

The effects of saltwater intrusion on germination success of standard and alternative crops

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ABSTRACT

Saltwater intrusion (SWI) is the landward movement of seawater, which can salinize coastal farmlands and affect crop yields. Germination is an especially vulnerable life history stage for crops. Salt stress reduces a seed's ability to absorb water (osmotic stress) and causes an ion imbalance within the seed (ionic stress), ultimately inhibiting germination and preventing crop production. Therefore, growing standard crops in the face of SWI can risk crop failure. If alternative crops are more tolerant of osmotic and ionic stress, they may be more suited to withstand SWI. Our objectives were to (1) determine how salt stress (ionic and osmotic stress) and osmotic stress levels affect seed germination in standard and alternative crops in a controlled environment experiment and (2) compare germination rates in the controlled environment with field trials. In our study region of the Eastern Shore of Maryland, standard crop species include corn, soybean, and wheat; alternative species and varieties include sorghum, chloride-excluding soybean, barley, and quinoa. Seeds of these species were germinated under different levels of salt and osmotic stress ranging from 0 to -4 MPa (0 – 111.1 dS m^{-1} , respectively) by manipulating concentrations of NaCl to alter salt stress and concentrations of polyethylene glycol to alter osmotic stress. Crop species differed greatly in germination tolerance to salt and osmotic stresses, however, there was no significant difference in percent germination between standard and alternative crop species. Standard soybean, chloride-excluding soybean, and quinoa were the only species to germinate in salt stress; standard soybean and chloride-excluding soybean had an equal tolerance during germination at -1.8 MPa (50.0 dS m^{-1}) and quinoa successfully germinated at -0.5 MPa (13.9 dS m^{-1}). However, wheat (standard) and barley (alternative) were the most tolerant of osmotic stress, successfully germinating at -2 MPa (55.6 dS m^{-1}). We also examined germination of sorghum and chloride-excluding soybeans in previously-established field trials for two years on two salt-intruded farms on the Eastern Shore of Maryland. One of the farms showed greater germination success (measured as seedling emergence) in chloride-excluding soybean than sorghum despite a small difference in salt stress ($\Delta -0.05$ MPa across the field). These results highlight the complexity of understanding how salt stress shapes germination processes in the real world and suggest temporal windows of low and high stress may have long-lasting consequences for crop performance.

1. Introduction

Soil salinization is a global problem affecting at least 75 countries and more than 20 % of global irrigated land (Ghassemi et al., 1995; Metternicht and Zinck, 2003; Qadir et al., 2014). The extent of soil salinization is projected to increase due to climate change, specifically as a result of more variable weather conditions and rising sea levels

(Zaman et al., 2018). For instance, salts may concentrate in soil as water evaporates during prolonged droughts (saltwater incursion; Ardón et al., 2013; Tully et al., 2019a). In arid regions, such as the San Joaquin Valley in the United States, the Aral Sea Basin in Central Asia, and the Murray-Darling Basin in Australia, fields can salinize following irrigation because of evaporation of the groundwater that is naturally high in salts (e.g. sulfate, sodium, and chloride; Wichelns and Qadir, 2015;

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Foster et al., 2018; Munns and Tester, 2008; Qadir et al., 2014). However, in coastal regions such as our focal region of the Eastern Shore of Maryland, soils are salinizing via saltwater intrusion (SWI). Saltwater intrusion, the landward movement of saltwater, is driven by rising sea levels, water withdrawals from coastal aquifers, the frequency and duration of droughts, hydrological connectivity of agricultural ditches to saline waterways, and the frequency of storm surges and tidal inundation (Tully et al., 2019a). Although a great deal of research has focused on the effects of salinization on crop performance (Zörb et al., 2019), SWI-induced salinization is a unique phenomenon composed of high salinity levels and periodic wet-dry cycles. Given that germination is one of the most stress-sensitive life history stages of a plant, it is anticipated that effects of salt stress on germination may pose a problematic bottleneck in crop performance for SWI locales (Del Vecchio et al., 2020).

Salt stress reduces a plant's ability to absorb water (osmotic stress) and causes an ion imbalance (e.g. ionic stress due to excess sodium [Na^+] and chloride [Cl^-]). Successful seed germination requires the absorption of water through the seed wall via diffusion and capillary action as water moves from outside the seed wall (higher water potential) to inside the seed wall (lower water potential; Woodstock, 1988). Salinity causes osmotic stress by lowering the water potential of the germination medium in comparison to the seed interior (Khan and Weber, 2008; Parihar et al., 2015) and slowing the absorption of water by the seed. The salt itself may also affect germination through ionic stress, which disrupts enzyme function, metabolism, hormonal signaling, and energy reserve utilization (Welbaum et al., 1990; Huang and Redmann, 1995; Parihar et al., 2015). The overall effect of salt (osmotic and ionic combined) stress on seeds is to delay and inhibit germination.

As an initial filter in plant establishment, germination is the first bottleneck after dispersal shapes plant distributions and can also limit selection on traits that might confer salinity tolerance at later plant life history stages (Donohue et al., 2010; Prabhakaran et al., 2019). Recent studies of salt stress effects on germination of coastal trees showed that most tree species cannot germinate in the elevated salinity levels that accompany SWI, with consequences for future forest community composition (Middleton, 2016; Woods et al., 2020).

Although plant physiologists recognize germination and establishment as one of the most sensitive stages of plant life history (Munns and Tester, 2008; Zörb et al., 2019; García Hernández et al., 2018), the majority of research on salt tolerance in plants has focused on seedling or mature plant life history stages rather than seeds. Most experimental studies of salt stress germinate seeds in a non-saline medium prior to study. These studies have revealed a variety of effects of salt stress post-establishment. Osmotic stress can cause immediate stomatal closure and rapid suppression of the rate of cell expansion and shoot growth (Munns and Tester, 2008). In the response of plants to salinity, ionic stress effects are seen over slightly longer timescales, after Na^+ and Cl^- accumulate at toxic concentrations within plant tissues (Parida and Das, 2005; Munns and Tester, 2008; Roy et al., 2014). Ionic stress can reduce photosynthesis, suppress growth rates, and cause premature leaf senescence (Munns and Tester, 2008). Overall, salt stress can suppress growth and limit plant productivity, ultimately resulting in plant death (Zhu, 2001; Parida and Das, 2005; Roy et al., 2014; Parihar et al., 2015). Our experimental design teases apart the isolated effect of osmotic stress and the combined osmotic and ionic stress (herein, salt stress; both Na^+ and Cl^-) conferred by saltwater (Igartua et al., 1994; Shani and Dudley, 2001).

Salt tolerance is the ability of a plant to complete its life cycle on a substrate with high concentrations of soluble salts (Parida and Das, 2005; Zörb et al., 2019). Plants utilize biochemical strategies to manage salt exposure, including selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves, and re-translocation of Na^+ and Cl^- from the leaves to the roots (Erdei and Taleisnik, 1993; Koyro, 1997; Netondo et al., 2004; Parida and Das,

2005; Roy et al., 2014). Halophytes (e.g. *Salicornia*, *Taeniatherum*, *Chenopodium* including the crop quinoa, *C. quinoa* Willd.) are plants adapted to growing in saline conditions and commonly exhibit salt tolerance through biophysical mechanisms (Shabala, 2013); and although alternative solutions have been generated, they have not been entirely suitable for some crops (Alzate et al., 2018). The seeds of halophytes are capable of avoiding or tolerating salinity stress: periods of dormancy or inactivity to weather stressful conditions, recovery from brief exposures to salinity, and seed heteromorphism (Gul et al., 2013). However, halophytes may also be sensitive to high levels of salinity during germination or seedling emergence stages, even if they can tolerate these levels (upwards of 25 dS m^{-1}) as a mature plant (Colmer and Flowers, 2008; Malcolm et al., 2003; Debez et al., 2004; Adolf et al., 2013; Panuccio et al., 2014; Zörb et al., 2019). On the other hand, many mature glycophytes (salt-intolerant plants; e.g. *Triticum*, *Maize*, *Nicotiana*) are unable to regulate ions entering the xylem at salinity levels $>4 \text{ dS m}^{-1}$ in mature stages because they do not have the biochemical mechanisms to cope with salt stress (Munns and Tester, 2008; Flowers and Colmer, 2015). Most common agricultural crops are glycophytes and exhibit 50–80% declines in productivity when salinity reaches between $4\text{--}8 \text{ dS m}^{-1}$ ($2\text{--}4$ ppt, respectively; Tanji and Kielen, 2002; Munns and Tester, 2008; Ventura et al., 2015; Zörb et al., 2019). Quinoa [*Chenopodium quinoa* Willd.] is a rare exception; it can tolerate salinity levels up to 40 dS m^{-1} (Adolf et al., 2013; Jacobson et al., 2003; Cisneros Almazan et al., 2020).

Plant breeders have been working for decades to develop crop varieties resistant to salt stress, in particular the conditions of arid and semi-arid regions where soils salinize due to irrigation with saline groundwater (Ashraf and Akram, 2009). However, breeding for overall salt-tolerance is difficult because it is a polygenic trait (Flowers, 2004; Flowers et al., 2010), and while the genes used for ion exclusion (e.g. Cl^-) are well-established and manipulated, the mechanisms of osmotic tolerance remain unknown (Roy et al., 2014; Munns and Tester, 2008). Interestingly, most crops marketed as “salt-tolerant” are selectively bred for ion-exclusion in order to resist the negative impacts of a specific ion, but do not afford overall salt-tolerance. Other crops, such as barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.), and rapeseed (*Brassica napus* L.; Table 1), are natural ion-excluders due to the activation of salt-induced genes, such as the *Lea* gene family and *HKT* gene family, which are responsible for the efficient transport of ions across vacuole and cell membranes (Chandra Babu et al., 2004; Roychoudhury and Chakraborty, 2013; Li et al., 2013; Gürel et al., 2016; Chen et al., 2019).

The Eastern Shore of Maryland has a low elevation (mean elevation of 2.7 m above sea level) and is hydrologically connected to saline waterbodies via an extensive ditch network, thus saline water is easily transported into ground- and surface water. The acceleration of sea level rise in the region is three times the global average (Sallenger et al., 2012). Large areas of agriculture have been affected by sea level rise and SWI, with 2% of farmland in one coastal county converting to tidal marsh in only 8 years (Gedan et al., 2020). In the region, we have commonly observed dramatic declines in the productivity of crops on salt-intruded fields. Standard crops in the region are corn [*Zea mays* L.], soybean [*Glycine max* (L.) Merr.], and wheat [*Triticum aestivum* L.]. Commercially-viable alternative crops for the region selected for this study were sorghum [*Sorghum bicolor* L.], a chloride-excluding soybean [*Glycine max* (L.) Merr.], barley [*Hordeum vulgare* L.], and quinoa [*Chenopodium quinoa* Willd.]. These alternative crops were selected based on reported salinity tolerances (Table 1) and farmer interest from the region.

Two management strategies employed by farmers in the region are to (1) continue to grow the standard crop rotations as long as they can until the land becomes un-farmable and to (2) cultivate crops more tolerant of SWI, which are also economically-appropriate for the region (alternative crops). The objectives of this study were: (1) to determine how osmotic and salt (osmotic and ionic) stress affect seed germination in standard

Table 1Salinity thresholds (dS m⁻¹ and ppt) at mature plant stage of focal plant species.

Common name	Botanical name	Crop group	Threshold (EC) dS m ⁻¹	Threshold (salinity) ppt	References
Corn	<i>Zea mays</i> L.	Standard	1.8	0.9	Tanji and Kielen, 2002
Soybean	<i>Glycine max</i> (L.) Merrill	Standard	5.0	2.7	Tanji and Kielen, 2002
Wheat	<i>Triticum aestivum</i> L.	Standard	6.0	3.3	Tanji and Kielen, 2002
Sorghum	<i>Sorghum bicolor</i> (L.) Moench	Alternative	6.8	3.7	Tanji and Kielen, 2002
Barley	<i>Hordeum vulgare</i> L.	Alternative	8.0	4.4	Tanji and Kielen, 2002
Chloride-excluding soybean cultivar	Chloride-excluding <i>Glycine max</i> (L.) Merrill	Alternative	10.6	6.0	Tanji and Kielen, 2002
Quinoa	<i>Chenopodium quinoa</i>	Alternative	40	18.9	Adolf et al., 2013

crops and alternative crops; and (2) to determine the germination success of alternative crop species planted on salt-intruded farm fields.

We hypothesized that: (1) in a controlled environment experiment, alternative crop species (sorghum, chloride-excluding soy, barley, and quinoa) will have a higher germination and faster rates of germination at elevated osmotic and salt stress levels compared to standard crop species (corn, soybean, and wheat); (2) in a field experiment, soybean bred for Cl⁻ ion exclusion will have higher in-field germination than sorghum.

2. Methods

2.1. Controlled environment experiment

2.1.1. Effect of osmotic and salt stress on crop germination

The standard crops in the Mid-Atlantic US, corn, soybean, and wheat have reported salinity thresholds of 2, 5, and 6 dS m⁻¹, respectively (Tanji and Kielen, 2002; Table 1), and are typically grown in rotation. These thresholds are far below salinity levels observed in the fields on the Eastern Shore of Maryland where this research was conducted (12 dS m⁻¹; Tully et al., 2019b). The selected alternative crop species, sorghum, chloride-excluding soybean, barley, and quinoa have higher reported salinity thresholds of 7, 11, 8, 40 dS m⁻¹, respectively (Table 1). A 10 × 7 factorial experiment was designed to test the effect of osmotic and salt stress on seed germination. At George Washington University (Washington, D.C., USA), ten osmotic potentials were created using mixtures of polyethylene glycol 8000 (PEG) and distilled water and ten salt potentials using mixtures of sodium chloride (NaCl) and distilled water. The ten water potentials were the same for osmotic and salt stress: 0, -0.2, -0.5, -0.8, -1.1, -1.4, -1.8, -2, -3, -4 MPa (Table 2). When PEG has a molecular weight greater than or equal to 6000, it cannot penetrate the cell wall of seeds (Carpita et al., 1979; Verslues et al., 1998), thus mimicking osmotic stress. The concentration of PEG used to create each solution was calculated using Eq. 1 (Hardege and

Table 2

Water potentials (MPa) used in the study and their equivalent electrical conductivity (dS m⁻¹), salinity (ppt), and sodium chloride (NaCl) concentration (mM).

Water potentials (MPa)	Electrical conductivity (dS m ⁻¹)	Salinity (ppt)	NaCl (mM)
0	0	0.0	0
-0.2	5.6	3.0	40.3
-0.5	13.9	8.2	100.9
-0.8	22.2	13.6	161.4
-1.1	30.6	19.3	222.0
-1.4	38.9	25.0	282.5
-1.8	50.0	32.9	363.3
-2	55.6	36.9	403.6
-3	83.3	57.3	605.4
-4	111.1	78.4	807.2

[†] To convert from water potentials (MPa) to electrical conductivity (dS m⁻¹), we used the following equation, EC (dS m⁻¹) = MPa ÷ -0.036 (U.S. Salinity Laboratory Staff, 1954).

[‡] The conversion from electrical conductivity (dS m⁻¹) to salinity (ppt) was based on the following equation, ppt = (dS m⁻¹)^{1.0878} * 0.4665, assuming the temperature was 25 °C (Williams, 1986).

Emmerich, 1990).

$$\psi = 0.130[PEG]^2T - 13.7[PEG]^2 \quad (1)$$

Where, Ψ is the water potential in MPa, PEG is grams of PEG per gram of water, and T is temperature in degrees Celsius.

The concentration of NaCl was calculated using Eq. 2 (Lang, 1967).

$$[NaCl] = \frac{\Psi}{2RT} \quad (2)$$

Where, Ψ is the water potential in atm, T is temperature in Kelvin, and R is the ideal gas constant $\left(0.8206 * \frac{L \cdot atm}{mol \cdot K}\right)$. In this study, T = 25 °C

because seeds in solution were kept in an incubator on a diurnal cycle of 30 °C for 12 h light and 20 °C for 12 h dark.

To mimic field conditions where seeds are regularly saturated with saltwater, 25 seeds per species were placed on one 85 mm Grade 1 Whatman filter paper, 11 µm pore size, in 100 × 20 mm glass petri dishes and moistened with 3.2 mL of deionized water, PEG solution, or NaCl solution. Each species x solution combination was replicated four times (total of 100 seeds per species x solution combination). The petri dishes were tightly covered and wrapped with parafilm to prevent evaporation and were incubated in a Percival Incubator (Percival Scientific, Perry, IA; Fig. S1).

If the filter paper appeared to be drying or there was no visible solution in the petri dish, we replaced the filter paper and added 3.2 mL of new solution to the petri dish. Due to the large seed size of corn, larger petri dishes and filter paper were used (150 and 125 mm diameter, respectively), and 7.8 mL of solution was added to fill the larger volume. Germination counts were made in ~6 h intervals in the first 48 h of experiment initiation and then in ~2–4 h intervals for the following two days. After four days, a daily single count was made until there was no new germination observed for 14 consecutive days. A seed was considered germinated if a radical was present.

We used germination data to calculate the time to 50 % germination (Eq. 3).

$$t_{50} = T_i + \frac{\left(\frac{N}{2} - N_i\right)(T_j - T_i)}{N_j - N_i} \quad (3)$$

Where t_{50} is the median germination time, N is the final number of germinated seeds, and N_i and N_j are the total number of seeds germinated in adjacent counts at time T_i and T_j , respectively (Farooq et al., 2005). Within a species seed lot or across different species, seeds can germinate at varying rates, and data is not normally distributed, making it challenging to draw comparisons between studies. Time to 50% germination is a measurement that provides a central tendency to the data (Scott et al., 1984; Ranal and de Santana, 2006).

2.2. Field experiment

2.2.1. Study location

In order to examine the effects of salinity on germination *in situ*, we utilized a previously-established field experiment located on the Eastern Shore of Maryland (Fig. S2). The experiment was conducted at two sites,

73 km apart. One site was near Crisfield, Maryland in Somerset Co. (37.983436°N, -75.854527°W) and a second was in Cambridge, Maryland in Dorchester Co. (38.5632°N, -76.0785°W; herein referred to as “Somerset farm” and “Dorchester farm”). The Somerset farm had predominantly Othello-Fallsington complex sandy loam soils and a mean elevation of 1 m above sea level. Somerset Co. receives 679 mm of rainfall on average with a mean temperature of 19.3 °C over the growing season (April to October; [National Oceanic and Atmospheric Administration, National Centers for Environmental Information, 2018](#); weather data for the study period reported in Table S1). The Dorchester farm had Elkton silt loam soils and a mean elevation of 1 m above sea level. Dorchester Co. receives 659 mm of rainfall on average and has a mean temperature of 20.6 °C over the growing season ([National Oceanic and Atmospheric Administration, National Centers for Environmental Information, 2018](#); weather data for study period reported in Table S1). Saltwater moves onto these agricultural fields via hydrologically connected ditch networks and through the groundwater ([Tully et al., 2019a](#)). The extensive agricultural ditch network designed to drain excess water from farms often now serves the reverse purpose by acting as a conduit for saltwater to reach the fields during high tides and storms ([Bhattachan et al., 2018](#); [Tully et al., 2019a](#)). Although there is not data on inundation for 2018 (a wet year), 2019 (a dry year from planting to germination survey) Somerset farm was inundated only once and Dorchester farm not at all (unpublished water level data).

The current standard crop rotation in the Mid-Atlantic region consists of corn and soybean with a winter wheat cover crop. Most farms in the area have been no-till for at least 40 years ([Huggins and Reganold, 2008](#)). No-till agriculture replaces deep inversion tillage (where ≥ 25 cm of the topsoil is disturbed) by using specialized seed drills to ensure good seed-to-soil contact. Most farmland in the region dates back prior to 1800 ([Benitez and Fisher, 2004](#)).

2.2.2. Experimental design

We leveraged an ongoing field trial of alternative crops to evaluate the *in situ* effects of salt on alternative crop germination success. Field trial plots were established in 2018 in a randomized complete block design with four replicates per treatment at each farm (Fig. S2). Treatments consisted of: (1) a natural recruitment control; and (2–4) a rotation made up entirely of alternative crops, sorghum-chloride-excluding soybean-barley, with each entry point present each year (total of three plots per block). Each plot was 3 m wide by 20 m long and established within 2 m of a field edge with evidence of SWI in reduced yield and bare soil. Plots were made intentionally long so as to span a natural salinity gradient, from high salinity near the field edge (0–5 m from the agricultural ditch) to low salinity towards the center of the field (15–20 m). There were four replicates of each treatment for a total of 16 plots per farm with 0.5 m buffers between plots. In this design, the *in situ* germination rate of a subset of the alternative crops tested in the laboratory – sorghum, chloride-excluding soybean, and barley – could be compared to their germination in the ideal conditions of the controlled environment.

Porous cup lysimeters (22 mm diameter; Soil Solution Access Tubes, Irrrometer Riverside, California, USA) were installed in March 2018 to 60 cm depth at 5 m (near salt source) and 15 m from the edge of the plot (far from salt source). Lysimeters were installed by removing soil to 60 cm with a 2 cm diameter soil probe. Soil and water slurries were made with the deepest soil, and poured the slurry into the hole before inserting the lysimeter to ensure good soil contact. Finally, lysimeters were sealed at the soil surface with a bentonite/clay mixture to avoid preferential flow of water down the side of the tube. Pilot studies confirmed that soil porewater collection was only possible following rain events that were greater than or equal to 6 mm, thus soil porewater was only collected following rain events of this level. For this study, the soil porewater collected on August 8, 2018 and June 21, 2019 were used. The day before sampling, lysimeters were purged of any water, and an internal pressure of -60 to -70 kPa was applied. Soil porewater

samples were collected, filtered (Whatman No. 42; 2.5 μ m), and stored in a freezer at -20 °C until further analysis for electrical conductivity. Electrical conductivity (EC) was measured on a Thermo Scientific Orion Versa Star Advanced Electrochemistry Meter probe (Waltham, MA).

Experimental plots were managed as active farms, with practices intended to maximize germination and yield. Prior to planting, plots were sprayed with glyphosate [N-(phosphonomethyl) glycine] (~0.91 kg active ingredient per acre) in early-May 2018. Sorghum (var. *Dekalb DKS 2805*) and chloride-excluding soybean seeds (Pioneer P42a52x) were sown once in mid-May 2018 using a 1.52-m Tye drill and again in mid-June 2018 due to poor germination as a result of heavy rainfall (328 mm) between May 16 and June 16, 2018. Sorghum and chloride-excluding soybean were planted in 38.1 cm rows at a rate of 197,600 seeds ha⁻¹ and 481,650 seeds ha⁻¹, respectively. Chloride-excluding soybean plots received 39 kg K ha⁻¹ in the form of potash (K₂SO₄) and sorghum received 84 kg ha⁻¹ as urea in late-June 2018. In early August 2018, chloride-excluding soybean was sprayed with glyphosate (~0.45 kg active ingredient per acre) and fomesafen sodium salt [5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-methylsulfonyl-2-nitrobenzamide] (~0.32 kg active ingredient per acre). All plots were sprayed with glyphosate [N-(phosphonomethyl) glycine] (~0.91 kg active ingredient per acre) in early-May 2019 to kill weeds. Sorghum and chloride-excluding soybean were sown in early-June 2019 using a 1.52-m Tye drill. Sorghum was planted with the same row width and seeding rate as in 2018. In 2019, chloride-excluding soybean was planted in 19 cm rows at a rate of 481,650 seeds ha⁻¹. Sorghum received 84 kg N ha⁻¹ as urea and chloride-excluding soybean received 67 kg K ha⁻¹ in the form of potash in early-June 2019. Due to poor germination as a result of drought conditions following planting, sorghum and chloride-excluding soybean were re-sown, with the same seeding rate, in late-June 2019.

2.3. Field emergence surveys

In late July 2018 and 2019, plots were surveyed in order to determine the percent germination in each plot. Seedling emergence of sorghum and chloride-excluding soybean was used as a proxy for field germination as they were planted as seeds, rather than considering whether a radical was present as done for the controlled environment experiment. On July 24, 2018 (30 days after planting), every sorghum plant was counted in each 0–5 m, 5–15 m, and 15–20 m segment of each plot. We also recorded the percent cover of chloride-excluding soybean in each plot as the stands were too dense to easily identify individuals without damaging plants. For surveys in 2019, three out of six rows were randomly selected from each plot and every sorghum and chloride-excluding soybean plant was counted along the row in each 0–5 m, 5–15 m, and 15–20 m plot segment on July 18, 2019 (30 days after planting). The 2019 counts for each plot segment were summed and multiplied by two as an estimate of all six rows and for comparison with 2018 counts.

2.4. Statistical analysis

2.4.1. Controlled environment experiment

To test for an effect of osmotic or salt stress on percent germination, linear mixed-effects (LME) models (*lme4* package for R; [Bates et al., 2015](#)) were used with water potential and species as fixed effects and replicate as a random effect (two LME models: one for osmotic stress and one for salt stress). Where there was a significant interaction between water potential and species in the LME models, we further investigated planned comparisons between standard and alternative crop species with Mann-Whitney tests for comparing non-parametric data (*stats* package in R; [R Development Core Team, 2019](#)). Specifically, differences between percent germination at each water potential for each salinity stress (osmotic or salt) in standard and alternative crop species occupying parallel points in a crop rotation were tested. These

corresponded to the following varietal or species pairs: barley and wheat, chloride-excluding soybean and standard soybean, sorghum and corn, and quinoa and every other species. Given a large number of comparisons generated, a Bonferroni-corrected α ($p < 0.0002$) was used to reduce Type I error (Sedgwick, 2012). Next, we tested for an effect of osmotic stress or salt stress on the time for each species to reach 50 % germination (Eq. 3) using LME models with water potential and species as fixed effects and replicate as a random effect. Where a significant interaction between water potential and species in the LME models was observed, we investigated the same planned comparisons for percent germination between standard and alternative crop species with Mann-Whitney tests for comparing non-parametric data. To test for differences in germination of the two crop categories (standard vs. alternative) to osmotic and salt stress, a Mann-Whitney test was run for each level of osmotic or salt stress. The standard crop category included corn, soybean, and wheat and the alternative crop category included sorghum, chloride-excluding soybean, barley, and quinoa. Given a large number of comparisons generated, a Bonferroni-corrected α ($p < 0.003$) was used to reduce Type I error.

To describe the shape of the germination-stress response curve, logistic regression models were fit to the data with stress levels as a fixed effect and germination of crop species as the response variable (total of 14 GLMs: seven crops by two types of stress, osmotic and salt). A Chi-square test was used to evaluate the goodness of fit of our models (Table 3).

2.4.2. Field experiment

At Somerset farm and Dorchester farm, salt stress was not manipulated. In order to evaluate the levels of salt stress in the field, EC (measured in dS m^{-1}) of soil porewater collected from lysimeters was used, with a total of 128 porewater samples. To test if there was a difference in porewater EC between 2018 and 2019 and the natural gradient in SWI for each farm, a LME model was used with distance (5 m vs. 15 m from plot edge) and year as the main effects and block as the random effect.

Percent germination (emergence used as a proxy) for each plot was calculated based on the known seeding rate and the number of plants surveyed (Eq. 4).

$$\text{field germination rate (\%)} = \frac{\# \text{ of plants in plot after } \sim 30 \text{ days}}{\text{seeding rate (seeds per plot)}} \quad (4)$$

To determine if sorghum germination differed between 2018 and

Table 3

Summary statistics of logistic regression model of germination of corn, soybean, wheat, sorghum, chloride-excluding soy, barley, and quinoa at osmotic and salt stress levels. The intercept is the log odds ratio when $x = 0$ (i.e. if the intercept > 0 , germination is more than 50 % likely if osmotic or salt stress level = 0).

	Intercept	Stress treatment (coefficient)	Pseudo- R^2	P-value
<i>Germination by osmotic stress</i>				
Corn	2.11	-5.66	0.94	<0.0001
Soybean	-1.06	-11.67	0.99	<0.0001
Wheat	5.15	-2.65	0.77	<0.0001
Sorghum	4.59	-7.32	0.96	<0.0001
Chloride-excluding soy	2.45	-13.73	0.99	<0.0001
Barley	2.77	-2.04	0.67	<0.0001
Quinoa	5.13	-8.37	0.97	<0.0001
<i>Germination by salt stress</i>				
Corn	2.44	-192.40	0.99	<0.0001
Soybean	-0.76	-1.90	0.61	<0.0001
Wheat	3.48	-200.30	0.99	<0.0001
Sorghum	2.31	-190.50	0.99	<0.0001
Chloride-excluding soy	0.42	-4.27	0.90	<0.0001
Barley	0.71	-167.30	0.99	<0.0001
Quinoa	1.63	-13.05	0.99	<0.0001

2019 and with distance from the saltwater source (5 m vs. 15 m) for each farm, a LME model was conducted with distance and year as main effects and block as a random effect.

We plotted the field percent germination on the same plots as controlled environment trials to investigate the concordance of field data with logistic models of osmotic and salt stress effects on germination. The germination responses in-field in relation to the 95 % confidence intervals of the model (on controlled environment germination response) were used to evaluate if in-field germination was better explained by osmotic or salt stress.

We used Box-Cox transformations (Box and Cox, 1964) prior to analysis when necessary to satisfy the assumptions of the statistical model. All analyses were conducted in the R environment for Macintosh (v1.2.1335).

3. Results

3.1. Controlled environment experiment: the effect of osmotic and salt stress on seed germination

We observed variable tolerance to osmotic stress between the seven species and varieties (Fig. 1 & Fig. S3). Barley and wheat were the only species to germinate at osmotic stress levels below -1.1 MPa. However, wheat had significantly higher percent germination compared to barley at 0, -0.2, -0.5, -0.8, -1.1, -1.8, and -2 MPa ($p < 0.05$). Surprisingly, standard soybean and chloride-excluding soybean also had very similar germination responses to osmotic stress. Quinoa was able to germinate to -0.8 MPa (osmotic stress) and had a significantly higher percent germination at -0.8 MPa than corn, sorghum, or barley.

Chloride-excluding soybean, standard soybean, and quinoa were the only species able to germinate under salt stress of any level. Chloride-excluding soybean was able to germinate at high salt stress levels (-2 MPa; 55.6 dS m^{-1}), which is equivalent to levels found in seawater, although at very low rates (1%; Fig. 1B). Standard soybean was also surprisingly tolerant of salt stress; it was able to germinate, albeit also at very low rates (4%), at -1.8 MPa (50.0 dS m^{-1}). We found no difference in percent germination between chloride-excluding soybean and standard soybean at any salt stress level. Quinoa was able to successfully germinate to -0.5 MPa (13.9 dS m^{-1}) at a low rate (3%). There was no difference in percent germination between either soybean and quinoa at any salt stress level.

When grouped, we found no difference in percent germination between the standard (corn, soybean, and wheat) and alternative (sorghum, chloride-excluding soybean, barley, quinoa) crop groups at any osmotic or salt stress level (Fig. 1A & B). In addition, there was no difference in time to 50% germination between the standard crop and the alternative crop groups at any osmotic or salt stress level (Fig. 2A & B).

3.2. Field experiment: the effect of saltwater intrusion on field germination compared to lab germination

At Somerset farm, the mean EC of soil porewater at 60 cm was $5.44 \pm 0.61 \text{ dS m}^{-1}$ (-0.2 MPa) across 2018 and 2019. There was no significant effect of distance from the saltwater source (5 m vs. 15 m from the plot edge) on soil porewater EC at Somerset farm. At Dorchester farm, the EC of soil porewater was significantly higher 5 m from the plot edge ($6.49 \pm 0.35 \text{ dS m}^{-1}$; -0.23 MPa) than at 15 m from the plot edge ($4.92 \pm 0.42 \text{ dS m}^{-1}$; -0.18 MPa; $p = 0.02$ for both years). There was no effect of year on the EC of soil porewater at either farm.

In 2019, Dorchester farm there was a significant difference in field germination between species, where percent germination of chloride-excluding soybean was two times higher than sorghum ($p = 0.001$). Sorghum germination was depressed in general that year at Dorchester farm; sorghum germination in 2019 was half of the level of 2018 at the site ($p = 0.0005$; Table 4A & B). At Somerset farm, sorghum germination did not significantly differ between 2018 and 2019. Nor did percent

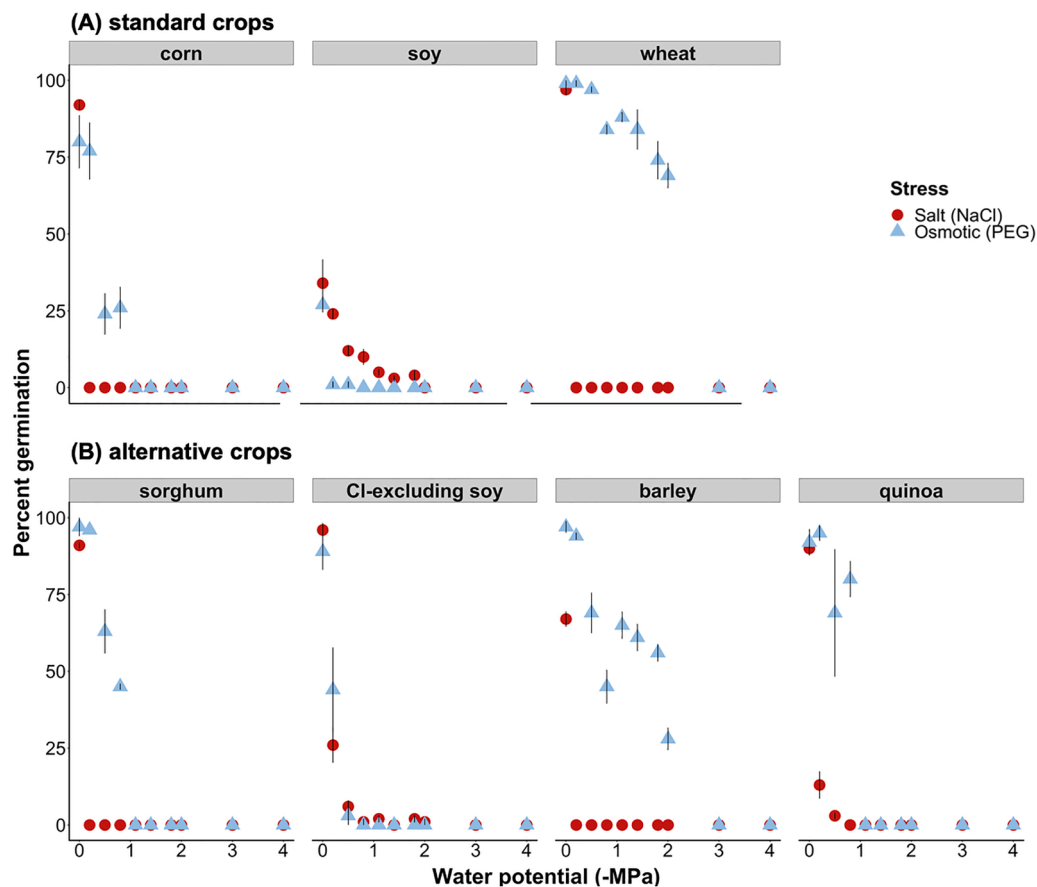


Fig. 1. Percent germination of (A) standard crops: corn, soybean, wheat, and (B) alternative crops: sorghum, chloride-excluding soybean [Cl-excluding soy], barley, and quinoa along a water potential gradient of salt stress (red circles) and osmotic stress (blue triangles). Error bars are standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

germination differ between sorghum and chloride-excluding soybean at Somerset farm in 2019 (Table 4B). There was no significant effect of distance from plot edge on either species on either farm in either year.

In our examination of the effects of osmotic and salt stress effects on in-field germination of sorghum in 2018 and 2019 and chloride-excluding soybean in 2019, we found that the two species were affected differently. Sorghum germination was best explained by salt stress levels (Fig. 3A). For chloride-excluding soybean, the confidence intervals of salt and osmotic stress response curves were overlapping, complicating interpretation. However, the confidence interval of the salt stress curve was very wide, whereas the confidence interval of osmotic stress was very narrow and aligned neatly with the in-field germination rates of chloride-excluding soybean (Fig. 3B). Therefore, we interpret that chloride-excluding soybean germination rates were best explained by osmotic stress.

4. Discussion

This is the first study to compare the germination response of standard and alternative crops to salt stress and to tease apart the osmotic and salt components of that stress. Furthermore, laboratory germination data was compared to field germination rates to understand how idealized germination rates translate into realized germination success. These experiments and analyses were designed to understand crop performance and to provide necessary information to farmers responding to SWI. We focused on the germination phase of crop species because it is the initial determinant of plant success. Additionally, grain farmers plant seeds (not seedlings), thus relying on successful germination to have productive yields, and germination failure is often a major

financial burden through reseeding due to the high cost of seeds. This may be even more true for alternative crops, as ion-excluding seed varieties generally cost more than standard crop seeds.

4.1. Standard vs. alternative crop species

Of the seven species tested, wheat (standard crop) and barley (alternative crop) had the highest germination tolerance to osmotic stress, able to germinate at significant levels at water potentials as low as -2 MPa (Fig. 1). Wheat and barley had the same time to 50% germination at all water potentials under osmotic stress, with the exception of -1.8 MPa where barley reached 50% germination faster than wheat. There were no large differences between wheat and barley, possibly because both species are cereal grains with natural ion-excluding traits (Chen et al., 2019; Chandra Babu et al., 2004). In Maryland, both crops are grown as winter cover crops (sown in the fall after corn or soy). Farmers can either terminate the crop in the spring (and receive a subsidy) or sell the grain for livestock silage or malting barley. Of note is that we did not include the most salt-tolerant crop varieties available to growers (e.g. Melusine, Sandalbar), but instead used crop varieties that farmers of the Mid-Atlantic region would currently purchase and plant (as determined in stakeholder meetings). It was important that our research was designed to answer questions relevant and applicable to the farmers in the region and under the current market and environmental conditions.

The most salt tolerant species in our study was one of the alternative species, quinoa. Quinoa successfully germinated under salt stress (-0.5 MPa) and osmotic stress (-0.8 MPa). Under salt stress, quinoa outperformed barley, wheat, corn, and sorghum (-0.2 to -0.5 MPa).

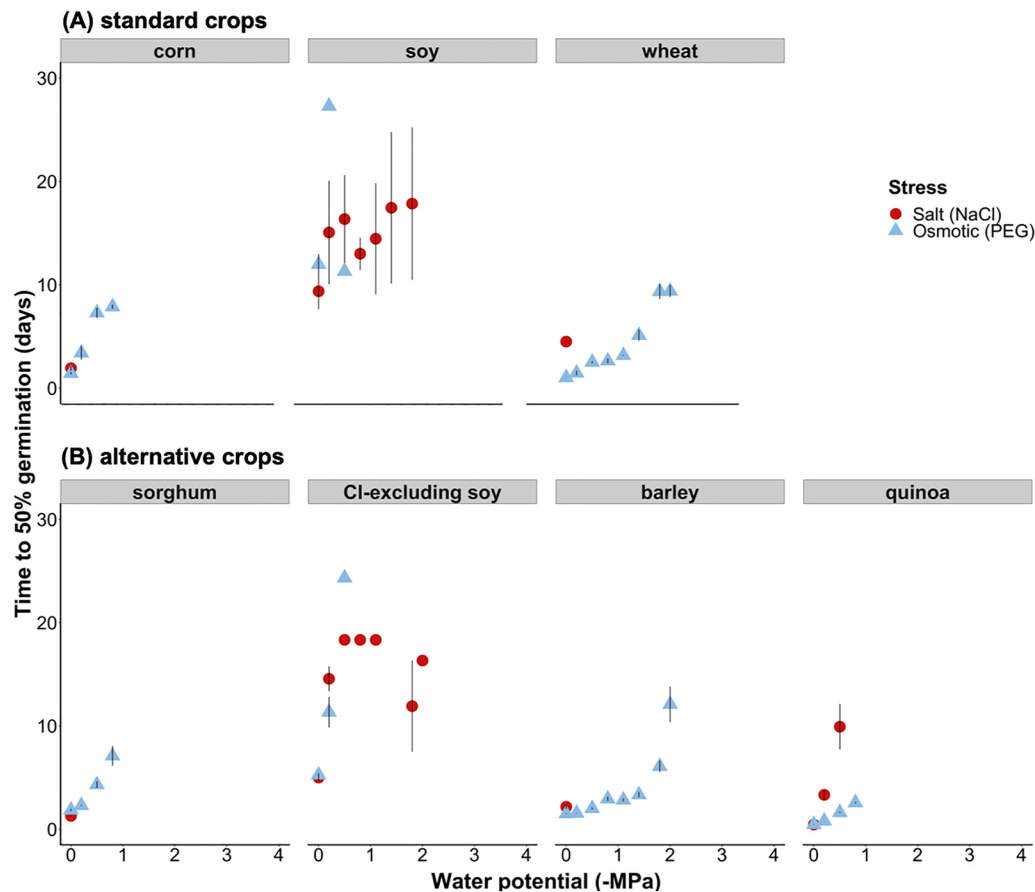


Fig. 2. Time to 50 % germination (hours) of all seven focal species – (A) standard crops: corn, soybean, wheat, and (B) alternative crops: sorghum, chloride-excluding soybean [Cl-excluding soy], barley, quinoa – along a water potential gradient of salt stress (red circles) and osmotic stress (blue triangles). Error bars are standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 4
Percent germination of (A) sorghum at Somerset and Dorchester farm in 2018 and (B) sorghum and chloride-excluding soybean at Somerset and Dorchester farm in 2019 field water potential (MPa; mean \pm SE).

A. 2018		Somerset farm		Dorchester farm	
Water potential (MPa)		Sorghum			
−0.18				61 ± 2.7	
−0.20		20 ± 2.4			
−0.23				45 ± 6.7	
B. 2019		Somerset farm		Dorchester farm	
Water potential (MPa)	Sorghum		Chloride-excluding soybean		
−0.18					24 ± 4.4
−0.20	12 ± 3.7		14 ± 1.7		54 ± 4.2
−0.23					26 ± 0.5
					43 ± 5.0

Additionally, quinoa outperformed barley, corn, and sorghum under osmotic stress at –0.8 MPa with a higher germination success and a faster time to 50 % germination. Quinoa germination in our trials was similar to levels observed in prior studies of NaCl effects on germination rates (Maas et al., 1983; Almansouri, 2001; Panuccio et al., 2014). Quinoa is a halophyte and tolerant of salinity levels approaching those of seawater (Adolf et al., 2013, 2012; Shabala et al., 2012). Furthermore, quinoa can grow in drought-prone and marginal soils (Jacobsen et al., 2003, 2005, 2007; Sun et al., 2014) such as those found on the Eastern Shore, MD. Based on its high germination success and rapid time to 50 % germination in these salt stress trials and high salinity tolerance at other life

history stages (Adolf et al., 2013, 2012; Shabala et al., 2012), it is worth exploring if it would be advantageous for farmers to plant quinoa on their salt-intruded farm fields. However, we did not include quinoa in the field experiment because our farmer partners stated they would not plant quinoa due to the cost of new equipment (e.g. new seed plates) and the non-existent market. Further research is needed to assess if there could be a market for quinoa in the region.

Markets on the Eastern Shore, MD support the cultivation of sorghum, chloride-excluding soybean, and barley, all of which past research indicated should have a higher salt tolerance than corn, soybean, and wheat (Rani et al., 2012; Munns et al., 2006; Munns and Tester, 2008; Table 1). For example, barley seeds are capable of absorbing Na^+ , which facilitates imbibition and germination under salt stress as water is able to pass through the cell wall (Zhang et al., 2010). Although we hypothesized the alternative crop species (e.g. sorghum, chloride-excluding soybean, barley, and quinoa) would have higher salt and osmotic stress levels than the standard crops (e.g. corn, soybean, and wheat), our data does not support this hypothesis. Instead, there was no difference in germination (Fig. 1A & B) or time to 50 % germination (Fig. 2A & B) between the alternative and standard crop species in the controlled environment experiment. Based on these observed germination tolerances to salt and osmotic stress, one might assume there would be no reason for a farmer to switch from the standard crops to alternative crops. However, stakeholder meetings have revealed that some farmers on the Eastern Shore, MD are switching from corn to sorghum on salt-intruded farm fields. We conclude from this action that salinity tolerance in later life history stages has yield benefits for sorghum over corn in salt-intruded conditions, a hypothesis that will be tested in future work.

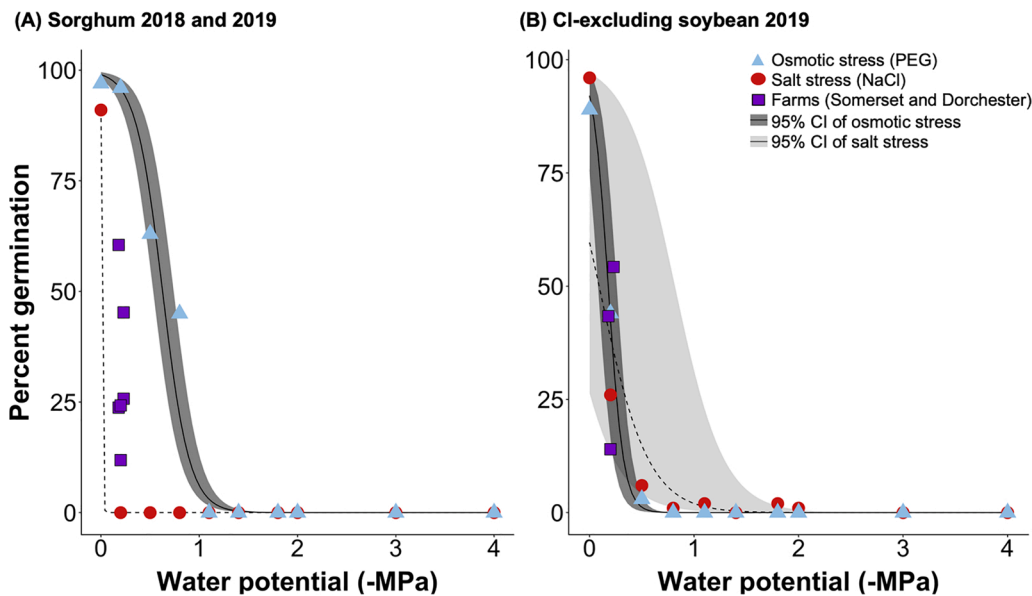


Fig. 3. Percent germination of field studies (both farms; purple squares) (A) sorghum in 2018 and 2019, and (B) chloride-excluding soybean in 2019 and controlled environment experiment. Osmotic stress indicated by blue triangles and salt stress indicated by red circles and both in units of -MPa. Response to osmotic stress is indicated by a solid black line fitted to logistic regression model and 95% confidence interval (dark grey shading). Response to salt stress is indicated by the dashed black line fitted to logistic regression model and 95% confidence interval (light grey shading). The large confidence interval for salt-tolerant soybean response to salt stress was driven by the standard error of the mean at water potential -0.2 MPa, which is why field germination of chloride-excluding soybean fell within the salt stress confidence interval as well as the osmotic stress confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4.2. Field germination of chloride-excluding soybean and sorghum

Despite small variation in salt stress in the field across all scales of comparison (0.05 MPa difference between plot edge and center at Dorchester farm, and no significant difference between sites or across years), there was high variation in in-field germination rates of sorghum and chloride-excluding soybean. This was particularly true at the Dorchester farm, where there were differences in germination success between the two species and between the two study years. Germination success at Dorchester farm in 2019 supported our hypothesis that chloride-excluding soybean would have higher germination than sorghum, which fits with our controlled environment trials that observed higher salt tolerance in chloride-excluding soybean than sorghum. Additionally, this result may include salinity tolerance of the plants at the seedling phase. Other studies have observed that sorghum is more sensitive to salinity in the seedling phase than soybean (Maas et al., 1986; Igartua et al., 1995; Hosseini et al., 2002).

In our field trials, extreme weather was even more important to germination success than salinity. For instance, Somerset and Dorchester farms required reseeded in both years due to poor germination as a result of heavy rains in 2018 and drought conditions in 2019. Between the time of seeding and counting in-field germination (June to July) in 2018, Somerset farm received 325 mm of rainfall; whereas in 2019, Somerset farm received 202 mm of rainfall. Dorchester farm received 328 mm of rain between June and July 2018, 65 mm more rainfall than June and July 2019 of 263 mm. Thus, greater sorghum germination at Dorchester farm in 2018 compared to 2019 may be a result of extreme weather (e.g. heavy rains promoting germination in the second seeding of 2018 or drought suppressing germination in the second seeding of 2019; Table S1).

As reseeding fields is an additional expense to farmers, planting during a window of time in which weather and soil conditions are favorable to germination success is key. Our field experimental results point to the importance of temporal windows with benign conditions to germination success in these environments. This will be an important area of future research, to understand more about the occurrence of benign and extreme weather during planting seasons and how this shapes the response of farmers to SWI.

4.3. Comparing controlled environment and field experiments

In-field sorghum germination was better explained by salt stress than osmotic stress alone (Fig. 3A), suggesting that sorghum germination was more sensitive to Na^+ and Cl^- toxicity than water availability (e.g. inundation and drought). Previous research shows sorghum has developed strategies to adapt to drought conditions (i.e. deep and extensive rooting; Tari et al., 2013), which aid in its success growing in semi-arid and arid regions (Bibi et al., 2012; Patané et al., 2013). Sorghum's response to inundation is less clear with some studies indicating flood tolerance (Ejeta and Knoll, 2007; Tari et al., 2013) and others flood-intolerance (Orchard and Jessop, 1984; Promkhambut et al., 2011). However, all of these studies report sorghum survival and/or productivity and no studies to date have reported sorghum seed germination (or seedling emergence) in response to flooding. Our data suggest that sorghum germination in-field was a response to changes in salt stress (Na^+ and Cl^- toxicity) rather than osmotic stress alone. Coastal farmers facing SWI have already started planting sorghum (*personal observation*), which we show may be tolerant to osmotic stress induced by SWI, and which the literature suggests tolerates climate variability well. Although sorghum was not salt-tolerant at the germination stage, it requires far less inputs than corn and therefore affords a lower financial risk to the farmer should the sorghum crop fail. Further research is needed to determine if sorghum is more tolerant than corn at later life history stages, which would support the promotion of sorghum cultivation in the region.

In contrast to sorghum, the decline in chloride-excluding soybean germination in-field was better explained by increasing osmotic stress rather than salt stress (Fig. 3B), suggesting that germinating chloride-excluding soybeans were more sensitive to reduced water availability than Na^+ and Cl^- toxicity. This is likely due to the fact that the cultivar planted was bred to exclude Cl^- (Pioneer Co®), making it more resistant to changes in ionic strength.

Other studies have found that soybean germination responds poorly to both drought (Dornbos et al., 1989) and flooding (Wuebker et al., 2001). Given the high interannual environmental variability of the Eastern Shore of MD, and the wet-dry fluctuations within the growing season, seeds must contend with both salt stress and freshwater inundation. Under these conditions, we expect highly variable germination success in soybean of any variety. In comparing the chloride-excluding

soybean in our field experiment to adjacent farms growing soybean, we and collaborators observed no immediate difference in germination success (Jarrod Miller, *personal communication*). Although the chloride-excluding cultivar shows resistance to salt stress, it cannot tolerate inundation and drought. Therefore, we suggest that there is little reason for farmers facing SWI to switch to chloride-excluding soybean.

4.4. Future research

The environmental variability of coastal environments and the interaction of hydrology and climate with SWI make it complex to understand how tolerance to osmotic and ionic components of salt stress will translate into field germination success. There is still work to do to understand how crops will respond to both salt stress and fluctuating wet-dry regimes. However, the fact that sorghum and chloride-excluding soybean show sensitivity to different stressors associated with SWI demonstrates that different crop species will have different susceptibilities that will translate into performance success. Furthermore, the performance of quinoa in the laboratory, and its high tolerance to both osmotic and salt stresses, suggests that it is worthwhile to initiate field trials with this species to investigate its viability in the region.

Our study focused only on germination and not crop yield. More research is needed to identify how salt tolerance at each life history stage translates into yield effects of SWI. The field experiment described herein will run for 3+ years to have a full rotation of the alternative crops and multiple insertion points for all crops in order to disentangle the relationship between species performance, inter-annual climate variability, and SWI impacts on yield. In field experiments, we are also exploring the possibility of halophytic and perennial species, *Spartina patens* and others, to be grown on salt-intruded farm fields as a means of ameliorating salt damage, providing a transitional forage or biomass crop, and promoting coastal wetland restoration.

5. Conclusion

The purpose of this study was to (1) determine how osmotic and salt stress levels affect seed germination in standard crops and alternative ion-excluding crops in a controlled environment experiment and (2) determine the germination success of alternative crops planted in salt-intruded farm fields on the Eastern Shore of Maryland. Overall, there was no significant difference in percent germination between the standard and the alternative crop species under osmotic or salt stress in controlled environmental conditions. Under field conditions, germination success was two times higher in chloride-excluding soybean than sorghum. The in-field germination patterns of sorghum and chloride-excluding soybean were explained by increasing salt stress and osmotic stress, respectively. The fact that sorghum and chloride-excluding soybean show sensitivity to different stressors associated with saltwater highlights the complexity and challenges of identifying crop species capable of germinating on salt-intruded farm fields. Farmers facing SWI have to decide whether to continue growing the standard crops or begin planting alternative crop species. Understanding the germination success of crop species experiencing SWI is critical to developing informed farm management strategies in coastal agricultural regions. This research provides the groundwork for assessing the success of an alternative cropping rotation on farms experiencing SWI.

CRediT authorship contribution statement

Elizabeth de la Reguera: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Funding acquisition. **Jacquelyn Veatch:** Investigation, Writing - review & editing. **Keryn Gedan:** Conceptualization, Methodology, Resources, Writing - original draft, Supervision. **Katherine L. Tully:** Conceptualization,

Methodology, Writing - original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2020.104254>.

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