Evaluation of the Impacts of Salinity on Biomass and Nutrient Levels of *Agave* Species with Agricultural Potential in Semiarid Regions

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Additional index words. century plant, Asparagaceae, *Agave parryi*, *Agave utahensis*, *Agave weberi*, hydroponics, salt stress

Abstract. Long used as a source of food, beverages, and fiber, *Agave* exhibits potential to be cultivated as a crop to produce alternative sweeteners, bioenergy, and a variety of other end uses. However, little is known regarding the productivity levels of *Agave* when grown in saline soils in semiarid regions. Hydroponic experiments were carried out to evaluate the effects of salinity on biomass accumulation and nutrient levels of young plants of *Agave parryi*, *Agave utahensis* ssp. *kaibabensis*, *Agave utahensis* ssp. *utahensis*, and *Agave weberi*. Salinity treatments (0.6, 3.0, 6.0, and 9.0 dS·m⁻¹) were imposed in each experiment. Both subspecies of *A. utahensis* were sensitive to salt treatments. In the higher salinity treatments, *A. utahensis* ssp. *utahensis* exhibited high mortality; both subspecies had lower plant dry weights. *Agave parryi* was more tolerant, but experienced a decrease in plant dry weight in the 9.0 dS·m⁻¹ treatment. The biomass of *A. weberi* was unaffected by any level of salinity. Calcium, Mg, S, and Mn levels decreased in both *A. parryi* and *A. weberi* at higher salinity levels. Potassium and P levels in *A. parryi* decreased in the higher salt treatments. Decreases in nutrients were not severe enough to cause any apparent nutrient deficiencies in *A. parryi* and *A. weberi*. *Agave parryi* and *A. weberi* tolerated salinity at higher levels than expected, and may show promise for cultivation in saline soils.

Throughout the world, groundwater levels have been in continual decline (Konikow, 2013; Wada et al., 2010), which could potentially lead to local and regional water shortages, especially in areas that require large water inputs to sustain crop production (Dominguez-Faus et al., 2009). Such limitations increase the need for identifying plant species that generate high yields despite constraints on water availability. Indeed, exploiting the crop potential of select *Agave* spp. could help reconcile disparities related to growing populations and dwindling water reserves in semiarid regions, such as the southwestern United States. Agaves employ the crassulacean acid metabolism (CAM) photosynthetic pathway, which maximizes water-use efficiency by shifting most CO₂

uptake to the night (Borland et al., 2009; Nobel, 2010; Yang et al., 2015). Cooler nighttime temperatures reduce the vapor pressure gradient between their leaves and the air, resulting in markedly lower transpiration rates as compared with C₃ and C₄ plants (Griffiths, 1988; Winter and Smith, 1996). Consequently, CAM confers the ability to agaves to be highly water-use efficient in hot, drought-prone environments.

Most agaves, however, lack sufficient cold hardiness to survive winters in high-elevation deserts (Nobel, 1996), but some species appear to be sufficiently cold hardy. *Agave parryi*, *A. utahensis*, and *A. weberi* can tolerate temperatures as low as -19.6, -17.5, and -9.8 °C, respectively (Nobel, 1984; Nobel and Smith, 1983; Parida and Das, 2005), suggesting they have potential to be grown in more northern regions of the southwestern United States at elevations up to 850 m. *Agave parryi* is native to the southwest, and is found in mountainous areas of central and northern Arizona, southwestern New Mexico, and northern Chihuahua (Minnis and Plog, 1976). *Agave utahensis* populates mountain

slopes of southern Utah, southern Nevada, northwestern Arizona, and southeastern California (Baldwin et al., 2012; Welsh et al., 1993). The native distribution of *A. weberi* ranges from southern Texas to San Luis Potosi and Tamaulipas, Mexico (eMonocot Team, 2012).

Traditionally, agaves were used for food, beverage, and fiber (Castetter et al., 1938). Nowadays, however, *Agave* shows promise as a bioenergy crop (Conlu et al., 2011; Davis et al., 2011; Escamilla-Trevino, 2012; Holtum et al., 2011; Nunez et al., 2011), which is underscored by the high productivity of *Agave mapisaga* and *Agave salmiana*. Both were reported by Nobel (1991) to have yielded 38 and 42 Mg·ha⁻¹·year⁻¹, respectively, which exceeds that of other feedstock crops, such as corn (*Zea mays*) (15–19 Mg·ha⁻¹·year⁻¹) (Dohleman and Long, 2009) and switchgrass (*Panicum virgatum*) (10–12 Mg·ha⁻¹·year⁻¹) (Heaton et al., 2008). In addition, the high biomass and putative cold hardiness of mature *A. weberi* (Gentry, 1982; Irish and Irish, 2000) plants suggest that this species could be produced as a bioenergy crop.

However, salinity could severely impede cultivation of *A. parryi*, *A. utahensis*, and *A. weberi* in many semiarid regions. In the United States, 8.5 million ha of land are considered saline or sodic (Massoud, 1976). Over half of this land area is located in the western United States (Bohn et al., 1985). Excess salinity can decrease water availability to plants because the osmotic pressure of the soil solution increases as the salt concentration increases, resulting in stunted plant growth (Abrol et al., 1988). In addition, high salt concentrations can reduce cell expansion in root tips and young leaves, leading to stomatal closure and reduced water uptake (Munns and Tester, 2008). Excessive salt absorption can cause plants to suffer ionic stress due to ion accumulation in shoots (Munns and Tester, 2008), leading other nutrient ions such as Ca²⁺, K⁺, and Mg²⁺ to become deficient because they have to compete with high NaCl uptake (Khan et al., 1999).

The impact of high salinity on yield of many C₃ and C₄ crops is well documented. Corn yield decreased by 23% at an electrical conductivity (EC) level of 3.4 dS·m⁻¹ (Katerji et al., 1996). Similarly, soybean (*Glycine max*) had a 56% decrease in yield at 6.7 dS·m⁻¹ (Katerji et al., 1998). Contrary to what is widely assumed, several succulent plant species do not exhibit a higher degree of resistance to salt stress than C₃ and C₄ plant species (Nobel and Berry, 1985). Cladodes of *Opuntia ficus-indica* decreased 40% in growth at 4.2 dS·m⁻¹ NaCl (Nerd et al., 1991). Pineapple (*Ananas comosus*) can grow in soil with an EC range from 3.0 to 6.0 dS·m⁻¹ before declining in growth (Ayers and Westcot, 1985). *Aloe vera* started to experience a decrease in leaf number and root dry weight at EC levels higher than 6.0 dS·m⁻¹ (Moghbeli et al., 2012).

The tolerance of *Agave* to salinity varies depending on species. Nobel and Berry (1985) found that EC levels above 3.0 dS·m⁻¹

Received for publication 18 June 2015. Accepted for publication 21 Nov. 2015.

We are thankful to three BYU undergraduate students, Cali Mc Murtrey, Yue Shen, and Westen Archibald, for helping us carry out this experiment.
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greatly decreased elongation of roots and shoots of *Agave deserti* seedlings. Schuch and Kelly (2008) found that *A. parryi* shoot and root dry weights decreased at an EC of $5.0 \text{ dS}\cdot\text{m}^{-1}$. In addition, 10-month-old *A. sisalana* plants exposed to $10 \text{ dS}\cdot\text{m}^{-1}$ NaCl and $10 \text{ dS}\cdot\text{m}^{-1}$ CaCl_2 had a reduction in dry weight of 40% after 5 months (El-Gamassy et al., 1974). In another study, the dry weight of *A. sisalana* decreased in EC levels of $6.3 \text{ dS}\cdot\text{m}^{-1}$, and decreased by 46% in EC levels of $25 \text{ dS}\cdot\text{m}^{-1}$ (El-Bagoury et al., 1993). However, in contrast to these findings, Miyamoto (2008) found that salinity did not have any impacts on *Agave americana* growth at levels up to $9.0 \text{ dS}\cdot\text{m}^{-1}$.

To gain a better understanding of how agaves respond to high salinity, more species need to be evaluated, particularly those of agricultural interest. The main objective of this study was to determine the impact of treatments ranging from low to high salinity had on productivity of young plants of *A. weberi*, *A. parryi*, and two subspecies of *A. utahensis*. If salinity severely impacts growth, cultivating or reestablishing these species for commercial purposes may not be feasible in dryland regions. This may particularly be the case in degraded, marginal lands where salinity exceeds normal thresholds. However, the tolerance of *Agave* to salinity appears to vary depending on species, suggesting that some species may be more tolerant to salinity than generally assumed. We hypothesized that *A. parryi*, *A. utahensis*, and *A. weberi* would be able to tolerate salinity levels up to $6.0 \text{ dS}\cdot\text{m}^{-1}$ before decreasing in productivity. On the basis of past research, $6.0 \text{ dS}\cdot\text{m}^{-1}$ appears to be the threshold at which growth and development of many *Agave* spp. begin to be impaired (El-Bagoury et al., 1993; Schuch and Kelly, 2008).

Materials and Methods

Experimental design, plant material, and location

Four separate species-level experiments were established to analyze the following *Agave* taxa: *A. parryi*, *A. utahensis* ssp. *kaibabensis*, *A. utahensis* ssp. *utahensis*, and *A. weberi*. The two subspecies of *A. utahensis* were evaluated in this study to gain a more comprehensive understanding of how the species responds overall to salinity. Because of limited plant availability, *A. parryi* and *A. weberi* plants used in the study were 24-week-old plants propagated through tissue culture (Rancho Tissue Technologies, Rancho Santa Fe, CA). Three-week-old *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* plants were grown from seed obtained from Phoenix Desert Nurseries (Phoenix, AZ). The study was conducted under greenhouse conditions at Brigham Young University in Provo, UT. Plants were grown under supplemental light (12 h daily) with average temperatures of $25 \pm 5^\circ\text{C}$ during the light period and $15 \pm 2^\circ\text{C}$ during the dark period. Relative humidity during the study period ranged from 6% to

75%, with a median value of 47%. Average photosynthetically active radiation was $287 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All plants were grown in hydroponics. Hydroponic culture of seedlings has been found to be effective for screening for salinity tolerance in agaves (Nobel and Berry, 1985), other species (Munns and James, 2003; Shaheen and Hood-Nowotny, 2005), and for other forms of environmental stress tolerance (Bouslama and Schapaugh, 1984). In addition, young plants were selected instead of mature plants for the experiments to assess the impacts of salinity at the most susceptible stage of plant growth and development. When grown as crops, most agaves are planted as young seedlings, bulbils, or small vegetative offsets (Davis et al., 2011; Holtum et al., 2011; Nobel, 1994).

Each experiment was arranged in a randomized complete block design. The first (*A. utahensis* ssp. *kaibabensis*) and second (*A. weberi*) experiments began on 15 Nov. 2012, and concluded after 75 d. The third (*A. parryi*) and fourth (*A. utahensis* ssp. *utahensis*) experiments began on 20 Feb. 2013 and ended after 60 d. A container (diameter = 23 cm, height = 23.5 cm, volume = 9643 cm^3) containing four plants was defined as the experimental unit. Four salinity treatments (0.6 , 3.0 , 6.0 , and $9.0 \text{ dS}\cdot\text{m}^{-1}$) were established, with each treatment being replicated four times, resulting in a total of 16 containers in each experiment. The $0.6 \text{ dS}\cdot\text{m}^{-1}$ treatment served as the control. Containers were randomly placed in four rows $\approx 30 \text{ cm}$ apart on a greenhouse bench.

Agave parryi. Sixty-four plants were thoroughly washed to remove soil particles from roots. Plants were then transferred to 16 aluminum foil-covered polyethylene containers (depth = 24 cm, width = 24 cm, volume = 7.6 L), containing 7.5 L dilute, modified Steinberg nutrient solution (Nichols et al., 2012; Steinberg, 1953). The containers were randomly arranged on a greenhouse bench. Each plant was inserted into neoprene net cup lids and placed inside 5.1-cm foam-net pots (Atlantis Hydroponics, College Park, GA). Each foam-net pot was placed inside an opaque plastic lid situated on top of each container. Air stones (AIR-1000, Top Fin, Phoenix, AZ) were placed at the base of each container to aerate the roots and solution. Plants were grown in the pretreatment solution for 14 d before transfer into treatments.

The initial nutrient concentrations used during the treatment period were the same as the pretreatment, except NaCl (Sigma-Aldrich, St. Louis, MO) concentration was adjusted to reach the desired EC level for each treatment at the same time. EC was measured using a HM Digital COM-100 EC meter (Atlantis Hydroponics). Salinity was supplied at four levels (0.6 , 3 , 6 , and $9.0 \text{ dS}\cdot\text{m}^{-1}$) of NaCl in nutrient solutions buffered at a pH of 6. Solution pH was initially adjusted and then maintained daily with sodium hydroxide and hydrochloric acid. Nutrient solutions in each container were replenished every 2 weeks. When replenishing the solutions, the whole container was

replaced with a new solution in deionized water to ensure that salinity level would remain relatively constant over the course of the experiments. Seedlings were harvested after 60 d in treatment. Shoots and roots were separated for further analysis.

Agave utahensis ssp. *kaibabensis*. Seeds were germinated by placing them on cheese-cloth placed on 4-mm stainless steel screens in 9.5-cm deep rectangular plastic trays. The screens were immersed with 2 L of diluted modified Steinberg solution. Germination and subsequent elongation of seedlings occurred over a 21-d period at $\approx 25^\circ\text{C}$. The pretreatment procedure applied to *A. parryi* was also used for *A. utahensis* ssp. *kaibabensis*. However, during the treatment period, nutrient solutions were replenished every 4 weeks instead of every 2 weeks. Seedlings were harvested after 75 d in treatment.

Agave utahensis ssp. *utahensis*. Seedlings of *A. utahensis* ssp. *utahensis* were propagated similarly to those of *A. utahensis* ssp. *kaibabensis*. They were also pretreated and treated with the same modified Steinberg solutions as the *A. parryi* plants, except that 128 seedlings comparable in size were transferred to opaque plastic lids placed on top of containers (eight plants per container). After a 14-d pretreatment period, 64 seedlings uniform in size were then selected out of the 128 seedlings and transferred to new containers (four plants per container) with opaque plastic lids. The treatment protocol for *A. parryi* was used for *A. utahensis* ssp. *utahensis* except nutrient solutions were initially replenished every 2 weeks for the first 30 d. They were then replenished every 4 weeks due to slow growth rates. Seedlings were harvested after 60 d in treatment.

Agave weberi. Plants of *A. weberi* were pretreated and treated with the same modified Steinberg solution as the *A. parryi* plants. However, during the treatment period, nutrient solutions were replenished 4 weeks after treatment started. Following the initial replenishment, nutrient solutions were replenished every 2 weeks. Seedlings were harvested after 60 d in treatment.

Mortality count, dry weight, and elemental analysis

At the end of each experiment, a mortality count was taken to determine the percent mortality of plants in each treatment. Shoots and roots of plants in all experiments were oven dried at 65°C for a minimum of 72 h to uniform dryness and then weighed. Shoots of *A. parryi* and *A. weberi* were subsequently ground using a mortar and pestle for elemental analysis. Shoots of *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* were not ground because there was not enough dry material to use for elemental analysis. To measure the concentrations of Ca, Mg, K, P, S, Zn, Cu, and Na in the shoots, 0.5 g of each sample was digested for 12 h in 5 mL nitric-perchloric acid and analyzed by inductively coupled plasma atomic emission spectroscopy (IRIS Intrepid II XSP; Thermo Electron Corporation, Franklin, MD). In

addition, 0.1 g of each sample was weighed in a tin capsule. The capsule was then combusted in a CHN analyzer (LECO CHN628 series; LECO Corporation, St. Joseph, MI) to estimate total C and N.

Statistical analysis

The average dry shoot, dry root, and nutrient concentrations were calculated from the four plants in each bucket for each of the species-level experiments. Statistical analyses were performed using Statistical Analysis System (SAS, version 9.3; SAS Institute, Cary, NC). Data for shoot dry weight, root dry weight, total dry weight, and nutrient uptake were analyzed using analysis of variance with mean separation using the Tukey–Kramer test at the 0.05 level of significance ($P \leq 0.05$). A control for normality for each analysis was accomplished with quantile–quantile plots for residuals, and by running the Shapiro–Wilk, Cramer–von Mises, and Anderson–Darling tests.

Because of the binary nature of the data, plant survival was analyzed with analysis of deviance using the glm() function in R [version 3.0.2 (R Development Core Team, 2013)]. A binomial distribution was specified for each generalized linear model (i.e., one for each subspecies). A series of Tukey contrasts using the multcomp package (Hothorn et al., 2008) were run to assess differences in survival among the treatments. The 0.6 dS·m⁻¹ (control) treatment was removed before this analysis as no mortality was noted within this treatment. This situation (i.e., all success or failure) is problematic with binomial data as standard errors are inflated to a point where statistical differences cannot be discerned.

Results

Mortality. Across salinity treatments, there were no differences in mortality of seedlings of *A. utahensis* ssp. *kaibabensis* ($P = 0.36$) (Table 1). As EC levels increased,

Table 1. Percent mortality of 3-week-old seedlings of *Agave utahensis* ssp. *kaibabensis* and *Agave utahensis* ssp. *utahensis* exposed to four levels of salinity in hydroponic culture under greenhouse conditions for 75 and 60 d, respectively. Standard errors follow mortality mean values.

Taxa and treatment	Percent mortality
<i>Agave utahensis</i> ssp. <i>kaibabensis</i>	
0.6 dS·m ⁻¹	31 ± 6 A ^z
3.0 dS·m ⁻¹	19 ± 6 A
6.0 dS·m ⁻¹	44 ± 16 A
9.0 dS·m ⁻¹	44 ± 12 A
<i>Agave utahensis</i> ssp. <i>utahensis</i>	
0.6 dS·m ⁻¹	0 ± 0
3.0 dS·m ⁻¹	27 ± 10 A
6.0 dS·m ⁻¹	50 ± 0 AB
9.0 dS·m ⁻¹	75 ± 10 B

^zDifferent letters indicate means that are significantly different from each other within species ($P \leq 0.05$, analysis of deviance test).

there was a corresponding increase in mortality of *A. utahensis* ssp. *utahensis* seedlings. Mortality in the 9.0 dS·m⁻¹ treatment was ≈25%, 50%, and 75% higher than in the 6.0, 3.0, and 0.6 dS·m⁻¹ treatments. The mortality of plants in the 9.0 dS·m⁻¹ treatment exceeded those in the 3.0 dS·m⁻¹ treatment. All *A. parryi* and *A. weberi* plants survived, regardless of treatment (data not shown).

Shoot, root, and total dry weight. Shoot, root, and total dry weights of *A. parryi* differed between plants in the control and 9.0 dS·m⁻¹ treatments. Plants in the control treatment had the highest dry weight values (Table 2).

Seedlings of *A. utahensis* ssp. *kaibabensis* in the 6.0 and 9.0 dS·m⁻¹ treatments had 1.5 to 2 times less shoot dry weight than those in the control treatment (Table 2). Roots in the 9.0 dS·m⁻¹ treatment had nearly 2.5 times less dry weight than of those in the control treatment (Table 2). Total dry weight of plants in the control treatment exceeded that found in the 6.0 and 9.0 dS·m⁻¹ treatments. Also, total dry weight of plants in the 3.0 dS·m⁻¹ treatment was higher than of those in the 9.0 dS·m⁻¹ treatment (Table 2).

Agave utahensis ssp. *utahensis* seedlings in the control treatment had more than two times as much shoot dry weight than those in the 9.0 dS·m⁻¹ treatment (Table 2). Shoot dry weight of control seedlings also were 1.8 times greater than seedlings in the 6.0 dS·m⁻¹ treatment (Table 2). Seedlings in the control treatment had 2.6 and 4.5 times more root dry weight, respectively, than seedlings in the 6.0 and 9.0 dS·m⁻¹ treatments (Table 2). Treatment differences in total dry weight followed that of shoot and root dry weights. There were no differences in shoot, root, or total dry weights of *A. weberi* among the four treatments (Table 2).

Table 2. Four separate experiments evaluating the effects 0.6–9.0 dS·m⁻¹ salinity levels had on shoot, root, and total dry weight of plants of four *Agave* spp. in hydroponic culture under greenhouse conditions. Twenty-four-week old plants of *Agave parryi* and 3-week-old seedlings of *Agave utahensis* ssp. *utahensis* were in treatment for 60 d, whereas 24-week-old plants of *Agave weberi* and 3-week-old plants of *Agave utahensis* ssp. *kaibabensis* were in treatment for 75 d. Standard errors follow mean values.

<i>A. parryi</i>	Shoot dry wt (g)	Root dry wt (g)	Total dry wt (g)
0.6 dS·m ⁻¹	1.63 ± 0.08 A ^z	0.54 ± 0.03 A	2.17 ± 0.11 A
3.0 dS·m ⁻¹	1.47 ± 0.08 AB	0.51 ± 0.03 AB	1.98 ± 0.11 AB
6.0 dS·m ⁻¹	1.30 ± 0.08 AB	0.49 ± 0.03 AB	1.79 ± 0.11 AB
9.0 dS·m ⁻¹	1.16 ± 0.08 B	0.40 ± 0.03 B	1.56 ± 0.11 B
<i>A. utahensis</i> ssp. <i>kaibabensis</i>			
0.6 dS·m ⁻¹	0.0387 ± 0.0036 A	0.0071 ± 0.0009 A	0.0459 ± 0.0041 A
3.0 dS·m ⁻¹	0.0328 ± 0.0036 AB	0.0057 ± 0.0009 AB	0.0386 ± 0.0041 AB
6.0 dS·m ⁻¹	0.0201 ± 0.0036 B	0.0045 ± 0.0009 AB	0.0246 ± 0.0041 BC
9.0 dS·m ⁻¹	0.0170 ± 0.0036 B	0.0029 ± 0.0009 B	0.0199 ± 0.0041 C
<i>A. utahensis</i> ssp. <i>utahensis</i>			
0.6 dS·m ⁻¹	0.0235 ± 0.0022 A	0.0058 ± 0.0010 A	0.0293 ± 0.0030 A
3.0 dS·m ⁻¹	0.0179 ± 0.0022 AB	0.0049 ± 0.0010 AB	0.0228 ± 0.0030 AB
6.0 dS·m ⁻¹	0.0131 ± 0.0022 B	0.0020 ± 0.0010 B	0.0151 ± 0.0030 B
9.0 dS·m ⁻¹	0.0113 ± 0.0022 B	0.0014 ± 0.0010 B	0.0127 ± 0.0030 B
<i>A. weberi</i>			
0.6 dS·m ⁻¹	2.93 ± 0.137 A	0.682 ± 0.211 A	3.61 ± 0.257 A
3.0 dS·m ⁻¹	2.93 ± 0.137 A	0.921 ± 0.211 A	3.85 ± 0.257 A
6.0 dS·m ⁻¹	2.77 ± 0.137 A	0.902 ± 0.211 A	3.67 ± 0.257 A
9.0 dS·m ⁻¹	2.44 ± 0.137 A	0.743 ± 0.211 A	3.18 ± 0.257 A

^zDifferent letters indicate means that are significantly different from each other within species (Tukey's test, $P \leq 0.05$, analysis of variance).

Nutrient concentrations. Carbon concentration of *A. parryi* in the 3.0 dS·m⁻¹ treatment exceeded that in the 9.0 dS·m⁻¹ treatment. Nitrogen concentration differed between the 3.0 and 6.0 dS·m⁻¹ treatments, with more N in the 6.0 dS·m⁻¹ treatment. There were no differences between the 0.6, 6.0, and 9.0 dS·m⁻¹ treatments. Dried shoot samples indicated differences in Ca, K, Mg, Mn, Na, P, and S (Table 3). Calcium, K, Mg, Mn, and S concentrations all decreased with an increase in salt treatment. Each of these elements had decreases in the 6.0 and 9.0 dS·m⁻¹ treatments relative to the control. Sodium levels increased correspondingly as the salt treatment increased. Phosphorus concentration was lower in the 9.0 dS·m⁻¹ treatment relative to the 3.0 and 6.0 dS·m⁻¹ treatments. There was also no difference in P concentration between the 0.6 and 9.0 dS·m⁻¹ treatments (Table 3). However, zinc concentration in the control treatment exceeded that of all other treatments.

There were no differences in C concentration between any of the treatments for *A. weberi*. Nitrogen concentration in plants in the control treatment was lower than of those in the other treatments (Table 3). There were no differences in K, P, Zn, and Fe among plants in the four treatments. However, Ca, Mg, S, and Mn all decreased in concentration as salinity increased, particularly in the 6.0 and 9.0 dS·m⁻¹ treatments. Copper concentrations were lower in plants in the control and 9.0 dS·m⁻¹ treatments compared with the 3.0 dS·m⁻¹ treatment. Sodium concentration increased as salinity treatment increased (Table 3).

Discussion

Growth of both subspecies of *A. utahensis* was reduced at high salt concentrations. The poor response of these plants to salinity may

Table 3. Nutrient concentrations of two separate species-level experiments evaluating the effects of salinity on nutrient uptake of 24-week-old plants of *Agave weberi* and *Agave parryi* in hydroponic culture under greenhouse conditions. Standard errors follow mean values.

Nutrient	C (%)	N (%)	K (%)	P (%)	Ca (%)	Mg (%)	S (%)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Na (ppm)
<i>A. parryi</i>												
0.6 dS·m ⁻¹	42.4 ± 0.3 AB ^z	2.09 ± 0.04 AB	2.11 ± 0.02 A	0.415 ± 0.003 AB	2.75 ± 0.05 A	0.71 ± 0.01 A	0.236 ± 0.005 A	109 ± 4 A	4.10 ± 0.24 A	18.2 ± 0.5 A	79.8 ± 16.8 A	1,326 ± 506 D
3.0 dS·m ⁻¹	43.1 ± 0.3 A	1.97 ± 0.04 B	2.07 ± 0.02 AB	0.418 ± 0.003 A	2.22 ± 0.05 B	0.63 ± 0.01 B	0.218 ± 0.005 AB	101 ± 4 A	3.74 ± 0.24 A	16.3 ± 0.5 B	74.5 ± 16.8 A	8,952 ± 506 C
6.0 dS·m ⁻¹	42.2 ± 0.3 AB	2.15 ± 0.04 A	2.01 ± 0.02 BC	0.422 ± 0.003 A	1.71 ± 0.05 C	0.55 ± 0.01 C	0.213 ± 0.005 BC	79 ± 4 B	3.96 ± 0.24 A	15.7 ± 0.5 B	117 ± 16.8 A	18,001 ± 506 B
9.0 dS·m ⁻¹	41.4 ± 0.3 B	1.99 ± 0.04 AB	1.94 ± 0.02 C	0.398 ± 0.003 B	1.44 ± 0.05 D	0.51 ± 0.01 C	0.190 ± 0.005 C	67 ± 4 B	3.83 ± 0.24 A	14.8 ± 0.5 B	81.3 ± 16.8 A	23,373 ± 506 A
<i>A. weberi</i>												
0.6 dS·m ⁻¹	44.5 ± 0.2 A	3.28 ± 0.21 B	2.08 ± 0.02 A	0.44 ± 0.02 A	2.87 ± 0.11 A	0.83 ± 0.03 A	0.35 ± 0.01 AB	84.7 ± 4.7 A	3.29 ± 0.24 B	28.6 ± 16.3 A	75.7 ± 3.7 A	1,086 ± 484 D
3.0 dS·m ⁻¹	43.9 ± 0.2 A	4.10 ± 0.21 AB	2.11 ± 0.02 A	0.52 ± 0.02 A	2.76 ± 0.11 A	0.78 ± 0.03 A	0.38 ± 0.01 A	89.0 ± 4.7 A	4.75 ± 0.24 A	29.6 ± 16.3 A	81.3 ± 3.7 A	9,917 ± 484 C
6.0 dS·m ⁻¹	43.8 ± 0.2 A	4.21 ± 0.21 A	2.06 ± 0.02 A	0.47 ± 0.02 A	1.81 ± 0.11 B	0.61 ± 0.03 B	0.31 ± 0.01 BC	54.0 ± 4.7 B	3.79 ± 0.24 AB	26.8 ± 16.3 A	77.9 ± 3.7 A	19,710 ± 484 B
9.0 dS·m ⁻¹	43.8 ± 0.2 A	4.11 ± 0.21 A	2.04 ± 0.02 A	0.45 ± 0.02 A	1.35 ± 0.11 B	0.55 ± 0.03 B	0.25 ± 0.01 C	39.5 ± 4.7 B	3.60 ± 0.24 B	27.7 ± 16.3 A	80.1 ± 3.7 A	22,910 ± 484 A

^zDifferent letters indicate means that are significantly different from each other within species and nutrient type (Tukey's test, $P \leq 0.05$, analysis of variance).

have been due to their exposure to high levels of salt stress as seedlings. Their ability to adapt to salt stress was low compared with the more established and putatively stress-tolerant *A. parryi* and *A. weberi* plants. Nobel and Berry (1985) reported that 12-d-old seedlings of *A. deserti* also performed poorly in high salinity, with a 50% decrease in root growth occurring at 5.6 dS·m⁻¹, and a similar decrease in shoot growth occurring at 9.3 dS·m⁻¹.

To date, research has not been carried out determining how mature plants of *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* respond to high salinity. However, using soil-based data collected by Nobel and Berry (1985), Hara (1992) estimated that EC values of soils, where *A. utahensis* plants naturally establish, range between 2.5 and 3.2 dS·m⁻¹. This possibly explains why the species is fairly intolerant of high salt concentrations. Another reason that *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* seedlings performed poorly may have been due to the inability of the plants to osmotically adjust to high salt levels. Mature *Agave* plants typically tolerate high salt concentrations through osmotic adjustment, where moisture content decreases and Na⁺ and Cl⁻ ions increase in the shoots (Peña-Valdivia and Sánchez-Urdaneta, 2009; Schuch and Kelly, 2008). *Agave* plants will also exclude salt from their leaves to adjust to salt stress. However, since the plants of both taxa in our study were only seedlings, their capacity to store salt was possibly limited. Although not directly measured, such adjustment mechanisms may have been at play with the larger *A. parryi* and *A. weberi* plants.

Data from the study suggest that *A. parryi* performs well in EC levels up to 6.0 dS·m⁻¹, which is in line with another study that analyzed the salinity tolerance of 1-year-old *A. parryi* plants grown under greenhouse conditions (Miyamoto, 2008). Miyamoto (2008) found that plant growth was not restricted within the range of 6–8 dS·m⁻¹, but was severely reduced at 9.4 dS·m⁻¹. Contrary to our findings, Schuch and Kelly (2008) found that relative to field-grown *A. parryi* plants exposed to low-salinity conditions (0.6 dS·m⁻¹), shoot and root dry weights of potted conspecific plants decreased by 33% when irrigated with water with an EC of 5 dS·m⁻¹. This suggests that even within species, some conspecific *Agave* plants may be more tolerant to salinity than others.

Agave weberi was notably tolerant to high salinity levels, with no difference in growth among treatments. Other species of agaves have also done well in high salinity. Miyamoto (2008) found that *A. americana* plants were considerably tolerant of high salt levels, and did not have any decreases in growth when irrigated with 9.4 dS·m⁻¹ water. In addition, soil samples taken from the root zones of field-grown *A. americana* had EC levels ranging between 7.0 and 8.0 dS·m⁻¹ (Hara, 1992; Nobel and Berry, 1985). Interestingly, EC levels of soils were found to be exceedingly high where mature

A. salmiana (13–16.0 dS·m⁻¹), *Agave lechuguilla* (17–20 dS·m⁻¹), and *Agave foudroydes* (44–47 dS·m⁻¹) plants were growing (Hara 1992; Nobel and Berry, 1985). Successful establishment at such high salinity levels suggests that at least some *Agave* spp. exhibit appreciable salt tolerance.

To our knowledge, studies evaluating optimal nutrient concentrations of *A. parryi* and *A. weberi* have not been carried out. Moreover, identifying nutrient deficiencies in *Agave* spp. can be difficult because visual symptoms are not always obvious (Ruiz-Luna et al., 2011). Indeed, Ruiz-Luna et al. (2011) observed that nutritional deficiencies in agaves may not be manifest for up to 12 months. As such, it was difficult to visually determine if plants in our study were suffering from nutrient deficiencies. However, by taking into consideration reported nutrient concentrations of ecologically similar *Agave* spp. (*A. americana*, *A. deserti*, *A. fourcroydes*, *A. lechuguilla*, *A. salmiana*, and *A. utahensis*) (Nobel and Berry, 1985), estimates can be made of nutrient threshold levels of both *A. parryi* and *A. weberi*. For *A. parryi* and *A. weberi*, N uptake differed between treatments, but did not appear to be associated with salinity level. *Agave weberi* had less N in the control group than the 6.0 and 9.0 dS·m⁻¹ treatments, whereas *A. parryi* had less N in the 3.0 dS·m⁻¹ treatment than the 6.0 dS·m⁻¹ treatment. The decrease in N in the lower treatments does not correlate with that found in other studies, where N tended to decrease with an increase in salinity (Al-Rawahy et al., 1992; Feigin et al., 1991; Pessarakli, 1991). However, salinity level does not necessarily affect overall N uptake (Maksimovic and Ilin, 2012). Salt-stressed plants may continue to accumulate N, even if the plants experience a reduction in yield or dry matter.

The decrease in N, however, did not appear to impair plant growth and productivity. Plant dry weight in the control and 3.0 dS·m⁻¹ treatments exceeded that in the 6.0 and 9.0 dS·m⁻¹ treatments. Compared with the average N content of six *Agave* spp. (1.19%) evaluated by Nobel and Berry (1985), the lowest N levels in *A. parryi* (1.97%) and *A. weberi* (3.28%) in our study were relatively high. This suggests that the plants in these treatments were likely not impaired by N deficiency.

In terms of the other essential nutrients that decreased, Ca appears to have been the only element that became deficient at high NaCl concentrations. Calcium levels in the 9.0 dS·m⁻¹ treatment of *A. parryi* (1.35%) and *A. weberi* (1.44%) were noticeably lower than of *Agave* spp. (4.16%) evaluated by Nobel and Berry (1985). Even in the control treatments, the Ca levels were still fairly low for *A. parryi* (2.75%) and *A. weberi* (2.87%) as compared with that reported by Nobel and Berry (1985). However, Lock (1962) reported that mature leaves of field-grown *A. sisalana* in Tanzania had similar levels of Ca (1.4%), indicating that not all *Agave* need

high Ca concentrations in their leaves to be productive. Furthermore, seedlings of *A. deserti* did not vary in growth with Ca levels ranging from 0.0008% to 0.02% (Nobel and Berry, 1985). *Agave* plants have been found to grow in soil Ca levels ranging from 0.015% to 0.01% (Nobel and Berry, 1985).

Although decreases of K and P were statistically significant in *A. parryi*, their low concentrations (1.97% K, 0.40% P) were still above the average values found by Nobel and Berry (1985). Furthermore, the lowest S concentrations in *A. parryi* (0.19%) and *A. weberi* (0.25%) were above that found in well watered and fertilized *A. angustifolia* (Ruiz-Luna et al., 2011). Despite the decrease in Mg and Mn in both *A. parryi* and *A. weberi*, the lowest Mg levels in *A. parryi* (0.52%) and *A. weberi* (0.55%) were relatively similar to the average of the six *Agave* spp. mentioned above (0.55%) (Nobel and Berry, 1985). Also, Mn levels in the highest NaCl treatments of *A. parryi* (65 ppm) and *A. weberi* (39 ppm) were considerably larger compared with the average (18 ppm) of the six species evaluated by Nobel and Berry (1985). Studies on micronutrient deficiency symptoms in *A. sisalana* indicated that deficiencies occurred below ≈ 10 ppm Mn, 2 ppm Cu, and 5 ppm Zn (Lock, 1962; Pinkerton, 1971). The plants in our study thus appeared not to have experienced micronutrient deficiencies. Despite a decrease in nutrient uptake at higher salt concentrations, actual physiological impairment to the plants appeared to be minimal, which is interesting considering that Na levels increased in both *A. parryi* and *A. weberi* plants as salt treatments increased (Table 3). These data corroborate with that observed by Schuch and Kelly (2008), but we do not know to what degree the plants may have excluded Na from their tissues, if at all. The presence of such a tolerance mechanism in agaves needs to be further explored.

The relations between salinity and mineral nutrition are extremely complex (Grattan and Grieve, 1999). Such complexity makes it difficult to predict which nutrients would be deficient in agaves growing in saline soils. Based on studies of other plants, it is common for most essential elements to decrease as salinity increases (Parida and Das, 2005). However, whether increased salinity leads to decreases in plant growth and productivity depends on the nutrient in question, salinity level, salt composition, plant species, and environmental factors (Grattan and Grieve, 1999). *Agave parryi* and *A. weberi* responded comparatively well to high concentrations of salinity, but to identify specific nutrient deficiency threshold levels for each species, long-term experiments need to be carried out evaluating each essential nutrient separately.

Conclusions

Based on the results of our study, it appears that several *Agave* spp. show variation in response to high levels of salinity. However, age and stage of development may play a factor in the degree of tolerance

expressed. *Agave utahensis* seedlings were very sensitive to high levels of salinity, with growth and survival greatly decreasing in higher salinity treatments. In contrast, *A. parryi* and *A. weberi* plants were relatively tolerant to high levels of salinity. Consequently, both species show potential to be grown in saline soils in semiarid regions as crops. Additional work, however, needs to be done to determine their degree of establishment and productivity under field conditions.

Literature Cited

- Abrol, I., J.S.P. Yadav, and F. Massoud. 1988. Salt-affected soils and their management. FAO, Rome.
- Al-Rawahy, S.A., J.L. Stroehlein, and M. Pessarakli. 1992. Dry-matter yield and nitrogen-15, Na⁺, Cl⁻, and K⁺ content of tomatoes under sodium-chloride stress. *J. Plant Nutr.* 15:341–358.
- Ayers, R.S. and D.W. Westcot. 1985. Water quality for agriculture. FAO, Rome.
- Baldwin, B.G., D.H. Goldman, D.J. Keil, R. Patterson, T.J. Rosatti, and D.H. Wilken. 2012. The Jepson manual: Vascular plants of California. University of California Press, Berkeley, CA.
- Bohn, H.L., B.L. McNeal, and G.A. O'Connor. 1985. Soil chemistry. Wiley, New York, NY.
- Borland, A.M., H. Griffiths, J. Hartwell, and J.A.C. Smith. 2009. Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *J. Expt. Bot.* 60:2879–2896.
- Bouslama, M. and W.T. Schapaugh. 1984. Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci.* 24:933–937.
- Castetter, E.F., W.H. Bell, and A.R. Grove. 1938. The early utilization and the distribution of agave in the American southwest. University of New Mexico Press, Albuquerque, NM.
- Conlu, R., D. Ha, and J. Shen. 2011. Exploring the potential of *Agave* as a biofuel crop on arid land. University of Nevada, Las Vegas, NV. 18 Dec. 2015. <http://digitalscholarship.unlv.edu/focs Ug_research/2011/april16/30/>.
- Davis, S.C., F.G. Dohleman, and S.P. Long. 2011. The global potential for *Agave* as a biofuel feedstock. *Glob. Change Biol. Bioenergy* 3:68–78.
- Dohleman, F.G. and S.P. Long. 2009. More productive than maize in the Midwest: How does *Miscanthus* do it? *Plant Physiol.* 150:2104–2115.
- Dominguez-Faus, R., S.E. Powers, J.G. Burken, and P.J. Alvarez. 2009. The water footprint of biofuels: A drink or drive issue? *Environ. Sci. Technol.* 43:3005–3010.
- El-Bagoury, O., M. El-Agroudy, and M. Shenouda. 1993. Effect of salinity levels on growth of six plant species. *Egypt. J. Agron.* 18:129–143.
- El-Gamassy, A., A.A. El-Rahman, M. Hassib, and M. Mandour. 1974. Vegetative patterns and water economy of *Agave sisalana* in saline soils. *Zeitschrift fuer Acker-und Pflanzenbau* 139:165–171.
- eMonocot Team. 2012. *Agave weberi* J.F.Cels ex J. Poiss. 11 June 2015. <<http://e-monocot.org/taxon/urn:kew.org:wcs:taxon:294351>>.
- Escamilla-Trevino, L.L. 2012. Potential of plants from the genus *Agave* as bioenergy crops. *BioEnergy Res.* 5:1–9.
- Feigin, A., E. Pressman, P. Imas, and O. Miltau. 1991. Combined effects of KNO₃ and salinity on yield and chemical composition of lettuce and Chinese cabbage. *Irr. Sci.* 12:223–230.
- Gentry, H.S. 1982. Agaves of continental North America. The University of Arizona Press, Tucson, AZ.
- Grattan, S.R. and C.M. Grieve. 1999. Salinity mineral nutrient relations in horticultural crops. *Sci. Hort.* 78:127–157.
- Griffiths, H. 1988. Crassulacean acid metabolism: A re-appraisal of physiological plasticity in form and function. *Adv. Bot. Res.* 15:43–92.
- Hara, Y. 1992. Physical and chemical soil properties affecting the growth habits of *Agave* species. Masters of Science, University of Arizona, Tucson, AZ.
- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of *Miscanthus*. *Glob. Change Biol.* 14:2000–2014.
- Holtum, J.A.M., D. Chambers, T. Morgan, and D.K.Y. Tan. 2011. *Agave* as a biofuel feedstock in Australia. *Glob. Change Biol. Bioenergy* 3:58–67.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical J.* 50:346–363.
- Irish, M. and G. Irish. 2000. Agaves, yuccas, and related plants: A gardener's guide. Timber Press, Portland, OR.
- Katerji, N., J.W. van Hoorn, A. Hamdy, F. Karam, and M. Mastrorilli. 1996. Effect of salinity on water stress, growth, and yield of maize and sunflower. *Agr. Water Mgt.* 30:237–249.
- Katerji, N., J.W. van Hoorn, A. Hamdy, M. Mastrorilli, and F. Karam. 1998. Salinity and drought, a comparison of their effects on the relationship between yield and evapotranspiration. *Agr. Water Mgt.* 36:45–54.
- Khan, M.A., I.A. Ungar, and A.M. Showalter. 1999. Effects of salinity on growth, ion content, and osmotic relations in *Halopyrum mucronatum* (L.) Stapf. *J. Plant Nutr.* 22:191–204.
- Konikow, L.F. 2013. Groundwater depletion in the United States (1900–2008). U.S. Geological Survey Scientific Investigations Report 2013–5079. United States Geological Survey, Washington, DC. 18 Dec. 2015. <<http://pubs.usgs.gov/sir/2013/5079/>>.
- Lock, G.W. 1962. Sisal: Twenty-five years' sisal research. Longmans, London.
- Maksimovic, I. and Ž. Ilin. 2012. Effects of salinity on vegetable growth and nutrients uptake, p. 169–187. In: T.S. Lee (ed.). Irrigation systems and practices in challenging environments. InTech, Rijeka, Croatia.
- Massoud, F. 1976. Basic principles for prognosis and monitoring of salinity and sodicity. *Proc. Intl. Salinity Conf.* Lubbock, TX.
- Minnis, P.E. and S.E. Plog. 1976. A study of the site specific distribution of *Agave parryi* in east central Arizona. *Kiva* 41:299–308.
- Miyamoto, S. 2008. Salt tolerance of landscape plants common to the Southwest. Texas Water Resources Institute, El Paso, TX.
- Moghbeli, E., S. Fathollahi, H. Salari, G. Ahmadi, F. Saliqehdar, A. Safari, and M.S.H. Grouh. 2012. Effects of salinity stress on growth and yield of *Aloe vera* L. *J. Med. Plants Res.* 6:3272–3277.
- Munns, R. and R. James. 2003. Screening methods for salinity tolerance: A case study with tetraploid wheat. *Plant Soil* 253:201–218.
- Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59:651–681.
- Nerd, A., A. Karadi, and Y. Mizrahi. 1991. Salt tolerance of prickly pear cactus (*Opuntia ficus-indica*). *Plant Soil* 137:201–207.
- Nichols, B.A., B.G. Hopkins, V.D. Jolley, B.L. Webb, B.G. Greenwood, and J.R. Buck. 2012.

- Phosphorus and zinc interactions and their relationships with other nutrients in maize grown in chelator-buffered nutrient solution. *J. Plant Nutr.* 35:123–141.
- Nobel, P.S. 1984. PAR and temperature influences on CO₂ uptake by desert CAM plants. *Adv. Photosyn. Res.* 3:193–200.
- Nobel, P.S. 1991. Achievable productivities of certain CAM plants: Basis for high values compared with C₃ and C₄ plants. *New Phytol.* 119:183–205.
- Nobel, P.S. 1994. Remarkable agaves and cacti. Oxford University Press, New York, NY.
- Nobel, P.S. 1996. Responses of some North American CAM plants to freezing temperatures and doubled CO₂ concentrations: Implications of global climate change for extending cultivation. *J. Arid Environ.* 34:187–196.
- Nobel, P.S. 2010. Desert wisdom/agaves and cacti: CO₂, water, climate change. iUniverse, New York, NY.
- Nobel, P.S. and W.L. Berry. 1985. Element responses of agaves. *Amer. J. Bot.* 72:686–694.
- Nobel, P.S. and S.D. Smith. 1983. High and low-temperature tolerances and their relationships to distribution of agaves. *Plant Cell Environ.* 6:711–719.
- Nunez, H.M., L.F. Rodriguez, and M. Khanna. 2011. *Agave* for tequila and biofuels: An economic assessment and potential opportunities. *Glob. Change Biol. Bioenergy* 3:43–57.
- Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* 60:324–349.
- Peña-Valdivia, C.B. and A.B. Sánchez-Urdaneta. 2009. Effects of substrate water potential in root growth of *Agave salmiana* Otto ex Salm-Dyck seedlings. *Biol. Res.* 42:239–248.
- Pessarakli, M. 1991. Dry-matter yield, N-15 absorption, and water-uptake by green bean under sodium-chloride stress. *Crop Sci.* 31:1633–1640.
- Pinkerton, A. 1971. Some micronutrient deficiencies in sisal (*Agave sisalana*). *Exp. Agr.* 7:113–122.
- Ruiz-Luna, J., V. Velasco-Velasco, R. Maldonado-Peralta, J. Enríquez-del Valle, G. Campos-Ángeles, G. Rodríguez-Ortiz, and P. Preciado-Rangel. 2011. Expression of nutrient deficiencies of nitrogen, phosphorus, potassium, sulfur and iron in *Agave angustifolia* Haw. *Acta Hort.* 947:339–345.
- Schuch, U.K. and J.J. Kelly. 2008. Salinity tolerance of cacti and succulents. College of Agriculture and Life Sciences, University of Arizona, Tucson, AZ. 18 Dec. 2015. <<http://hdl.handle.net/10150/216639>>.
- Shaheen, R. and R.C. Hood-Nowotny. 2005. Carbon isotope discrimination: Potential for screening salinity tolerance in rice at the seedling stage using hydroponics. *Plant Breed.* 124:220–224.
- Steinberg, R.A. 1953. Symptoms of molybdenum deficiency in tobacco. *Plant Physiol.* 28:319–322.
- Wada, Y., L.P.H. van Beek, C.M. van Kempen, J.W.T.M. Reckman, S. Vasak, and M.F.P. Bierkens. 2010. Global depletion of groundwater resources. *Geophys. Res. Lett.* 37:L20402.
- Welsh, S.L., N.D. Atwood, S. Goodrich, and L.C. Higgins. 1993. A Utah flora. Brigham Young University, Provo, UT.
- Winter, K. and J.A.C. Smith. 1996. An introduction to crassulacean acid metabolism. Biochemical principles and ecological diversity, p. 1–13. In: K. Winter and J.A.C. Smith (eds.). *Crassulacean acid metabolism*. Springer Berlin, Heidelberg, Germany.
- Yang, X., J.C. Cushman, A.M. Borland, E.J. Edwards, S.D. Wullschlegel, G.A. Tuskan, N.A. Owen, H. Griffiths, J.A.C. Smith, H.C. De Paoli, D.J. Weston, R. Cottingham, J. Hartwell, S.C. Davis, K. Silvera, R. Ming, K. Schlauch, P. Abraham, J.R. Stewart, H.-B. Guo, R. Albion, J. Ha, S.D. Lim, B.W.M. Wone, W.C. Yim, T. Garcia, J.A. Mayer, J. Petereit, S.S. Nair, E. Casey, R.L. Hettich, J. Ceusters, P. Ranjan, K.J. Palla, H. Yin, C. Reyes-Garcia, J.L. Andrade, L. Freschi, J.D. Beltran, L.V. Dever, S.F. Boxall, J. Waller, J. Davies, P. Bupphada, N. Kadu, K. Winter, R.F. Sage, C.N. Aguilar, J. Schmutz, J. Jenkins, and J.A.M. Holtum. 2015. A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. *New Phytol.* 207:491–504.