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Salinity tolerance of germinating alternative oilseeds

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ABSTRACT

Integrating oilseed crops into rotations can improve soil health benefits, nutrient retention, and pollinator provisions. Field margins represent areas where incorporation of oilseeds is feasible. However in the northern Great Plains, field margins can oftentimes be areas of saline soil, which can impact seed germination and seedling establishment. Therefore, a replicated growth chamber experiment was used to determine winter camelina (*Camelina sativa*), winter pennycress (*Thlaspi arvense* L.), echium (*Echium plantogineum*), cuphea (*Cuphea viscosissima X Cuphea lanceolata*), and calendula (*Calendula officinalis*) tolerance to germinating under saline conditions. A total of 50 seeds, replicated 3 times were germinated in petri dishes saturated with NaCl, CaCl, and Na_2SO_4 solution at 0, 0.2, 2, 4, 8, and 16 dS m^{-1} in an incubator at constant 20 °C. Fully germinated seeds were counted and removed daily for 7 days, followed by every other day for a total of 21 days. Final germination percent, corrected germination rate index, and germination velocity were calculated. Germination percent, corrected germination rate index and germination velocity were negatively affected by increases in salinity for camelina, pennycress, cuphea, and calendula. Echium germination was not impacted by salt or salinity level. Sodium based salts were more detrimental for camelina, pennycress, and calendula. Camelina and cuphea germination was tolerant to salinity, with average salinity thresholds of 8.0 and 3.1 dS m^{-1} and a 25% germination decline at 35.3 and 11.0 dS m^{-1}, respectively. Pennycress and calendula germination was moderately tolerant to salinity with average salinity thresholds of 5.9 and 2.7 dS m^{-1} and a 25% germination decline at 9.4 and 7.7 dS m^{-1}, respectively. These oilseeds show potential for adoption in saline soils.

1. Introduction

Alternative oilseed crops represent potential feedstocks for biofuel production, industrial products such as plastics and resins, cosmetic applications, as well as human and animal consumption (Hojilla-Evangelista et al., 2015; Dose et al., 2017). Additionally, oilseeds have been shown to provide multiple benefits on the landscape such as nectar and pollen resources for beneficial flower visiting insects such as managed and wild pollinators, natural enemies of pest insects, and species of conservation concern like the Monarch butterfly, *Danaus plexippus* L. (Eberle et al., 2014a,b; Eberle et al., 2015; Thom et al., 2016; Thom et al., 2017). When planted as cover crops, the oilseeds camelina [*Camelina sativa* (L.) Crantz] and pennycress (*Thlaspi arvense* L.) can scavenge surplus nitrogen and phosphorus left in the soil following maize (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], or wheat (*Triticum* spp.) production showing promise for reducing pollution of lake, river, and groundwater systems by fertilizers (M. Thom, unpublished data).

In order to meet the demands of a growing world population, agricultural lands need to be utilized to simultaneously produce food, feed, fiber, and fuel. Field margins, which are typically areas of lower productivity, represent an area where producers can grow alternative crops such as oilseeds without much displacement of cash crops produced for grain or feed. Field margins and edges are also recognized as important sources of pollinator-friendly habitat in an otherwise unfriendly agricultural landscape. When margins are sown with mixtures of nectar and pollen-producing plants, they attract a greater abundance and diversity of wild bees and hoverflies (Carreck and Williams, 2002; Pywell et al., 2005; Carvell et al., 2007; Wratten et al., 2012; Blaauw and Isaacs, 2014; Kremen and M'Gonigle, 2015). Single species plantings in field margins also attract a diversity of bees, flies, and butterflies (Carreck and Williams, 2002). There is also evidence that these pollinator plantings can help mitigate runoff and protect against soil erosion (Wratten et al., 2012).

In Red River Valley, located in portions of North Dakota, South Dakota, and Minnesota, USA, the soils in field margins can be salt-

Abbreviations: EC, electrical conductivity; CGRI, corrected germination rate index; GV, germination velocity

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affected (Skarie et al., 1986). In the United States, saline soils are classified as having an electrical conductivity (EC) of $\geq 4\, dS\, m^{-1}$, a sodium adsorption ratio of < 13, and a pH of < 8.5 (Richards, 1954). Salt-affected soils negatively impact crop growth by altering osmotic balance and inducing specific ion toxicity (Bernstein and Hayward, 1958; Sarig and Steinberger, 1994; Zahran, 1997). Salinity in this area is naturally occurring as a mixture of Ca^{2+}, Mg^{2+}, and Na^{+} $-Cl^{-}$ and $-SO_4^{2-}$ salts. Land use coupled with climatic changes have resulted in a rise of the local water table which has deposited these salts within the rooting zone (Lobell et al., 2010). Although saline salts are naturally occurring in the region, salinity can be exacerbated by drainage ditches, which represent areas of groundwater recharge and discharge.

Although the areas of salt-affected soils from ditches in an individual field may be small (Skarie et al., 1986), alternative management practices to remediate soil salinity are needed on the estimated 800,000 ha of land in the Red River Valley (Ulmer et al., 2007). These salt-affected areas may be an attractive location for producers to grow alternative oilseed crops as planting and harvesting are compatible with existing equipment (Gesch et al., 2014; Berti et al., 2016). However, little information regarding alternative oilseed crop tolerance to salinity is known. Therefore our objectives were to determine the effects of several types of salinity (NaCl, CaCl, Na_2SO_4) on the total germination and germination rate of winter camelina, winter pennycress, echium (*Echium plantogineum* L.), cuphea (*Cuphea viscocissima* x *Cuphea lanceolata* W.T. Aiton), and calendula (*Calendula officinalis* L.).

2. Materials and methods

Winter camelina ('Joelle'), winter pennycress ('Beecher'), echium, cuphea ('PSR-23'), and calendula ('Carola') were used in this study. All seeds used in this study were reproduced at the Swan Lake Research Farm near Morris, Stevens County, Minnesota (45.68°N, 95.80°W) USA. Camelina and pennycress are self-pollinating, whereas cuphea, echium, and calendula tend to be strongly cross-pollinating species. Winter camelina was obtained from North Dakota State University Extension. The calendula and echium seeds originated from Technology Crops International (Winston-Salem, NC, USA) whereas pennycress was obtained courtesy of Terry Isbell (USDA-ARS-National Center for Agricultural utilization Research, Peoria, IL, USA). Cuphea was grown for several generations at the Swan Lake Research Farm, but was originally obtained courtesy of Steven Knapp (Knapp and Crane, 2000). Each oilseed species was germinated at five EC levels representing 0.2, 2, 4, 8, and 16 $dS\, m^{-1}$. The actual EC levels for each salt solution can be found in Table 1. Seeds were also germinated in ultrapure water (Barnstead e-Pure, ThermoFisher Scientific, Waltham, MA, USA) which acted as a no salinity control (EC 0). Salt solutions were made with pure concentrations of NaCl, CaCl, and Na_2SO_4 mixed with distilled water. A total of 50 seeds were placed in 100 × 150 mm petri dishes lined with blue blotter paper (Anchor Paper Company, St. Paul, MN, USA). A 5 mL volume of salt solution was added to the petri dishes to saturate the blotter paper. Additional salt solutions were added every day for the first week followed by every 2–4 days thereafter to maintain moisture. The study design was a completely randomized design with three replications. Petri dishes were stacked three-high and were re-randomized within the growth chamber after each day that germinated seeds were counted and removed. The entire experiment was repeated for a second time, resulting in a total of 6 observations of each oilseed in salt and EC combinations in a factorial arrangement. Germination temperature was maintained at 20 °C with a relative humidity of 60% (± 2%) using a Percival R&D I-36VL model (Percival Scientific, Inc., Perry, IA, USA). Dark conditions (24 h dark) were utilized for this study as described by Huang and Redmann (1995).

Table 1
The representative and actual electrical conductivity (EC) level of each salt solution used to germinate alternative oilseeds.

Salt	Representative EC level, $dS\, m^{-1}$	Actual EC level, $dS\, m^{-1}$
NaCl	0.2	0.17
	2	1.85
	4	3.91
	8	8.11
	16	16.17
CaCl	0.2	0.13
	2	1.92
	4	3.95
	8	7.87
	16	15.99
Na_2SO_4	0.2	0.14
	2	2.03
	4	3.91
	8	7.96
	16	16.02

Seeds were considered fully germinated when the plumule and radicle were fully visible. Germinated seeds were counted and removed every day for the first 7 days, and every-other day for an additional 2 weeks. Petri dishes were randomized within the germinator every time seeds were counted to minimize within chamber errors. A tetrazolium test (AOSA, 2010) was not utilized to determine viable but non-germinated seeds at the conclusion of the experiment as several of the species tested in this experiment exhibit dormancy and the tetrazolium test is non-conclusive for dormant seeds.

Several metrics were calculated to determine the effects of salt type and electrical conductivity on seed germination as described in Schmer et al. (2012). The daily germination percentage data was fitted to a logistic function using Eq. (1).

$$G = \frac{a}{1 + \exp\,(b - c \text{ x } \ln(d))} \tag{1}$$

Where G is the daily percent of germinated seeds, a is the maximum germination percentage, b is the day when the midpoint of the curve occurs, c is the slope of the germination curve, and d is days (Schimpf et al., 1977; Lafond and Baker, 1986). The final germination percent for each petri dish was also calculated as was the corrected germination rate index (*CGRI*), which takes each individual petri dish into account. The *CGRI* was calculated for each species, salt, and electrical conductivity level using Eq. (2).

$$CGRI = \left(\gamma\left(\frac{T_L - T_G}{T_L}\right) \times \frac{1}{\text{Germ \% of single dish}}\right) \times 100 \tag{2}$$

Where γ is the percent of germinated seeds per a single petri dish, T_L is the length of the trial in days, T_G is the first day of an observed seed germination in a single petri dish, and Germ% is the final germination percent of a single petri dish. Finally, the germination velocity (*GV*) was calculated to determine the speed of germination from Eq. (3).

$$GV = \Sigma\frac{G}{t} \tag{3}$$

Where t is the length of the germination trial in days.

Nonlinear regression, using Proc NLIN in SAS 9.4 (SAS Institute, Inc. Cary, NC, USA) was used to fit the germination logistic function and determine values for a, b, and c across salt type. To determine the effects of salt and EC level on oilseed germination, the final germination percent, *CGRI*, and *GV* were analyzed using analysis of variance procedures using Proc GLM. Mean separation was carried out using least significant difference with a Tukey adjustment. Species, salt, and EC were all considered fixed factors, and repetition was considered a random factor. Simple linear regression analysis, as a means to integrate the variance among EC levels, was used to determine the tolerance of each species to each salt type and the threshold electrical conductivity level where 25 and 50% reductions in germination occurred. All statistical significance was performed at the $p < 0.05$ level.