Poor Emergence of *Brassica Species* in Saline-Sodic Soil Was Improved by Biochar Addition

Thandiwe Nleya *, Sharon A. Clay, Unius Arinaitwe *

Posted Date: 17 February 2025

doi: 10.20944/preprints202502.1276.v1

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Article

Poor Emergence of *Brassica Species* in Saline-Sodic Soil Was Improved by Biochar Addition

Thandiwe Nleya *, Sharon Clay and Unius Arinaitwe

Department of Agronomy, Horticulture, and Plant Science, South Dakota State University, Brookings, SD 57007

* Correspondence: thandiwe.nleya@sdstate.edu

Abstract: Salt-affected soil areas are increasing in the Northern Great Plains (NGP) with patches occurring in some of the most productive croplands. High electrical conductivity (EC) and sodium and/or sulfate concentrations of saline-sodic areas impede growth and yield of 'normal' [corn (*Zea mays*)/soybean (*Glycine max*)] rotational crops and more appropriate management systems are needed. Brassica sp. and amendment application, such as biochar, may provide management alternatives for these areas. In two greenhouse studies, 1) 10 canola (*Brassica napus*) genotypes were evaluated for emergence in non-saline ($EC_{1:1}$ = 0.62 dS m^{-1}), moderately saline-sodic (EC = 5.17 dS m^{-1}), and highly saline-sodic ($EC_{1:1}$ = 8.47 dS m^{-1}) soils and 2) 10 canola genotypes and three other brassicas (*Brassica juncea*/*B. oleracea*) were evaluated for emergence and biomass in non-saline or highly saline-sodic soils with or without two 5% biochar (hardwood or softwood) amendments. Canola emergence at 28 days after planting (DAP) in moderately and highly saline-sodic soils was less than 12% for most genotypes, although one had 37% emergence. The hardwood biochar improved Brassica sp. emergence (42%) from the saline-sodic soil compared to nonamended soil (29%), although shoot biomass was similar among treatments 60 DAP. These findings suggest that specific salt-tolerant canola genotypes may be an alternative crop for NGP saline-sodic areas. Florida broadleaf mustard, typically used for forage, had the greatest emergence (52%) in the saline-sodic soil and may be a suitable cover crop for these areas. In addition, hardwood biochar application may aid in plant establishment.

Keywords: *Brassica species*; saline soils; saline-sodic soils; hardwood biochar; softwood biochar; phytoremediation

Introduction

Currently, about estimated 1 billion hectares (30% of arable soils worldwide) are affected by salt stress, resulting in crop losses of $27 billion annually [1, 2]. In the Northern Great Plains (NGP) of North America, marine sediments buried below glaciated soils are a source of salts that cause saline seeps due to rising water tables [3]. In South Dakota, salinity and sodicity lead to approximately $26.2 million in annual economic losses across over 113,300 acres in the northeastern and central counties of the state [4]

Maize (*Zea mays* L.) (47%), soybean (*Glycine max* L.) (41%), and wheat (*Triticum aestivum*) (11%) are the most common crops in eastern South Dakota [3]. Maize yield losses can start when saturated paste electrical conductivity (EC_e) values are about 1.7 dS m^{-1}, with additional 12% yield losses for each 1 dS m^{-1} above that value [5]. Currently, around 14% of corn farmers are witnessing some yield detriment due to ECe levels exceeding this value, with indications that soil ECs are on the rise [6]. Soybean and wheat have higher EC_e threshold values, 5.0 and 6.0, respectively, [5], but even these crops are at risk for yield losses to the presence of a subsurface natric soil horizon that continue to supply salts to surface The elevated osmotic potential associated with saline-sodic soils restricts water availability to plants, thereby hindering growth [7]. Additionally, the high sodium concentration

 doi:10.20944/preprints202502.1276.v1

contributes to soil dispersion and sealing while increasing the likelihood of water erosion. Salts can also be transported by water to previously unaffected regions, leading to an expansion of saline-sodic zones, which in turn elevates soil EC levels and diminishes future soil productivity, even far from the initial source of the issue [8].

Traditional remediation/restoration strategies for NGP saline-sodic soils, such as leaching of soil salts using non-saline water, chemical amendments such as gypsum and elemental sulfur, installing tile drains, and mechanical methods [e.g., deep tillage (ripping) to improve water infiltration], have been unsuccessful [6, 8, 9] in either reducing the affected areas or lowering the EC. However, establishing salt-tolerant plants in saline-sodic areas may help restore soil functionality and health to near barren areas [3, 10-12], slow the expansion of saline seeps, and provide incomes to the farmers [6, 13, 14]. Previous studies on plant tolerance to saline-sodic soils in South Dakota dryland areas have evaluated native plants [4, 15], and perennial grasses [3, 10, 16] with less attention given to annual crops that are have significantly greater economic importance.

The *Brassica spp* like canola (*Brassica napus*), mustard (*B. juncea*), and carinata (*B. carinata*) have shown promise in studies as viable options for salt-tolerant annual crops [13, 17, 18]. Some canola genotypes and other Brassicas have been reported to have an EC_e threshold of 9.7 [5, 19], much higher than the rotational crops now commonly grown in these areas. The *Brassica spp*. could be used as either cash (canola and carinata) or cover (brown mustard or Florida mustard) crops, although evaluation is needed since tolerance to higher EC_e values varies by species and genotypes. For example, amphidiploids species of *Brassica spp*, such as canola and Florida broadleaf mustard (*Brassica juncea*), are relatively more salt tolerant compared to Brassica diploid species [20, 21]. An added advantage of *Brassica species* is that they have glucosinolate compounds with antimicrobial activity in the leaves and, therefore, have suppressive effects of major soilborne pathogens that cause root rots such as *Pythium* and *Rhizoctonia spp* [22, 23]. While microbial suppression may be desirable in some soil types, although it may be undesirable in saline-sodic soils that may have low microbial populations [12] with further suppression being undesirable for long-term ecosystem function restoration [24].

The often-suggested chemical amendments of gypsum and elemental sulfur application have had minimal impact on soil chemical and physical properties of NGP saline-sodic soils [6, 8]. Soils of South Dakota are often saturated with calcite and have high sulfate levels (2170 mg SO_4^{-2} kg^{-1}) making the amendments valueless. However, biochar has attracted considerable attention as a soil amendment to remediate and improve physical, chemical, and biological properties of degraded soil [25] and promote plant growth [26]. Despite the potential of biochar as the amendment for saline-sodic soils, biochar has not been evaluated on South Dakota saline-sodic soils.

Several types and sources of biochar are available, with pyrolysis temperature, time, and pressure, and source of feedstock impacting the final product [27-30]. For example, hardwood feedstock pyrolyzed at high temperature produces high carbon char with higher amounts of aromatic structures and greater cation exchange capacity, whereas soft and non-wood feedstocks, such as crop residues, manures and straw biomass, produce biochar with lower carbon content [29] more aliphatic compounds and lower sorption capacity [31]. Biochar produced from pinewood chips at 500 ^oC under slow pyrolysis had 817 g kg^{-1} C content, whereas poultry manure char pyrolyzed at the same temperatures had about half the carbon content (only 400 g kg^{-1} C) [29]. Biochar produced from herbaceous feedstock also tends to contain lower proportions of mesopores and macropores and exhibit smaller surface area compared with biochar pyrolyzed from woody biomass which also influences the activity when added to the soil [30].

A review of the impact of soil biochar amendment on crop yields [32] indicated mixed results with yields ranging from -28 to +39% compared to non-amended soils, although there was an overall average yield increase of 10%. Some studies reported negative effects of biochar on soil organic carbon, nutrient mineralization and uptake, and soil microbial activity [27, 30], whereas others showed negative affects to seed germination, due to the release of volatile organic compounds [32], and plant growth [33]. However, biochar has been reported to reduce salinity impacts on soil's EC_e

 doi:10.20944/preprints202502.1276.v1

[34], by sorbing sodium and sulfate, which can be present in very high amounts in NGP saline-sodic soils [3]. These changes in soil properties may improve plant emergence, growth, and development.

Due to the variation in *Brassica spp*. salt and EC_e tolerances, and differences in biochar performance in past studies, it is worthwhile to screen genotypes and chars produced from various wood sources under controlled conditions to determine if positive results to seedling emergence and plant growth could be obtained prior to large-scale field experiments. Therefore, the objectives of these greenhouse studies were to (i) evaluate emergence of *Brassica spp* (canola, and mustards genotypes) in a South Dakota saline-sodic soil, and (ii) evaluate emergence and shoot biomass of canola and mustard genotypes in the saline-sodic soil amended with two biochar types (softwood and hardwood).

Materials and Methods

2.1. Soil Description

Soils used in this study were collected from Clark, SD (44°42'9.68" N and 97°54'40.9" W) from a field described in detail in several previous papers [3, 10, 11]. The upslope (non-saline) soil is a Forman-Cresbad loam, which is characterized as a well-drained, fine-loamy, mixed, superactive, frigid Calcic Argiudoll. The footslope (saline-sodic) soil is a Cresbard-Cavour loam with the Cavour series characterized as a fine, smectitic, frigid, Calcic Natrudoll.

Soils for Experiments 1 and 2 were collected in the same field but at different landscape locations and having slightly different field treatments. Experiment 1 soils were collected at three landscapes; upslope (non-saline/productive), backslope (moderately saline/saline) and foot slope (highly saline/saline) in a field area with no plant growth (areas were barren). Experiment 2 soils were taken where perennial grasses had been planted and established for two seasons at two positions, (upslope, productive 2, and foot slope, saline-sodic 2).

Soil for each experiment were collected at multiple areas within each landscape to about 5 cm depth and bulked by position. These samples were thoroughly mixed but not dried or ground. Subsamples prior to planting were analyzed by a commercial soil testing laboratory (Ward Laboratories Inc., Kearney, NE) (Table 1) for $EC_{1:1}$, soil organic matter (loss on ignition method), $pH_{1:1}$, NO_3-N , M3-P (Mehlich-3 soil phosphorus) [35], K, sulfate, Na, Ca, Mg and % Ca, Mg and Na (which is similar to the sodium adsorption ratio (SAR)) [36]. The sub-sample for biological characterization were held at -4° C until analyzed for microbial biomass using phospholipid fatty acid analysis (PLFA) procedure (Ward Laboratories Inc. Kearny, Nebraska).

Table 1. Chemical properties for productive (1 and 2), moderately saline-sodic (1), and saline-sodic (1 and 2) soils used in Experiments 1 (Upper) and 2 (Bellow) conducted in the greenhouse at South Dakota State University in 2020 and 2021. The K, Ca, Mg, and Na were extracted with ammonium acetate and the sum of bases were the summation of these bases. The %Ca, %Mg, and % Na were 100× each extractable cation and the sum of bases. .

Soil type	pH	EC (1:1)	SOM (LOI)	NO_3-N	M-3 P	K	SO_4
Experiment 1	1:1	**dS m^{-1}**	**g kg^{-1}**	……	**mg kg^{-1}**	.....	.....
Productive soil 1	7.5	0.62	49	41.2	15	357	142
Moderately saline-sodic 1	6.9	5.17	44	82.8	28	204	2742
Highly saline-sodic 1	6.8	8.47	43	123.9	22	189	3514
Soil type	Ca	Mg	Na	Sum bases	Ca	Mg	Na
		mg kg^{-1}		**cmol$_c$ kg^{-1}**	……	**%**.....	.....
Productive soil 1	2735	408	54	18.2	74	19	1
Moderately saline-sodic 1	2066	1164	2346	30.8	33	32	33
Highly saline-sodic 1	1614	1236	4286	39.3	21	26	47

Soil type	pH	EC (1:1)	SOM	NO_3-N	M-3 P	K	SO_4

 doi:10.20944/preprints202502.1276.v1

	1:1	dS m^{-1}	g kg^{-1}	……....mg kg^{-1}.....			
Experiment 2							
Productive soil 2	6.5	1.85	53	49.3	26	112	786
Saline-sodic 2	6.9	5.16	44	71.1	22	144	2315
Soil type	Ca	Mg	Na	Sum bases	Ca	Mg	Na
		mg kg^{-1}		cmol$_c$ kg^{-1}	……%.....		
Productive soil 2	2484	873	207	25.5	48	29	4
Saline-sodic 2	2329	1311	1970	32.1	36	34	27

Abbreviations: $EC_{1:1}$, electrical conductivity measured from a 1:1 solution of soil/water; SOM, soil organic matter analyzed using Loss on Ignition (LOI); M-3P, P extracted by the Mehlich P-3 test.

2.2. Experimental Description

Experiments were conducted at the Plant Science Greenhouse facility for South Dakota State University (44.3° N; 96.8° W). Supplemental light was used to maintain 12-hr day/night cycles for both experiments with a day/night temperature was approximately 26/15 ^{O}C but varied from 20 to 27^{o}C and 10 to 20^{o}C, respectively. Canola is a cool-season plant and the temperature range in the study was ideal for seedling emergence and plant growth.

In Experiment 1, 10 canola genotypes (Table 2) were evaluated for seedling emergence in each of the three soils (productive 1, moderately saline-sodic 1, highly saline-sodic 1) described above in October 2020. Each of the pots contained about 300 g of soil and were seeded with eight seeds per pot at 0.5 cm depth. Each soil by canola type was replicated four times (n=4). This study was done once in October 2020.

Table 2. Genotypes evaluated in the greenhouse experiments 1 and 2 at Brookings, SD.

Genotype	Source/Company	Maturity‡
NCC101S[1,2]†	Photosyntech	Early
DKTF91SC[1,2]	DEKALB Canola	Early
DKTF92SC[1,2]	DEKALB Canola	Late
CS2500[1,2]	Meridian Seeds	Med
InVigor L140P[1,2]	BASF Corporation	Med/Late
InVigor L233P[1,2]	BASF Corporation	Early
DKL7114BL[1,2]	DEKALB Canola	Early
CS2100[1]	Meridian Seeds	Early
CS2300[1]	Meridian Seeds	Late
CS2600[1]	Meridian Seeds	Early/Med
DKTF96SC[2]	DEKALB Canola	Med
DKLL82SC[2]	DEKALB Canola	Early
DKTFLL21SC[2]	DEKALB Canola	Early
African cabbage[2]	Green Cover Crops®, Nebraska	-
Brown mustard[2]	Green Cover Crops®, Nebraska	-
Florida broadleaf mustard[2]	Green Cover Crops®, Nebraska	-

†Genotypes [1,2]Used in both experiments, [1]Used in experiment 1 only, [2]Used in experiment 2 only.‡ Based on seed company ratings for maturity.

In Experiment 2, seedling emergence and shoot biomass of 13 Brassica genotypes (Table 2) were evaluated in a productive soil 2 and a saline-sodic soil 2, described in Table 1, and amended with three biochar treatments [no biochar, 5% (w/w) softwood biochar, or 5% (w/w) hardwood biochar]. The biochars were produced by pyrolysis of pine (*Pinus spp*) for the softwood and maple (*Acer spp*) for the hardwood using carbon optimized gasification technique at about 350^{o}C for 30 min in a commercial pyrolysis kiln [Advanced Renewable Energy Technology International (ARTi), Prairie City, IA]. Biochar subsamples of each type were dried at 65 ^{o}C for 36 hours to moisture free weight and sent to Ward Laboratories Inc., Nebraska for chemical analysis (Table 3). The methods used for analysis are described in the publication WardGuide-Master-20211118.pdf available at (wardlab.com).

 doi:10.20944/preprints202502.1276.v1

Table 3. Chemical properties for biochar used in Experiment 2.

Biochar Type	Surface Area	pH	N	P	K	S	Ca	Mg	Zn	Fe	Mn	Na	C
	$m^2\ g^{-1}$							$g\ kg^{-1}$					
Softwood	376	7.9	1.28	0.1	1.2	0.3	1.78	0.6	0.04	3.9	0.1	0.01	821
Hardwood	18	8.2	2.39	0.2	3.3	0.7	5.14	0.8	0.04	3.3	0.3	0.00	787

Note: The biochar was sent to Ward Laboratory, NE for chemical analysis. The methods used can be found in the publicationWardGuide-Master-20211118.pdf available at (wardlab.com) .

The Brassica genotypes consisted of 10 canola genotypes (all hybrids), seven of which were selected from Experiment 1 based on their performance (Table 2) and three new entries DKTF96SC, DKLL82CC, DKTFLL21SC (DEKALB Canola) added to replace those that performed poorly (Table 2). Seeds of three open pollinated *Brassicas spp* [African cabbage (*B. carinata*), Florida broadleaf mustard, and Brown mustard (*B. juncea*)] were obtained from Green Cover Seeds® Nebraska (varietal names were not stated) and included in Experiment 2.

About 300 g soil or soil + amendment (5% w/w) were placed into 500-ml pots, watered thoroughly, and seeded with eight seeds of each genotype at a depth of 0.5 cm. Each species by salinity level by biochar treatment (Experiment 2) was replicated three times and the entire experiment was replicated in time (January to February 2021, first replication & March to April 2021, second replication). Similar results were observed for both replications and combined for analysis.

2.4. Measurements

In Experiment 1, seedlings emerged were counted in each pot every 7 days up to 28 days after planting (DAP) when the experiment was terminated. In Experiment 2, the seedlings emerged were counted and removed about every 7 days, except for one plant from the first observation that was selected to grow through 60 DAP. The 7-day interval was chosen because seedling emergence is delayed in saline-sodic soils [few plants were present at the first observation point in the soil with the highest salinity (Table 5). The percentage of emerged seedlings for each treatment in each experiment was calculated. In Experiment 2, the one remaining plant in each pot was cut at soil surface at 60 DAP and weighed to determine fresh weight. The plant was then dried at 60°C to constant weight to quantify dry weight per plant.

Table 5. Seedling emergence (%) from 7 days after planting (DAP) to 28-DAP in productive 1, moderately saline-sodic 1 and highly saline-sodic 1 soils of Experiment 1 conducted in the greenhouse at South Dakota State University, SD in 2020.

Soil type	**Productive Soil 1**				**Moderately saline-sodic soil 1**				**Highly saline-sodic soil 1**			
DAP	7-DAP	14-DAP	21-DAP	28-DAP	7-DAP	14-DAP	21-DAP	28-DAP	7-DAP	14-DAP	21-DAP	28-DAP
Genotype												
NCC101S	25.0 c	50.0 c	50.0 d	62.5 e	0.0 c	25.0 a	25.0 a	37.5 a	4.2	20.8	25.0 a	37.5 a
DKTF91SC	37.5 bc	87.5 a	95.8 a	100.0 a	4.2 bc	12.5 b	16.7 bc	25.0 b	4.2	12.5	12.5 bc	12.5 b
DKTF92SC	50.0 b	50.0 c	50.0 d	62.5 e	12.5 a	12.5 b	25.0 a	25.0 b	0.0	4.2	12.5 bc	12.5 b
CS2500	45.8 b	62.5 b	62.5 c	79.2 bc	8.3 ab	8.3 b	12.5 c	12.5 c	4.2	8.3	8.3 bcd	16.7 b
L140P	45.8 b	66.7 b	70.8 b	87.5 b	0.0 c	12.5 b	25.0 a	37.5 a	0.0	12.5	16.7 ab	16.7 b
L233P	45.8 b	62.5 b	75.0 b	83.3 bc	4.2 bc	8.3 b	12.5 c	12.5 c	0.0	0.0	0.0 d	0.0 d
DKL7114BL	33.3 bc	50.0 c	62.5 c	75.0 cd	0.0 c	12.5 b	20.8 ab	25.0 b	0.0	4.2	12.5 bc	12.5 b
CS2100	45.8 b	50.0 c	50.0 d	75.0 cd	0.0 c	12.5 b	12.5 c	25.0 b	0.0	4.2	12.5 bc	12.5 b
CS2300	66.7 a	66.7 b	62.5 c	66.7 de	0.0 c	4.2 b	12.5 c	25.0 b	0.0	12.5	12.5 bc	12.5 b
CS2600	50.0 b	66.7 b	75.0 b	87.5 b	0.0 c	4.2 b	12.5 c	25.0 b	0.0	4.2	4.2 cd	4.2 c
Mean	*44.6*	*61.3*	*65.4*	*77.9*	*2.9*	*11.2*	*17.5*	*25.0*	*1.2*	*8.3*	*11.7*	*13.8*
P-value	*0.003*	*<0.001*	*<0.001*	*<0.001*	*0.004*	*0.002*	*<0.001*	*<0.001*	*0.639*	*0.124*	*<0.001*	*<0.001*
CV	*20*	*5.7*	*4.6*	*6.7*	*125*	*41.1*	*18*	*1.8*	*316*	*94*	*43.2*	*25*

 doi:10.20944/preprints202502.1276.v1

Abbreviations: DAP - Days After Planting. Mean values within columns followed by the same lowercase letters are not significantly different at $P < 0.05$. Where no letters are included next to the mean value, no significant difference were observed.

2.5. Statistical Analysis

Data analysis was conducted using the analysis of variance (ANOVA) following linear mixed model for a randomized block design in R version 3.4.3 using the package "doe bioresearch" [37]. In Experiment 1, soil type and canola genotypes were fixed, and replication was a random factor. In Experiment 2, soil type, biochar, and plant genotypes were fixed effects, and replication was a random factor. Significant soil type by biochar interaction was observed for seedling emergence, hence data for each salinity level were analyzed separately. Fisher's Least Significant Difference (LSD) was used to compare the differences among treatments within salinity level at the 95% confidence level.

3.0. Results

3.1. Soil and Biochar Analysis

The chemical properties for the soils used in the two experiments differed by experiment due to the time and plant presence differences when the soils were collected (Table 1). Highly saline-sodic soil 1 had the highest $EC_{1:1}$ (8.47 dS m^{-1}) followed by moderately saline-sodic 1 (5.17 dS m^{-1}) and productive soil 1 (0.62 dS m^{-1}). In addition to the high EC, Na content ranged from 54 mg kg^{-1} in the productive soil 1 to 4286 (highly saline-sodic 1) mg kg^{-1}, with the moderately saline-sodic soil 1 having 2346 mg kg^{-1}. The moderate and highly saline-sodic 1 soils also had higher sulfate and magnesium contents compared to productive soil 1 (Table 1). The EC values, and sodium and sulfate contents of the highly and moderately saline-sodic soils 1 areas are sufficient to seriously hamper growth of most NGP annual crops [5], as well as other typical plant species (e.g., *Asclepias speciosa, Gaillardia aristata, Pascopyrum smithii*) [15] for the area. Indeed, these areas were barren or near barren when the soils were collected.

Saline-sodic soil 2 had an $EC_{1:1}$ of 5.16 dSm^{-1}, like the moderately saline-sodic soil 1. The $EC_{1:1}$ of productive soil 2 was 1.85 dS m^{-1}, which was higher than productive soil 1 and could have been due to salt movement up the hill. Sodium and sulfate also were higher in productive soil 2 than in productive soil 1. Saline-sodic soil 2 had more Na and sulfate than productive soil 2 (Table 1).

Both biochars were slightly alkaline, and had very low concentrations of macro and micronutrients, except for iron (Table 3). The greatest difference between the biochars was that the softwood biochar had over 20 times greater surface area than the hardwood biochar (Table 3). The greater surface area of the softwood biochar would be expected to have more active sites and enhance cation exchange, and water holding capacity [38]

3.1.1. Microbial Community Structure

Total microbial biomass differed by soil type, with productive soil 1 and 2 averaging 2.4 μg g^{-1} soil, whereas moderately saline-sodic soil 1 had 42% less biomass, about 1.4 μg g^{-1} and the highly saline-sodic soil 1 and saline-sodic soil 2 averaged about 1 μg g^{-1} (Table 4). While the total biomass differed, the PLFA analysis indicated that the relative amounts of functional groups were similar across soil types. Undifferentiated biomass made up 45 to 60% of the total microbial biomass for each soil (Table 4).

 doi:10.20944/preprints202502.1276.v1

Table 4. Phospholipid fatty acid (PFLA) biological characterization of the soils used in Experiments 1 and 2 conducted in the greenhouse at South Dakota State University in 2020 and 2021.

Microbial Functional Groups	Productive soil 1	Moderately saline-sodic soil 1	Highly saline-sodic soil 1
	Experiment 1		
	PFLA microbial biomass (µg/g soil)		
	.....	.....	.....
Total Bacteria	**0.810 (41) †**	**0.623 (42)**	**0.367 (40)**
Gram (+)	0.613 (31)	0.474 (32)	0.328 (36)
Actinomycetes	0.161 (8)	0.130 (9)	0.114 (12)
Gram (-)	0.196 (10)	0.149 (10)	0.039 (4)
Rhizobia	0	0	0
Total fungi	**0.51 (3)**	**0.042 (2.7)**	**0.006 (0.7)**
AMF	0	0.017 (1.2)	0
Saprophytes	0.051 (3)	0.024 (1.6)	0.06 (0.7)
Protozoa	0	0	0
Undifferentiated	**1.134 (57)**	**0.821 (55)**	**0.537 (59)**
Total	**2.454**	**1.486**	**0.910**
	Experiment 2		
	Productive soil 2	**Moderately saline-sodic soil 2**	**Saline-sodic soil 2**
	PFLA microbial biomass (µg/g soil)		
	.....	.....	.....
Total Bacteria	**1.245 (49)**	**-**	**0.462 (40)**
Gram (+)	0.914 (36)	-	0.401 (35)
Actinomycetes	0.321 (13)	-	0.116 (11)
Gram (-)	0.330 (13)	-	0.060 (5.3)
Rhizobia	0	-	0
Total fungi	**0.137 (5.4)**	**-**	**0.023 (2.1)**
AMF	0.066 (2.6)	-	0.015 (1.3)
Saprophytes	0.071 (2.8)	-	0.009 (0.8)
Protozoa	0	-	0
Undifferentiated	**1.135 (45)**	**-**	**0.664 (57)**
Total	**2.517**	**-**	**1.149**

†Numbers in parenthesis are the percentage of the total biomass the functional group represents.

Bacteria, which can be beneficial or deleterious to plant growth depending on species [39], dominated the differentiated biomass in all soils, accounting for 40 to 49% of the total biomass, whereas fungi comprised the remaining <1 to 5% of the total biomass. Of the bacterial biomass, gram-positive bacteria comprised about 75% of the total differentiated biomass. Actinomycetes, which are reported to accelerate soil organic breakdown and phosphate solubilization [40], comprised about 32% of the gram-positive total biomass. Gram-negative bacteria, some species of which have been shown to be free-living N-fixing [41], whereas others can be pathogenic [42], comprised 25% of the total bacteria biomass. Although not examined in this study, specific species increases and decreases of bacteria in these soils have been shown to be highly impacted through salinity/sodicity selection [11].

Saprophytes, which help decompose organic matter, made up 50 to 100% of the fungi functional group. Arbuscular mycorrhizal fungi (AMF), which aid in nutrient recycling and mediation of response to environmental stress [43] was not present in two soil types (productive 1; highly saline-sodic 1) but was present in all other soils, comprising about 1% of total microbial biomass.

 doi:10.20944/preprints202502.1276.v1

3.2. Experiment 1 Canola Genotype Screening

Total seedling emergence and time of emergence differed by genotype and soil type (Table 5). In general, total emergence after 28 days was lower and time to emergence was longer for moderately and highly saline-sodic soils compared to productive soil 1. In the productive soil about 45% of the seedlings had emerged by 7 DAP, whereas in the moderately and highly saline-sodic soils, average number of emerged seedlings were 2.9% and 1.2%, respectively. By 28 DAP, the average emergence was 78, 25, and 14%, respectively, for productive 1, moderately saline-sodic 1, and highly saline-sodic 1 soils.

Emergence differences were also noted by genotype. CS2300 in the productive soil had the highest emergence 7 DAP (67%) but no other seedlings from this genotype emerged by 28 DAP. This can be compared with the emergence of DKTF91SC, which had a low % germination (37%) 7 DAP, but 100% by 28 DAP. In the moderately saline-sodic soil 1, DKTF92SC and CS2500 had the highest emergence percentage (12.5 and 8.3%, respectively) 7 DAP, with 6 other genotypes having no emerged seedlings. By 28 DAP, all genotypes had some emerged seedlings, with the highest percentage (37.5%) for L140P and NCC101S. In the highly saline-sodic soil 1, seven of the genotypes had no emerged seedlings 7 DAP, and the three genotypes with seedlings present (CS2500, DKTF-91SC, NCC101S) had only 4.2% emerged. By 28 DAP, L233P, which had emerged in the moderately saline-sodic soil 1, had no seedlings present in the highly saline-sodic soil 1. NCC101S, which had the greatest emergence in the moderately saline-sodic soil 1, also had the greatest emergence (37.5%) in the highly saline-sodic soil 1 28 DAP.

3.3. Experiment 2 Brassica growth and biochar amendment

When analyzed by soil type, total seedling emergence was influenced by biochar treatment in the saline-sodic soil 2 ($p = 0.017$) but biochar did not influence emergence in the productive soil 2 (Table 6). Hardwood biochar addition in the saline-sodic soil 2 had greater emergence (42%) than no biochar (29%), whereas the softwood amendment was similar to both the no biochar and hardwood amendments (37%).

Table 6. Final seedling emergence, and shoot dry weight of plants at 60 days after planting for canola, carinata, and mustard genotypes in productive, and saline-sodic soils in Experiment 2 conducted in the greenhouse at South Dakota State University, SD in 2021.

	Productive soil 2		**Saline-sodic soil 2**	
Genotype	Seedling emergence	Shoot dry weight	Seedling emergence	Shoot dry weight
	%	**g/plant**	**%**	**g/plant**
NCC101S	70.4	12.4	48.2 ab	3.7
DKTF91SC	88.7	9.2	30.2 cd	2.6
DKTF92SC	66.4	8.9	42.2 c	4.3
CS2500	65.9	8.7	20.2 e	3.1
L140P	75.7	10.4	32.2 cd	5.0
L233P	71.4	8.7	48.2 ab	3.5
DKL7114BL	73.6	8.3	25.2 cd	2.5
DKTF96SC	77.5	7.6	34.2 cd	2.3
DKLL82SC	85.2	8.5	32.2 cd	2.4
DKTFLL21SC	67.1	6.0	38.2 c	1.9
African cabbage	55.6	12.4	29.2 d	2.3
Brown mustard	83.0	10.0	33.2 cd	5.5
Florida broadleaf mustard	75.9	8.3	52.2 a	5.0
		Biochar		
Control (no biochar)	75.0	8.5	29.2 b	3.1
Softwood Biochar	71.0	10.5	36.5 ab	4.1
Hardwood Biochar	74.5	8.3	41.6 a	3.2
		P-value		
Biochar	**0.696**	**0.733**	**0.017**	**0.501**
Genotype	**0.248**	**0.476**	**0.051**	**0.305**

 doi:10.20944/preprints202502.1276.v1

Biochar x genotype	**0.608**	**0.608**	**0.595**	**0.786**

Mean values within each column sharing same lower-case letters are not statistically significant at P<0.05. Where no letters are included, no significant differences were observed.

None of the genotypes in the saline-sodic soil 2 had as much emergence or biomass as those grown in productive soil 2 (Table 6). Seven genotypes from Experiment 1 were used in Experiment 2. Emergence percentages were similar, except the greater percentage of seeds emerged in the saline-sodic soil 2 for all genotypes except NCC101S and CS2500. L233P, which had no seedlings emerged in highly saline-sodic soil 1, had 48.2% emergence in the saline-sodic soil 2. This difference may be due to the lower sulfate and sodium content of saline-sodic soil 2. Florida broadleaf mustard in the saline-sodic soil 2 had high emergence (52%) at 60 DAP.

4. DISCUSSION

Although saline-sodic soils can have high soil water content in field situations [3], the high soil salt content in the seed-soil contact zone draws water from the seed, lowering available moisture for hydrolyzing seed endosperm contents [44, 45] resulting in seedling desiccation due to reverse osmosis [46]. Similarly, under greenhouse conditions, high salt content in the soil lowers osmotic potential, reducing availability of water for seed germination and seedling emergence [44, 45] and as observed in the current study, seedling emergence was reduced. Even though we observed that watering the plants flushed some of the salts out of the pots, as observed by white crusting at the base of the pot, the amount of salt remaining in the soil was high enough to impact seedling emergence. These results in the current study are consistent with other studies using canola [44, 47, 48] where negative associations of seedling emergence and soil salt content were observed.

All Brassica species used in this study are amphidiploids and have been reported to be relatively more salt tolerant compared to diploid species [17, 49]. However, salt tolerance threshold levels within amphidiploids vary [49] with, for example, canola (*B. napus*) reported as more salt tolerant than Brown mustard (*B. juncea*). The genetic variation, therefore, explains part of the variation in salt tolerance among Brassica species used in the current study. This assertion is also consistent with Francois [47] who reported the threshold for growth in saline soil to be an EC_e of 10 dS/m^{-1} for most canola genotypes. In the current study on average, only 36.0% of the seeds emerged at an $EC_{1:1}$ of 5.16 $dS\ m^{-1}$. However, as $EC_{1:1}$ increased, the % emerged seedlings declined. It must be remembered that the EC_e value is determined on a saturated paste, whereas the $EC_{1:1}$ is quantified on a soil slurry. To convert the $EC_{1:1}$ to the EC_e, the $EC_{1:1}$ value needs to be multiplied by 1.8 to 2.1, depending on soil type [50, 51]. This means that if the soils used in this study had been analyzed by the saturated paste method, which is expensive, labor intensive, and not done in commercial labs, the EC_e of our saline-sodic soil would be about 12 $dS\ m^{-1}$ [50] and pH 6.9, which is then comparable to the EC_e threshold of 10 $dS\ m^{-1}$, reported by Francois [47].

When we separated the entries by species, *B. napus* emergence averaged 35.1% (ranging from 20.2% to 48.2 % for 10 entries). Emergence for the two *B. juncea* species averaged 42.2% ranging from 33.2 % (Brown mustard) and 52.2% (Florida broadleaf mustard). The interaction of canola genotype/*Brassica spp*. with soil salinity indicates that although emergence was not 100% under saline-sodic conditions, carefully chosen genotypes/species may be useful to plant, to provide stability from roots and vegetative shoots to the saline-sodic areas. For example, Green Cover (https://store.greencover.com/) categorizes African cabbage and Florida broadleaf mustard tolerance to saline soils as very good and fair, respectively, and recommend them for cover cropping in areas impacted by salinity.

Biochar application enhanced seedling emergence in the current study. This is likely due to biochar sorbing Na from the saline soils [26, 27] or due to its capacity to increase soil water (with lower ionic content) to reduce the salinity stress [52, 53]. We did not analyze the biochar-treated soil in this specific study to determine changes in soil properties. However, a related experiment conducted under similar conditions showed that biochar application reduced Na by 33.9% (from

 doi:10.20944/preprints202502.1276.v1

1151 to 761 mg kg^{-1}), Ca by 12% (from 1502 to 1322 mg kg^{-1}), sulfate-S by 48.7% (from 933 to 478 mg kg^{-1}), and $EC_{1:1}$ by 48.6% (from 3.5 to 1.8) (Bhattarai et al., unpublished data) supporting the above notion. Besides, our findings are supported by prior studies with biochar that showed a reduction in the soil EC, and improved soil aggregation and oxygen exchange [34, 54].

Although softwood biochar had over 20-fold the surface area of the hardwood biochar (able 3), the hardwood biochar had the greatest impact on emergence in the saline-sodic soil. The small surface area of the hardwood biochar likely improved soil porosity, aeration and improved water and nutrient availability, also reducing osmotic stress, resulting in better conditions for seedling emergence [55]. The impacts of biochar on the chemical and physical properties of soil are observed in both the characteristics of the biochar itself [26] and the properties of the soil [56, 57]. In a comparative study conducted by Singh, et al. [58], hardwood and softwood biochars were evaluated for their effects on soil characteristics, as well as on plant growth and maize yield at three different application rates. The findings indicated that hardwood biochar resulted in a reduction in soil bulk density, an enhancement in soil porosity, and an increase in vegetative biomass when compared to the control group that received no biochar.

Microbial biomass and diversity are important in maintaining soil health and ecosystems sustainability [12, 59]. The relative proportion of soil bacterial and fungal biomass is an indicator of how long it will take to rebuild the soil structure [59, 60], because fungi release exudates important in the formation and strengthening of soil aggregates [59, 61]. The low microbial biomass in the saline-sodic soil types used in this study indicate that carbon and nutrients will have lower turnover rates, and these soils would provide no buffering to plants for abiotic and biotic stresses [24]. This highlights the importance of the current study and others aimed at identifying plant species for remediating these salt-impacted soils as presence of plants increase the fungi to bacteria ratio (from 0.063 to 0.094) [3] suggesting that vegetative remediation will improve soil microbial diversity and ultimately rebuild the soil structure of these degraded soils.

5. CONCLUSION

Seedling emergence decreased with increasing $EC_{1:1}$ value. Seedling emergence delayed with soil salt composition. The impact of biochar application on seedling emergence varied depending on biochar type and Brassica genotype. The Brassica genotypes with greatest percent emergence were Florida broadleaf mustard and NCC101S. These two genotypes could be further investigated for their physiological and genotypic salt tolerance traits. Using biochar to support plant establishment in saline-sodic soil needs further investigation due to the mixed results.

Data Availability: All the data and supporting materials for this study are available within the paper and the referred materials for the methods and the studies that were conducted in the past on the study site at South Dakota State University

Conflict of Interest: No conflicts of interest. This research was conducted following the established research ethical standards, and no funders, industry or collaborative relationships that would affect the outcomes of this study.

Abbreviations list:

NGP: Northern Great Plains
dS m^{-1}: DeciSiemens per meter
DAP: Days after planting.
Ece: Soil electrical conductivity
SO_4 kg^{-1}: Sulfate per kilogram.
g kg^{-1} C: Grams of carbon per kilogram of the sample
SD: South Dakota
K: Potassium.
Na: Sodium
Ca: Calcium

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Mg: Magnesium.
SAR: Sodium accumulation ratio
PLFA: Phospholipid fatty acid analysis.

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