an intermediate-flowering to long-day cultivar (Kays and Kultur, 2005) as we estimated the flowering induction photoperiod in southern California (Lat. 33.9°58′24″N) to have been 14 h:18 min (The longest summer day in 2014 was 14 h:25 min, corresponding to 17 days before flower buds were noticeable on June 20. Although flowering of this species can be substantially influenced by planting date, location has a lesser effect with short-day clones flowering when the inductive photoperiod is approximately 13 h:30 min. (mid-August in southern California)(Kays and Kultur, 2005).

Jerusalem artichoke plants form stolons (elongated thick roots) at early flowering; then tubers (the enlarged end of a stolon) develop faster after shoots start to wither. Our 'Stampede' plants already presented fairly well developed tubers at full bloom (July 18 harvest). Although stresses are said to accelerate senescence (Denoroy, 1996), salinity did not affect the timing for this cultivar to flower, to start, or to complete tuber development.

On September 4 (128 DAP, 29 days after blooming ended), we harvested the remaining (half) plants to evaluate the effect of salinity on tuber yield. At this time most leaves were desiccated, indicating that plant cycle was completed. No visual effect of salinity was noticed at the completion of the crop cycle. Thus, in southern California, 'Stampede' planted in late April will have fully formed tubers 128 DAP (4.2 months). Although, if planted a month earlier, this cultivar could generate more shoot biomass, it is unknown if tuber yield would be significantly increased, as tuber yield is related to blooming and photoperiod. As lower tuber yields are associated with excessive lateness of the cultivar related

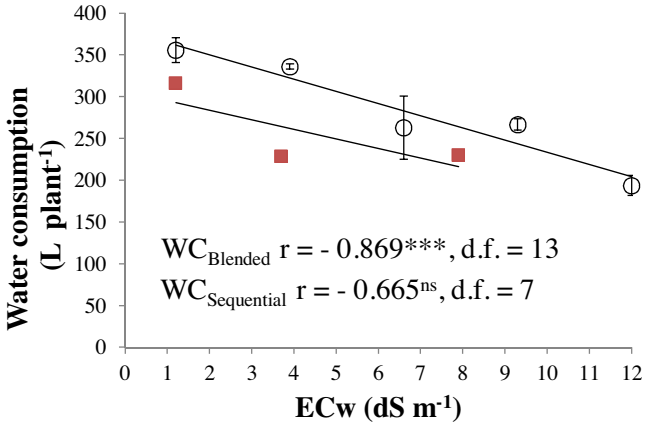

Fig. 2. Water consumption (WC) related to water management strategy, including blended waters (○) with electrical conductivity of irrigation water (EC_w) ranging from EC_w = 1.2 to EC_w = 12 dS m^{-1} and sequential irrigation waters ( ) of EC_w = 1.2 dS m^{-1} (100%$_{LSW}$ + 0%$_{HSW}$), 3.7 dS m^{-1} (77%$_{LSW}$ + 23%$_{HSW}$), and 7.9 dS m^{-1} (38%$_{LSW}$ + 62%$_{HSW}$). LSW = 1.2 dS m^{-1} and HSW = 12 dS m^{-1}. *** Significance for the Pearson's correlation coefficient for the pertinent degrees of freedom (n-2); ns not significant.

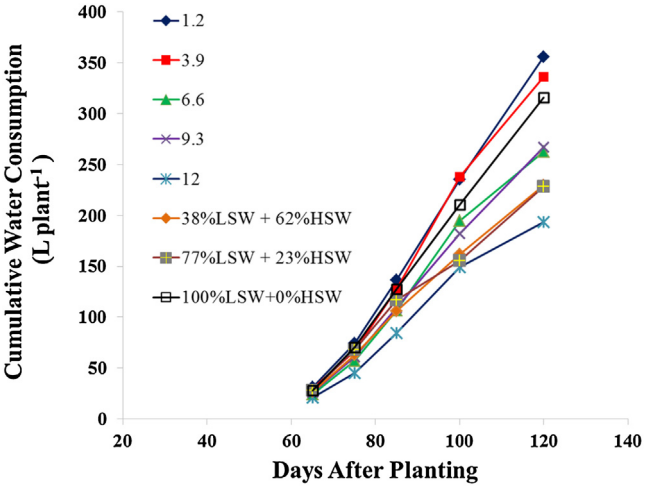

Fig. 3. Cumulative water consumption related to water management strategy, including blended waters with electrical conductivity of irrigation water (EC_w) ranging from EC_w = 1.2 to EC_w = 12 dS m^{-1} and sequential irrigation waters of EC_w = 1.2 dS m^{-1} (100%LSW + 0%HSW), 3.7 dS m^{-1} (77%LSW + 23%HSW), and 7.9 dS m^{-1} (38%LSW + 62%HSW). LSW = 1.2 dS m^{-1} and HSW = 12 dS m^{-1}.

to the local climate (Denoroy, 1996), an early cultivar such as 'Stampede' can achieve good tuber yield in southern California and other similar Mediterranean climates.

3.2. Water consumption

Total water consumption per plant decreased significantly with increased salinity for treatments with both blended (EC ranging from 1.2 to 12 dS m^{-1}) and sequential managements (Fig. 2). As expected, the sequential water use treatments had comparable water use to the control, up to the time irrigation water changed from lsw to hsw. The sequential water treatments had lower water use than the equivalent (corresponding EC averages) blended water treatments (Fig. 2). Regarding the cumulative water consumption over time, plants used more water as they grew (Fig. 3). When water consumption was separated in periods (45–65DAP, 66–75DAP, 76–85DAP, 86–100DAP, and 101–120DAP) water consumption increased with time in an approximately linear fashion

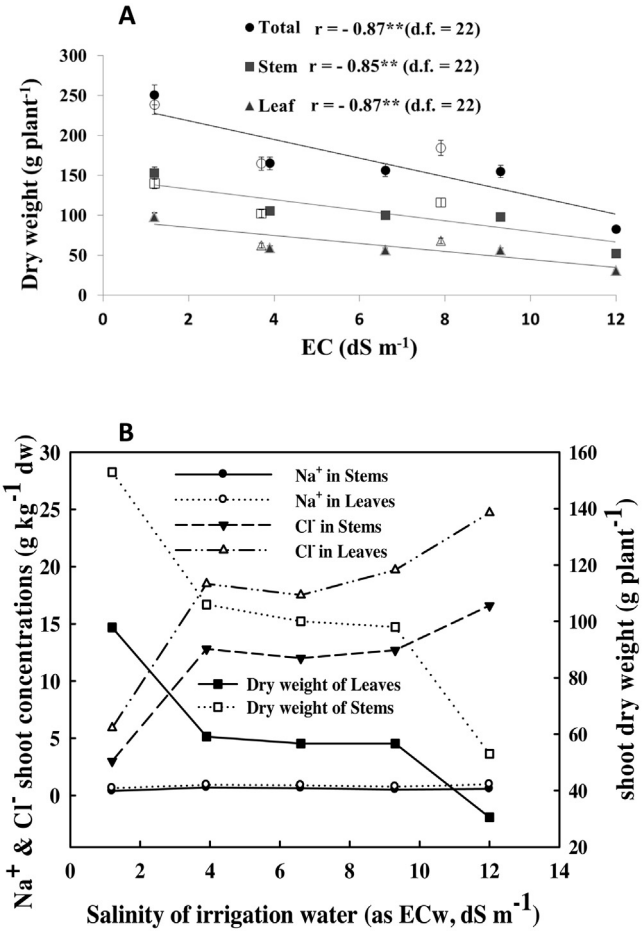

Fig. 4. A) Linear regression equations relating leaf (▲, WL), stem (■, WS) and total shoot dry matter (●, WT) of Jerusalem artichoke to salinity levels from blended LSW + HSW and sequential treatments of 100%LSW (EC_w = 1.2 dS m^{-1}), 77%LSW + 23%HSW (EC_w = 3.7 dS m^{-1}), and 38%lsw + 62%hsw (EC_w = 7.9 dS m^{-1}) for leaf (△), stem (□), and total shoot dry matter (○). ** Significance for the Pearson's correlation coefficient for 23 ◦ of freedom (n-2). B) The inverse response of stem and leaf dry weight to chloride tissue accumulation. Concentration of chloride (Cl^-) and sodium (Na^+) in stems and leaves (based on dry weight) of plants irrigated with blended saline waters.

for the control (EC_w = 1.2 dS m^{-1}), the blended water treatment with EC_w = 3.9 dS m^{-1}, and the sequential treatment using 100% lsw (EC = 1.2 dS m^{-1}). The remaining two sequential treatments using 38% lsw + 62% hsw (ECw = 3.7 dS m^{-1}) and 77% lsw + 23% hsw (ECw = 7.9 dS m^{-1}) had cumulative water consumption almost as low as the blended water treatment of ECw = 12 dS m^{-1}. This indicates that growth was impaired when lsw was replaced by hsw with ECw = 12 dS m^{-1} and that, even though plants adjusted and resumed growth, their growth (measured by the water consumption) was much inferior to that of plants with equivalent salinities provided by blended waters throughout the cycle.

3.3. Sodium and chloride concentration in shoots and roots

Based on the analyses of Na^+ and Cl^-, we determined that there were significant treatment effects only for the Na^+ concentration in Jerusalem artichoke roots and tubers. There was no effect of salinity in the concentration of Na^+ in either leaves or stems (Table 2, Fig. 4).

The concentration of Na^+ in Jerusalem artichoke roots and tubers increased with salinity of blended waters, but not with salinity of sequential treatments. Roots and tubers from sequential treatments had similar and non-significant Na^+ concentrations, and at levels similar to the ones found in tubers and roots of plants irri-

Table 2
Mean values for concentrations of macronutrients, sodium, and chloride in Jerusalem artichoke leaves, stems, roots, and tubers in response to electrical conductivity of waters (EC_w) of blended salinity and of sequential water management.

EC (dS m^{-1}) Blended	N Leaves	P	K^+	Ca^{2+} (g kg^{-1})	Mg^{2+}	Total-S	Na^+	Cl−
1.2	*40.2 ± 0.5a	2.6 ± 0.2a	42.9 ± 3.8a	*25.8 ± 4.9a	*5.1 ± 0.3a	*4.3 ± 0.9a	0.64 ± 0.2a	*5.9 ± 0.9e
3.9	*36.3 ± 2.4a	*2.5 ± 0.1a	36.4 ± 0.3a	*20.1 ± 0.6a	*5.0 ± 0.2a	*2.9 ± 0.2a	*0.92 ± 0.1a	*18.5 ± 1.1abcd
6.6	*41.1 ± 0.5a	*2.7 ± 0.1a	42.8 ± 5.6a	*26.3 ± 3.7a	*5.1 ± 0.3a	*3.4 ± 0.2a	0.89 ± 0.1a	*17.5 ± 1.1bcd
9.3	*38.6 ± 0.8a	*2.6 ± 0.2a	*44.1 ± 5.0a	*27.3 ± 5.7a	*5.3 ± 0.4a	*3.3 ± 0.1a	0.76 ± 0.3a	*19.7 ± 0.6abcd
12	*40.7 ± 1.2a	*2.7 ± 0.2a	37.4 ± 0.7a	*22.1 ± 2.6a	*6.1 ± 0.6a	*3.4 ± 0.2a	*0.97 ± 0.1a	*24.7 ± 1.1a
Sequential								
1.2 ($100_{LSW} \pm 0_{HSW}$)	*43.1 ± 2.6a	2.6 ± 0.3a	*40.5 ± 3.7a	*24.6 ± 4.0a	*5.4 ± 0.3a	4.8 ± 1.3a	0.54 ± 0.3a	12.0 ± 3.8de
3.7 ($77_{LSW} \pm 23_{HSW}$)	*38.4 ± 0.9a	*2.7 ± 0.1a	*45.0 ± 5.4a	*33.1 ± 5.7a	*5.3 ± 0.3a	7.3 ± 2.7a	0.76 ± 0.3a	*14.9 ± 1.9cd
7.9 ($38_{LSW} \pm 62_{HSW}$)	*39.0 ± 1.8a	2.4 ± 0.3a	46.7 ± 6.0a	*38.6 ± 5.7a	*5.5 ± 0.2a	*5.0 ± 1.5a	*0.89 ± 0.1a	22.6 ± 0.5ab
Blended	Stems							
1.2	13.4 ± 1.9a	1.3 ± 0.4a	26.9 ± 5.3a	6.6 ± 0.9a	1.3 ± 0.2a	0.72 ± 0.1a	0.39 ± 0.2a	3.0 ± 0.5b
3.9	11.4 ± 1.9a	1.0 ± 0.2a	29.6 ± 4.9a	5.9 ± 0.6a	1.2 ± 0.1a	0.72 ± 0.1a	0.70 ± 0.1a	12.8 ± 1.5ab
6.6	11.3 ± 0.7a	0.9 ± 0.1a	28.9 ± 3.1a	6.3 ± 0.7a	1.1 ± 0.1a	0.78 ± 0.1a	0.64 ± 0.1a	12.0 ± 1.3ab
9.3	14.6 ± 3.5a	1.0 ± 0.2a	26.4 ± 2.4a	7.2 ± 2.2a	1.5 ± 0.5a	0.91 ± 0.2a	0.52 ± 0.1a	12.7 ± 1.8ab
12	12.7 ± 0.9a	1.2 ± 0.1 a	31.5 ± 3.6a	6.6 ± 1.6a	1.8 ± 0.1a	0.89 ± 0.1a	0.58 ± 0.1a	16.6 ± 1.1a
Sequential								
1.2 ($100_{LSW} \pm 0_{HSW}$)	12.6 ± 0.1a	1.2 ± 0.1a	22.8 ± 0.9a	5.0 ± 0.4a	1.0 ± 0.1a	0.71 ± 0.1a	0.56 ± 0.1 a	2.7 ± 1.0b
3.7 ($77_{LSW} \pm 23_{HSW}$)	11.7 ± 0.7a	1.3 ± 0.1a	23.6 ± 1.0a	6.0 ± 0.6a	1.2 ± 0.1a	0.67 ± 0.1a	0.37 ± 0.1 a	9.1 ± 0.5ab
7.9 ($38_{LSW} \pm 62_{HSW}$)	11.4 ± 1.5a	1.5 ± 0.4a	28.3 ± 5.6a	7.1 ± 1.8a	1.2 ± 0.2a	0.67 ± 0.1a	0.59 ± 0.1 a	14.6 ± 4.0a
Blended	Roots							
1.2	13.8 ± 0.6a	*0.86 ± 0.1a	31.0 ± 2.8a	*9.2 ± 0.3a	*1.9 ± 0.1a	*2.4 ± 0.02c	*3.6 ± 0.3d	*3.31 ± 0.2d
3.9	11.1 ± 2.1a	*0.44 ± 0.5a	*21.8 ± 0.4ab	*7.6 ± 1.5a	*1.9 ± 0.3a	*3.7 ± 0.5bc	13.7 ± 4.3cd	*16.4 ± 1.3c
6.6	13.6 ± 1.6a	*0.34 ± 0.2a	24.1 ± 2.1ab	*7.3 ± 0.7a	*1.9 ± 0.2a	*5.1 ± 0.2b	*16.9 ± 0.1bcd	*17.3 ± 1.8c
9.3	13.3 ± 2.5a	*0.63 ± 0.4a	24.4 ± 1.8ab	*9.8 ± 0.8a	*2.3 ± 0.1a	*9.0 ± 0.4a	*34.0 ± 5.1a	*25.6 ± 2.4ab
12	9.5 ± 2.8a	0.56 ± 1.0a	*15.8 ± 2.3b	5.7 ± 2.1a	1.7 ± 0.1a	*6.4 ± 1.1ab	*27.9 ± 0.2abc	*20.1 ± 0.2abc
Sequential								
3.9 ($75_{LSW} \pm 25_{HSW}$)	*8.4 ± 1.0a	*0.81 ± 0.9a	*16.4 ± 0.1b	*10.3 ± 1.4a	2.4 ± 0.1a	*7.6 ± 0.9ab	*30.9 ± 2.6ab	*22.4 ± 1.8abc
6.6 ($50_{LSW} \pm 50_{HSW}$)	*8.8 ± 1.0a	*0.80 ± 0.3a	*18.7 ± 1.0b	*10.2 ± 1.1a	*2.5 ± 0.1a	*6.8 ± 0.3ab	*30.9 ± 0.7ab	*24.1 ± 1.7abc
9.3 ($25_{LSW} \pm 75_{HSW}$)	11.0 ± 2.0a	*0.57 ± 0.5a	20.9 ± 1.5b	*7.7 ± 0.3a	*2.5 ± 0.1a	*5.9 ± 0.5b	*29.9 ± 1.7ab	*26.8 ± 0.5a
Blended	Tubers							
1.2	13.4 ± 0.3a	2.2 ± 0.1a	24.2 ± 0.3a	1.1 ± 0.1a	1.0 ± 0.1a	1.2 ± 0.1d	0.4 ± 0.1d	2.3 ± 0.1d
3.9	12.9 ± 0.2a	1.9 ± 0.1a	25.3 ± 0.4a	1.2 ± 0.1a	1.0 ± 0.1a	1.6 ± 0.1dc	1.9 ± 0.1cd	6.3 ± 0.2cd
6.6	13.3 ± 1.1a	1.7 ± 0.2a	24.7 ± 1.5a	1.3 ± 0.1a	1.0 ± 0.1a	2.1 ± 0.3bcd	3.0 ± 0.2bcd	7.6 ± 0.6bcd
9.3	12.9 ± 0.3a	1.8 ± 0.2a	24.7 ± 1.3a	1.3 ± 0.1a	1.2 ± 0.1a	2.7 ± 0.3abc	6.2 ± 1.4ab	11.7 ± 1.4 ab
12	13.1 ± 0.2a	1.8 ± 0.1 a	25.0 ± 0.9a	1.6 ± 0.1a	1.5 ± 0.1a	3.4 ± 0.1a	8.4 ± 0.3a	13.5 ± 0.9a
Sequential								
3.9 ($75_{LSW} \pm 25_{HSW}$)	12.9 ± 0.3a	2.0 ± 0.2a	27.2 ± 0.5a	1.8 ± 0.4a	1.5 ± 0.2a	2.7 ± 0.3abc	5.1 ± 1.3abc	10.2 ± 1.5abc
6.6 ($50_{LSW} \pm 50_{HSW}$)	12.9 ± 0.2a	2.0 ± 0.1a	26.6 ± 0.4a	1.9 ± 0.1a	1.6 ± 0.1a	2.8 ± 0.3ab	6.1 ± 0.6abc	12.6 ± 1.1ab
9.3 ($25_{LSW} \pm 75_{HSW}$)	14.6 ± 0.4a	2.2 ± 0.1a	27.2 ± 1.8a	1.8 ± 0.1a	1.6 ± 0.2a	2.6 ± 0.3abc	6.0 ± 1.1abc	14.4 ± 1.2a

Values are means of three replications. In each organ group, values followed the same letter (a, b) are not significantly different at p = 0.05. *Indicates significant difference ($p < 0.05$) between values for the same nutrient between leaves and stems or between roots and tubers. LSW = 1.2 dS m^{-1}, HSW = 12 dS m^{-1}.

gated with blended waters of 9.3 and 12.0 dS m^{-1} 100% of the cycle (Table 2). Concentrations of root Na^+ were 3 to 9 times higher than in tubers, with differences decreasing as salinity increased. Concentration of Na^+ in tubers and roots were higher than in leaves and stems, but differences were more accentuated between roots and aerial organs, with Na^+ being 4 to 40 times more concentrated in roots than leaves and 6 to 60 more concentrated in roots than stems. Regarding sequential treatments, there was also significantly more (5 to 6 times) Na^+ in roots than in tubers (Table 2). There was a positive and significant correlation between Na^+ concentration in both roots and tubers and salinity levels of blended waters (Fig. 3). It is clear that roots of 'Stampede' accumulate more Na^+ than any other organ, including tubers. The low levels of Na^+ in stems and leaves suggest the existence of a Na^+ exclusion or efflux mechanism that prevents its transport to (or accumulation in) shoots. Similarity, the levels of Na^+ among sequential treatments indicates that, regardless of how soon or later irrigation occurs, roots will readily uptake Na^+ to the highest level of that specific organ. Thus, regardless of the same high Na^+ concentration in waters of 12 dS m^{-1}, delivered through 100% of the crop cycle or as in the HSW of the sequential treatments, concentrations of Na^+ in each organ were similar at the highest levels of salinity (Table 2). Data suggests that Na^+ concentration can double in roots and tubers when salinity increases above 6.6 dS m^{-1} in blended waters and that using sequential irrigation will not change that. Although tuber Na^+ was higher than that of leaves and stems, it was 1/3 to 1/9 that of roots, suggesting that tubers have some mechanism for exclusion of Na^+ as they differentiate from roots.

Regarding the levels of Cl^-, there was significantly more Cl^- in leaves from plants irrigated with EC_w = 3.9 dS m^{-1} and higher than with 1.2 dS m^{-1}, and its concentration in roots was significantly higher (twice as high in average) than in tubers, but similar between roots and leaves. Levels of Cl^- between stems and tubers were also similar (Table 2).

A similar exclusion mechanism as that found for Na^+ was also observed between tubers and roots for Cl^- as tubers had on average half the Cl^- concentration of the roots (Table 2). However, Cl^- concentrations increased in all organs with salinity, being higher in leaves and roots (Table 2). In addition, after root Na^+ and Cl^- reached a certain average level (28–31 g kg^{-1} and 20–27 g kg^{-1}, respectively), their concentrations in roots tended to stabilize indicating that the roots will try to exclude Na^+ and Cl^- after a certain concentration has been reached (Table 2). Accumulation of Na^+ had a poor correlation with salinity in leaves and stems reflecting the low Na^+ concentration in these organs, despite salinity increase.

Our results showing increased Na^+ uptake and accumulation in Jerusalem artichoke with increased salinity agree with similar results reported for a number of other species, such as *Helianthus annum* (Ashraf and Tufail, 1995) and *Atriplex patula* (Ungar, 1996), both Asteraceae as Jerusalem artichoke, but also in *Cucumis melo*

(del Amor et al., 1999), *Capsicum anuum* (Aktas et al., 2006a; Savvas et al., 2007), and cherry tomato (*Lycopersicon sculentum* var. *cerasiforme*) (Maggio et al., 2007). However, our results differ from those of others (Long et al., 2009) who reported that Na^+ increased with salinity in all organs of five cultivars of Jerusalem artichoke, indicating that 'Stampede' might cope with salinity by sequestering Na^+ in roots and keeping it out of leaves.

Chloride concentrations increased significantly with salinity in all organs, being higher in leaves and roots, but without reaching a maximum in leaves, stems, and tubers at the maximum salinity tested, indicating that these organs reach toxic levels of Cl^- at salinity higher than $EC_w = 12\ dS\ m^{-1}$ (Table 2). In salt-sensitive plants Na^+ and/or Cl^- is transported to aerial parts and accumulate in leaves resulting in visual toxicity symptoms (e.g., Cl^- in strawberry leaves). Our results also agree with those of Newton et al. (1991) who reported a linear increase in Cl^-, but not Na^+, in all Jerusalem artichoke organs with increasing salinity.

3.4. Organ accumulation of macro and micronutrients

There was no significant effect of sequential water management, or of salinity, on the concentrations of any macro or micronutrient in any organ analyzed, except for a significant decrease of K^+ in roots and a significant increase of Zn in leaves (Tables 2, A1). Regarding salts, concentration of Na^+ increased in roots and tubers, and Cl^- significantly increased in every organ as salinity increased. However, plants maintained adequate concentrations of all macro and micronutrients in roots, stems, and leaves and no antagonism between Na^+ and K^+ or Cl^- and NO_3^- (measured as N) was observed. Results suggest that reduced growth and tuber yield was due to higher Cl^- tissue levels rather than to the lack of nutrients. Plants also accumulated large concentrations of Na^+ in the roots, followed by tubers, but not in shoots.

Sodium sequestration in roots (major sink) allowed the plant to maintain high ratios of K^+/Na^+ and Ca^{2+}/Na^+ (and ratios of Na^+/Ca^{2+} lower than 4.0) in leaves and stems (Table 2), and to avoid imbalances in macro and micronutrients needed for plant growth and development. The concentrations of macro and micronutrients in organs of Jerusalem artichoke indicate that leaves are the major above-ground sink for N, K^+, Ca^{2+}, and Mg^{2+}, while roots are the main underground sink for Ca^{2+} and S (Table 2). In fact, past research indicated that both leaves and stems can act as major aboveground sinks of nutrients in Jerusalem artichoke, but that these nutrients are relocated to tubers (rather than to seeds) to assure proper formation and nutritional reserve of tubers as clonal propagation organs (Swanton and Cavers, 1989).

The ability of plants to maintain high levels of K^+ and Ca^{+2} in shoots, while keeping shoot Na^+ levels low, is one of the key mechanisms contributing to expression of high salt tolerance (Aktas et al., 2006a; Yoshida, 2002). In addition, K^+ salts (except KCl) applied to leaves of salt-stressed sunflower (*Helianthus annuus*) improved growth and photosynthesis (Akram et al., 2009). However, although salt-tolerant accessions of sunflower accumulated less Cl^- and more K^+ in leaves than salt-sensitive accessions under salt-stress conditions (Ashraf and Tufail, 1995), 'Stampede' accumulated high concentrations of Cl^- in leaves without any corresponding decrease in macro or micronutrients. While there is no published information regarding the genetic variability of Jerusalem artichoke to salt tolerance or the role of K^+/Na^+ ratio in identification of salt-tolerant genotypes, salinity stress ($12\ dS\ m^{-1}$) applied to 64 genotypes of safflower (*Carthamus tinctorium*, Asteraceae) reduced capitula per plant and seeds per capitulum in 25 and 21%, respectively, but only reduced the weight of 1000 seeds by 10% (Yeilaghi et al., 2015). Both the low leaf Na^+ and high K^+/Na^+ ratio of safflower reported by those authors are in agreement with our results.

Regarding average micronutrient composition (in $mg\ kg^{-1}$), there was no salinity effect (for blended or sequential treatments) for Fe (ranged from 157 to 291 in leaves, 23–52 in stems, 426–893 in root, and 61–95 in tubers), Cu (8.4–11.5 in leaves, 3.0–4.5 in stems, 7.0–14.5 in roots, and 1.6–3.7 in tubers), Mn (63.5–79.0 for leaves, 6.5–10.3 for stems, 23–46 for roots, and 3.5–4.9 for tubers), and Zn (26–35 for stems, 32.6–57.4 for roots, and 16–19 for tubers). However, leaf Zn levels increased significantly (157%) between control and the highest salinity level, without reaching a toxic level to the plant and ranging from $63.3\ mg\ kg^{-1}$ (control) to $162\ mg\ kg^{-1}$ at $ECw = 12\ dS\ m^{-1}$ (Table A1). Concentrations of Zn in leaves had a positive and highly significant (at $P < 0.01$) correlation ($R^2 = 0.956^{**}$) with salinity. In salinity stressed pepper plants, when Zn was added to a Zn-deficient soil, shoot Zn content was linked to lower shoot Na^+ concentrations and better plant growth, compared to plants under Zn deficiency (Aktas et al., 2006b). Recently, proper Zn nutrition was also reported to keep Na^+ levels down and to improve reactive oxygen scavenging in shoots of wheat seedlings under salinity stress (Xu et al., 2014). In our plants, elevated leaf Zn did not reach toxic levels but did not lower leaf Na^+. Zn is an important cofactor of some proteins and antioxidant enzymes (e.g., Cu/Zn superoxide dismutase or SOD, and zinc-finger proteins) that dismutate superoxide radicals into H_2O_2 and oxygen. Superoxide radicals are toxic by-products of oxidative metabolism and are produced in excess during abiotic stresses, such as salinity. Lee et al. (2001) hypothesized that salt stress may induce more severe oxidative stress in leaves than in roots, and salt stress in arabidopsis significantly increased the expression of the zinc-finger protein Zat12 in leaves while Zat12-knock-out mutants had their root growth significantly decreased under salinity stress (Davletova et al., 2005). In our case, considering that Na^+ was low and K^+/Na^+ ratio high in leaves, increased leaf Zn could have resulted from oxidative stress triggered by Cl^- accumulation in leaves.

In general, there was no difference in the concentrations of macro or micronutrients between blended and sequential treatments, and levels of nutrients in sequential treatments (when higher than the control) were similar to those of the blended water treatments of $9.3\ dS\ m^{-1}$.

Pessarakli and Tucker (1988) reported an insufficient absorption of N (70% less) by tomato plants irrigated with high-NaCl nutrient solution, indicating antagonistic absorption between NO_3^- and Cl^- or depolarization of the cell membrane by Na^+ (Suhayda et al., 1990). However, our results with *H. tuberosus* do not corroborate their results as leaf N remained unchanged in all organs (Table 2), but agree with those of Semiz et al. (2013) who reported that the concentrations of N and other macronutrients in pepper plants remained high in all organs despite increasing salinity.

3.5. Growth parameters

Plant response to salinity is generally described in terms of relative yield as a function of root-zone salinity, expressed as electrical conductivity of the solution (EC_s) in contact with the roots (Maas and Hoffman, 1977), or EC_w in this work. Statistically-significant decreases in leaf, stem, and total shoot (leaf + stems) dry matter, relative to the control, were found only at the highest salinity level ($EC_w = 12.0\ dS\ m^{-1}$) of blended waters, while all parameters had similar values at the intermediate blended water treatments with EC_w ranging from 3.9 to $9.3\ dS\ m^{-1}$ (Table 3). Because the harvest of shoot biomass occurred just before irrigation 14, only the first 13 irrigations were considered for evaluation of shoot biomass. The proportions of water for the sequential water use pertinent to shoot biomass accumulation are thus 100% LSW + 0% HSW, 77% LSW + 23% HSW, and 38% LSW + 62% HSW, with corresponding average salinities with EC_w of 1.2, 3.7 and $7.9\ dS\ m^{-1}$, respectively.

Table 3
Effect of water management strategy and salinity on average leaf, stem and total dry matter of Jerusalem artichoke. Treatments were blended waters from 1.2 to 12 dS m^{-1} or sequential irrigation management with low-salinity water (lsw, EC_w = 1.2 dS m^{-1}) followed by high-salinity water (hsw, EC_w = 12 dS m^{-1}) for irrigation. Sequential management consisted of changing water from lsw to hsw after plants were irrigated with lsw water for 100%, 77%, or 38% of total irrigations.

Treatment (EC_w in dS m^{-1})		Average shoot dry matter (g $plant^{-1}$)			
		Leaf	Stem (treatment average)		Total DM (Mg/ha)
1.2		98 a	153 a	251 a	13.9 a
3.9		59 ab	106 ab	165 ab	9.2 ab
6.6		57 ab	100 ab	157 ab	8.7 ab
9.3		57 ab	98 ab	155 ab	8.6 ab
12		30 b	53 b	83 b	4.6 b
	Seasonal EC_w				
100% LSW + 0%HSW	1.2	99 a	140 a	240 a	13.3 a
77% LSW + 23%HSW	3.7	63 ab	102 ab	165 ab	9.2 ab
38% LSW + 62%HSW	7.9	68 ab	116 ab	185 ab	10.3 ab
LSD		*47*	*84*	*128*	*7.2*

**,* significantly at P < 0.01 and 0.05, respectively. Values are means of three replications. In each column, values followed the same low-case letter are not significantly different at p = 0.05. LSD = least standard deviation.

For shoot biomass, only the sequential treatment 100% LSW + 0% HSW (EC_w = 1.2 dS m^{-1}) was significantly higher than the blended treatment of EC_w = 12 dS m^{-1} (Table 3). Thus, for shoot biomass accumulation, there was no advantage of using LSW followed by HSW, sequentially, as compared to blended waters of similar salinity.

The decreases in shoot parameters were similar with a decrease in dry matter of 35% when salinity increased from 1.2 to 3.9 dS m^{-1} and with an average decrease of 67% when salinity of blended waters increased from 1.2 to 12 dS m^{-1} (Table 3). Results indicate that Jerusalem artichoke is sensitive to salinity for at least 77% of its vegetative stage and that there was no advantage in sequential irrigation when up to 77% of the vegetative stage was irrigated with lsw. However, after total shoot biomass dropped 34% from control salinity levels, shoot biomass was maintained at similar levels with salinities of irrigation water up to 9.3 dS m^{-1}. In a field situation, this irrigation water salinity threshold could be as low as 4.5 dS m^{-1} depending on the leaching regime employed.

Although significant differences for leaf, stem, and total shoot dry matter were only found between controls for blended (EC_w = 1.2 dS m^{-1}) and sequential 100% lsw + 0% hsw and the highest salinity blended treatment, the Pearson correlation coefficients (d.f. = 22) for all three parameters clearly show that salinity had a significant and negative impact on shoot biomass accumulation of Jerusalem artichoke (Fig. 4A).

Our results agree with those of previous studies that report that salinity reduced Jerusalem artichoke photosynthetic capacity and carbohydrate synthesis, leading to a decrease in plant dry matter accumulation (Conde et al., 1991; Maggio et al., 2007; Xiao-Hua et al., 2011), but did not specify which ion was causing these reductions. Our work with 'Stampede' strongly suggests that the decrease in total shoot (leaves + stems) dry matter was caused by Cl^-, not Na^+ (Fig. 4B). This figure shows an inverse relation between the concentrations of Cl^- in shoots and shoot dry weight, while Na^+ remained unchanged in both shoot and stem (Fig. 4B). In addition, our data clearly show that the shoots have some mechanism of Cl^- control at salinities ranging from 3.9 to 9.3 dS m^{-1}, as Cl^- remained stable in shoots and shoot dry matter yield was maintained until salinity increased to EC_w = 12 dS m^{-1} (Fig. 4B, Table 2).

Plant height was significantly reduced for all time periods, mainly between the LSW control (1.2 dS m^{-1}) and the HSW treatments of EC_w of 9.3 and 12 dS m^{-1}, with average reductions of 21% and 36%, respectively (Table 4). Although there was no significant difference in plant height at any time period between sequential treatments and blended treatments of similar salinities (seasonal calculated salinity), average plant heights for plants irrigated 100% of the cycle with waters of 9.3 dS m^{-1} were always shorter than for plants irrigated with the sequential treatment with average EC 7.9 (38% LSW + 62% HSW). This indicates that, at early developmental stages, plant growth was more vigorous if the first 40% of the irrigations were done with LSW (1.2 dS m^{-1}), before switching irrigation to HSW.

The lack of significant increase in height between 65 DAP and 95 DAP indicates that plants had reached their maximum heights at 65 DAP, or anywhere in between 55 DAP and 65 DAP. This can be explained by the fact that plants were 13 days into the blooming stage (started at 53 DAP) and growth slows down or stops shortly after blooming in herbaceous plants (Table 4). Thus, plants cultivated for shoot biomass (destined for biofuels or animal fodder) should be harvested before or at the onset of flowering.

The same trend observed for plant height was observed for stem diameter (data not presented) and leaf area at all evaluation dates, both significantly affected by salinity. There was also no difference among sequential treatments or between sequential and blended with similar salinity for leaf area. For all measurement points (60 to 110 DAP, there was an average leaf area reduction of 61.5% between the low- and high-salinity water treatments (Table 5). Leaf area for the management strategy 100% lsw + 0% hsw was within 15% of the average leaf areas of plants cultivated with low-salinity control applied 100% of the crop cycle as expected, as both treatments had the same salinity. Our results on leaf area reduction with salinity agree with those reported for *Amaranthus tricolor* cultivated under salinity stress (Wang and Nii, 2000). Also, Medeiros and coworkers (2014) reported that the leaf area of melon plants was reduced by irrigation with high-salinity water at the initial growth stage, due to the highest sensitivity of melon plants to salt at this phenological stage. Also, they reported that when salinity increased from 0.6 to 4.8 dS m^{-1}, from the onset of flowering to harvest, leaf area was not affected by salinity. However, we recorded leaf area reductions due to salinity increase at every stage and data collection date (Table 5).

In general, all the shoot growth data suffered significant reduction in response to salinity, mainly seen between control and the high salinity treatment, with some to no significant difference at the salinities of 3.9, 6.6, and 9.3 dS m^{-1}. We believe that the fairly similar data for all shoot growth parameters at those salinities relate to the Cl^- tolerance mechanism discussed above for shoot dry mater (Fig. 4B). This indicates that this plant, if submitted to saline irrigation, can produce similar shoot biomass at ECw ranging from 4 to 9 dS m^{-1}.

3.6. Tuber yield

Tuber yield (Table 6) was reduced by salinity of blended waters, similarly to the effects on height, leaf area, and stem diameter.

Table 4
Effect of salinity and water management strategies on Jerusalem artichoke plant height at 45, 55, 65 and 95 days after planting (DAP). Treatments were blended waters from 1.2 to 12 dS m^{-1} or sequential irrigation management with low-salinity water (LSW, EC_w = 1.2 dS m^{-1}) followed by high-salinity water (HSW, EC_w = 12 dS m^{-1}) for irrigation. Sequential management consisted of changing water from LSW to HSW after plants were irrigated with LSW water for 100%, 77%, or 38% of total irrigations.

Treatments		Average plant height (cm)			
(EC_w in dS m^{-1})		45 DAP	55 DAP	65 DAP	95 DAP
1.2		84.7 a	138.4 a	154.7 a	156.1 a
3.9		73.9 a	130.2 ab	149.9 a	148.4 ab
6.6		61.3 ab	117.2 ab	139.8 ab	140.3 abc
9.3		62.7 ab	109.6 bc	127.8 b	126.7 c
12		49.1 b	85.6 c	109.0 c	107.0 d
	Seasonal EC_w				
100%LSW+0%HSW	1.2	80.0 a	133.4 ab	143.4 a	140.6 abc
77%LSW+23%HSW	3.7	72.2 ab	127.3 ab	140.6 ab	139.4 abc
38%LSW+62%HSW	7.9	77.0 a	128.7 ab	139.8 ab	136.7 bc
LSD		24.4	24.3	15.1	17.9
Pearson's c.c. (r)		***0.94*****	***0.97*****	***0.98*****	***0.98*****

Values are means of three replications. Values followed by the same letter (a, b) in columns are not significantly different for $p \geq 0.05$. ECw = Electrical conductivity of water. LSD = least standard deviation.

Table 5
Effect of water management strategy and water salinity on average leaf area per plant (n = 6) at 60, 70, 80 and 110 days after planting (DAP) for blended waters with EC_w ranging from 1.2 to 12 dS m^{-1} and for sequential treatments with calculated seasonal EC ranging from 1.2 to 7.9 dS m^{-1}. Treatments were blended waters from 1.2 to 12 dS m^{-1} or sequential irrigation management with low-salinity water (lsw, EC_w = 1.2 dS m^{-1}) followed by high-salinity water (hsw, EC_w = 12 dS m^{-1}) for irrigation. Sequential management consisted of changing water from lsw to hsw after plants were irrigated with lsw water for 100%, 77%, or 38% of total irrigations.

Treatments (EC_w in dS m^{-1})		Average leaf area (cm^2 $plant^{-1}$)			
		60 DAP	70 DAP	80 DAP	110 DAP
1.2		7363 a	7437 a.	7734 a	12749 a
3.9		4737 ab	6053 ab	7035 a	7296 ab
6.6		4183 ab	5446 ab	6360 ab	6389 ab
9.3		3890 ab	4940 ab	5003 ab	6050 ab
12		2579 b	2996 b	3172 b	4795 b
	Seasonal EC_w				
100%LSW + 0%HSW	1.2	6908 a	7442 a.	7120 a	10812 a
77%LSW + 23%HSW	3.7	7224 a	7335 ab	5324 ab	7870 ab
38%LSW + 62%HSW	7.9	3572 ab	4672 ab	5780 ab	6627 ab
LSD		*4235*	*4222*	*3711*	*6943*

** and * means significance at P < 0.01 and 0.05, respectively. Values are means of three replications. In each column, values followed the same letter (a, b) are not significantly different to p = 0.05. ECw = electrical conductivity of water. LSD = least standard deviation.

Table 6
Effect of water management strategy and water salinity on tuber yield in grams per plant and megagrams per hectare (Mg ha^{-1}), and total soluble solids (TSS as°Brix) of Jerusalem artichoke. Treatments were blended waters from 1.2 to 12 dS m^{-1} or sequential irrigation management with low-salinity water (LSW, EC_w = 1.2 dS m^{-1}) followed by high-salinity water (HSW, EC_w = 12 dS m^{-1}) for irrigation. Sequential management consisted of changing water from LSW to HSW after plants were irrigated with LSW water for 75%,50 %, or 25% of total irrigations.

Treatment (EC_w in dS m^{-1})		Average tuber yield and TSS		
		g $plant^{-1}$	Mg ha^{-1}	°Brix (%)
1.2		1663 ± 224	92 ± 12	24.0 cde
3.6		1486 ± 126	83 ± 7	29.4 ab
6.6		1488 ± 311	83 ± 17	31.4 a
9.3		1048 ± 139	58 ± 8	26.3 bcd
12		885 ± 187	49 ± 10	22.9 cde
	Seasonal EC_w			
75%LSW + 25%HSW	3.9	1719 ± 513	96 ± 28	27.1 abc
50%LSW + 50%HSW	6.6	1422 ± 357	79 ± 20	22.5 de
25%LSW + 75%HSW	9.3	1672 ± 102	93 ± 6	20.4 e

Values are means of three plants per tank and three replications (n = 9). In each column, values followed the same letter (a, b) are not significantly different to p = 0.05. No letter means no statistical significance. ECw = electrical conductivity of water. Crop yield per area (Mg/ha) was calculated based on a plant density of 55,556 plants per hectare.

Although reduction in tuber yield per plant was not significant due to the large plant-to-plant variability, there was a clear tendency of tuber yield to decrease with increased salinity with average reductions from control to EC_w = 3.9 and 6.6 dS m^{-1} (both by 11%), control to EC_w = 9.3 dS m^{-1} (37%) and to EC_w = 12 dS m^{-1} (47%). Regarding the sequential treatments, the average tuber yield (90 Mg ha^{-1}) obtained with sequential treatments was similar to that of the blended LSW control (92 Mg ha^{-1}), indicating that it is possible to save fresh water using the sequential irrigation strategy if the goal is tuber production (Table 6). Regression analysis showed that EC_w of the blended saline waters had a significant inverse linear correlation with tuber yield (r = −0.97**, d.f. = 14), which decreased as salinity increased (data not shown).

'Stampede' plants were at full bloom at the July 18 harvest (for shoot biomass evaluation) and tubers at the initial development stage. Once plants matured and shoots withered (September 4, 128 DAP), tubers were well developed and weighed on average 42 g each, although size was not uniform for this cultivar, regardless of salinity (Fig. 5).

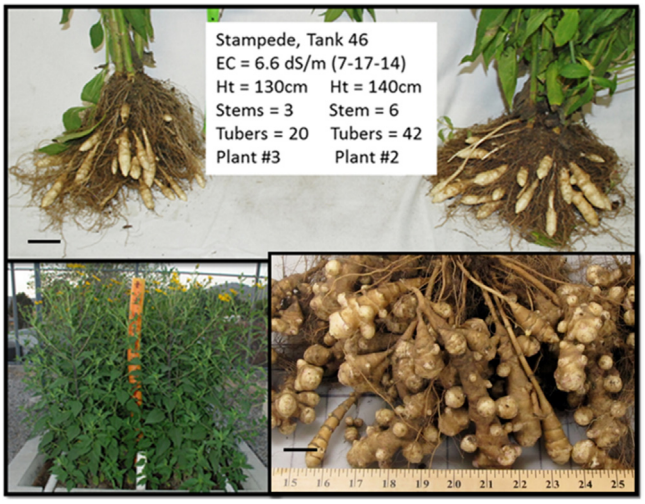

Fig. 5. 'stampede' plants irrigated with blended waters of $EC_w = 6.6\ dS\ m^{-1}$ and harvested on 7-18-2014 for shoot biomass evaluation. Top picture showing tuber development at 80 DAP of 2 of 3 plants harvested from same tank; bar = 5 cm. Canopy (bottom left) at full bloom. Right bottom, fully developed tubers of a plant from the same tank and treatment harvested on September 4 (128 DAP), weighing on average 1.4 kg/plant; bar = 2.54 cm.

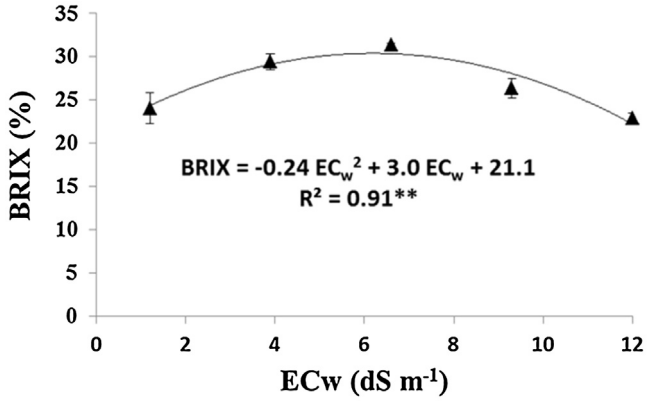

Fig. 6. Second order regression for the °Brix of tubers in response to water salinity (EC_w) levels from mixture of low-salinity water and high-salinity water. ** Significant at $p < 0.01$.

Newton et al. (1991) assessed the salt tolerance of irrigated Jerusalem artichokes (*Helianthus tuberosus,* L., unknown cv.) and classified it as moderately sensitive when cultivated on a heavy clay loam soil. Their relative tuber yield was expressed using the Maas and Hoffman (1977) salt tolerance equation: $Y = 100 - 9.62\ (EC_e - 0.4)$. The EC_e of the soil in our sand tanks can be calculated from the relationship $EC_e = 0.472\ x\ EC_{iw}$ (Cornacchione and Suarez, 2015). The relative tuber yield expression based on our data is thus $Y = 100 - 10.0\ (EC_e - 0.566)$, which is in remarkable agreement with Newton et al. (1991) from their field study (where it is more difficult to characterize the water uptake or mean soil EC_e). The EC_e at which the tuber yield of 'Stampede' would decrease by 50% is around 5.7, which is similar to the EC predicted by Newton et al. (ECe = 5.6), placing 'Stampede' in the moderately sensitive category of Maas and Hoffman (Maas and Hoffman, 1977).

Assuming that a loss of 25% in relative production is the loss that is economically acceptable, the relative tuber yield results suggest that EC_e should be below $3.1\ dS\ m^{-1}$. Very few papers compared water management strategies using blended saline waters *vs*. sequential applications (fresh water followed by highly saline water) for a single crop. Also, alternating (cyclic) use of saline water from drainage (average $EC_w = 4.5\ dS\ m^{-1}$) with fresh water ($EC_w = 0.6\ dS\ m^{-1}$) resulted in slightly lower tomato fruit yield than mixing both waters (Malash et al., 2008). These authors suggested that cyclical use of fresh with saline water may expose the plant to cyclic osmotic stresses, and concluded that exposing the tomato crop to one osmotic stress using blended water might allow the crop to adjust better to salinity stress than alternating saline water with fresh water. However, Medeiros et al. (2014) reported that melon irrigated with fresh water ($0.61\ dS\ m^{-1}$) for two days followed by saline water ($EC_w = 4.8\ dS\ m^{-1}$) for one day provided the highest fruit production, reducing by 33% the amount of good quality water in irrigation. In our study, we utilized sequential use with fresh followed by saline because published literature suggests that the initial crop stages are the most sensitive to salinity. In addition, we avoid the adverse effect of numerous changes in osmotic pressure (and possible osmotic shock) and the logistic problems of cyclic water use.

Although losses in shoot biomass were much greater with salinity (EC_w) levels of 3.9 (−34.3%), 6.6 (−37.5%), and 9.3 (−38%) $dS\ m^{-1}$, tuber biomass losses of only 11% were obtained with both $EC_w = 3.9$ and $EC_w = 6.6\ dS\ m^{-1}$, while losses of 14% (50% lsw + 50% hsw) to none were achieved with sequential management (Table 6). These results indicate that this cultivar can produce 89–100% of its tuber potential if HSW ($12\ dS\ m^{-1}$) is provided from 53 DAP on. We attribute this benefit of the sequential strategy on the fact that even if all growth stages are equally sensitive to salinity, applying the salinity in the latter stages of growth allows for dilution of the salt into the relatively large existing biomass.

3.7. Total soluble solids (°Brix)

Salinity treatments had a statistically significant effect on total soluble solids content (TSS or °Brix) of Jerusalem artichoke tubers, ranging from 20.4 to 29.4% for the 25% LSW + 75% HSW sequential treatments and $3.9\ dS\ m^{-1}$ salinity level (blended water treatment), respectively (Table 6). Compared with LSW control, tubers from plants irrigated with $EC_w = 12.0\ dS\ m^{-1}$ were not statistically different in TSS. On the other hand, the regression analysis of °Brix as a function of salinity from blended saline waters (Fig. 6) resulted in a significant quadratic relation between °Brix and salinity. The increase in °Brix up to $6.6\ dS\ m^{-1}$, followed by a decline after that, suggests that increased soluble sugars may be involved in osmoregulation to counteract Na^+ accumulation in tubers up to a certain salinity level. Similar increase in glucose from control up to $7.5\ dS\ m^{-1}$, and then a decrease with $EC = 12.5\ dS\ m^{-1}$ was reported in leaves of *Olea europaea* (Petridis et al., 2012). An increase in soluble sugars (sucrose, glucose, and fructose) was also reported in *Aster tripolium* (a.k.a. *Tripolium pannonicum*), a halophyte belonging to *Helianthus tuberosus* family (Asteraceae) and irrigated with control and sea-water salinities of 50% and 75%. However, sugar levels decreased at 100% seawater for plants treated with both ambient and high CO_2 (Geissler et al., 2009). Fructans accumulate in the vacuole, similarly to Na^+, and can also embed themselves deeper into cell membranes contributing to membrane stabilization during drought and cold stress (Valluru and Van den Ende, 2008). After fructans (50–60% tuber DW), sucrose was the most concentrated sugar in tubers (10% DW), accounting for most of the soluble free sugars.

3.8. Inulin, soluble sugars, and starch

Inulin-type fructans (inulin) concentration in tubers ranged from 491 to $612\ mg\ g^{-1}$ on dry weight (Fig. 7A) and accounted for 80 to 91% of the total non-cell wall carbohydrate reserves (that is the total free soluble sugars fructose, glucose, and sucrose plus inulin and starch) in the tubers. Salt stress did not affect fructans on a dry weight (DW) basis (Fig. 7A). Also, salinity did not

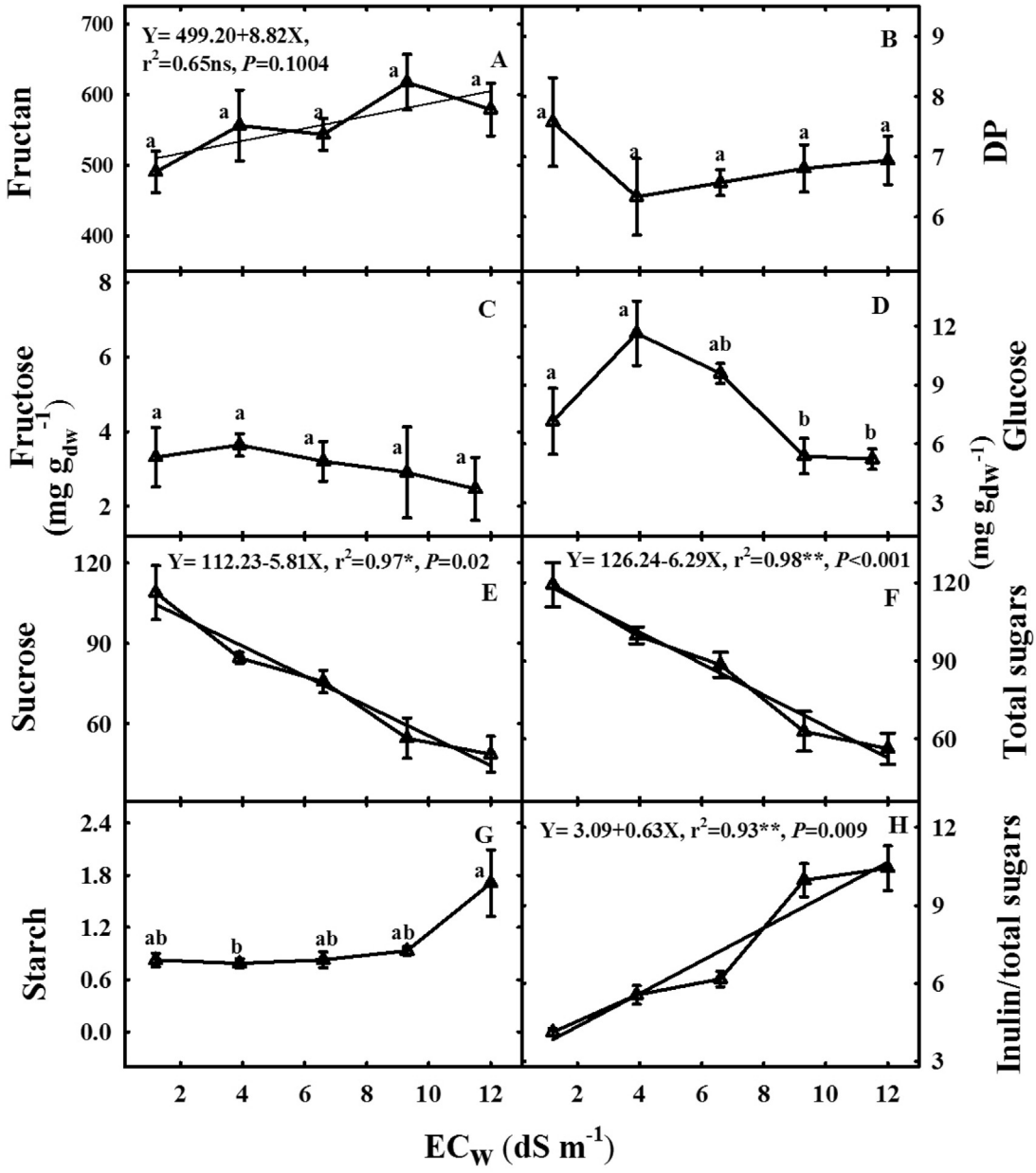

Fig. 7. Concentrations (mg g^{-1} dry weight) of inulin-type fructans (A), degree of polymerization (DP) (B), free fructose (C), free glucose (D), free sucrose (E), total sugars as free glucose + fructose + sucrose (F), starch (G), and the ratio of inulin over total sugars (H) of Jerusalem artichoke (cv. Stampede) tubers from plants irrigated with blended waters of different electrical conductivities (EC_w). Values are means, n = 3 (tanks), and bars represent ±1 standard error. **, *significant at $p \leq 0.01$, and at $p \leq 0.05$, ns = not significant.

affect inulin degree of polymerization (DP), which ranged from 6 to 8 units under the whole EC_w salinity range of blended waters (Fig. 7B). Inulins are the major form of carbohydrate accumulated in tubers of Jerusalem artichoke and have also been cited as a rich, but neglected, source of antioxidants (Stoyanova et al., 2011; Van den Ende and Valluru, 2009) and prebiotics (Slimestad et al., 2010). Slimestad et al. (2010) also mentioned that fructans with DP < 10 are easier to ferment by bifidobacteria and better as prebiotics. Our inulin-type fructans had DP < 10, indicating their suitability as feedstock for biofuels and for the value-added food market. Although we cannot dispute the antioxidant role of these sugars inside of the cell, our *in vitro* ORAC test resulted in low antioxidant activity (in μmol TE g^{-1}) for several sugars, including the ones mentioned here such as glucose, fructose, inulin (both inulins from Alfa Aesar, Cat. number A18425, and from TCI America, CAS number 9005-80-5), and sucrose, which ranged from 4 to 13 μmol TE g^{-1} for hydrophilic ORAC (results not shown). Thus, we cannot imply that these sugars, including inulin, worked to balance oxy/redox status in the cells by neutralizing ROS scavengers triggered by salinity.

The increase in approximately 25% in inulin in response to salinity may be associated with an osmotic protection mechanism triggered by salinity. When comparing two Jerusalem artichoke cultivars in response to salinity, Long et al. (2010) reported that inulins and total sugars were always higher (40–70%) in the cultivar that was tolerant to salinity. Our work agrees with those authors as inulin tended to increase with salinity up to $EC_w = 6.3$ dS m^{-1} and total sugars (inulin + sucrose + glucose + fructose) amounted to approximately 66% of tuber DW at the highest salinity ($EC_w = 12$ dS m^{-1}) tested (Fig. 7). This indicates that sugars such as sucrose and fructans (both inulins and levans) may be used to maintain the osmoticum and cell membrane integrity under saline

stress as previously postulated (Stoyanova et al., 2011), and that mild salinity may increase the tuber fructan content.

Free fructose and glucose concentrations in the tubers were relatively low compared with inulin and sucrose, and ranged from 2.5–3.6 and 5.2–11.6 mg g^{-1}, respectively, under the whole EC_w range (Fig. 7C, D). Overall, there was no salt effect on fructose and glucose concentrations except that glucose concentration was significantly ($p \leq 0.05$) lower at the EC_w values of 9.3 and 12.0 dS m^{-1} compared to those ranging from 1.2 to 6.6 dS m^{-1} (Fig. 7C, D). Free sucrose concentration was relatively high and ranged from 49 to 109 mg g^{-1}, decreasing significantly ($p = 0.02$) with increasing salinity, with a significant negative linear relation with salinity ($r^2 = 0.97^*$) (Fig. 7E). At $EC_w = 12.0$ dS m^{-1}, sucrose concentration dropped by 55%, compared to tuber from control plants. Mainly due to this inverse sucrose-EC_w negative correlation, the total free sugar concentration (sucrose + fructose + glucose) also had a highly significant ($P < 0.001$, $r^2 = 0.98^{**}$) inverse correlation with salinity (Fig. 7F).

Starch concentration in tubers of Jerusalem artichoke was very low (0.8–1.7 mg g^{-1}), but unaffected by salinity, except for a small increase at $EC^w = 12.0$ dS m^{-1} (Fig. 7G). Starch in potatoes ranges from 350 to 500 mg g^{-1}DW (Freitas et al., 2012). While the salt stress altered the tuber's carbon partitioning into different entities of its carbohydrate reserves, there was a linear increase of ratio of inulin concentration over the total free sugar concentration, which increased significantly ($p = 0.009$, $r^2 = 0.93$). This ratio increased from 4.1 to 10.4 as salinity increased from 1.2 to 12.0 dS m^{-1} (Fig. 7H). As the concentrations of fructose (<5 mg g^{-1}) and glucose (<10 mg g^{-1}) in tubers were low, this increased ratio in response to salinity was caused by the concomitant increase in inulin (>490 mg g^{-1}) and decrease in sucrose (from approximately 110 to 50 mg g^{-1}).

The significant decrease in sucrose levels with salinity, could be due to its reduced transport and synthesis in response to salinity, but also due to its consumption to maintain tuber cell osmoticum, and to stabilize cell membranes and proteins (Matros et al., 2015). Sucrose concentrations were on average 10–16 times higher than those of glucose and 16–35 times higher than fructose (Fig. 7).

3.9. Oxygen radical absorbancy capacity (ORAC) and total phenolics (TP)

There was no effect of salinity on tuber antioxidant capacity measured by both ORAC and TP, although there was a trend for both ORAC and TP values to increase with salinity before salinity reached 12 dS m^{-1} (Fig. 8A,B). This suggests that the increased concentration of both Na^+ and Cl^- in tubers can trigger a biochemical response that increased tuber antioxidants accumulated in the vacuoles and decreased sugars that accumulate in the cytosol (such as sucrose). Although the linear regression between antioxidants (measured by both ORAC and TP) and salinity had a high correlation coeficient (r^2), in the salinty range tested, their r^2 were not significant. Also, when we correlated both tuber ORAC and TP with the concentrations of tuber Na^+ and Cl^- in the precise salinity range of $EC_w = 3.9$ to 9.3 dS m^{-1} (where plant growth was maintained while levels of Cl^- in stems and shoots also remained stable – Fig. 4B), the coeficient of correlations were very high, but not significant at $p \leq 0.05$ ($r^2 = 0.98$, $p = 0.102$ and $r^2 = 0.97$, $p = 0.114$) for the linear regression between ORAC vs. Cl^- and ORAC vs Na^+, respectively. However, significant correlation coeficients resulted between TP vs. Na^+ and TP vs. Cl^- ($r^2 = 1.00^*$, $p = 0.021$ and $r^2 = 1.00^{**}$, $p = 0.008$), respectively (Fig. 8C). These linear regression coeficients suggest that, in that specific salinity range, the mechanism by which the plant maintained stem and leaf weight, leaf area, and stem diameter probably involved non-enzymatic antioxidants in addition to osmotic balance. Other antioxidants involved could be phenolics, based on

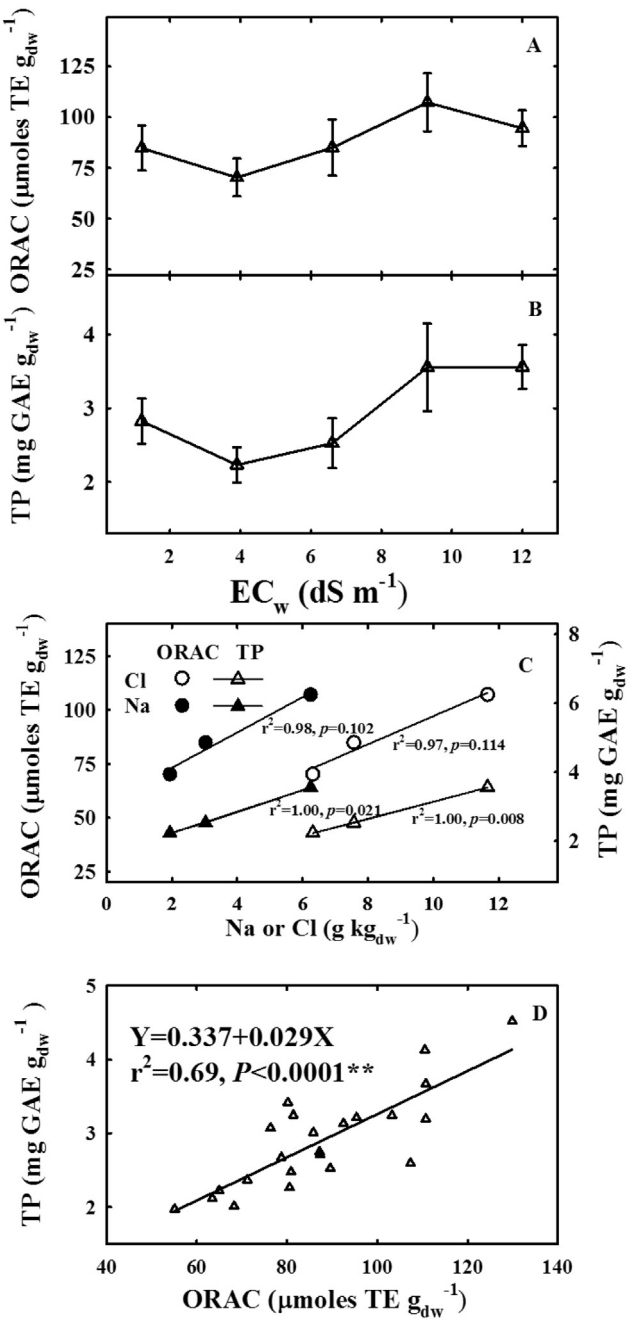

Fig. 8. Oxygen radical absorbance capacity (ORAC) (A), total phenolics (TP) (B), the linear regression between tuber ORAC and TP (C) vs. Na^+ and Cl^- tuber concentrations at the ECw range of 3.9 to 9.3 dS m^{-1}, and correlation between ORAC and TP of tubers (D). Values are means, n = 3 (9 plants from 3 different lysimeter tanks combined into one replicate/tank), and bars represent ±1 standard error. ***significant at $p \leq 0.001$.

the highly significant ($p < 0.001$) correlation between ORAC and TP in tubers (Fig. 8D), which indicates that phenolics in tubers (not inulin or sucrose) are responsible for tuber antioxidant capacity. Salinity was reported to increase leaf antioxidant enzymes and non-enzymatic antioxidants in saflower plants exposed to salinity stress (Gengmao et al., 2015; Salem et al., 2014). Considering that antioxidant flavonoids, phenolics, and inulin accumulate in the vacuole, where salts also accumulate, it has been speculated that these antioxidant enzymes and compounds are part of the defense mechanism of plants against salt stress (Agati et al., 2011; Fini et al., 2011; Matros et al., 2015; Peshev et al., 2013; Van den

Ende and Valluru, 2009). If antioxidants are produced in response to increasing levels of ROS, triggered by increased Na^+ and Cl^- concentrations in the roots, these ROS could work as signaling molecules, in turn triggering responses in shoots that would lead to increased antioxidants that would help ROS-redox homeostasis and help plants cope with salinity stress to a certain point. Roots accumulated almost four times more Na^+ than tubers, and 30–50 times more than leaves and stems, respectively, and are known to increase antioxidant enzymes faster than shoots in response to ROS triggered by salinity (Petridis et al., 2012). As for the antioxidant capacity per se, tubers had hydrophilic ORAC ranging from 75 to 100 μmol TE g^{-1}, 3–4 times higher than the total antioxidant capacity (sum of hydrophilic + lipophilic ORAC) found in several types of sweet potatoes (Teow et al., 2007), 1.4 to 2.0 times higher than the hydrophilic ORAC of Russet potatoes, and similar to the average ORAC values for several wild cultivars of Andean potatoes (Andre et al., 2007). Its high tuber and shoot biomass yield and its inulin-type fructan content makes Jerusalem artichoke far superior to corn stover, rice straw, sugarcane bagasse, wheat straw, and hemp as a natural feedstock for bioethanol per hectare (Gunnarsson et al., 2014).

4. Conclusions

Although Jerusalem artichoke has been reported as a salt-tolerant crop, its shoot and tuber yield were affected by high salinity of irrigation water. After the initial osmotic shock, plants irrigated with the highest salinity treatment adjusted and then resumed growth, but never achieved same growth and tuber yield as plants irrigated with waters of lower salinity throughout the crop cycle. According to shoot and tuber yield, Jerusalem artichoke cv. Stampede could be classified as moderately-sensitive (for shoot yield) to moderately-tolerant (for tuber yield) to salinity, respectively, with plants being more tolerant to salinity at later stages of tuber development than during the vegetative stage. At the highest salinity (EC_w = 12 dS m^{-1}) tested, tuber yield was less affected than shoot biomass yield (47% vs 67%, respectively), and at moderate salinity (EC_w = 6.6 dS m^{-1}) tuber yield decreased only by 11%, while shoot biomass decreased by 37%, indicating that roots and tubers have a higher tissue tolerance to salinity than shoots.

Low transport of Na^+ from roots to shoots and adequate transport and accumulation of macro and micronutrients in roots and shoots are strategies used by the plant to grow and develop under salinity stress. Accumulation of Cl^- in all organs, mainly in leaves and roots, point to Cl^- as the main toxic ion to the crop and the ion responsible for the decreased plant growth. The significant decrease of tuber sucrose and glucose and the stability of both tuber inulin and antioxidant capacity suggest that sugars (including inulin) and antioxidants may synergize to maintain cell osmotic potential and membrane integrity under salinity stress. The high correlation between TP and salinity between EC_w = 3.9 and 9.3 dS m^{-1}, where plant sustained growth and biomass accumulation, strongly suggests that tuber non-enzymatic antioxidants (but not sugars) are involved in neutralizing ROS triggered by Cl^- accumulation in leaves and roots.

Sequential irrigation using LSW for 75% of the irrigation events, followed by HSW for the last 25% of the crop cycle and blended waters of EC_w = 3.9 dS m^{-1} allowed tuber yield similar to that of control plants. Thus, this study confirms that blended and sequential water uses are viable strategies to save fresh water and use recycled waters where fresh water is scarce. Plants under all levels of salinity maintained their mineral nutrients and their tuber inulin-type fructans content, fructans DP, and antioxidant capacity. Opposite to what was stated for other crops, increased root Na^+ did not lead to decreased levels of Ca^{2+} or K^+, and increased shoot Cl^- did not decrease shoot NO_3^-, as reflected by N concentration.

'Stampede' is an early cultivar that adapted well to moderate salinity of blended irrigation waters and illustrates that water quality, exclusion of toxic ions, and maintenance of antioxidant status in tissues are major factors to consider if the crop is to be cultivated under saline conditions.

As at least 50% of tuber DW is inulin-type fructans, at 5.6 plants ha^{-1} it produced 83 tons of tubers, or 41.5 tons of inulin ha^{-1} at EC_w = 6.6 dS m^{-1} and, at EC_w = 1.2 dS m^{-1}, it produced 92 tons of tubers, or 46 tons of inulin-type fructans ha^{-1}. Besides its main importance as a carbon source for biofuels, inulin-type fructans are raw materials for non-caloric, prebiotic, and soluble fibers for the food/health industry and for animal feed. These features make *Helianthus tuberosus* a great industrial crop for the semi-arid and Mediterranean climate areas that are afflicted by soil and water salinity, and are unsuitable for agricultural crops. Jerusalem artichoke has been a neglected crop in North American, but our results clearly show that the plant has attributes that make it a valuable agricultural, biofuel, and value-added crop for North America and other regions where fresh water is a limiting factor for agriculture.

Contributions of authors

Drs. Jorge Ferreira, Nildo Dias, and Donald Suarez acted on the experimental design, data interpretation, and first drafts. Dr. Dias, aided by three students from UCR (Riverside, CA), performed the data collection. Dr. Dias did the statistics and tables on growth and water use, and wrote part of the results on growth parameters. Dr. Xuan Liu did all biochemical analysis for sugars, inulin, and antioxidant capacity of tubers, worked on data analysis, figures and tables, and wrote part of materials and methods. Dr. Suarez discussed the results on water use and helped Dr. Ferreira complete final revisions. Dr. Ferreira wrote most of the manuscript, compiled versions from co-authors, and discussed the effects of salinity on phenological aspects, organ accumulation of sugars, antioxidants, Na^+ and Cl^- ions, and mineral nutrients.

Role of the funding source

This work was funded by the U.S. Salinity Laboratory, USDA-ARS, by CAPES, and CNPq (Brazilian Ministry of Education), and by the Federal Rural University of the Semi-Árido, Mossoró, RN, Brazil. The study design, data interpretation, and writing of the manuscript were based on the scientific judgement of the authors, with no input from funding sources.

Conflict of interest

The authors declare no conflict of interest. The mention of proprietary brands and names is solely for the convenience of the reader and does not imply endorsement of the authors or of the USDA over similar products. The USDA is an equal-opportunity employer.

Acknowledgements

To Manju Pudussery and Nahid Vishteh for leaf and water analysis, Potato Farm, Austin, CO, USA, for the 'Stampede' tubers; to Alan Pacheco, Tessa Ries, and William Yee for data collection; to DPJ Arquitetura e Engenharia Ltda. (Belém, Brazil) and the Architect Imara de Oliveira for Fig. 1.

Appendix A.

Table A1
Concentration of micronutrients in aerial and underground organs of Jerusalem artichoke in response to electrical conductivity of waters (EC_w) of blended salinity and of sequential water management.

EC_w(dS m−1)	Fe (mg kg^{-1})	Cu	Mn	Zn
Blended			Leaves	
1.2	*280 ± 15 a	10.6 ± 4 a	*67.8 ± 7 a	63.3 ± 16 c
3.9	157 ± 53 a	*11.1 ± 1 a	*59.8 ± 3 a	*91.5 ± 11ab
6.6	*291 ± 40 a	*9.9 ± 1 a	*68.3 ± 4 a	*133 ± 18 ab
9.3	*237 ± 32 a	11.5 ± 3 a	*59.0 ± 12 a	*143 ± 11 ab
12	*221 ± 37 a	*11.4 ± 1 a	64.7 ± 24 a	*162 ± 10a
Sequential				
1.2($100_{LSW} \pm 0_{HSW}$)	176 ± 98 a	8.6 ± 3 a	*63.5 ± 10 a	64.9 ± 31 bc
3.7($77_{LSW} \pm 23_{HSW}$)	*252 ± 48 a	*10.6 ± 1 a	*79.2 ± 3 a	*85.3 ± 7 bc
7.9($38_{LSW} \pm 62_{HSW}$)	234 ± 15 a	8.4 ± 3 a	*71.3 ± 2 a	*92.1 ± 16ab
Blended			Stems	
1.2	40.6 ± 6 a	3.8 ± 1 a	10.3 ± 1 a	30.8 ± 4 a
3.9	27.7 ± 2 a	4.5 ± 1 a	8.4 ± 1 a	31.7 ± 2 a
6.6	30.5 ± 3 a	3.3 ± 1 a	7.6 ± 0.1 a	32.6 ± 4 a
9.3	52.0 ± 16 a	3.9 ± 1 a	9.3 ± 3 a	35.2 ± 9 a
12	27.3 ± 1 a	3.6 ± 1 a	8.6 ± 4 a	35.5 ± 3 a
Sequential				
1.2($100_{LSW} \pm 0_{HSW}$)	25.4 ± 1 a	3.8 ± 0.7a	9.1 ± 1.9a	26.8 ± 2 a
3.7($77_{LSW} \pm 23_{HSW}$)	27.8 ± 2 a	4.1 ± 0.6a	7.1 ± 1.2a	27.6 ± 2 a
7.9($38_{LSW} \pm 62_{HSW}$)	23.0 ± 1 a	3.0 ± 0.4a	6.5 ± 0.3a	28.7 ± 4 a
Blended			Roots	
1.2	*872 ± 132 a	*12.6 ± 1 a	*29.1 ± 2.1a	*46.9 ± 2 a
3.9	603 ± 189 a	11.3 ± 3 a	*27.6 ± 6.7a	*46.8 ± 6 a
6.6	*620 ± 177 a	*10.1 ± 1 a	*28.3 ± 2.5a	*48.1 ± 3 a
9.3	426 ± 272 a	*10.1 ± 1 a	*28.0 ± 1.8a	*47.8 ± 3 a
12	459 ± 163 a	6.9 ± 2 a	25.1 ± 12.5a	32.6 ± 7 a
Sequential				
3.9 ($75_{LSW} \pm 25_{HSW}$)	*790 ± 114 a	*9.9 ± 2 a	46.1 ± 18.3a	*52.3 ± 5 a
6.6 ($50_{LSW} \pm 50_{HSW}$)	*893 ± 227 a	14.5 ± 6 a	*35.8 ± 5.9a	*57.4 ± 9 a
9.3 ($25_{LSW} \pm 75_{HSW}$)	463 ± 63 a	*9.6 ± 2 a	*23.2 ± 2.7a	*43.0 ± 3 a
Blended			Tubers	
1.2	82.6 ± 15 a	2.0 ± 0.1a	4.9 ± 1 a	16.2 ± 1 a
3.9	80.5 ± 6 a	3.7 ± 1 a	3.6 ± 1 a	18.8 ± 2 a
6.6	61.5 ± 23 a	2.5 ± 0.1a	3.5 ± 1 a	15.1 ± 1 a
9.3	71.3 ± 9 a	2.6 ± 1 a	3.8 ± 0.2a	16.5 ± 1 a
12	83.0 ± 8 a	3.2 ± 0.3a	4.1 ± 1 a	19.3 ± 0.1a
Sequential				
3.9 ($75_{LSW} \pm 25_{HSW}$)	68.3 ± 13 a	2.8 ± 1 a	4.0 ± 2 a	17.6 ± 1 a
6.6 ($50_{LSW} \pm 50_{HSW}$)	95.0 ± 25 a	1.8 ± 0.2a	4.5 ± 1 a	16.1 ± 1 a
9.3 ($25_{LSW} \pm 75_{HSW}$)	83.7 ± 16 a	1.6 ± 0.1a	4.2 ± 0.1a	18.5 ± 1 a

Values are means of three replications. In each organ group, values followed the same letters are not significantly different at $p > 0.05$. *Indicates significant difference ($p \leq 0.05$) between values for the same nutrient between leaves and stems or between roots and tubers. LSW = 1.2 dS m^{-1}, HSW = 12 dS m^{-1}.

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