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# Evaluation of salt-tolerant forages for sequential water reuse systems

## III. Potential implications for ruminant mineral nutrition

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### Abstract

Reuse of drainage waters is an attractive management option that has been proposed for many irrigated agricultural areas. In California's San Joaquin Valley (SJV), however, drainage effluents are not only saline, but may also contain potentially toxic trace elements such as selenium and molybdenum. Crop suitability for reuse systems depends on the influence the sodium sulfate-dominated waters have on biomass production, plant sustainability, and mineral elements that are critically important for forage quality.

Ten promising forage crops were grown in greenhouse sand cultures irrigated with synthetic drainage waters dominated by  $\text{Na}_2\text{SO}_4$  with an EC of either 15 or 25 dS/m each containing 500  $\mu\text{g/L}$  Se and Mo as  $\text{SeO}_4^{2-}$  and  $\text{MoO}_4^{2-}$ . Plant material was analyzed three times for mineral content and selected trace elements that may have a profound influence on ruminant health.

Trace element concentrations indicate Se toxicity is of little concern, but that high concentrations of both Mo and S in the herbage may lead to Cu deficiency in ruminants. Similarly, high K/Mg and K/(Ca + Mg) ratios in many of the legume and grass forages, respectively, indicate that there may be potential for development of sub-normal Mg levels (hypomagnesaemia) in ruminants. However, each of these disorders can be avoided or corrected with dietary supplements. The most concern regarding

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ruminant nutrition based on these data is sulfur toxicity. Sodium-sulfate dominated drainage waters will likely elevate forage S concentrations to levels that might cause excessive sulfide concentrations in the rumen and potentially lead to serious neurological disorders affecting animal health.

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## 1. Introduction

Naturally occurring trace elements in soils and groundwaters in the San Joaquin Valley (SJV) of California add an additional dimension to the management of saline drainage waters (van Schilfhaarde, 1990). Selenium (Se) and molybdenum (Mo) are trace elements of particular interest in regards to irrigation of forages. These elements occur in relatively high concentrations at many locations in the geochemically mobile and biologically available forms as selenate and molybdate (Deverel et al., 1984; Phillips and Meyer, 1993).

High sulfate-containing drainage water in itself could be problematic regarding forage quality. There is a narrow margin between sulfur concentrations in forages that are desirable and those that can be harmful for ruminants (Underwood and Suttle, 1999). Forages containing 3–4 g S/kg dry wt. were found to decrease appetite and growth rate of sheep and cattle by as much as two to three-fold (Kandylis, 1984) and may lead to neurological disorders caused by cerebrocortical necrosis (polioencephalomalacia or PEM) (Gould et al., 1991).

In this greenhouse sand-culture study, we irrigated ten forages with sodium sulfate-dominated waters with high levels of selenate and molybdate prepared to simulate saline drainage effluents with compositions typical of those present in the SJV. The objective of this portion of the study was to evaluate forage mineral concentration, compare those values with published standards based on the maximum tolerable concentration (MTC) and identify potential concerns regarding ruminant nutrition with emphasis on Cu, Mo, Se, sulfur and K/Mg and K/(Ca + Mg) ratios.

## 2. Materials and methods

The ten forage species chosen for this study were alfalfa (*Medicago sativa* L.) cvs. 'Salado' and 'SW 9720', narrowleaf trefoil (*Lotus glaber* Greene), broadleaf trefoil 'Big' (*L. ulginosus* Schk.), kikuyugrass (*Pennisetum clandestinum* Hochst. Ex Chiov.) cv. 'Whittet', alkali sacaton (*Sporobolus airoides* Torr.), paspalum (*Paspalum vaginatum* Swartz) cvs. 'Polo' and 'PI 299042', tall wheatgrass (*Agropyron elongatum* (Host) Beauv.) cv. 'Jose', and bermudagrass (*Cynodon dactylon* (L.) Pers.) cv. 'Tifton'. The experiment was conducted from 17 July 2000 to 1 Aug 2001. Growth conditions and experimental details are given in a companion paper (Grattan et al., 2004).

After each of the first three harvests, Ca, Mg, K, S, Cu, and Mo were determined on nitric-perchloric acid digests of the tissues by inductively coupled plasma optical

emission spectrometry. The method described by Briggs and Crock (1986) was followed for analysis of tissue Se. Statistical analyses were performed by analysis of variance with mean comparisons at the 95% level based on Tukey's studentized range test.

### 3. Results and discussion

Plant performance can be adversely affected by salinity-induced nutrient imbalances that result from the effect of salinity on nutrient availability, competitive uptake, transport and partitioning within the plant (Grattan and Grieve, 1999). In light of the unique composition of SJV drainage water, such interactions are of particular concern and are the basis for a companion article from this study (Grieve et al., 2004). With the exception of sulfur and ion ratios affecting ruminant nutrition, major ions will not be discussed further in this paper.

#### 3.1. Potential for tetany

Tetany (hypomagnesaemia) is a serious metabolic disorder in ruminants that is affected by forage species and mineral composition (McDowell and Valle, 2000). Bio-availability of magnesium in the rumen is dependent on the intake concentration and the concentration of K in the forage. High K in the forage decreases Mg absorption in the rumen and can potentially lead to tetany (McDowell, 1985; Spears, 1994). The ratio of  $K/(Ca + Mg)$  in grasses, calculated as mole charge per kg, has been suggested as a good indicator for the vulnerability to tetany. The frequency of hypomagnesaemic tetany cases increased when ratios exceeded 2.2 (Grunes et al., 1970). There is evidence that low levels of Ca in the forage can also increase the risk of tetany (Grunes and Welch, 1989). Because legumes tend to have higher concentrations of Ca in shoot tissue than grasses, the K/Mg ratio is a more appropriate indicator of proneness to tetany when evaluating mineral concentrations in legumes (Gross and Jung, 1978; Baligar et al., 2001).

Concentrations of Mg and K varied among forages and harvests (Grieve et al. 2004). Concentrations of Mg in most of the forages we evaluated ranged from 2 to 5 g/kg dry wt. Alfalfa, tall wheatgrass 'Jose', and alkali sacaton tended to fall in the lower portion of this range. Grunes and Welch (1989) indicate that forage concentrations should at least be 2.5 g Mg/kg dry wt. for lactating cattle when concentration of K in the forage is also high (i.e. 30 g K/kg dry wt.). Concentrations of K in our forages are considered high (16–48 g/kg dry wt.) (Grieve et al., 2004) and therefore the K/Mg and  $K/(Ca + Mg)$  ratios were affected.

The  $K/(Ca + Mg)$  equivalent ratio in the grasses we tested (Table 1) usually fell well below critical level of 2.2 [i.e., the ratio above which increases the potential for tetany (Grunes and Welch, 1989)]. Kikuyugrass and tall wheatgrass, however, did not fall below this level until the third harvest. Kikuyugrass was found to have a low metabolic energy value compared to the other forages (Robinson et al., 2004) and was already eliminated as a potentially useful forage based on this quality criterion. Tall wheatgrass, on the other hand,

Table 1

Shoot K/Mg ratios (alfalfas and trefoils) and K/(Ca + Mg) ratios (grasses) for the first three harvests at the two salinity levels (means and standard errors)

	Legumes (K/Mg)				Grasses (K/(Ca + Mg))					
	Salado alfalfa	SW9720 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose Tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot K/Mg or K/(Ca + Mg) ratios (equivalent ratio)										
Harvest 1										
EC 15	3.0 (0.2)	3.9 (0.4)	0.8 (0.1)	3.5 (0.1)	1.1 (0.1)	0.9 (0.1)	2.8 (0.1)	1.2 (0.0)	3.2 (0.2)	1.2 (0.1)
EC 25	2.2 (0.1)	2.5 (0.1)	0.5 (0.0)	2.3 (0.3)	1.1 (0.1)	0.8 (0.1)	2.7 (0.1)	1.1 (0.0)	2.8 (0.2)	1.4 (0.1)
Harvest 2										
EC 15	4.5 (0.4)	4.6 (0.3)	0.7 (0.1)	3.4 (0.3)	1.6 (0.1)	1.7 (0.1)	2.5 (0.1)	1.1 (0.1)	2.9 (0.1)	1.4 (0.1)
EC 25	2.1 (0.2)	2.3 (0.1)	–	2.0 (0.1)	1.7 (0.0)	1.7 (0.1)	2.8 (0.1)	1.1 (0.0)	3.5 (0.2)	1.3 (0.1)
Harvest 3										
EC 15	3.7 (0.3)	3.8 (0.1)	–	2.1 (0.2)	1.5 (0.1)	1.5 (0.2)	1.7 (0.1)	0.5 (0.0)	1.7 (0.1)	1.5 (0.1)
EC 25	1.7 (0.1)	1.8 (0.1)	–	1.4 (0.1)	1.7 (0.1)	1.8 (0.1)	1.9 (0.1)	0.6 (0.1)	1.6 (0.1)	1.4 (0.2)

All ratios are based on equivalent concentrations in the forages.

was found to have high nutritional value (Robinson et al., 2004) in addition to high biomass production and high salt tolerance. Therefore, it would be advisable to monitor Mg nutritional status of animals grazing in tall wheatgrass pastures irrigated with saline drainage waters.

The K/Mg equivalent ratio in the legumes (Table 1) differ from the grasses because the value was largely influenced by the salinity level. As salinity increased, the K/Mg ratio decreased. At the lower salinity level, a level more appropriate for alfalfa due to its lower salt-tolerance, the K/Mg ratio ranged from 3 to 4.6. Gross and Jung (1978) indicated that alfalfa had a higher K/Mg ratio than other legumes tested including ‘birdsfoot’ trefoil implying that ruminants consuming alfalfa may be at higher risk for developing tetany than if they consumed other legumes. We also found alfalfa to generally have a higher K/Mg ratio than broadleaf trefoil, a forage that did not perform well under salinity stress, or narrowleaf trefoil by the third harvest. No criteria are available to identify the critical K/Mg ratio in legumes (Gross and Jung, 1978).

It is possible that the high K/Mg and K/(Ca + Mg) ratios in the legumes and grasses reported here may be partly related to the conditions of the sand-tank system. High water content (i.e. near field capacity) over an extended period, such as these forages experienced due to frequent irrigations in the sand system, was found to increase these ratios (Grunes and Welch, 1989 re-evaluating data by Karlen et al., 1980). Increased volumetric water content more than doubled the K/(Ca + Mg) ratio. Therefore under field conditions with longer irrigation intervals, the ratios may be quite different than those we found in our controlled experiment.

### 3.2. Sulfur

Very little attention has been given to the influence of salinity on sulfur uptake and accumulation in crops (Grattan and Grieve, 1999). The salt-stressed forages present several species-specific patterns of shoot-S accumulation (Table 2). Sulfur in the legumes increased in response to the two-fold increase in substrate-SO<sub>4</sub>. Total-S patterns in the grasses differed with species. The paspalum varieties accumulated the most S (250–430 mmol/kg dry wt.) while “Jose” tall wheatgrass accumulated the least (100–120 mmol/kg dry wt.). Differences in external SO<sub>4</sub> generally did not affect total-S in the paspalum varieties, ‘Jose’ tall wheatgrass or alkali sacaton. Sulfur relations in bermudagrass and kikuyugrass, on the other hand, were unusual in that shoot-S in earlier harvests was higher in the 15 dS m<sup>-1</sup> treatment containing 58 mM SO<sub>4</sub> than those in the 25 dS m<sup>-1</sup> treatment containing 104 mM SO<sub>4</sub><sup>2-</sup>.

Although cattle can tolerate more S from natural feed ingredients than from supplemental sulfate (McDowell, 1985), forages grown under irrigation with high-sulfate waters should be evaluated carefully for S effects on animal nutrition. Dietary S above 0.30–0.40% dry wt. (94 to 125 mmol/kg dry wt.) may be toxic to ruminants through interactions with essential micronutrients (McBride et al., 2000). The shoot-S concentrations in forages at both salt levels came close to or exceeded the maximum tolerable concentrations (MTC) of 125 mmol/kg dry wt. Perhaps the greatest concern with ruminants consuming excessive S is development of excessive quantities of sulfides potentially leading to PEM (Gould et al., 1991).

Table 2  
Shoot S concentration (mmol/kg dry wt.) for the first three harvests at the two salinity levels

	Salado alfalfa	SW972 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot S concentration (mmol/kg dry wt.)										
Harvest 1										
EC 15	164b	126b	394b	105a	303a	251a	109a	212a	185a	140a
EC 25	213a	211a	732a	166a	397a	311a	115a	206a	156b	122b
Harvest 2										
EC 15	138b	131b	796	144b	393a	228a	102a	194a	154a	150a
EC 25	173a	180a	–	224a	428a	256a	101a	204a	132b	136a
Harvest 3										
EC 15	195b	171b	533	214a	384a	256a	104b	198a	144a	143a
EC 25	288a	289a	–	217a	383a	268a	116a	205a	142a	131a

Means followed with the same letters are not significantly different at the 5% level.

### 3.3. Cu–Mo–S relations

The availability of forage Cu to the animal is dependent not only on the absolute Cu concentration in the forage tissue but the accompanying Mo and S content as well. Copper availability decreases as both the S and Mo concentration increases by the formation of unabsorbable complexes with thiomolybdates (Suttle, 1991; Spears, 2003). For example, sheep consuming forage with a modest 12–20 mg Cu/kg dry wt. may exhibit symptoms of Cu toxicity if the tissue is low in both S and Mo. On the other hand, this same forage with the same Cu concentration may cause Cu deficiency if the tissue is accompanied by high levels of both S and Mo (Suttle, 1991).

Increased salinity had very little impact on forage Cu concentration (Table 3). In a few instances increased salinity reduced Cu concentration, the most notable case was the first cutting of bermudagrass. The copper concentration in our forages are at least equal to or greater than average concentrations found in field-grown forages (Minson, 1990). Shoot Cu concentrations were generally higher in the grasses than in the legumes. Concentrations in legumes ranged from 5 to 14 mg/kg dry wt. whereas in grasses, concentrations ranged from 10–66 mg/kg dry wt. Nevertheless due to the accompanying high concentrations of both Mo and S, our data indicate that ruminants could eventually suffer from Cu deficiency, regardless of forage, should they be dependent solely on a diet of this quality without Cu supplementation (Suttle, 1991).

Mo concentrations varied considerably among the forages tested (Table 4). The legumes (trefoils and alfalfa cultivars) accumulated much more Mo than did the grass forages tested. It has long been recognized that pasture legumes can accumulate more molybdenum than grasses (Barshard, 1948; Johnson, 1966). The legumes, being strong Mo accumulators, allowed shoot Cu:Mo ratio to fall below the minimum recommended level, e.g. 2:1 for prevention of Cu deficiency in ruminants (Table 5). With the exception of 'Narrowleaf' trefoil at the third harvest, increasing salinity (i.e. increased sulfate) did not reduce total Mo in the plant tissue.

Another important point is that Cu availability in fresh herbage, such as that consumed by grazing animals, is considerably lower than that in dry forages (Minson, 1990). Copper availability is also influenced by sulfide concentrations in the rumen of grazing animals. This has important implications if animals are grazing on fields irrigated with saline drainage water rather than fed the forage after it is dried and baled.

Since antagonistic ions such as sulfur and molybdenum were found in our forage tissue in high concentrations, it is possible that the fraction of Cu available to the animal would be reduced substantially should animals be fed with forage of this quality. Quantitative relationships have been proposed as a means of estimating the availability of dietary Cu (Minson, 1990; MacPherson, 2000), but they vary depending upon the ruminant and forage type. It is suggested that when such antagonistic ions are present at relatively high levels that the best way of assessing animal Cu status is not by analysis of the forage sample but rather by monitoring the animals themselves through tissue samples such as liver biopsies (Mortimer et al., 1999). Therefore, our results indicate that ruminants fed forages, particularly legumes, irrigated with saline SJV drainage water should be monitored to determine if Cu feeding supplements are necessary.

Table 3  
Shoot Cu concentration (mg/kg dry wt.) for the first three harvests at the two salinity levels

	Salado alfalfa	SW9720 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot Cu concentration (mg/kg dry wt.)										
Harvest 1										
EC 15	12.9a	10.7a	5.6a	5.8a	15.2a	31.8a	17.7a	15.7a	19.9a	66.5a
EC 25	14.3a	9.6a	4.5a	6.1a	13.3a	22.3b	15.9a	11.4a	20.9a	17.0b
Harvest 2										
EC 15	7.1b	7.6a	12.4	6.5a	16.7a	15.4a	10.6a	13.7a	15.2a	18.9a
EC 25	8.5a	8.4a	–	6.8a	15.1a	23.0a	10.5a	14.6a	8.5a	25.1a
Harvest 3										
EC 15	5.9a	5.6a	–	12.4a	16.2a	19.9a	12.8a	13.9a	10.6a	20.6a
EC 25	6.6a	6.6a	–	8.0b	18.4a	23.4a	13.5a	15.6a	12.2a	16.0a

Means followed with the same letters are not significantly different at the 5% level.



Table 4  
Shoot Mo concentration (mg/kg dry wt.) for the first three harvests at the two salinity levels

	Salado alfalfa	SW9720 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot Mo concentration (mg/kg dry wt.)										
Harvest 1										
EC 15	136.6a	42.4a	128.7a	37.6a	3.0a	2.1a	3.0a	3.5b	5.0a	4.4a
EC 25	2.5b	1.9b	144.0a	42.8a	3.1a	2.4a	3.2a	5.6a	3.8a	2.3b
Harvest 2										
EC 15	13.2b	14.1b	128.4	49.4a	2.8a	1.7a	3.9b	1.2a	3.4a	4.8a
EC 25	23.9a	27.3a	–	57.0a	3.0a	1.7a	5.5a	1.0a	2.6a	3.8a
Harvest 3										
EC 15	6.8b	6.0b	–	99.5a	2.9a	1.9a	2.8b	1.9a	2.3a	6.8a
EC 25	23.2a	30.3a	–	49.8b	3.2a	1.9a	4.5a	1.4b	2.3a	3.6a

Means followed with the same letters are not significantly different at the 5% level.

Table 5  
Shoot Cu/Mo concentration ratios (based on mg/kg dry wt.) for the first three harvests at the two salinity levels

	Salado alfalfa	SW9720 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot Cu/Mo ratio										
Harvest 1										
EC 15	0.1b	0.3b	0.04a	0.16a	5.2a	16.0a	6.0a	4.3a	4.0a	15.6a
EC 25	5.9a	5.1a	0.03a	0.14a	4.4a	9.2b	5.0a	2.0a	5.3a	7.5b
Harvest 2										
EC 15	0.5a	0.5a	0.1	0.1a	5.9a	9.6a	2.7a	11.2a	4.6a	4.0a
EC 25	0.4b	0.3b	–	0.1a	5.0a	13.1a	1.9b	15.8a	3.3a	6.4a
Harvest 3										
EC 15	0.9a	0.9a	–	0.1a	5.7a	10.3a	4.6a	7.6a	4.6a	4.4a
EC 25	0.3b	0.2b	–	0.2a	5.7a	12.8a	3.0a	11.0a	5.3a	4.7a

Means followed with the same letters are not significantly different at the 5% level.

Table 6  
Shoot Se concentration (mg/kg dry wt.) for the first harvest at the two salinity levels

	Salado alfalfa	SW9720 alfalfa	Big trefoil	Narrow trefoil	Polo paspalum	PI 299042 paspalum	Jose tall wheatgrass	Alkali sacaton	Kikuyu grass	Bermuda grass
Shoot Se concentration (mg/kg dry wt.)										
Harvest 1										
EC 15	1.3a	1.0a	2.2a	0.7a	1.6a	0.6b	0.9a	1.7a	1.8a	0.7a
EC 25	0.9b	0.9a	1.5a	0.7a	1.5a	1.1a	0.2b	0.8b	0.9b	0.4b
Harvest 3										
EC 15	1.3a	1.1a	–	1.4a	2.2a	1.5a	0.6a	1.1a	1.1a	0.8a
EC 25	1.2a	1.3a	–	0.6b	1.3a	1.0b	0.3b	0.6b	0.6b	0.5b

Means followed with the same letters are not significantly different at the 5% level.

### 3.4. Selenium

Selenium concentrations were generally influenced by salinity (Table 6). As the salinity increased from 15 to 25 dS/m, the external sulfate concentration increased from 112 to 195 mmol/L. This increase in sulfate presumably caused a significant reduction in shoot Se concentration in the grasses 'Jose' tall wheatgrass, alkali sacaton, kikuyugrass and bermudagrass. Increased salinity either had no effect or mixed effects, depending upon harvest date, for the other species tested.

Selenium concentrations in the forages were low (e.g.  $\sim 1\text{--}2\text{ mg kg}^{-1}$  dry wt.) and would pose little health risk to livestock based on reported toxicity values. This concentration range is above what is regarded as inadequate ( $<0.03\text{ mg/kg}$  dry wt.) and below the maximum tolerable concentration ( $2.0\text{ mg/kg}$  dry wt.) (Minson, 1990). High sulfate not only reduces Se in the plant but also reduces Se bioavailability in the rumen (Tanji et al., 1988; Spears, 2003).

## 4. Concluding Remarks

Forage mineral quality was evaluated by relating nutrient ion concentrations and ratios we found in our forages to those where nutrient disorders, both deficiencies and toxicities, are possible or likely to occur in ruminants based on findings in the literature.

Trace element concentrations and ratios in our study indicate that there is little concern regarding Se toxicity but the high concentrations of both Mo and S in the plant tissue may pose a problem regarding Cu deficiency in ruminants (Ward, 1978). Although the potential for sulfur and Mo-induced Cu deficiency is higher with the legumes, this potential exists regardless of the forage type. Therefore the Cu status in the animals needs to be monitored and supplements may be needed.

The potential for tetany may exist if some of these forages, particularly alfalfa and 'Jose' tall wheatgrass, were irrigated with SJV drainage water and ruminants were not monitored for Mg nutrition. However, the ratios of K/Mg and K/(Ca + Mg) may be lower in field conditions than found here. Moreover, hypomagnesaemia can readily be corrected with Mg supplements.

Based on our study, sulfur toxicity may be the greatest concern regarding ruminant nutrition. It is a condition that can not be corrected readily by a dietary supplement. The sodium-sulfate nature of this drainage water will likely elevate forage S concentrations, possibly to levels that could cause excessive sulfide concentrations in the ruminal environment. If this condition were severe enough, it could lead to PEM or polioencephalomalacia. Regardless, the forage and animals need to be monitored for S status and nutritional health.

An ideal forage for use in saline water reuse systems would be one with high biomass production potential, high salt-tolerance, and high forage quality. The forage species tested performed differently in terms of absolute biomass accumulation, biomass accumulation relative to salinity level, and the concentration of various ions accumulated in the aboveground tissues. At 25 dS/m, tall wheatgrass, 'PI 299042' paspalum, and bermudagrass accumulated biomass at the greatest rate (see Grattan et al., 2004), followed

closely by the alfalfas and kikuyugrass. Kikuyugrass produced well under these conditions, but its forage quality was among the lowest (Robinson et al., 2004). Forages of good to high quality from an organic, nutritive perspective were the two alfalfa varieties, 'PI 299042' paspalum, narrow leaf trefoil, bermudagrass, tall wheat grass and 'Polo' paspalum. The forage mineral nutrient quality of 'Jose' tall wheatgrass was desirable is that it accumulated the least sulfur. Although the alfalfa cultivars, narrow leaf trefoil and 'PI 299042' paspalum grew well under these controlled conditions, their performance will likely decline at higher salinity because these cultivars were found to be the more salt-sensitive than the others tested. Based on our study and satisfying all three criteria (i.e. high biomass, high salt-tolerance and high forage quality), tall wheatgrass 'Jose' and bermudagrass emerge as the top forage candidates from those we tested followed closely by 'PI 299042' paspalum.

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