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Irrigation Water Salinity Effects On Germination and Emergence of Six Halophytes

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Abstract

Low availability of surface water has exacerbated the use of groundwater for irrigation in New Mexico. About 75% of the groundwater in NM is brackish and prolonged application without treatment could increase soil salinity. Reverse Osmosis (RO) treats brackish groundwater but disposal or reuse of RO concentrate is a problem. The objective of this study was to test germination and emergence of six halophyte species under a water salinity gradient. Four irrigation water treatments were tap water (EC 0.8 dS/m), brackish groundwater (EC 5.0 dS/m), RO1 concentrate water (EC 8.0 dS/m), and RO1 concentrate mixed with NaCl (RO2; EC 10 dS/m). Experiments with six halophytes species (*Atriplex canescens*, *Hordeum vulgare*, *Lepidium alyssoides, Distichlis stricta*, *Panicum virgatum*, *xTriticosecale)* were carried out for 30 days in a greenhouse. *H. vulgare* and *xTriticosecale* had no significant difference in percent germination with higher germinations under salinity treatments, and other species showed similar germinations under higher salinity treatments. On the other hand, *D. stricta* seeds displayed lower germinations under higher salinity treatments. Results of the emergence percentage showed that *H. vulgare* and *xTriticosecale* had no significant difference with higher emergence under higher salinity treatments, while other species showed similar emergence percentage under higher salinity treatments. In contrast, *L. alyssoides* and *A. canescens* seeds showed lower emergence percentage under higher salinity treatments. Increasing irrigation water salinity increased mean germination time for all species except *L. alyssoides*, but did not affect the percentage germination significantly except for *A. canescens*. Increasing irrigation water salinity increased mean emergence time for all species except *L. alyssoides,* but did not affect the percent emergence for *H. Vulgare, xTriticosecale,* and *P. virtagum* species significantly. Results showed that *H. vulgare* (barley), *xTriticosecale* (triticale), and *P. virtagum* (switchgrass) are candidate species for irrigation with brackish groundwater and RO1 concentrate in water scarce areas. However, their survival and growth should be further tested in different soils.

Keywords: Halophytes; Saline water; Germination; Irrigation; *xTriticosecale*; *H. vulgare*; *P. virtagum*

Introduction

In New Mexico, surface water is declining so ground water is increasing used for agricultural irrigation demands [1,2]. According to the NM State Engineer's Office, 46% of water for human consumption has been supplied from groundwater, and the remaining 54% from the surface water. Agriculture water uses about 79% of total surface and groundwater, 8% is used for the public water supply, 7% is lost to evaporation, and 6% is used for self-supplied consumption that include 0.33% industrial, 1% commercial, 1% domestic wells, 2% is for power, 1% is for mining, and 1% for livestock [3]. Almost 75% of groundwater in New Mexico is brackish with electrical conductivities (EC) greater than 3 dS/m [4]. Desalination of brackish groundwater (salinity level is greater than freshwater however less saline than seawater) produces both drinkable, low saline water, and high saline or high saline-sodic wastewater concentrate [5]. However, after groundwater is desalinated, environmentally safe disposal or reuse of RO (reverse osmosis) concentrate that is generated from an inland desalination unit is a crucial problem in New Mexico and the southwestern U.S.

It is a well-known fact that saline water can have a direct impact on the germination and emergence of halophytes. Some of these halophytes species (*Atriplex canescens*, *Hordeum vulgare*, *Lepidium alyssoides, Distichlis stricta*, *Panicum virgatum*, *xTriticosecale*) are found in West Mesa in New Mexico. However, prolonged application of brackish water without treatment could increase soil salinity. Experimental study could encourage use of saline water for sustainable agricultural demand.

A halophyte is a plant that grows, flowers and produces seeds under saline conditions [6]. As author indicates, the word halophyte is used to express all plant growth in an environment with more than 0.5% NaCl water. According to this research, halophytes are able to survive when watered with high salinity water. On the other hand, salinity has an essential effect on planting and germination of halophyte seeds because it affects germination, imbibition, and root elongation. Halophytes can be separated from glycophytes by their tolerance of saline conditions. For instance, seeds of the halophytes *A. prostrata* and *A. patula* were evaluated under numerous NaCl solution treatments [7]. As Al-Seedi [8] reported that the germination percentage of *H. vulgares* seeds decreased by applying concentrated water in levels 6, 9, 12, 15 ds/m. Additionally, Muhammad and Hussain [9] tested seeds of *Lepidium sativum* L., *Linum usitatissimum* L., Plantago ovata Forssk., and *Trigonella foenum-graecum* L. to check the influence of NaCl salinity on the germination. The ANOVA revealed that the effects of various concentrations of NaCl on the seed germination were not statistically significant. In terms of food security, halophytic forage and seed products could be used for animal feed or as a salt substitute in animal fodder [10]. In another study, Glenn and Brown [11] indicated that halophytes generally have high protein content, ranging from 10 to 20% of dry matter as well as

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high salt content (15 to 50% salts on the leaf dry matter). In a study on *H. vulgare* L., it was shown that percentage germination decreased under saline treatments which are 0.5, 3.0, 6.0, 9.0, 12.0 dS/m [12]. In the Flores et al. [1] study, the germination of six halophyte species was tested for 22 days with four saline treatments (EC 0, 0.6, 4.0, and 10.0 dS/m), and results indicated that percentage germination were similar within a species across salinity treatments, with the exception of *L. alyssoides* which showed increased germination under high salinity. Flores et al. [1] also reported that except for *A. canescens*, all species showed delays in germination in response to increasing water salinity. For this study, we hypothesize that reverse osmosis (RO) concentrate water can be reused for irrigating halophytes that can be used as a salt substitute in animal fodder for ensuring food security, and since tested species are halophytes, germination and emergence percentages will not be influenced by irrigation water salinity. The objective of the experimental study was to test the germination and emergence of halophytes under an irrigation water salinity gradient.

Materials and Methods

Experimental detail

This greenhouse experiment focuses on the effects on germination and emergence of halophytes irrigated with tap water, brackish groundwater, RO1 concentrate water, and RO2 concentrate water with NaCl. Six halophyte species were: *Atriplex canescens* (Pursh) Nutt. (fourwing saltbush), *Hordeum vulgare* L. (barley), *Lepidium alyssoides* A. Gray (mesa pepperwort), *Distichlis stricta* (Torr.) Rydb. (Inland salt grass), *Panicum virgatum* L. (switchgrass), and *xTriticosecale* Wittm. (triticale). Except for L. *alyssoides,* all seeds were supplied by Curtis & Curtis Inc. in Clovis, NM. This experiment was implemented in two well defined phases which are germination and emergence. These phases were carried out in the greenhouse at the Fabian Garcia Science Center in Las Cruces, New Mexico (32.2805°N latitude and 106.770°W longitude, elevation 1186 m above sea level). This experimental study was conducted in the greenhouse because the New Mexico Department of Agriculture does not allow land application of water with EC > 4 dS/m.

Water treatments

Brackish groundwater and RO concentrate was obtained from the Brackish Groundwater National Desalination Research Facility (BGNDRF) located in the Tularosa Basin, Alamogordo, New Mexico (32°52'N, 105°58'W). All water samples were analyzed for EC and pH by first calibrating Oakton EC Testr 11 Dual Range and Oakton pH Testr 20 probes, respectively. The measurements were recorded by directly placing probes in water samples according to EPA method 150.1 for pH and EPA 120.1 for EC [13,14]. The concentrations of Mg^{2+}, Na^{+}, and Ca^{2+} ions were determined by analyzing water samples in a PerkinElmer Optima 4300 DV (Dual View) ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) according to EPA 200.7 method and sodium adsorption ratio (SAR) was subsequently determined using the following equation [15,16]:

$$SAR = \frac{\left[Na^{+}\right]}{\sqrt{\frac{\left(\left[Ca^{2+}\right]\left[Mg^{2+}\right]\right)}{2}}} \quad (1)$$

where $[Na^{+}]$: The concentration of sodium ion (meq/L), $[Ca^{(2+)}]$: The concentration of calcium ion (meq/L), and $[Mg^{(2+)}]$: The concentration of magnesium ion in the sample (meq/L).

Saline water has high concentrations of dissolved salts (cations and anions). Salt could be sodium chloride (NaCl); however, it can have dissolved calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfate (SO_4^{2-}), bicarbonate (HCO_3^{-}), and other compounds. Salinity of water is specified as total dissolved solids (TDS); however, salinity is also determined by measuring the electrical conductivity (EC) of water. TDS is related to EC by the equations: TDS (mg/L)=640*EC (dS/m) [17]. It is stated in the U.S. Department of Agriculture that if EC of water is greater than 4.0 dS/m, it is defined as saline. If SAR is greater than 13, water is specified as sodic water [17].

Four water treatments selected for this study were: greenhouse of Fabian Garcia Science Center irrigation tap water for the control (EC=0.8 dS/m, SAR=2.22, pH=7.31), BGNDRF brackish groundwater (EC=5 dS/m, SAR=4.49, pH=7.44), BGNDRF RO1 concentrate (EC=8 dS/m, SAR=5.92, pH=7.35), and RO1 concentrate with NaCl (RO2) (EC=10 dS/m, SAR=10.73, pH=7.43). The RO concentrate from BGNDRF was calcium dominant; however, in NM ground water has variable amounts of Ca and Na. Therefore, NaCl was added to create another salinity treatment. These four treatments were selected to create a salinity gradient with tap water as a control.

Germination experiment

For the seeds of *A. canescens*, Twitchell [18] discovered that taking 30 g of seeds and soaking in 3 L of water for two hours, followed by rinsing with 3 L of distilled water, and air drying the seeds for seven days decreased seed dormancy. This method was used to prepare the seeds of *A. canescens* for germination.

Irrigation water salinity effects on germination of halophytes were conducted using an experimental set up containing 72 petri dishes [3 (replicates) × 4 (treatments) × 6 (species)] for each of the two runs. The germination study was conducted using Petri dishes (100 mm diameter, 16 mm height) which were lined by placing four Whatmann # 3 blotting filter paper (90 mm) in each dish. 25 seeds were placed on the blotting paper in each dish and saturated with 3 mL of the treatment water. Each petri-dish was tightly sealed with Para ''M'' laboratory film to avoid evaporation loss. In order to avoid reduction in weight/moisture of petri dish, about 1 mL of the water was added to each dish once every six days to replenish the moisture loss.

Germinated seeds were removed from petri-dish once the length of the radicle exceeded the length of the seed. Ungerminated seeds remained in the dish until the conclusion of the study and their viability was determined using the imbibed crush test. The experiment was conducted on the bench in the greenhouse and by observing seeds every day for 30 days. The number of remaining seeds and the number of germinated seeds were counted daily for 30 days. The petri-dishes were arranged in a completely randomized design with three replications of each combination of treatments.

Parameters used to assess the irrigation water salinity effects were: germination percentage, mean germination time, germination index, Timson's index, and Timson index modified [19].

Germination percentage is the total percentage of viable seeds in a sample that complete the experimental germination process. This quantity is determined by taking the sum of the germinated seeds, dividing by the total number of seeds (germinated and viable, non-germinated) in the study, and multiplying by 100 to gain a percentage. As a result, germination percentage values range from 0-100%.

$$Germination(\%) = \frac{\sum_{i=1}^{k} n_i}{S} * 100 \quad (2)$$

where: n_i: The number of seeds newly germinated on the i^{th} observation, k: The last day of germination, S: The total number of seeds (germinated and non-germinated) in the experiment. The mean germination time (MGT) is determined by following equation.

$$MGT = \sum_{i=1}^{k}(n_i t_i) / \sum_{i=1}^{k}(n_i) \quad (3)$$

Where: t_i: The number of days from the start of experiment to the i^{th} observation, n_i: The number of seeds newly germinated on day i, and k: The last day of germination.

The germination index (GI) is an index that states the rate of seeds that germinate in a given time period for a given sample of seeds. It contains the non-germinated seeds in the calculation, which makes the calculation dependent on the seed sample size.

$$GI = \frac{\sum_{i=1}^{k}(31 - t_i)(n_i)}{S} \quad (4)$$

Where: 31 is the total number of days spent in this germination test plus 1, S is the number of seeds used in the experimental study, t_i:The number of days from the start of the experiment to the i^{th} observation, n_i: The number of seeds newly germinated on day i, and k: The last day of germination.

Timson's index (T) elucidates the number of seeds that germinate against time. However, it is limited because it is only appropriate when the germination percentages of the seed samples are cognate.

$$T = \sum_{i=1}^{k} \mathrm{gi}(\mathrm{K} - \mathrm{j}) \quad (5)$$

Where: g_i : The number of seeds newly germinated in the time interval i, K: The total number of time intervals, and j=i –1.

Timson's index modified (T_{mod}) is adjusted to take into account the final germinations of the samples by dividing Timson's index by the total number of germinated seeds. This minimizes the effect of the germination percentage.

$$Tmod = \frac{T}{\sum_{i=1}^{k} \mathrm{gi}} \quad (6)$$

Where: T: The Timson's index, K: The total number of time intervals, and g_i: The number of seeds newly germinated in the time interval i.

Emergence experiment

An emergence study was also conducted in the greenhouse at Fabian Garcia Science Center in Las Cruces, New Mexico (32.2805°N latitude and 106.770°W longitude at an elevation of 1186 m above sea level). Irrigation water salinity effects on the emergence of halophytes were determined by using an experimental set up containing 72 cylindrical pots (3 replicates × 4 treatments × 6 species) for each of the two separate runs.

The soil sample for the emergence experiment was collected from West Mesa, New Mexico because some halophytes (mainly atriplex) are growing in this area. Initially, soil was air dried and then the sample was sieved through a 4 mm sieve. Afterwards, the soil sample was sterilized in an oven at a temperature of 85°C for 30 minutes. In the emergence experiment, cylindrical pots of 6.5 cm diameter and 25 cm length were used. The perforated cylindrical pots were packed by first putting cheese cloth at the base of the pot to prevent soil loss and then placing gravel to allow for free drainage. The pots were packed with soil at 5 cm depth increments to obtain a homogenous profile.

In the emergence experiment, knowledge of physical properties of the soil is crucial because it plays an important role in regulating plant growth, air flow, compaction and irrigation water management. The soil bulk density was determined as the ratio of dry soil mass to its total volume. The total volume also contains pore volumes (water filled and air filled) [20].

$$\text{Soil bulk density} = \frac{\text{mass of soil solids}}{\text{Total volume of soil}} = \mathrm{g/cm^3} \quad (7)$$

The soil sample was dried in the oven at a temperature of 105°C for 24 hours. Afterwards, the final mass was considered as the mass of dry soil bulk density. In the experiment, cylindrical pots with gravel were weighed initially and then were weighed after packing with the dry soil.

At the end of packing, each column was initially leached with tap water to remove any salts present in the soil. Leachate water coming out of the bottom was collected and EC was measured. After the leachate sample ECs decreased to about 1 dS/m in approximately 6 irrigation days (Figure 1), they were irrigated with brackish water and RO concentrate to raise the soil EC to that of irrigation water (5.00, 8.00, and 10.00 dS/m). It took about 6 irrigation days to raise the EC of soil to each of the irrigation water treatment levels (Figure 2).

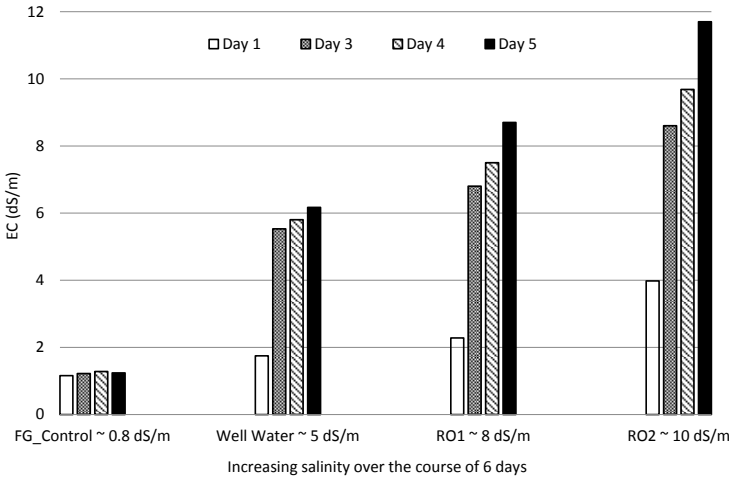

Figure 1: Decreasing salinity over the course of 6 days. The pots were leached with control water until leachate salinity decreased to about 1 dS/m.

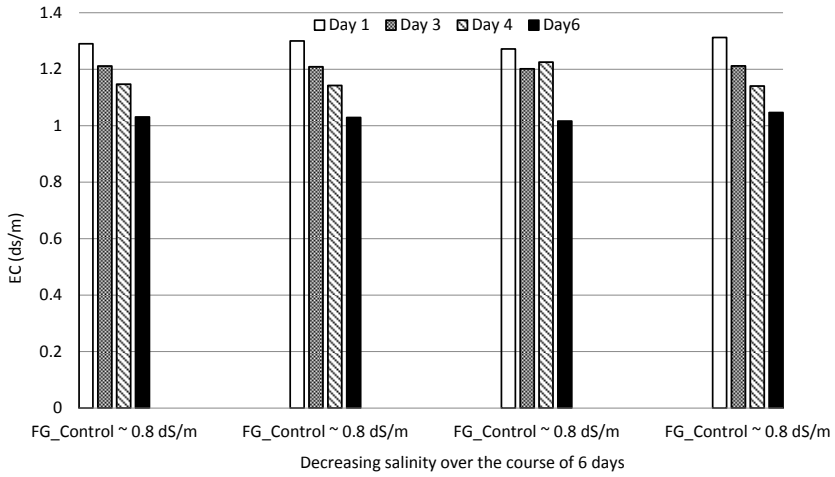

Figure 2: Increasing leachate ECs by irrigating with different water treatments to raise the EC of soil to each of the irrigation water treatment levels (5.00, 8.00, and 10.00 dS/m).

Type of Water	Mg (meq/L)	Ca (meq/L)	Na (meq/L)	K (mg/L)	SAR	EC (dS/m)	pH
Control	1	3.4	3.3	6.2	2.2	0.8	7.3
Groundwater	17.1	22.5	20	4.5	4.5	5	7.4
RO1 Conc.	40	51.8	40.1	10.5	5.9	8	7.4
RO2 Conc.	39.9	51.2	72.4	14.3	10.7	10	7.4

Table 1: Treatment analysis of waters used in the experimental study: water from the Fabian Garcia (FG) greenhouse in Las Cruces, NM; brackish groundwater from Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, NM; RO1 concentrate from BGNDRF; RO2 concentrate from BGNDRF (mixed with NaCl). *Conc. states concentrate.

Each cylindrical pot was sown by placing five halophyte seeds at a 1-2 cm soil depth. The experiment was a completely randomized design with three replications for each combination of irrigation water treatments. Emergence was recorded by making daily observations, and final emergence was recorded 30 days after sowing the seeds. Irrigation water treatments were continuously applied at an interval of 2-3 days, based on the change in pot weights.

Parameters used to evaluate irrigation water salinity effects were: emergence percentage, mean emergence time, emergence index, Timson's index, and Timson index modified. These indices were calculated using the same formula as for germination indices.

Statistical analysis

The cylindrical pot emergence experiments for two runs and the petri dish germination experiments for both runs were arranged in a completely randomized design with three replicates by generating random numbers using Microsoft Excel-2013. Since there are natural variations in the halophyte species selected for this research, the data were analyzed using one-way analysis of variance (ANOVA) using SAS (SAS Institute-2013) for identifying significant differences at $p<0.05$.

Results

The water samples were analyzed for EC, pH, and SAR and results of the analysis are presented in Table 1. The ECs for the greenhouse, groundwater, RO concentrate, and RO2 concentrate waters were obtained as 0.8, 5, 8, 10 dS/m, respectively. The SAR was <4 for the greenhouse water, but >4 for the well, RO concentrate, and RO2 concentrate waters. SAR was <12 and thus the groundwater, RO concentrate, and RO2 concentrate water treatments were saline but not sodic (Table 1).

Irrigation water salinity and germination

Analysis of the final germination percentage showed that two of the six plant species studied, *H. vulgare* and *xTriticosecale,* had no significant difference statistically (Table 2). Also, the *P. virgatum* seeds had no significant difference with germination under salinity treatments. The *A. canescens* seeds had significant differences with germination under salinity treatments. The *L. alyssoides* seeds were the only plant species that showed a difference in the final germinations with higher germination percentages for the higher salinity treatments. On the other hand, the *D. stricta* seeds were the one species that displayed lower germination percentages under salinity treatments (Figure 3).

Although the final germination percentages across the treatments were comparable within selected species, there is evidence of variability among treatments between the onset of germination and the final seed germination, indicating a delay for some species. This is supported by results from the MGT, GI, T, and T_{mod} comparisons for each species found in Tables 2 and 3. Significant differences were noted for the MGT of all species except *H. vulgare* and *xTristicosecale.* For *L. alyssoides,* the difference corresponded to a P-value of <0.004 and *A. canescens* had a P-value of <0.001. The largest differences were noted for *P. virgatum*, and *D. stricta* which all had P-values corresponding to <0.0001. The GI only showed differences for the one species which is *H. vulgare with a* P-value of <0.0013. Much like the GI, T also only showed significant differences for the one plant species which is *H. vulgare* (P-value of <0.0024). $T_{mod,}$ like the weighted MGT, takes into account the cumulative germination of the seed sample and showed significant differences for four of the six species: *H. vulgare* (P-value of <0.0479), *P. virgatum* (P-value of <0.0001), *A. canescens* (P-value of <0.0100), and *D. stricta* (P-value of <0.0001).

Species	Treatments	Germination Percentage	MGT
	(dS/m)	(%) ± SE	(day) ± SE
H. Vulgare	EC 0.8	98.00 ± 0.89 a	5.79 ± 0.87
	EC 5	96.67 ± 1.23 a	5.99 ± 0.26
	EC 8	97.33 ± 0.84 a	6.55 ± 0.35
	EC 10	82.67 ± 6.34 b	7.90 ± 0.51
×*Triticosecale*	EC 0.8	92.67 ± 3.64	4.60 ± 0.17
	EC 5	88.00 ± 4.95	4.76 ± 0.16
	EC 8	89.33 ± 4.70	4.86 ± 0.20
	EC 10	90.67 ± 3.21	5.10 ± 0.23
P. virgatum	EC 0.8	74.00 ± 4.23	8.29 ± 0.31 c
	EC 5	74.67 ± 9.10	8.79 ± 0.16 c
	EC 8	61.33 ± 11.58	10.00 ± 0.15 b
	EC 10	55.33 ± 7.19	11.39 ± 0.15 a
A. canescens	EC 0.8	36.7 ± 4.55 ba	9.93 ± 0.36 b
	EC 5	26.7 ± 3.68 b	11.89 ± 0.36 a
	EC 8	41.67 ± 2.44 a	11.17 ± 0.24 a
	EC 10	38.67 ± 3.53 a	11.35 ± 0.16 a
L. alyssoides	EC 0.8	44.67 ± 11.66	5.61 ± 0.40 b
	EC 5	56.67 ± 16.57	7.59 ± 0.73 a
	EC 8	53.33 ± 17.96	5.02 ± 0.24 b
	EC 10	53.33 ± 17.39	5.48 ± 0.30 b
Distichlis stricta	EC 0.8	16.00 ± 2.31	9.89 ± 0.21 d
	EC 5	16.67 ± 2.40	12.01 ± 0.49 c
	EC 8	16.00 ± 4.73	13.94 ± 0.25 b
	EC 10	10.67 ± 1.33	15.15 ± 0.40 a

Table 2: Effect of salinity on germination parameters of seeds of *Hordeum vulgare, xTriticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta*. Abbreviations: MGT, mean germination time; Within an index and within a species, treatments followed by different letters were significantly different at $p<0.05$. Results are means and standard errors (SE) of six replicates.

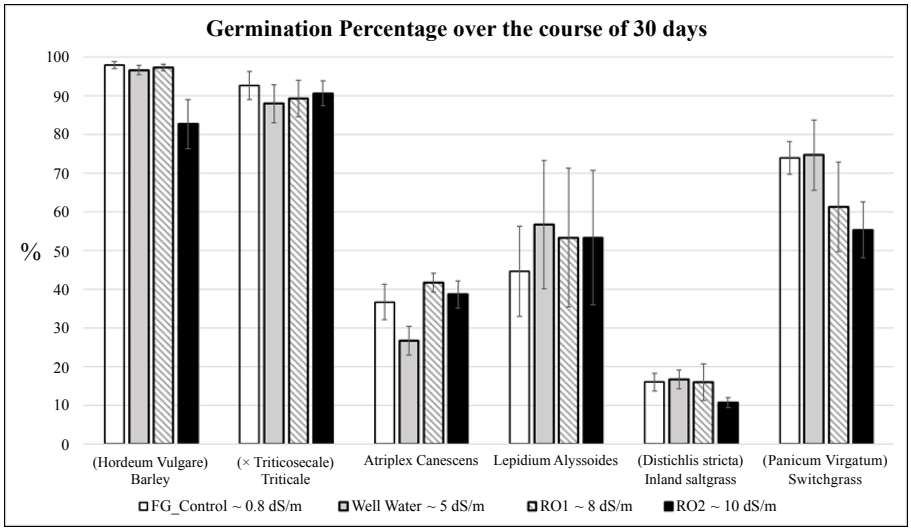

Figure 3: Germination percentage of halophytes seeds of *Hordeum vulgare, ×Triticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta* over 30 days, under different salt concentration treatments: EC 0.8, 5, 8, 10 dS/m. Results are means of six replicates across two runs.

Irrigation water salinity and emergence

Analysis of the final emergence percentage showed that three of the six species studied, *H. vulgare*, *xTriticosecale,* and, *P. virgatum,* had no significant difference under salinity treatments (Table 4). The *L. alyssoides* and the *D. stricta* seeds were the species that showed significant differences among water salinity treatments. For *L. alyssoides*, higher percentage emergence was observed for saline treatments than the control while for *D. stricta* seeds, it was exactly opposite with highest percent emergence taking place in control treatment (Table 4). On the other hand, *A. canescens* seeds were the only species that showed a difference in final percent emergence with the highest emergence under EC10 (Figure 4). Although the final emergence percentages across the treatments were comparable within species, there is evidence of variability among treatments between the onset of emergence and the final seed emergence, indicating mostly a delay for some species. This was supported by results of the MET, EI, T, and T_{mod} comparisons for each species (Tables 4 and 5). Significant differences were noted for the MET of all species. For *H. vulgare,* the difference corresponded to a P-value of <0.0002. The largest differences

Species	Treatments	GI	T	Tmod
	(dS/m)	(seeds day-1) ± SE	(% day) ± SE	(day) ± SE
H. vulgare	EC 0.8	24.69 ± 0.76 a	688.42 ± 9.41 a	28.12 ± 0.44 a
	EC 5	24.18 ± 0.45 a	676.83 ± 9.93 a	27.98 ± 0.13 a
	EC 8	23.79 ± 0.40 a	674.58 ± 7.31 a	27.72 ± 0.17 ba
	EC 10	19.10 ± 1.59 b	559.17 ± 43.92 b	27.03 ± 0.25 b
×*Triticosecale*	EC 0.8	24.46 ± 0.97	664.83 ± 26.11	28.70 ± 0.08
	EC 5	23.11 ± 1.38	629.92 ± 36.44	28.62 ± 0.08
	EC 8	23.32 ± 1.16	637.67 ± 32.66	28.57 ± 0.10
	EC 10	23.49 ± 0.92	645.00 ± 23.82	28.47 ± 0.11
P. virgatum	EC 0.8	15.46 ± 1.60	496.92 ± 29.14	26.85 ± 0.16 a
	EC 5	16.53 ± 1.93	496.00 ± 59.39	26.62 ± 0.08 a
	EC 8	12.81 ± 2.36	397.83 ± 74.40	25.98 ± 0.07 b
	EC 10	10.85 ± 1.42	350.08 ± 45.55	25.31 ± 0.08 c
A. canescens	EC 0.8	7.55 ± 1.02	238.50 ± 29.69	26.03 ± 0.18 a
	EC 5	5.12 ± 0.74	185.42 ± 19.38	24.78 ± 0.40 b
	EC 8	7.31 ± 0.82	233.50 ± 24.85	25.42 ± 0.12 ba
	EC 10	7.60 ± 0.69	244.83 ± 22.30	25.32 ± 0.08 b
L. alyssoides	EC 0.8	11.56 ± 3.13	317.60 ± 84.32	28.22 ± 0.20
	EC 5	13.77 ± 4.25	391.70 ± 117.20	27.20 ± 0.36
	EC 8	13.95 ± 4.74	389.60 ± 133.22	27.48 ± 1.28
	EC 10	13.65 ± 4.50	377.30 ± 123.58	28.25 ± 0.15
D. stricta	EC 0.8	3.36 ± 0.46	104.00 ± 14.70	26.07 ± 0.10 a
	EC 5	3.18 ± 0.48	180.08 ± 65.88	25.00 ± 0.25 b
	EC 8	2.73 ± 0.82	96.17 ± 28.54	24.05 ± 0.12 c
	EC 10	1.82 ± 0.22	70.42 ± 8.82	23.43 ± 0.20 d

Table 3: Effect of salinity on germination parameters of seeds of *Hordeum vulgare, xTriticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta*. Abbreviations: GI, germination index; T, Timson's index; T_{mod}, Timson's modified index. Within an index and within a species, treatments followed by different letters were significantly different at $p<0.05$. Results are means and standard errors (SE) of six replicates.

Species	Treatments	Emergence Percentage	MET
	(dS/m)	(%) ± SE	(day) ± SE
H. vulgare	EC 0.8	93.33 ± 4.22	6.13 ± 0.20 c
	EC 5	86.66 ± 6.67	6.26 ± 0.17 cb
	EC 8	96.67 ± 3.33	7.08 ± 0.36 b
	EC 10	96.67 ± 3.33	8.38 ± 0.43 a
×*Triticosecale*	EC 0.8	93.33 ± 4.22	5.13 ± 0.30 b
	EC 5	90.00 ± 4.47	5.17 ± 0.12 b
	EC 8	90.00 ± 6.83	5.55 ± 0.11 b
	EC 10	86.67 ± 4.22	7.28 ± 0.33 a
P. virgatum	EC 0.8	73.33 ± 6.67	8.67 ± 0.13 c
	EC 5	66.67 ± 12.29	9.27 ± 0.46 c
	EC 8	60.00 ± 8.94	10.42 ± 0.28 b
	EC 10	70.00 ± 6.83	12.11 ± 0.12 a
A. canescens	EC 0.8	26.67 ± 4.22 b	11.00 ± 0.29 b
	EC 5	26.67 ± 4.22 b	12.33 ± 0.17 a
	EC 8	36.67 ± 3.33 b	12.17 ± 0.25 a
	EC 10	53.33 ± 6.67 a	12.82 ± 0.18 a
L. alyssoides	EC 0.8	33.33 ± 4.22 b	6.92 ± 0.24 b
	EC 5	40.00 ± 0.00 a	9.67 ± 0.33 a
	EC 8	40.00 ± 0.00 a	7.17 ± 0.25 b
	EC 10	40.00 ± 0.00 a	7.58 ± 0.30 b
D. stricta	EC 0.8	56.67 ± 6.15 a	12.57 ± 0.22 d
	EC 5	50.00 ± 4.47 ba	13.39 ± 0.24 c
	EC 8	43.33 ± 3.33 ba	14.42 ± 0.16 b
	EC 10	40.00 ± 5.16 b	15.44 ± 0.16 a

Table 4: Effect of salinity on emergence parameters of seeds of *Hordeum vulgare, ×Triticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta*. Abbreviations: MET, mean emergence time. Within an index and within a species, values followed by different letters were significantly different at $p<0.05$. Results are means and standard errors (SE) of six replicates.

were noted for *xTristicosecale, P. virgatum*, *A. canescens, L. alyssoides,* and *D. stricta* which all had P-values corresponding to <0.0001. The EI only showed differences for the two plant species which were *A. canescens* (P <0.0039), and *D. stricta* (P <0.0167). On the other hand, T only showed significant difference for the one species which is *A. canescens* (P <0.0027). $T_{mod,}$ like the weighted MET, takes into account the cumulative emergence of the seed sample and showed significant difference for all species. For *H. vulgare,* the difference corresponded to a P-value of <0.0002. The largest differences were noted for *xTristicosecale, P. virgatum*, *A. canescens, L. alyssoides,* and *D. stricta* which all had P-values corresponding to <0.0001.

The difference between mean germination time and mean emergence time of *Hordeum vulgare*, *xTriticosecale,* and *Atriplex canescens* seeds over the course of 30 days is shown in Figure 5 while the difference between mean germination time and mean emergence time of the *Lepidium alyssoides, Distichlis stricta,* and *Panicum virtagum* seeds over the course of 30 days is presented in Figure 6.

Mean germination time and mean emergence time of halophyte seeds of *Hordeum vulgare*, *xTriticosecale, Atriplex canescens, Lepidium alyssoides, Distichlis stricta, and Panicum virgatum* over 30 days is presented in Figure 7 under different salt concentration treatments.

Discussion

This research examined the reuse of RO concentrate to irrigate existing or grow new halophyte species in the West Mesa or similar areas of New Mexico. Therefore, the experiment was conducted using halophyte species which have high tolerance for high salinity treatment. The analysis of the control (Greenhouse water) and three saline water treatments showed that the test solutions (BGNDRF brackish groundwater, BGNDRF RO concentrate water, and BGNDRF RO2

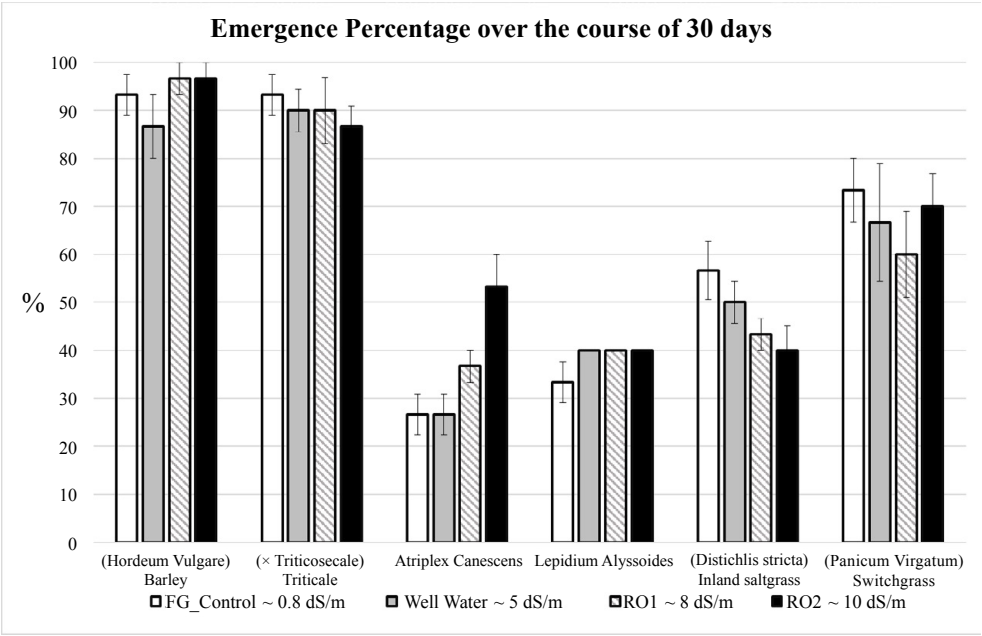

Figure 4: Emergence percentage of halophytes seeds of *Hordeum vulgare, xTriticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta* over 30 days, under different salt concentration treatments: EC0.8, 5, 8, 10 dS/m. Results are means of six replicates across two runs.

Species	Treatments	EI	T	Tmod
	(dS/m)	(seeds day-1) ± SE	(% day) ± SE	(day) ± SE
H. vulgare	EC 0.8	23.2 ± 1.0	130.33 ± 5.74	27.94 ± 0.10 a
	EC 5	21.4 ± 1.6	120.75 ± 9.21	27.87 ± 0.09 ba
	EC 8	23.1 ± 0.9	132.75 ± 4.83	27.46 ± 0.18 b
	EC 10	21.9 ± 0.9	129.58 ± 4.64	26.81 ± 0.22 c
×*Triticosecale*	EC 0.8	24.1 ± 0.9	132.58 ± 5.50	28.44 ± 0.15 a
	EC 5	23.3 ± 1.2	127.92 ± 6.53	28.42 ± 0.06 a
	EC 8	22.9 ± 1.8	127.08 ± 9.78	28.23 ± 0.05 a
	EC 10	20.6 ± 1.1	118.58 ± 5.87	27.37 ± 0.17 b
P. virgatum	EC 0.8	16.4 ± 1.5	97.75 ± 8.85	26.66 ± 0.07 a
	EC 5	14.4 ± 2.6	87.67 ± 15.96	26.37 ± 0.23 a
	EC 8	12.4 ± 1.9	77.42 ± 11.63	25.79 ± 0.14 b
	EC 10	13.2 ± 1.3	87.33 ± 8.54	24.95 ± 0.06 c
A. canescens	EC 0.8	5.3 ± 0.8 b	34.00 ± 5.38 b	25.50 ± 0.14 a
	EC 5	5.0 ± 0.8 b	33.08 ± 5.19 b	24.83 ± 0.08 b
	EC 8	6.9 ± 0.6 b	45.58 ± 4.02 b	24.92 ± 0.12 b
	EC 10	9.7 ± 1.2 a	65.50 ± 8.05 a	24.59 ± 0.09 b
L. alyssoides	EC 0.8	8.8 ± 0.8	50.42 ± 4.49	27.54 ± 0.12 a
	EC 5	8.5 ± 0.1	52.33 ± 0.33	26.17 ± 0.17 b
	EC 8	9.5 ± 0.1	54.83 ± 0.25	27.42 ± 0.12 a
	EC 10	9.4 ± 0.1	54.42 ± 0.30	27.21 ± 0.15 a
D. stricta	EC 0.8	10.4 ± 1.1 a	70.00 ± 7.55 a	24.72 ± 0.11 a
	EC 5	8.8 ± 0.8 ba	60.75 ± 5.41 ba	24.31 ± 0.12 b
	EC 8	7.7 ± 0.7 bc	55.50 ± 4.98 ba	23.79 ± 0.08 c
	EC 10	6.2 ± 0.8 c	46.50 ± 5.94 b	23.28 ± 0.08 d

Table 5: Effect of salinity on emergence parameters of seeds of *Hordeum vulgare, ×Triticosecale, Panicum virgatum, Atriplex canescens*, *Lepidium alyssoides*, and *Distichlis stricta*. Abbreviations: EI, emergence index; T, Timson's index; T_{mod}, Timson's modified index. Within an index and within a species, values followed by different letters were significantly different at $p<0.05$. Results are means and standard errors (SE) of six replicates.

concentrate water) were four distinct treatments that varied in both EC and SAR. Although the pH was lower for the control treatment, the water treatments had no significant difference for the pH.

Irrigation water salinity and germination

The five response variables noted above were analyzed for each halophyte species across saline water treatments. For the five response variables, significant differences were distinguished in all species statistically but for different indices and to different extents. Because germination percentage, GI, and T are all calculated based on the total number of seeds used in the experiment, results stated corresponding differences within a species. The weighted MGT and T_{mod} tests take

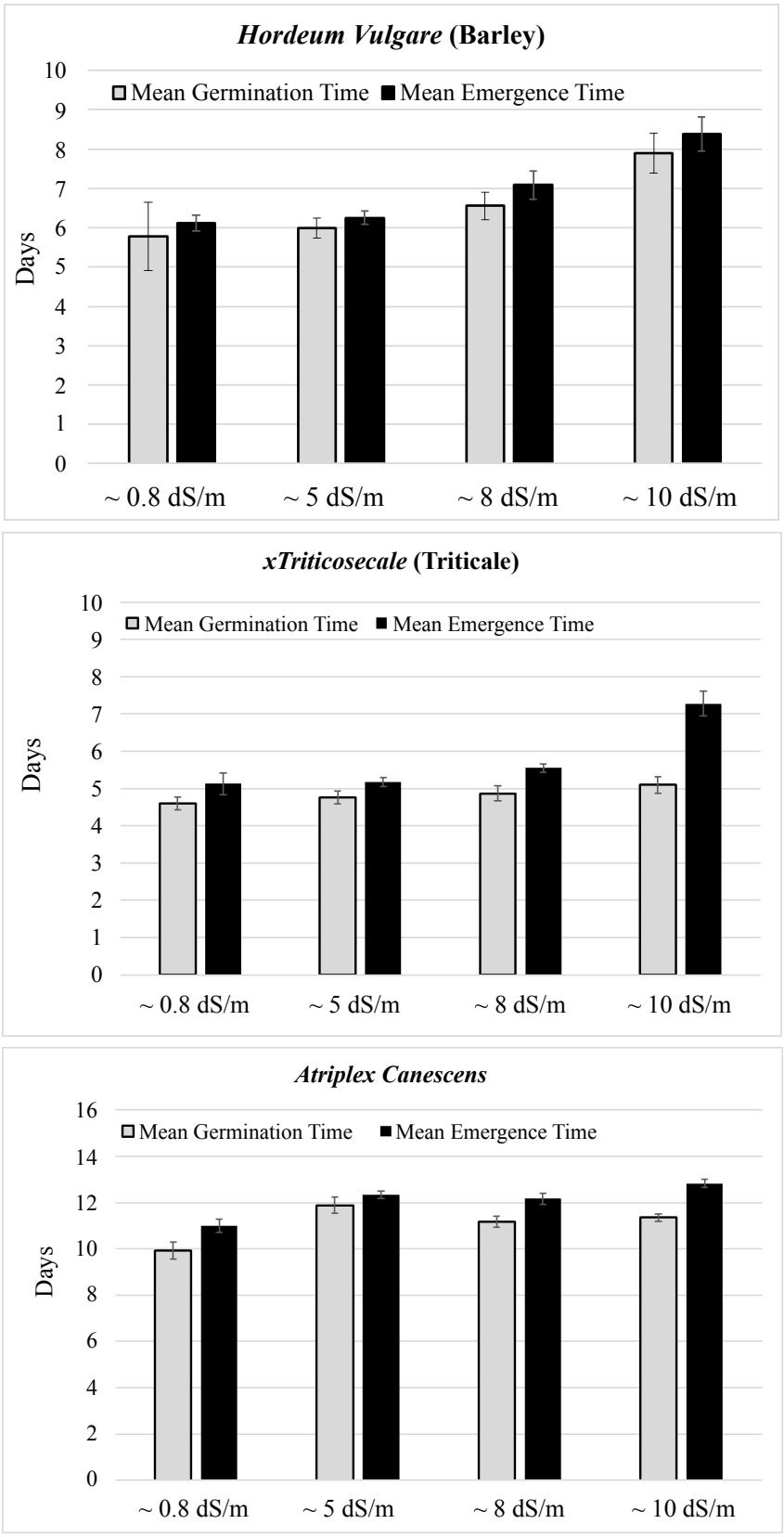

Figure 5: Mean germination and emergence time of the (a) *Hordeum vulgare*, (b) *xTriticosecale*, and (c) *Atriplex canescens* seeds over the course of 30 days. Results are means and standard errors (SE) of six replicates.

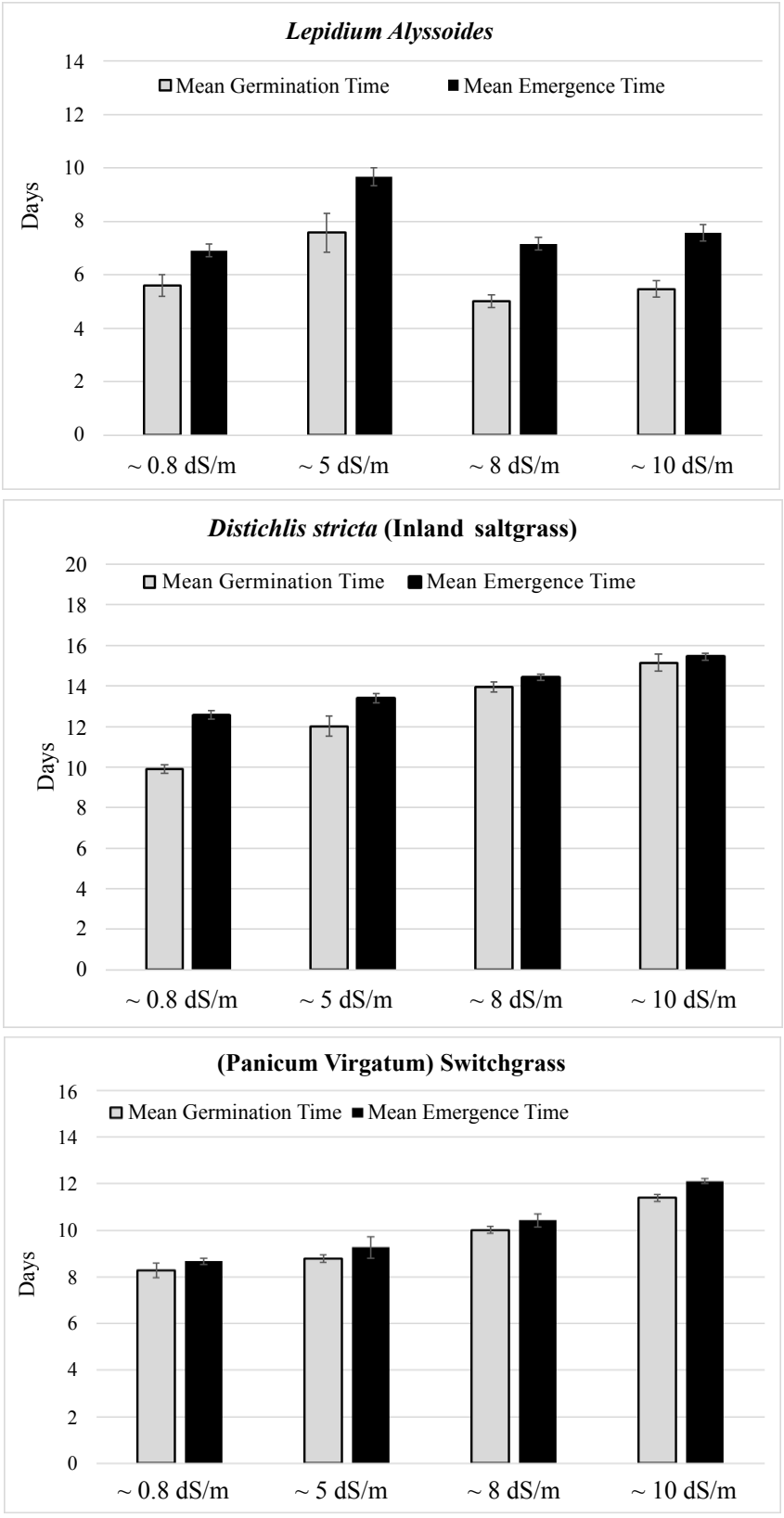

Figure 6: Mean germination and emergence time of the (a) *Lepidium alyssoides,* (b) *Distichlis stricta,* and (c) *Panicum virtagum* seeds over the course of 30 days. Results are means and standard errors (SE) of six replicates.

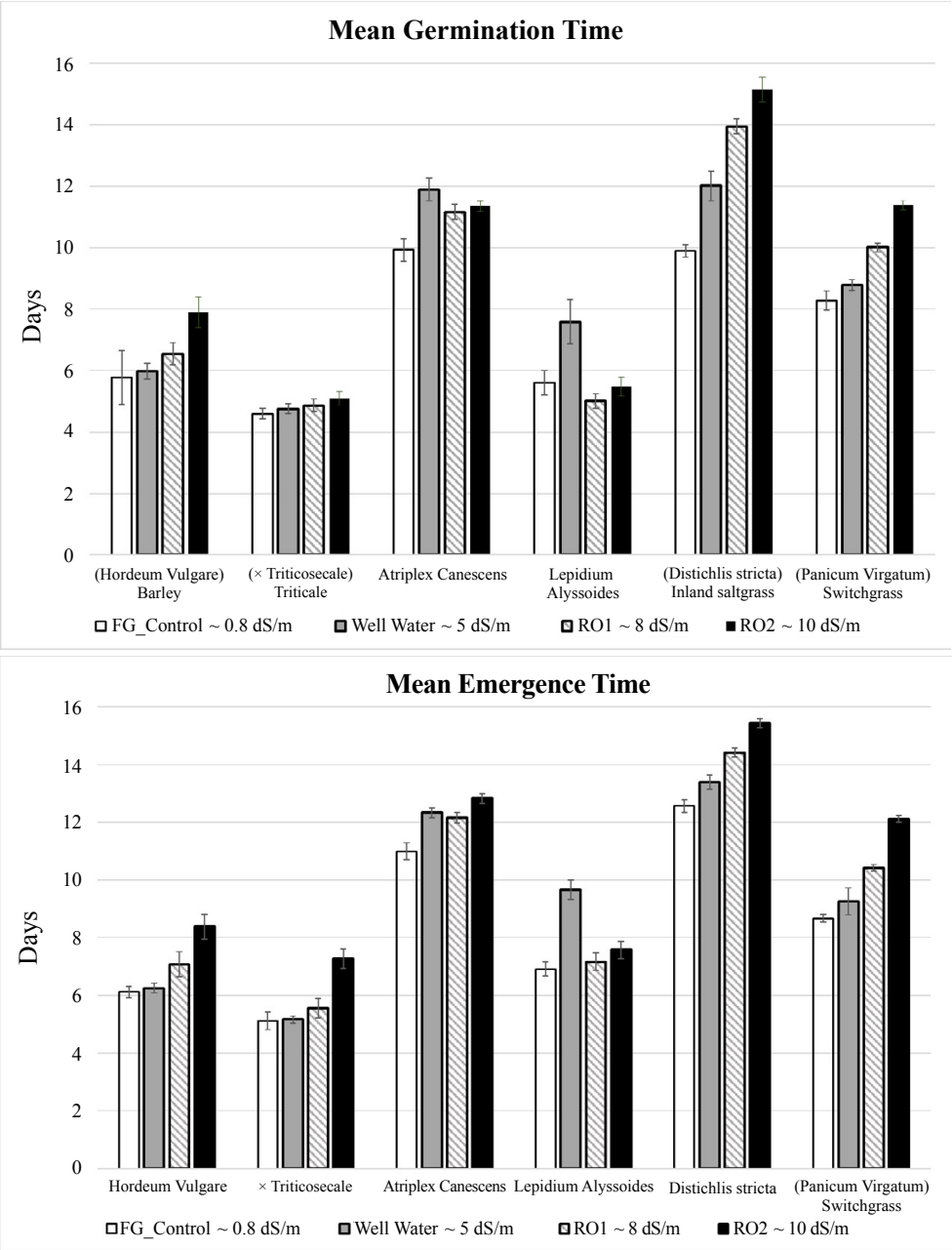

Figure 7: (a). Mean germination time and (b). Mean emergence time of halophytes seed of *Hordeum vulgare*, *xTriticosecale, Atriplex canescens, Lepidium alyssoides, Distichlis stricta, and Panicum virgatum* over 30 days, under different salt concentration treatments: EC 0.8, 5.8 and 10.0 dS/m. Results are means of six replicates across two runs.

into account the final cumulative germination percentage of the seed samples, and therefore, the extend of the significant differences were more pronounced for these two indices. *H. Vulgare* was most prone to a change in water treatment with a significant difference observed in all five indices, while *xTriticosecale* was the least with no significant differences noted of five calculated indices. *A. canescens* had the second highest number of significant differences with three of the five indices displaying significance. *P. virgatum* and *D. stricta* seeds had significant differences for MGT and T_{mod}. *L. alyssoides* contained a significant difference solely in MGT. All the indices aside from the germinations are related to the germination time of a species. These results illustrate that for all species (except *L. alyssoides*) the germination process at the population level was delayed by increasing saline treatments. The final germination percentage showed no significant differences for the species (except *H. Vulgare* and *A. canescens*) expressing that the germination percentage was not affected by the water treatments.

A delay in germination was observed for the *H. vulgare* seeds under the salinity treatments (P-values of <0.0095 for final germination percentage, <0.0013 for GI, <0.0024 for T, <0.0479 for T_{mod}; however, no significant difference in the MGT was noticed. Hussain et al. [21] found that germination decreased as salinity increased, with a 24-35% reduction from complete germination (100%) at an EC of 9.26 dS/m. The current study agreed with Hussain et al. [21] and indicated that

final germination percentage was 82.7% at the EC of 10 dS/m while it was 98.0% at the EC of 0.8 dS/m.

There was no significant difference among the five response variables noted for *xTriticosecale.* Although the final germination percentage had no significant difference, *xTriticosecale* seeds were delayed for the mean germination time with salinity water treatments. This is in accordance with two studies on the salt tolerance of *xTriticosecale,* where a delay in germination of the seeds was observed with increasing salinity [22,23]. In the Shalaby et al. [22] study, *xTriticosecale* was the only species to germinate (70.7%) at NaCI concentration corresponding to an EC of 27.6 dS/m which indicated that species is highly salt tolerant and supported results of this study.

A difference in the MGT was obtained for *P. virtagum* across saline treatments (P<0.0001). The results agreed with Ries and Hofmann [24] that the effect of salt treatments on percent germination is time dependent for *P. virgatum* as is inland salt grass species. In a second study on *P. virtagum,* it was found that percentage seed germination and rate generally decreased as salinity increased, but these differences were not noticeable for treatments under 15 dS/m [25]. This is consistent with the current study because the final germination percentage and germination rate of *P. virgatum* decreased as salinity of water treatments increased. Another study found that although saline treatment can lower germination in *P. virgatum,* it was possible for some species to tolerate the salt in spite of the initial inhibition. This study also agreed with Ries and Hofmann [24] where *P. virgatum* seeds have lower germination percentage and delay under higher salinity treatments.

For a characteristic dormancy, *A. canescens* seeds are frequently pretreated before the study of germination is started. Dewinging is applied frequently to promote germinations as well as methods such as heat desiccation and soaking [18,26,27]. However, germination studies for *A. canescens* differ even when similar pretreatments are applied. Both Potter et al. [27] and Weawer and Jordan's [26] studies used heat desiccation to pretreat seeds for their germination experiments. Potter et al. [27] observed increasing germination for treatments with an osmotic potential of 0.0 to -0.8 MPa and Weaver and Jordan [26] observed no effect on germination with increasing salinity (for KNO_3 solutions ranging from 0.02 M to 0.16 M). However, the current experimental study did not agree with Weaver and Jordan [26] and a significant effect on the final germination percentage was noted. The current study agreed with Ries and Hofmann [24] with MGT and T_{mod} showing significant differences illustrating the salt solution effect on germination time and rate for *A. canescens.* Prior studies reported that *A. canescens* was a suitable plant under saline conditions, and the results of this study are in agreement with that.

Muhammad and Hussain [9] found that there was no significant difference between the final germination percentage on *L. sativum* under 0.8 dS/m and brackish water treatments from 5 dS/m to 10 dS/m. Similarly, the current study showed that *L. alyssoides* seeds had no significant differences across treatments for the final percentage germination. On the other hand, the *L. alyssoides* seeds were significantly different in one germination time indices, MGT, (P<0.0038), stating that in addition to the difference in germination percentage for *L. alyssoides,* germination time was dependent on water salinity.

The final germination percentage of *D. stricta* did not differ significantly across the water treatments in a study by Shahba et al. [28], who found that germination decreased significantly under higher salinity treatments. The Shahba et al. [28] experimental study expressed that a 10% difference in germination percent and a 4% decrease in germination speed (the percentage of germinated seeds per time period at a given time period, similar to the GI) were observed when salinity increased from 0 to 15 dS/m. In addition, the highest EC tested in this study was 10 dS/m and the difference in germination may not be different enough to determine a discrepancy for values less extreme than 15 dS/m. The current experimental study agreed with Shahba et al. [28] where the germination process was observed across the saline water treatments for the MGT as well as the T_{mod} (P<0.0001).

This study showed that these six species, *H. Vulgare, xTriticosecale, P. virtagum, A. canescens, L. sativum, D. stricta,* can survive the germination process with these waters and are adequate candidate species for RO wastewater irrigation on land application sites. However, one potential problem that appears for assessing appropriate salt tolerance species for wastewater irrigation is that it has been shown that germination and seedling stage salinity tolerance is not necessarily the same as it is for later stages and vice versa [22,23,28]. Because this study solely dealt with the germination stage of growth, further investigation is required for other plant life cycle stages.

Irrigation water salinity and emergence

The five response variables stated in the Table 3 were examined for each halophyte species across saline water treatments. For the five response variables, significant differences were distinguished in all species statistically but for different indices and to different extents. Because emergence percentage, EI, and T are all calculated based on the total number of seeds used in the experiment, results of these stated corresponding differences within a species. The weighted MET and T_{mod} take into account the final cumulative emergence percentage of the seed samples, and therefore, the extent of the significant differences were more pronounced for these two indices. *D. stricta* and *A. canescens* seeds were more susceptible to a change in water treatment with a significant difference observed in all five calculated indices while *xTriticosecale, H. Vulgare,* and *P. virgatum* had the least significant differences for MET and T_{mod}. *L.alyssoides* had the second highest number of significant differences with three of the five indices displaying significance. All the indices aside from the emergence percentages are related to the emergence time of a species. These results illustrated that for all species (except *L. alyssoides*) the emergence process at the population level was delayed by increasing saline treatments. The final emergence percentage showed no significant differences for the species (except *A. canescens, L. alyssoides,* and *D. stricta*) expressing that the emergence percentage was not affected by the water treatments.

A delay in emergence was observed for the *H. Vulgare* seeds under the salinity treatments (P <0.0002 for mean emergence time, and T_{mod}); however, no significant difference in the final emergence percentage, EI, and T was noticed. A study by Al-Seedi [8], noted that there is a significant difference between control water treatment and saline water treatments on the effect of salinity on the percentage and rate of emergence of barley (*Hordeum vulgare)* seedlings. A gradual decrease was noticed with increasing salinity treatments of 3, 6, 9, and 12 dS/m. Final percentage emergence is 52% at an EC of 12 dS/m. Final percentage emergence reported were 100, 100, 100, 92 and 78% at EC of control, 6, 9, 12 dS/m, respectively [8].

There was no significant difference among the five response variables noted for *xTriticosecale* except the delay in the mean germination time with increasing water salinity. This agreed with two studies on the salt tolerance of *xTriticosecale,* where a delay in

seed germination was observed with increasing salinity [22,23]. In the Shalaby et al. [22] study, *xTriticosecale* was the only species to germinate (to 70.7% completion) at NaCI concentrations corresponding to an EC of 27.6 dS/m which indicates that species is highly salt tolerant and can account for the resistance to salinity shown in the current study.

A difference in emergence time was stated for *P. virtagum* across saline treatments as indicated by a P-value of <0.0001 for the MET. For the emergence experiment, the current study also had the same treatment for *A. canescens* as for germination tests to not have long term dormancy for emergencies. There was a significant difference among the five response variables noted above for *A. canescens* and MGT increased with increasing salinity. On the other hand, the *A. canescens* seeds were the only species that showed a difference in final emergence with higher emergences reported for the higher salinity treatments.

Final percentage emergences for *D. stricta* differed significantly across the water treatments and emergence decreased significantly under higher salinity treatments. In addition, the current experimental study showed that a delay in the mean emergence time was observed across the saline water treatments ($p<0.0001$).

Conclusions

This study was conducted to test the germination and emergence of six halophyte species under an irrigation water salinity gradient. Increasing irrigation water salinity increased mean germination time for all species except *L. alyssoides*, but did not affect the percentage germination significantly for the tested species except *A. canescens*. Increasing irrigation water salinity increased mean emergence time for all species except *L. alyssoides,* but did not affect the percentage emergence for *H. Vulgare, xTriticosecale,* and *P. virtagum* species significantly. All six species are candidate species for the irrigation with high salinity water. Irrigation with RO concentrate can augment inland desalination in arid and semi-arid water scarce areas with significant amount of available brackish water.

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