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# Evaluating Canola Genotypes for Growth and Yield under Different Environmental Conditions in South Dakota

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# EVALUATING CANOLA GENOTYPES FOR GROWTH AND YIELD UNDER DIFFERENT ENVIRONMENTAL CONDITIONS IN SOUTH DAKOTA

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I would like to dedicate this work to my parents, Mbabazi Novence and Kahonda Elidard, and my sister Kyobutungi Superia. This work would have not been possible without your love, care, and blessings. I am lucky to have you all in my life.

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TABLE OF CONTENTS

# LIST OF ACRONYMS

LIST OF FIGURES

# LIST OF TABLES

# APPENDICES

# ABSTRACT

## EVALUATING CANOLA GENOTYPES FOR GROWTH AND YIELD UNDER DIFFERENT ENVIRONMENTAL CONDITIONS IN SOUTH DAKOTA

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Canola *(Brassica napus L*) and carinata (*Brassica carinata A. Braun*) are potential oilseed crops for diversifying cropping systems and expanding into marginal lands impacted by saline and sodic soils in South Dakota (SD). However, genotypes that are high yielding, with high agronomic adaptability and stability over diverse environments, and salt tolerant have not been selected. One field experiment was conducted at two environments (Brookings - eastern SD and Pierre - western SD) from 2019 to 2020 to evaluate genotypes for growth and yield stability. Three greenhouse experiments were conducted to evaluate genotypes for salt tolerance in soils varying in electrical conductivity (EC), and with or without amendments (biochar and composted manure). The field experiment in 2019 evaluated ten canola and three carinata genotypes, whereas in 2020, twelve canola genotypes were evaluated. The experimental design was RCBD with treatments replicated four times. The earliest genotype to flower was NCC101S reaching 50% flowering at 41 and 36 days after planting (DAP) in 2019 and 2020, respectively. All carinata genotypes were later flowering reaching 50% flowering at >48 DAP. Seed yield for all genotypes averaged 1809 and 1740 kg $ha^{-1}$ at Brookings and (1384 and 858 kg $ha^{-1}$) at Pierre in 2019 and 2020, respectively. Similarly, seed oil concentration was greater at Brookings (410 g $kg^{-1}$) than at Pierre (356 g $kg^{-1}$) at Pierre in 2019. Environment was the most dominant cause of variation among genotypes, explaining 73.3%, 67.7%, 45.2% and 45.7%, of the variations in biomass yield, pods $plant^{-1}$, 1000-seed

weight, and seed yield, respectively, while genotype by environment interactions (GEi) explained most of the remaining variation. Data indicated that four genotypes, CS2300, DKTF92SC, CS2500, and NCC101S were stable with good yield in the four environments.

For greenhouse experiments, the first experiment evaluated ten canola and three carinata genotypes in three soils with EC ranging from non-saline (0.62 mmho/cm), moderately saline (5.17 mmho/cm) and highly saline (8.47 mmho/cm). The experimental design was an RCBD with treatments replicated three times. In the second experiment, two types of biochar (softwood and hardwood were introduced in each soil type at a rate of 5% by volume with an unamended control (no biochar) for each soil and 10 canola and three mustard genotypes were planted at a rate of eight seeds $pot^{-1}$. The four most promising genotypes identified in experiment two (African cabbage, Brown mustard, DKTF91SC and NCC101S) were evaluated in the third experiment. In this experiment composted manure was added to each soil-biochar combination (as in second experiment) at rated of 0, 30 and 50% by volume. Treatments were arranged in a split-plot design with soil salinity level as the main plot and biochar, composted manure rate and genotype arranged in factorial design within soil salinity level. Seedling emergence had a negative relationship with soil salt content with an average of 65.1%, 17.7% and 11.2% of emerged seedlings in non-saline, moderately saline and highly saline soils in experiment one. The genotypes with the greatest seedling emergence in moderately saline soils were L140P and NCC101S (29.2%), whereas NCC101S in the highly saline soil was the best at 29.2%. Averaged over biochar and genotypes, application of composted manure improved seedling emergence, number of leaves $plant^{-1}$ and leaf chlorophyll content (SPAD values) as compared to treatments with no composted manure, irrespective of soil salt content (salinity level). However, application of composted manure

interacted with biochar type to influence number of leaves plant$^{-1}$ and (SPAD values) in moderately saline and highly saline soil but not in non-saline soils. The interaction for number of leaves plant$^{-1}$ was due to better response in moderately saline and highly saline soils amended with softwood biochar compared to soil with no biochar or amended with hardwood biochar to increasing composted manure rate with the greatest number of leaves obtained at the highest rate of 50%. For SPAD values, the interaction between biochar and composted manure was due to high variability in SPAD values response to applied composted manure among biochar treatments. In terms of genotype, there was high variability with all genotypes showing improvement with composted manure application in moderately saline and highly saline soils with or without biochar amendment, but with the best observed in soils amended with softwood biochar. These findings suggest that canola has a potential to become an alternative spring broadleaf oilseed crop for diversifying cropping systems in SD. However, more research is required for this crop to determine the best management practices in saline-sodic soils.

# EVALUATING CANOLA GENOTYPES FOR GROWTH AND YIELD UNDER DIFFERENT ENVIRONMENTAL CONDITIONS IN SOUTH DAKOTA

## INTRODUCTION

Canola is the registered trademark of the Canola Council of Canada (CCC) for seed, oil, and meal derived from rapeseed cultivars low in erucic acid and low in glucosinolates (Mag, 1983). Canola and carinata are two oilseed crops from the rapeseed family that are thought to have originated from a cross where the maternal donor was closely related to diploid species of *B. oleracea* and *B. rapa and B. juncea* that are now cultivated in many parts of the world (Cardone et al., 2003; Licata et al., 2017; Paula et al., 2019; Seepaul et al. 2021b). Canola oil is primarily used for human edible products within a wide range of uses including cooking oil, margarine, salad dressing and shortening (U.S. Canola Association, (USCA)) (USCA, 2007; CCC, 2016b). Canola oils are in high demand due to their low saturated fatty acids, moderate levels of vitamin E, and K, and high omega-3 fatty acids. Besides the human edible market, canola oil meal is used in livestock feed and can be used to produce biodiesel fuel, bio lubricants and bioplastics (USCA, 2007; CCC, 2016b).

The European Union is the world's largest producer of canola, followed by China and Canada (Myers, 2018). The U.S. produces approximately one third of the canola oil used in the country, providing an opportunity to significantly expand production of the U.S. crop to meet domestic demand. The US Northern Great Plains (NGP) region is the leading producer of canola in the U.S with North Dakota producing over 85% of the US total volume (USCA, 2020).

The dominant cropping system in SD includes the corn-soybean rotation in the eastern part of the state, whereas winter wheat rotations dominate the state's western cropping region

and involves a single crop with about 14-month fallow period (Padbury et al., 2002; Tobin et al., 2020, O'Brien et al., 2020). However, many wheat farmers are interested in the alternative cropping system due to poor return on the investment considering the current cropping system. Canola and carinata hybrids are increasingly used as cover and rotation break crops in wheat, soybean to corn-based cropping systems (Mohammadi and Rokhzadi, 2012; Arcand et., 2014; Foyjunnessa et., 2018; Doolette et al., 2019) and several studies have investigated canola and carinata in different regions of USA to increase crop adoption with the purpose of diversifying rotations, increasing domestic production and reducing import costs (Starner et al., 2002; Pan et al., 2016; George et at., 2017) but less is known about their agronomic performance in different environmental conditions in SD.

Saline and sodic soils are of great challenge to crop production in SD most especially in Beadle, Brown, and Spink Counties where an estimated 113,312 hectares are saline, leading to about $26.2 million in losses annually (USDA NRCS, 2019). Currently most acreages impacted by salinity/sodic soil conditions lies barren with little plant life, providing no income to producers, no habitat for wildlife or pollinators, and is a threat to neighboring upland areas through wind and water dispersal. Methods to remediate these soils are expensive (Birru et al., 2019) compared with use of salt tolerant crops (Flowers et al., 1977; Munns, 2002). However, salt tolerant crops for the NGP have not yet been identified.

Although canola is reported to be salt tolerant crop (Kumar, 1984; Wright et al., 1997; Zheng et al., 1998; Ashraf and McNeilly, 2004), its growth and yield is negatively associated with salinity (Francois, 1994). However, organic amendments such as biochar and composted manure are potential ameliorants in saline soil that can improve physical, chemical, and biological properties (Huang and Redmann, 1995; Feng et al., 2020; Obia et al., 2020; Song et

al., 2020), but these need to be evaluated since their performance varies with composition, application rates and soil conditions. More so, biochar performance varies based on biomass type and pyrolysis conditions.

Canola crops fit well in rotation with wheat and other small grains because it shares similar growing season like wheat, with spring canola like spring wheat, planted in early spring and harvested in the late summer. There are over 510,314 ha dedicated to wheat production in SD (USDA NASS, 2017) therefore, there is an excellent potential for canola production in rotation with wheat. Inserting a broadleaf crop into a wheat-based rotation has been shown to provide benefits to the subsequent wheat crop. This "rotational effect" has been well documented for legumes (Williams et al., 2014) as well as for canola (Smith et al., 2004). Thus, canola can provide important ecosystem services and can be non-competitive with existing crops if strategically integrated into existing rotations, and in the marginal lands. Canola is well-suited to NGP production due to its short growing period and could support wheat production in the western part of the state by intensifying and diversifying crop rotations, reducing pest incidence, improving nutrient cycling, providing food for pollinators, and maintaining soil quality. By restoring saline/sodic soils to a more stable environment, producers will be given the opportunity to increase ecosystem services and restore lost productivity. Carinata is also an oilseed crop from the rapeseed family that is said to be drought tolerant (Kumar, and Singh, 1998; Seepaul et al., 2019). Because of that it has received attention for its potential to become an alternative oilseed crop for production in the semi-arid regions of the NGP of USA (Enjalbert et al. 2013; Seepaul et al., 2021a). However, it's not used in food due to its long chain erucic acid and high concentrations of glucosinolates, but it is an alternative bio-jet fuel feedstock.

Previous research on canola under SD growing conditions is very limited and less is known about the adaptation and performance of canola genotypes to diverse environmental conditions typical of SD and performance in saline and sodic soils. Based on the knowledge gap mentioned above, the objectives of the current study were (i) to evaluate canola genotypes for growth and yield in different agroeco-zones with varying precipitation, temperature, and tillage systems in SD and (ii) evaluate different canola and carinata genotypes for tolerance to saline-sodic soils, and (iii) evaluate canola and mustard genotypes for emergence and growth in saline-sodic amended with biochar and composted manure under greenhouse conditions.

# CHAPTER 1

# EVALUATION OF CANOLA AND CARINATA GENOTYPES FOR GROWTH, YIELD AND GE INTERACTION.

## LITERATURE REVIEW

Canola is the second largest oilseed crop in the world after soybean (Myers, 2018). It is an amphidiploid member of Brassica family that resulted from the cross between *B. oleracea L*. and *B. rapa* (Raymer et al., 1990; Downey and Rimmer, 1993; Raymer, 2002; Chen et al., 2010) a cytogenetic relationship that was described as the "U triangle" (Nagaharu, 1935). The triangle depicts the monogenomic diploids such as *B. rapa (*A genome, n=10), and digenetic species *B. carinata* (BC genome, n=17), *B. juncea* (AB genome, n=18) and *B. napus* (AC genome, n=19) (Vaughan, 1977; Downey and Rimmer 1993; Gulden et al., 2008). The term "canola" is a registered trade name by the Western Canadian Oilseed Crushers Association (WCOCA) representing rapeseed cultivars/varieties that are low in erucic acid content (<2 percent), and less than 30 micromoles $gram^{-1}$ of glucosinolates in the seed (Eskin and McDonald, 1991). This standard is now used popularly in the world on all canola-quality rapeseed products. Canola-quality rapeseed is in demand due to its healthy oils rich in omega-3 fatty acids for use in cooking. After oil extraction, the remainder of the seed (canola meal) is processed into livestock feed. Canola meal has lower protein when compared to soybean meal (36-39% for canola vs 45-48 % for soybean), but canola meal can still be substituted for soybean meal in livestock, poultry, and fish feed. It has high levels of lysine and arginine amino acids and is rich in vitamins and essential minerals (Gauthier et al., 2019).

The European Union is the world's largest producer of canola, followed by China and Canada (Myers, 2018). The U.S. produces approximately one-third of the canola oil used in the country, providing an opportunity to significantly expand canola production to meet

domestic demand. The Northern Great Plains (NGP) is the biggest canola producing region in the US. In 2019, 809,000 hectares were planted to canola in the US, yielding on average 2,224 kg $ha^{-1}$ for a total production of 1.5 billion kilograms of canola (USDA NASS, 2019). North Dakota is the leading canola producer with 650,000 ha, with Oklahoma, Idaho, Minnesota, Montana, and Washington producing the rest (USDA NASS, 2019). In SD, canola is generally a new crop with only five canola farms that were reported by Census of Agriculture in 2017 (USDA NASS, 2017). Canola has been shown to fit well in rotation with wheat and other small grains (Bushong et al., 2012). It has similar growing season like wheat, with spring canola, like spring wheat, planted in early spring and harvested in the late summer. There are over 510,300 ha dedicated to wheat production in SD (USDA NASS, 2017) therefore, an excellent potential land for canola production in rotation with wheat.

Since canola is not widely grown in SD, there is a need for more information on its adaptation to different environmental conditions to facilitate its integration into existing cropping systems. South Dakota has agro-ecologically diverse farming regions characterized by high temperatures, lower precipitation, and conservation tillage practices in the western region, and humid conditions with higher precipitation and mostly conventional tillage systems in the eastern region. The adaptation and performance of canola genotypes and or its relatives to these environments are largely influenced by their agronomic traits. These traits can be categorized as growth and yield traits and the important ones include emergence and stand establishment, days to flowering (DFL), plant height (PH), lodging (LR), biomass yield (BY), days to maturity (DM), harvest index (HI), number of pods $plant^{-1}$ (pods $plant^{-1}$) number of seeds $pod^{-1}$, seed oil concentration (OC), and seed yield (SY) (CCC, 2017).

Canola lifecycle begins at seed germination and emergence, which is affected by seedbed preparation and tillage practices (CCC, 2017; Willenborg et al., 2004; Assefa et al., 2014), abiotic stresses such as soil and air temperature conditions and moisture including freezing and or drought (CCC, 2017; McCauley, 2016; Zheng et al., 1998; Harker et al., 2015; Hwang et al., 2014). After emergence, biotic stresses such as soil-borne pathogens and insects can affect seedling at early stages of growth (Willenborg et al., 2004; Kaur and Bishnoi, 2011; Sharma et al., 2015; Beres et al.,2016), although seed treatments were reported to increase stand establishment (Willenborg et al., 2004; Sharma et al., 2015). For example, cold soils can delay emergence up to 20 days, resulting into non-uniform stands, and reducing growth and yield by 34 to 84% which is variable depending on genotype (McCauley, 2016). Canola has epigeal emergence and this exposes it to higher abiotic stress at early seedling stages compared to other crops (Koenig et al., 2011). So proper timing of planting under favorable conditions is critical (McGregor, 1987; Koenig et al., 2011).

Hybrid vigor can influence germination and emergence, for example Assefa et al. (2014) found that hybrids with large seeds emerged more vigorously compared to open pollinated genotypes. In a study evaluating tillage and planting practices for spring canola performance, Young et al. (2012) reported higher yield under no-till, compared to conventional till. On the other hand, Showalter (2017) reported 11 to 29% increase in stand establishment under high residue reduced tillage compared to no residue in a water-limited environment and attributed this to the fact that no-till can conserve moisture in the seedbed in dry environments.

Plant stands influence plant height, lodging rates, nutrient partitioning in plant and seed yield. Canola is very plastic and can attain optimum yield from stands ranging from as low as 10 plant $m^{-2}$ to stands as high as 200 plants $m^{-2}$ (McGregor, 1987; O'Donovan, 1994; Yantai et

al., 2016). French et al. (2016) reviewed the performance of canola under different stands (10 to 180 plants $m^{-2}$) in 24 studies over four years under different moisture ranges and tillage systems, using hybrids and open pollinated cultivars. The economic optimum plant density ranged from 7 to 180 plants $m^{-2}$ and higher for high precipitation zone and lower for low precipitation zone, but the difference in yield ranged from 0.3 to 1 % within each zone. This was because canola can compensate for stand losses up to 69% by branching, and increasing pods $plant^{-1}$, and seeds $pod^{-1}$ (Hosseini, 2006; Assefa et al., 2014; Yantai et al., 2016). Higher plant populations increase intraspecific competition resulting in taller plants with thin stems, fewer branches, and less pods. This increases competition among plants which increases potential for water and nutrient stress that can inhibit growth and development (Ma et al., 2015).

Under normal conditions and optimum stands, canola can attain an average height ranging from 75 to 175 cm on average (CCC, 2017) and produce about six primary branches which are among the contributor traits to yield. These variations are influenced by seeding rates, established stands, moisture, and variety or genotype (Johnson and Hanson, 2003). Canola plants at low-density can have thicker stems that are more resistant to lodging (Wu and Ma, 2016; CCC, 2017) compared with high-density plants that are more prone to lodging (CCC, 2017; Dahiya et al., 2018). Reduction in plant height is a desirable trait in canola that can increase branching, pod area index and seed yield (Hua et al., 2014).

Lodging increases chances of disease infection that reduces the photosynthetic capacity of the stems and pods. This results into weaker plants, fewer flowers and pods and reduced seed yield. More so, lodging increases harvesting time and energy costs, and reduces yield (McGregor, 1987; Wu and Ma, 2016). The primary causes of plant root lodging are over-

crowding of plants, wet soil, drought, and excess nitrogen in soil which can reduce seed yield by 16 to 80% (Islam and Evans, 1994; CCC, 2017).

Canola has indeterminate flowering (Koenig et al., 2011; Seepaul et al., 2019), and therefore can continue to produce branches and putting on more flowers and pods if nutrients and space are not limiting, but it has a determinate growth (central stem is limited by the development of the floral reproductive structure, and therefore does not lengthen indefinitely). Moisture and heat stress above 25 $^{o}$C reduce pollen fertility, pod formation, and seed development which also reduces seed weight, seed, and oil yield in canola (Assefa et al., 2014; Harker et al., 2015). For example, high temperatures were found to increase flower abscission and result into fewer, and smaller formed pods plant$^{-1}$ (Nuttal et al., 1992; Angadi et al., 2000; Singh et al., 2014). Temperatures at or higher than 27 $^{O}$C during reproductive growth stages increase pod abortion, and maximum heat (35/18 $^{o}$C, maximum/minimum temperature) and moisture stress during pod development can cause 77% loss of seed yield, but if this happens during flowering, the yield loss can be 58% (Gan et al., 2004). Canola genotypes that flower early (before late June/early July) with efficient pod set may reduce seed yield and oil concentration penalty associated with high temperatures during flowering and seed filling.

Seed size and number of seeds pod$^{-1}$ have a strong association with pods plant$^{-1}$, pod length and pod size which are all influenced by the genotype and the environmental conditions (Ivanovska et al., 2007, Zhang and Flottman, 2016). In canola, the number of pods plant$^{-1}$ can be varying from 60 to 200 (CCC, 2016a) and is influenced by the availability of photosynthate during pod development. Lack of N supply at growth reproductive stage also will result in fewer smaller pods with fewer, lighter seeds, especially in the later secondary and top branches (Angadi et al., 2003). Substantial stress (abiotic) at pod expansion stage leads to shorter pods

and missing seeds (CCC, 2016a; CCC, 2017) which both negatively impacts yield. Canola seed size ranges from 2g 1000-seeds$^{-1}$ for small seed cultivars to 7.5g 1000-seeds$^{-1}$ for large seed size cultivars (Gusta et al., 2004; Hwang et al., 2014; Elliott et al., 2008; Harker et al., 2015; Brill et al., 2016). Canola hybrids have larger seed size compared to open pollinated genotypes (Harker et al., 2015). Seed size or 1000-seed weight can be used as a selection parameter among canola genotypes (Elliott et al., 2008) since this is a mutagenic trait.

Canola seed oil content is influenced both by genotype and environmental conditions with oil content for some genotypes reaching up to 60% (CCC, 2017). Drought stress terminates endosperm development, which directly influences seed size, and seed oil content, however, this varies from genotype to genotype. Availability of nutrients and moisture during seed development can influence seed oil content. Jackson (2000) reported a range of 370 to 510 g kg$^{-1}$ oil content in seeds with the variation attributed to differences in genotype, environmental conditions, and management factors. Comparable to this, Zhang and Flottmann (2016) found a close relationship between yield and biomass production among Australian canola hybrids in a high yield environment relative to a moisture stressed zone.

The final seed yield in canola is a product of key yield components (pods plant$^{-1}$, seeds pod, and biomass yield). However, this is influenced by growing conditions that affect biomass accumulation, days to flowering, days to maturity, pods and seed development and final seed and oil yield (Zhang and Flottmann, 2016). Canola yield is highly variable based on genotype and environmental growing conditions (Assefa et al., 2014). Over the years, canola yield has been on an increasing trend due to innovative research on high yielding genotypes. In 2016, for example average canola seed yield in United States was 1976 kg ha$^{-1}$, (USDA NASS, 2017) which increased to 2164 kg ha$^{-1}$ in 2019 (USCA, 2020). Seed yields vary from environment to

environment. Rahimi-Moghaddam et al. (2021) investigated irrigation regimes, temperature and water stress using the Agricultural Production Systems siMulator (APSIM) model with the known high yielding canola genotypes in Iran and found that canola can yield up to 3761 kg $ha^{-1}$ under a cooler temperate environment and 1885 kg $ha^{-1}$ under a water stressed hot climate with mid maturing genotype yielding higher than the rest.

Yield of canola cultivars can also be affected by pod shatter which is a negative yield trait that varies among genotypes and environments. Wang et al. (2007) evaluated *B. napus, B. juncea*, *S. alba* and *B. napus* cultivars from a cross between *B. rapa* and *B. napus*, pod shatter was least at 4% in *B. juncea* and as high as 61% in hybrids. Newer cultivars however have lower pod shatter rates (Raman et al., 2014) due to intensive breeding and selection for pod shatter resistance. Pod shattering trait in canola is genetically inherited (Raman et al., 2014; Braatz et al., 2018) and is less controlled by the environment (Kadkol et al., 1989).

Carinata is a non-food oilseed crop from rapeseed family that can be used as a feedstock for bio-jet fuel. Carinata is said to be drought tolerant (Kuma and Singh, 1998; Seepaul et al., 2019). Because of that it has received attention for its potential to become an alternative oilseed crop for production in the semi-arid regions of the Northern Great Plains of USA (Enjalbert et al. 2013; Seepaul et al., 2021a). However, carinata is not used in food products due to its long chain erucic acid and high concentrations of glucosinolates. Three carinata genotypes were included in the study for comparison with canola genotypes.

Since canola performance varies greatly depending on genotype, environmental conditions, and their interactions (Gunasekera et al., 2006), it is critical that genotype evaluations be conducted in areas intended for its production to enhance selection of best performing well adapted ones. The objective of the current study was to evaluate canola

genotypes for growth and yield in different agroeco-zones with varying precipitation, temperature, and tillage systems in SD. From the literature above, we hypothesize that canola growth and yield traits will differ by genotype which will vary among environments.

## MATERIALS AND METHODS

A two-year study was conducted at Aurora Research farm-Brookings (44.6º N, 90.3º W) and Dakota Lakes Research farm-Pierre (44.3° N, 100.3° W) in 2019 and 2020. The soil type at the Brookings site was Brandt series characterized by fine silty, super active, frigid calcic hapludolls (Malo, 2003). At the Pierre site, study was conducted on a Dorna silty loam soil (coarse-silty over clayey, superactive, mesic Fluventic Haplustolls). The previous crop was winter wheat in Brookings and corn in Pierre. Pre-planting soil analysis results for the two locations and years are given on Table 1.1.

The experimental design was a randomized complete block (RCBD) with treatments consisting of 10 canola and three carinata genotypes in 2019 and 12 canola genotypes in 2020 (Table 1.2). The individual plot size was 1.62 x 9.14 meters (14.86 $m^2$). In 2019, the planting dates were 3$^{rd}$ May at Pierre and 18$^{th}$ May at Brookings. In 2020, the planting dates were 28$^{th}$ April at Pierre and 8$^{th}$ May at the Brookings location. Planting was done using a seven-row Hege 500® (Wintersteiger-Austria). Each plot had seven rows, 22 cm apart. Seeding rate was based on seed size with a target population of 148 plants $m^{-2}$ which is approximately 600,000 plants $acre^{-1}$.

Both years, 112 kg $ha^{-1}$ N and 22 kg $ha^{-1}$ S in the form of urea (46% N) and ammonium sulfate (21% N and 24% S) mixture was applied in a split application as recommended to ensure continuous supply of N. The first application occurred at planting and the second

application occurred around the bolting stage (30-35 DAP). The fertilizer was broadcast manually using an automatic hand-held spreader to ensure even application.

Weeds were managed with pre-emergence application of Prowl $H_2O$ (Pendimethalin, BASF, Research Triangle, NC) herbicide at the rate of 2.8 L $ha^{-1}$ applied 5 cm deep approximately 15 days prior to planting in both years. After crop emergence, Poast (Sethoxydim, BASF, Research Triangle, NC) herbicide was applied at the rate of 2.1 L $ha^{-1}$ four weeks after planting to control grassy weeds. Broadleaf weeds were managed by hand pulling from within each plot as required.

Days to flowering (50% of flowers open within each plot) and days to maturity (50% of plant with pods turned yellow within each plot) were recorded for each plot at Brookings. At physiological maturity (when 90% of the pods $plot^{-1}$ have reached a brown color and 50% of the plants parts have started turning color), average plant height was determined by measuring height of five random plants within each plot from soil line to the top of the plant. Lodging notes were taken and rated on a scale of 0 to 9 (0= no lodging, 9= completely lodged) (Passioura, 1977; Johnson and Hanson, 2003; Jan et al., 2016; Mahmood et al., 2018). Pod shattering notes were taken based on percent of pods shattered at the time of harvest within each plot (Spence et al., 1996; CCC, 2017). Plant samples were cut from a 30 x 30 cm area on each plot to determine yield traits. A subsample of 10 plants from each sample was used to determine number of pods $plant^{-1}$ and seeds $pod^{-1}$. The sample was then dried for 7 days at 60 $^{o}C$ to moisture free weight and weighed to determine total biomass yield. The seeds were then threshed from the sample and weighed. Thousand seed weight was computed by counting one thousand clean dry seeds and taking their weight using a high precision scale (Sartorius Model TE3 13S-DS).

At physiological maturity, Roundup® (Glyphosate, Monsanto) was applied at the rate of 2.24 L $ha^{-1}$ to burn-down the plants. Plots were harvested using a Kincaid 8XP® crop research combine (Kincaid Equipment and Manufacturing-Haven, KS) with the assistance of the H2 High-Capacity Grain Gage® (Juniper Systems Inc.- Juniper, UT) and seed yield was obtained from clean seeds and recorded as kg $ha^{-1}$. Seeds were dried to stable moisture under open air drying for 7 days at 60 $^{o}$C.

Twelve samples of 50 g each from the harvested seeds from each genotype were dried again to moisture free weight, sent to SGS Mid-West Seed Services, Inc. in Brookings, SD for oil content analysis using a hexane solvent extraction method. The results of this analysis were used to calibrate the NMR instrument (minispec mq, Bruker-Billerica, MA) for oil content analysis and then the rest of the samples were analyzed using the NMR instrument. Oil yield was calculated by multiplying the total seed yield by the oil concentration (percent basis).

*Statistical analysis*

Growth and yield traits data from the four site-years were initially combined and analyzed together using analysis of variance (ANOVA) for RCBD in RStudio (version 4.1.0) using the package "agricolae" (De Mendiburu, 2017). Fisher's Least Significant Difference (LSD) was used to compare the differences among treatments at the 95% confidence level. This analysis showed significant location/environment by genotype interactions for most traits evaluated, hence each site-year data was analyzed separately. Pearson correlation analysis was conducted using a four site-year combined data to determine relationships and associations among traits and trait profiles of genotypes (Yan et al., 2000; Yan, 2001; Yan et al., 2007; Yan and Frégeau-Reid, 2018).

The additive main effect and multiplicative interaction analysis (AMMI) which is a principal component-based method (Purchase et al., 2000; De Mendiburu, 2017; Ajay et al., 2019) in R studio version 4.0.1 was used to validate the effect of genotype, environment, and their interactions on expression of a selected set of four yield traits across four site-years using eight genotypes that were present in all four site-years. If the interaction between genotype and the environment was significant, these were further evaluated in detail using the principal components procedure and constructing AMMI biplots based on principal components that explained the most variability of the traits (Purchase et al., 2000; De Mendiburu, 2017; Ajay et al., 2019). Genotypes located close to the horizontal line are characterized by stable yields and adaptability irrespective of the environmental conditions. Stability ranking of genotypes was further done based on criteria described by Lin et al. (1986) whereby the genotype is stable if its variance ($\sigma^2$) over a range of environments is small and considering the sum of stability index (Becker and Leon, 1988; Crossa, 1990; Kang, 1988, Kang et al., 1991; Purchase et al., 2000). The genotype having lower AMMI stability value (ASV), rank of AMMI stability value (rASV), and lower yield stability index (YSI) is stable across environments (Purchase et al., 2000; Mut et al., 2010).

## RESULTS AND DISCUSSION

### *Environmental conditions*

Rainfall and temperature data for the study location were accessed from Mesonet at South Dakota State University (SDSU), (Mesonet, 2020), and the 30-year average data from 1985 to 2015 was accessed from National Weather Services used for comparison (Tables 1.3 and 1.4). The experimental period (2019–2020) was characterized by varied weather conditions at different stages of spring canola genotypes growth and development. The year

2020 was warmer and drier than 2019 at both locations compared to the long-term average weather. In 2019, the growing season precipitation at Brookings was 1.7 mm more than the long-term average. In the month of June, the precipitation was 43.9 mm less than the long-term average whereas July was 51.4 mm higher than long-term average. Heavy rainfall in July (total 132 mm) soaked root zone reducing anchorage that increased plants susceptibility to lodging. In 2020 at Brookings, total seasonal precipitation was 168.6 mm lower than the long-term average. Again, June was 43.7 mm drier than the long-term average whereas the July precipitation was close to the long-tern average.

At Pierre in 2019 seasonal precipitation was 102.9 mm lower than the long-term average. During flowering period in June, precipitation was 80.1 mm lower than the long-term average whereas in July the precipitation was slightly greater (+4.7 mm) than the long-term average. The dry weather conditions constrained crop growth during the pod and seed development period. Pierre was even drier in 2020 with the whole growing season precipitation of 114.3 mm lower than the long-term average and with both June and July drier than the long-term average (Table 1.3).

Temperature varied between the two environments and among the four site-years of the study (Table 1.4). At Brookings in 2019, maximum temperatures during June/July, which is the reproductive growth and therefore heat-sensitive period for canola, were comparable to the long-term average, whereas in 2020, maximum temperatures were 2.8 and 0.3 $^{o}$C higher than the long-term average. In April, which is the planting period, maximum temperatures were 11.1 and 12.5 $^{o}$C higher than the long-term average in 2019 and 2020, respectively. At the Pierre location in 2019 both June and July were slightly cooler than the long-term average. In 2020, June was warmer than the long-term average whereas July was cooler (Table 1.4). In

general, the Brookings location was cooler, wetter, and much more comparable to the long-term average in 2019 compared to 2020, and cooler in both years than the Pierre location. The Pierre location had more consecutive days of extreme hot days (above 25 $^{o}$C) compared with the Brookings location (Table 1.5).

Lower than optimum precipitation in April and May of 2020 at both locations resulted in reduced early growth and vigor which affected overall agronomic trait expressions, while heat stress during June and July constrained flowering, pod setting, seed development and seed filling which reduced seed yield and oil concentration. These results were similar to those reported by Morrison et al. (2016) in heat stress treatments. Drought was more severe at Pierre due to elevated temperatures resulting in reduced plant growth, pollen fertility, pods plant$^{-1}$, seeds pod$^{-1}$, and consequently leading to lower seed yield and seed oil content for all genotypes compared to Brookings location.

*Plant height and lodging*.

Plant height data were collected in three of four site-years, these data were not collected at Brookings in 2019 due to excessive lodging. Genotypes differed in plant height (Table 1.6 to 1.8). At Pierre in 2019, the tallest genotype was CS2300 with this genotype taller than all other canola and carinata genotypes except DH140251 (Table 1.6). At Pierre in 2020, the same genotype CS2300 was significantly taller than all others (Table 1.7). The shortest genotype was NCC101S (96.1 cm and 85.7 cm tall) at Pierre in 2019 and Brookings in 2020, respectively. The average canola height was 111.3 cm in 2019 and 93.3 cm in 2020 likely due to higher precipitation and less heat stress in the 2019 growing season (Tables 1.3 and 1.4). Volkov et al. (2006) also reported a reduced growth in Arabidopsis due to heat and moisture stress. Elferjani and Soolanayakanahally (2018) also reported that canola plant height averaged

145.8 cm under normal conditions (control) but when cultivars were subjected to heat and moisture stress treatments, plant height reduced drastically. Canola genotypically is shorter when compared to carinata (CCC, 2016a) and average height can be in the rage of 75 to 175 cm, which is consistent with the findings on plant height in this study. Taller plants tend to partition a lot of nutrients to biomass production, increasing susceptibility to lodging due to increased gravitational point and thinner nonlignified stem tissues.

Data for stem lodging were collected at the Brookings site only. In both years lodging was significantly influenced by genotype (Tables 1.7 and 1.9). In 2019, the most lodged genotype was CS2600 (7.8) while the least lodged genotype was CS2500 (1.8). In 2020, the most lodged genotype was DKTFLL21SC (8.5) which was the greatest overall, while the least lodged genotype was CS2300 (0.3). Canola plants lodged more in 2019 compared to 2020 likely due to higher moisture in the rooting zone that reduced anchorage, and plants were taller in 2019 leading to lodging (Wu and Ma, 2016). In the current study, taller genotypes lodged more than shorter ones, which is consistent with Alberti et al. (2019) who found positive correlation of plant height and lodging under similar South Dakota growing conditions. Likewise, Pan et al. (2016) found that lodging severity was strongly associated with plant height among canola hybrids. Favorable growing conditions in 2019 at early plant development resulted in tall, vigorous plants. This was followed by a mid-season heavy storms during the post-flowering to pod filling period in late July to August, creating conducive environment for stem and root lodging. Related to this, Zhang and Flottmann (2016) reported higher rates of lodging among Australian canola hybrids following heavy precipitation compared to a drier year. The Brookings location in 2019 was wetter, and plants were heavier with more pods plant$^{-1}$ and higher biomass making them susceptible to lodging.

*Days to flowering and days to maturity*

Days to flowering data were collected at the Brookings location only. Genotypes differed significantly in number of days to flowering (Tables 1.7 and 1.9). The earliest genotype to flower was canola NCC101S reaching 50% flowering at 41 and 36 DAP, in 2019 and 2020, respectively. The latest genotype was carinata, DH140251 (51 DAP) in 2019 and canola genotype CS2300 at 44 DAP in 2020. The three carinata genotypes (A120, DH140251 and DH069485) flowered late compared to canola genotypes, reaching 50% of flowering at >48 DAP in 2019. This variation in flowering between canola and carinata was also reported by Getinet et al. (1996) who observed a 5 to 19 delay in days to flowering by double haploid Ethiopian mustard genotypes compared to canola genotypes. On average, all canola genotypes flowered 2 to 4 days earlier in 2020 compared to 2019 which was likely due to early drought and mid-season heat stress that initiated early transitioning from vegetative to reproductive growth. Similar findings have also been reported by Tesfamariam et al. (2010) who reported early flowering due to stress, although this response varied from genotype to genotype under different environments. Canola is very sensitive to heat and moisture stress, and it is more devastating if flowering for spring canola coincides with high temperatures, especially in late June to early July. This often results in flower abortion and poor pod set, which negatively impact seed yield and oil concentration.

The days to maturity data were collected at the Brookings location only and genotypes differed in days to maturity (Tables 1.7 and 1.9). In 2019, canola genotype NCC101S matured earlier than all other genotypes (Table 1.9) while the carinata genotype A120 was the latest genotype to mature. All three carinata genotypes matured later compared to most canola genotypes. In 2020, the earliest genotype was DKTF91SC reaching maturity at 79 DAP, but

this was like most other genotypes except four canola genotypes (CS2300, CS2600, DKLL82SC and L140P). Over the 2-year period, the number of days to maturity ranged from 79 to 98. This is a slightly narrower range than 82 to 122 days reported by Yantai et al. (2016) for canola hybrids grown at 16 different locations. The variations in number to days to maturity were attributed to large moisture gradient among multiple locations/environments. There were only two location/environments in the current study (Brookings 2019 and 2020). Likely, high moisture in 2019 increased nutrient availability to plants which consequently increased vegetative growth and extended days to maturity by at least 4 days among genotypes compared to the slightly drier year of 2020.

*Number of pods plant$^{-1}$, seeds pod$^{-1}$, and 1000-seed weight*

Canola genotypes differed in number of pods plant$^{-1}$ only at Brookings in 2020 (P = 0.014) (Table 1.7). The genotype with greatest number of pods plant$^{-1}$ was CS2300 (57 pods plant$^{-1}$) while the genotype with least number of pods plant$^{-1}$ was DKTF91SC (18 pods plant$^{-1}$). On average, plants had more pods in 2019 at Brookings (82 pods plant$^{-1}$), and at Pierre (67 pods plant$^{-1}$) than in 2020 at Brookings (35 pods plant$^{-1}$), and at Pierre (24 pods plant$^{-1}$). This was not surprising since more than average rainfall is reported to enhance plant growth, promote branching and increasing the number of pods plant$^{-1}$. Pods plant$^{-1}$ in the current study ranged from 18 to 106 among all genotypes. This is in the lower end of the average range of 60 to 200 pods plant$^{-1}$ reported by CCC (2017). This is likely due to a combination of factors. For example, in 2020 precipitation was lower than optimum and temperatures higher than average at both locations. In 2020, we observed even lower pods plant$^{-1}$, (24 and 35 pods plant$^{-1}$) at Brookings and Pierre, respectively. The effect of heat and moisture stress on Brassica crops have been reported in different studies to negatively impact their growth and yield

(Nuttal et al., 1992; Angadi et al., 2000; Singh et al., 2014). Higher temperatures greater than 25 $^{0}$C during flowering can prevent canola plants from forming pods, (Gan et al., 2004).

Data for number of seeds pod$^{-1}$ were collected at both locations in 2020 (Tables 1.7 and 1.8). The number of seeds pod$^{-1}$ varied among genotypes at Brookings (P = 0.050), and at Pierre (P = 0.008). At Brookings, the number of seeds pod$^{-1}$ ranged from 7 (DKTF91SC) to 16 (CS2300) with a mean of 12 seeds pod$^{-1}$ whereas at Pierre the number of seeds pod$^{-1}$ ranged from 7 seeds pod$^{-1}$ (DKLL82SC) to 16 seeds pod$^{-1}$ (CS2300) with an average of 11 seeds pod$^{-1}$. These results indicate that the number of seeds pod$^{-1}$ are more stable across environmental conditions. In canola the number of seeds pod$^{-1}$ can range from 15 to 40, depending on the genotype and environmental conditions (CCC, 2016a). Harsh environmental conditions such as drought during critical growth stages has been shown to reduce seed development in canola (Gan et al., 2004; Mirzaei et al., 2013), especially under higher plant population (Angadi et al., 2000) due to elevated competition for moisture. In the current study, average established plant stands were greater at Brookings (168 plant m$^{-2}$) compared to 135 plant m$^{-2}$ at Pierre during the same growing season (data not shown) and greater than optimum plant population for canola production of 50 to 80 plants m$^{-2}$ (CCC, 2017). These greater plant populations and harsher environmental conditions likely explain the lower-than-average number of seeds pod$^{-1}$ observed in the current study. Furthermore, heat and drought stresses increase flower abortion, reduce the number of formed pods and seeds (Gan et al., 2004, Assefa et al., 2014) which contribute negatively to yield.

Canola seed size varied among genotypes at Brookings in 2019 (P<0.000) and 2020 (P = 0.045), and at Pierre in 2019 (P = 0.008) (Tables 1.6, 1.7, and 1.9). In 2019 at Brookings, seed size ranged from 2.8 g 1000 seeds$^{-1}$ (L140P) to 5.3 g 1000 seeds$^{-1}$ (DH140251) with a

mean of 3.5 g 1000 seeds$^{-1}$. At Pierre in 2019, seed size ranged from 2.1 g 1000 seeds$^{-1}$ (DKTF92SC) to 5.2 g 1000 seeds$^{-1}$ (DH140251) with a mean of 3.4 g 1000 seeds$^{-1}$. Seed size was smaller in 2020 ranging from 1.9 g 1000 seeds$^{-1}$ (L233P) to 3.3 g 1000 seeds$^{-1}$ (CS2500) with a mean of 2.7 g 1000 seeds$^{-1}$. Carinata genotypes had larger seeds than canola genotypes (Tables 1.6 and 1.9). The 1000-seed weight is an important genotype-specific trait (Thompson et al., 1977), so this can be used as a selection parameter among canola genotypes (Elliott et al., 2008). This trait is key in determining seeding rates, and has influence on seedling emergence rates, vigor, and growth. Canola seed development and expansion is influenced by the genotype (McGregor, 1987) as well as environmental conditions (Assefa et al., 2014; Harker et al., 2015). The partitioning and accumulation of growth assimilates into the seed endosperm determines seed size, and this can vary depending on availability of nutrients and moisture during critical seed development and maturation stages. Under optimal conditions, canola seed size can be in the range of 2 to 7.5 g 1000 seeds$^{-1}$ (Elliott et al., 2008; Hwang et al., 2014). If we consider canola genotypes only, canola seeds in this study are in the lower range of average seed size likely due to stress conditions during seed development.

*Biomass yield, harvest index, and seed yield*

Genotypes differed in biomass yield at Brookings location in 2019 (P = 0.013), and in 2020 (P = 0.033) (Tables 1.7 and 1.9). In 2019, biomass yield ranged from 1673 g m$^{-2}$ (DKL7114BL) to 2499 g m$^{-2}$ (L233P) with an average of 1967 g m$^{-2}$. In 2020, the biomass yield was much lower ranging from 329 g m$^{-2}$ (DKT 96SC) to 1237 g m$^{-1}$ (CS2300) with an average of 793 g m$^{-2}$. On average, biomass yield was 2.5 times greater in 2019 (1967 g m$^{-2}$) than in 2020 (793 g m$^{-2}$). The earlier maturing genotypes produced less biomass in a drier year of 2020 compared to a cool, wetter year of 2019. Under excess nutrients and moisture later in

the growing season, canola can continue to accumulate significant amounts of biomass even after pod set is complete due to its indeterminate growth, while under terminal water stress, biomass production is terminated early in favor of pod and seed development (Zhang and Flottmann, 2016). Biomass production has a linear association with seed yield, although variations exist depending on plant density, moisture, and nutrient availability to plants during the growing season. Biomass accumulation is the main driver to yield of canola hybrids under favorable conditions (Laza et al., 2003; Zhang and Flottmann, 2016). This literature together with analysis herein strongly suggest that drought and heat stress (Tables 1.3 to 1.5) are responsible for variations in biomass yield among genotypes evaluated.

Seed yield varied significantly among genotypes at all site-years (Tables 1.6 to 1.9). In 2019 at Brookings, seed yield ranged from 1182 kg $ha^{-1}$ (DH069485) to 2349 kg $ha^{-1}$ (DKTF91SC) with a mean of 1809 kg $ha^{-1}$ which was the greatest yield among all site-years. At the Pierre location during the same growing season (2019), seed yield ranged from 1001 kg $ha^{-1}$ (A120) to 1687 kg $ha^{-1}$ (CS2100) (Tables 1.6 and 1.9). The three carinata genotypes were the lowest yielding at both locations in 2019. In 2020, canola seed yield at Brookings ranged from 1104 kg $ha^{-1}$ (DKTFLL21SC) to 2964 kg $ha^{-1}$ (L140P) with a mean of 1740 kg $ha^{-1}$. At Pierre in the same year, seed yield was much lower ranging from 504 kg $ha^{-1}$ (L233P) to 1375 kg $ha^{-1}$ (CS2600) with a mean of 858 kg $ha^{-1}$ (Tables 1.7 and 1.8). Poor yield at Pierre was due to early drought stress and damage by chinch bugs (*Blissus leucopterus*) during early growth, and heat stress during reproductive growth. The drought and heat stress, and insect damage adversely affected the earlier flowering and maturing genotypes more that the later genotypes. Moisture and heat stresses have been reported in some studies to negatively impact seed yield

and oil concentration in Brassica crops (Tayo and Morgan, 1975; Morrison and Stewart, 2002; CCC, 2017).

Seeds yield in Brassica crops is a function of pods plant$^{-1}$, seeds pod$^{-1}$, 1000-seed weight, and biomass yield which are key yield components (Angadi et al., 2003; Zhang and Flottmann, 2016). In the current study we found a significant positive correlation of seed yield with seeds pod$^{-1}$ and pods plant$^{-1}$. In addition, environmental conditions can affect the genotypes adaptability to growth and yield. Other studies reported higher yields under irrigation compared to non-irrigated controls under extremes of drought and heat stress (Taylor et al., 1991; Mohtashami et al., 2020), suggesting that supplemental irrigation at the Pierre location in 2020 would have reduced yield penalties associated with drought and heat stress.

*Oil concentration and oil yield*

Genotypes differed in seed oil concentration at Brookings and at Pierre in 2019 and at Brookings in 2020 (Tables 1.6, 1.7, and 1.9). At Brookings in 2019, oil concentrations ranged from 263 g kg$^{-1}$ (A120) to 516 g kg$^{-1}$ (CS2600) while in 2020, the range in oil concentration among genotypes was narrower from 305 g kg$^{-1}$ (DKTFLL21SC) to 469 g kg$^{-1}$ (L140P) (Table 1.9). At Pierre seed oil concentration was lower ranging from 216 g kg$^{-1}$ (NCC101S) to 437 g kg$^{-1}$ (CS2100) in 2019 (Table 1.6). Oil concentration in seed was greater in 2020 compared to 2019, and greater in canola genotypes than carinata genotypes. Seed oil concentrations in canola ranged of 370 g kg$^{-1}$ to 510 g kg$^{-1}$ under optimum field conditions and was 340 g kg$^{-1}$ under drought stress conditions with only 332 mm of precipitation (Jackson, 2000). Canola oil content is also influenced by the environmental conditions that affect seed endosperm development and maturation (Zhang and Flottmann, 2016). At Pierre in 2019 the total growing season precipitation was 102.9 mm lower than the long-term average while in the same year at

Brookings, weather was much more comparable to the long-term average, all explaining the lower oil concentration for most genotypes at Pierre (356 g $kg^{-1}$) when compared to the same genotypes at Brookings (414 g $kg^{-1}$) in 2019. Low oil concentration in semi-arid environments was attributed to accelerated growth and short growing season as reported by Getinet et al. (1996) which negatively impacted proper seed development, maturation and ultimately reduced seed oil accumulation.

Genotypes differed in oil yield at Brookings in 2019 and 2020, and at Pierre in 2019 (Tables 1.6, 1.7, and 1.9). Oil concentration and oil yield was not measured at Pierre in 2020 since drought stress impacted seed development resulting in extremely small and shriveled seeds. In 2019 at Brookings oil yield ranged from 327 kg $ha^{-1}$ to 1178 kg $ha^{-1}$ with a mean of 771 kg $ha^{-1}$. At Pierre in the same year, oil yield was much lower ranging from 150 kg $ha^{-1}$ to 542 kg $ha^{-1}$ and a mean of 361 kg $ha^{-1}$. At Brookings in 2020, oil yield ranged from 323 kg $ha^{-1}$ (DKTFLL21SC) to 1405 kg $ha^{-1}$ (L140P). Carinata genotypes had lower oil yield at both locations in 2019 in comparison with canola genotypes. Periods of high temperatures and low soil moisture during flowering and seed-filling periods in canola have been shown to reduce seed oil concentrations (Morrison and Stewart, 2002), which helps explain the low oil yield among all genotypes at Pierre compared to Brookings.

*Association between growth and yield traits*

Pearson correlation was used to determine the relationship between growth and yield traits. Number of days to flowering was significantly correlated with days to maturity ($r = 0.69$, $P<0.000$), biomass yield ($r = 0.61$, $P<0.000$), 1000-seed weight ($r = 0.28$, $P = 0.006$), and seed yield ($r = 0.22$, $P = 0.034$) (Table 1.10). Similarly, days to maturity had a strong positive association with biomass yield ($r = 0.80$, $P<0.000$), and 1000-seed weight ($r = 0.49$, $P<0.000$).

However, days to maturity had no relationship with seed yield (Table 1.10). This is contrary to findings by Zhang et al. (2010) who found a positive correlation of genotype's days to maturity and seed yield. In the NGP, early flowering for spring canola is a desirable trait and flowering for late maturing genotypes often coincides with higher temperatures causing flower abscission, poor pod setting and seed development which negatively impacts on seed yield. However, in the presence of late-season precipitation, later flowering cultivars resume growth and produce more biomass, lengthening days to maturity without increasing seed yield, which agrees with Zhang and Flottmann (2016) who found a lengthened growth and delayed maturity under high precipitation zone compared to a lower precipitation zone using the same genotypes.

The number of pods plant$^{-1}$ had a positive correlation with biomass yield ($r = 0.84$, $p<0.000$), days to maturity ($r = 0.77$, $P<0.000$), 1000-seed weight ($r = 0.34$, $P<0.000$), and seed yield ($r = 0.38$, $P<0.000$). This means that late maturing genotypes produced more biomass, which lengthened days to flowering and partitioned more assimilates to growth and hence more pods plant$^{-1}$. The number of seeds pod$^{-1}$ were negatively correlated with 1000-seed weight ($r = -0.29$, $P = 0.05$), and positively correlated seed yield ($r = 0.41$, $P<0.000$) but with no relationship with biomass yield, pods plant$^{-1}$, days to flower and days to maturity (Table 1.10). Lack of moisture and nutrient supply at reproductive growth stages can constrain pod and seed development, resulting in fewer pods and lighter seeds explaining the negative correlation between seeds pod$^{-1}$ and 1000-seed weight. This is a common problem among late flowering genotypes under late drought and in early flowering genotypes during early drought (Angadi et al., 2003). However, is important to note that higher precipitation late in the growing season combined with soil nutrient availability can result in excessive vegetative growth and increased

number of pods plant$^{-1}$ with lighter small seeds. This assertion is supported by Zhang et al., 2010 who found no association of seeds m$^{-2}$ with seed pod$^{-1}$ and 1000-seed weight among late maturing Australian canola hybrids.

Biomass yield had lower but significant correlations with seed yield ($r = 0.26$ $P<0.000$) and 1000-seed weight ($r = 0.34$, $P<0.000$) (Table 1.10). Increase in biomass accumulation results in elevated canopy photosynthesis, resulting in greater assimilate partitioning into seed development (Zhang and Flottmann, 2016) resulting in increased yield. However, under drought stress, plants partition less assimilates into pod and seed development hence the lower correlations observed in the present study. This agrees with the analysis of variance (Table 1.6 to 1.9) where most genotypes at Pierre location accumulated higher biomass without partitioning the assimilate into seed development.

*Genotype-by-Environment Interaction (GEi).*

The results of the AMMI analysis for four traits of eight canola genotypes that were present at all four-site years of this study are presented in Table 1.11. Environment effects were significant for all four traits. Environment explained 73.3% of the variation in biomass yield, 67.7% of the variation in pods per plant, 45.2 % of the variation in 1000-seed weight and 45.7% of the variation in seed yield. Genotypic effect was not significant for all four traits and explained the least variation ranging from a low of 1.7 % in biomass yield to a high of 5.6 % in the 1000-seed weight. On the other hand, GE interactions were significant for three (biomass yield, pods plant$^{-1}$, seed yield) of the four traits and explained the remainder of the observed variation ranging from 6.8% for 1000-seed weight to a high of 18.1% for seed yield. Acclimatizing a new cultivar in a new environment for optimum yield, demands that both must interact (GE interaction) to influence yield (Gunasekera et al., 2006) although this may

complicate selection for multiple environments (Shafii and Price, 1998). Unlike in the study by Nowosad et al. (2017) where the GE interaction was significant only for canola seed oil content, GE interactions were highly significant (P<0.000) for the three out of the four traits in the current study. The only exception was for the 1000-seed weight trait that was influenced only by environment. Although canola seed size is a mutagenic trait, (Hwang et al., 2014; Elliott et al., 2008; Harker et al., 2015; Brill et al., 2016) the lack of genotypic effect on 1000-seed weight was surprising as seed size was variable among canola genotypes.

Canola growers are mostly interested in seed yield and yet it is a function of many components with the most important being 1000-seed weight (seed size), pods plant$^{-1}$ and biomass accumulation (Zhang and Flottmann, 2016). The GE interaction for these traits and seed yield were further analyzed in detail using AMMI-1 biplot following the criteria described by De Mendiburu (2017); Ajay et al. (2019). The results showed that GE interactions had the greatest effect on biomass yield with values of three principal components (PC's), PC1, PC2 and PC3 significant (Table 1.11). Genotype x environment interaction also had a great impact on seed yield with two first principal components, PC1 and PC2 values significant. While first two PC1 and PC2 explained >80% of observed GE variation for all four traits, it was PC1 that accounted for most of the observed variation, ranging from 57.1 % for 1000-seed weigh to 83.2% in pods plant$^{-1}$ (Table 1.11).

We further analyzed the nature of the GE interaction focusing on PC1. The traits were plotted on the x-axis and the PC1 scores on the y-axis (Figure 1.1 a-d), (Ararsa et al., 2015; Załuski et al., 2020). On average, seed yield was greatest for genotypes L140P (6) and lowest for L233P (7) (Fig 1-1a). This agrees with the ranking of yield in Table 1.12. In terms of environmental adaptability (Fig 1.1 a-d), Brookings 2019 (11) and Pierre 2019 (21) were very

similar and had the greatest seed yield whereas Pierre 2020 (41) was the harshest environment for seed yield (Fig 1.1a) due to lower than long-term average precipitation and higher temperatures during the growing season (Table 1.2 to 1.4). Pierre 2019 (21) and Brookings 2020 (31) differed greatly in terms of genotypes adaptability. Pierre 2020 (41) was very discriminating, as indicated by longer distance from the origin. The genotypes CS2300 and CS2500 were adapted to this environment with greater stability as indicated by closeness to the yield tester axis (YTA), as compared to L140P (6) which was also adapted to the Pierre 2019 but was unstable. The Brookings 2020 (31) environment was less discriminating with four genotypes (NCC101S, DKTF92SC, CS2100 and L233P) uniformly and relatively stable under this environment. The two canola genotypes L140P (6) at Pierre 2019 (21) and DKTF91SC (4) at Brookings 2020 (31) produced the greatest seed yield under the two favorable environments which were cooler, and wetter compared to Pierre 2020 (41), therefore contributing greatly to the GE interactions (Figure 1.1 a). The Pierre 2020 environment (41) was the harshest in terms of growing conditions and very discriminating compared to other three other environments with no genotype adapted to this environment.

On average, biomass yield was greatest for genotypes L233P (7) and lowest for DKTF91SC (4) (Fig 1.1b). This agrees with the ranking of genotype's biomass yield in Table 1.12. In terms of environments, Brookings 2019 (11) and Brookings 2020 (31) were very similar and were the greatest yielding environments in terms of biomass yield whereas Pierre 2019 (21) and Pierre 2020 (41) were least favorable environment for biomass yield (Fig 1.1b). The two canola genotypes NCC101S (8), and L233P (7) produced the greatest biomass yield under the two favorable environments of Brookings 2019 and 2020 which were cooler, and wetter compared to Pierre 2019 (21) and 2020 (41) (Fig 1.1 b). However, the same two

genotypes (NCC101S and L233P) and CS2300 (2) were the most unstable genotypes contributing to the observed GE interaction as indicated by the fact that they are the farthest from the tester axis.

For pods plant$^{-1}$, the two Brookings environments (Brookings 2019 (11) and Brookings 2020 (31) were similar in terms of growing conditions, although 2019 (11) was more discriminating compared to Brookings 2020 (31) (Fig 1.1c). The three environments, Brookings 2019 (11), Pierre 2019 (21) and Pierre 2020 (41) were characterized by varied growing conditions as shown by their placement in relation the center of origin (Fig 1.1c). This contributed greatly to GE interactions for pods plant$^{-1}$ with Brookings 2019 (11) being the most favorable for pod production while the dry, hot growing environment of Pierre 2020 (41) being the least favorable for pod production. Pods plant$^{-1}$ was greatest for L233P (7) and lowest for L140P (6) (Fig 1.1c) agreeing with the ranking of pods plant$^{-1}$ in Table 1.12. This means that same genotype that accumulated the greatest biomass yield (Fig 1.1b) produced the greatest number of pods plant$^{-1}$. These results agree with earlier report suggesting a close relationship between biomass yield and pods plant$^{-1}$ (Zhang and Flottmann, 2016) and supports the high and significant correlation between biomass yield and pods plant$^{-1}$ observed in the current study (Table 1.10).

Unlike the other three traits (biomass yield, pods plant$^{-1}$, seed yield), seed size was greatly influenced by the environment but did not show GE interactions. The four environments were highly discriminating for seed size as indicated by extreme projections from the tester axis. This means that each environment was very distinct in growing conditions that impacted seed size. Canola seeds were larger at Brookings 2019 (11) and Brookings 2020 (31) both environments with favorable growing conditions in terms of precipitation and

temperature (Fig 1.1d). Seeds were smaller at Pierre location in both years. The genotype with the largest seed size was CS2500 (3) while DKTF92SC (5) had the smallest seeds. Environmental conditions such as moisture and temperature influences seed formation and development (Assefa et al., 2014) although this may be a function of genotype since variations in seed size exist between hybrid and conventional cultivars (Hwang et al., 2014; Harker et al., 2015).

*Genotypes stability indices*

In addition to the AMMI-1 biplot we analyzed the eight genotypes for stability using the AMMI stability procedures as described by Kang, (1988) and Purchase et al. (2000). With this method the genotype is stable if its variance ($\sigma^2$) (ASV) over a range of environments is small (Lin et al., 1986; Purchase et al., 2000). The AMMI stability value (ASV) represents the variance and the lower the (ASV), the more stable the genotype. Thus, according to ASV-values, the ranking of the genotypes for stability in the four environments from most stable to least stable are NCC101S, DKTF92SC, CS2100, L233P, CS2300, CS2500, DKTF91SC, and L140P (Table 1.12). However, based on seed yield, the ranking of genotypes in from highest to lowest yield are as follows: L140P, CS2300, CS2500, DKTF91SC, DKTF92SC, NCC101S, CS2100, and L233P (Table 1.12). The most stable genotypes with high seed yield were CS2300, DKTF92SC, CS2500, DKTF91SC and NCC101S agreeing with the results from the GE biplots analysis (Figure 1.1a). When the stability ranking for seed yield was compared to stability for the other three traits, the three genotypes (L233P, DKTF92SC and CS2100) stood out as being stable in seed yield as well as in the three other traits. However, when considering stable canola genotypes with high yield, the top genotypes are CS2300 (late maturing), CS2500, (Intermediate), DKTF92SC, and NCC101S (early maturing) (Table 1.12).

# CONCLUSIONS

Even though canola can be adapted to diverse environments, genotype performance varied from one environment to another. Greatest seed yield was observed at the Brookings environments which had higher precipitation and cooler temperatures compared to the Pierre environments (2019 and 2020). Environment was the most dominant cause of variation among genotypes, explaining 73.3%, 67.7%, 45.2% and 45.7%, of variations in biomass yield, pods plant$^{-1}$, 1000-seed weight, and seed yield, respectively whereas GE interactions explained most of the remaining variation. Data from the four site years indicated that four genotypes, CS2300, DKTF92SC, CS2500, and NCC101S were stable over the four environments and had good yields. The overall findings indicate that canola can be an alternative spring broadleaf oilseed crop for diversifying the cropping systems in SD.

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Table 1.1. Pre-planting soil chemical characterization at Aurora farm-Brookings and Dakota Lakes farm-Pierre experimental study sites during 2020 growing season

| Location | Depth | Rotation | pH [a] | EC [b] | OM [c] | $NO_3$ [d] | P [e] | K [f] | Na [g] | S [h] |
|---|---|---|---|---|---|---|---|---|---|---|
| | cm | | | $dS/m^{-1}$ | % | $kg\ ha^{-1}$ ..... | | $mg\ kg^{-1}$ ..... | | |
| Brookings | 0-15 | WH | 6.5 | 0.21 | 4.4 | 32.1 | 10 | 86 | 11 | 13.8 |
| Brookings | 15-61 | WH | 6.6 | 0.14 | 2.9 | 37.1 | 4 | 44 | 11 | 7.2 |
| Pierre | 0-15 | WH | 6.4 | 0.26 | 1.7 | 15.1 | 24 | 629 | 12 | 11.2 |
| Pierre | 15-61 | Corn | 7.4 | 0.29 | 1.6 | 23.0 | 12 | 417 | 16 | 6.7 |

Abbreviations: WH=Winter wheat

[a] Potential of hydrogen concentration

[b] Soil electrical conductivity

[c] Soil organic matter

[d] Soil nitrates ($NO_3$ -N)

[e] Soil phosphorous

[f] Soil potassium

[g] Soil sodium content

[h] Sulfur

Table 1.2. Genotypes evaluated in 2019 and 2020 field experiments at Brookings and Pierre locations

| Treatments in 2019 field experiment | | | | | Treatments in 2020 field experiment | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Entry** | Name | Crop | Source | Maturity | **Entry** | Name | Crop | Source | Maturity |
| **1** | NCC101S | Canola | Caldbeck Consult | Early | **1** | NCC101S | Canola | Caldbeck Consult | Early |
| **2** | CS2300 | Canola | Meridian Seeds | Med/Late | **2** | CS2100 | Canola | Meridian Seeds | Med/Late |
| **3** | CS2500 | Canola | Meridian Seeds | Med/Late | **3** | CS2300 | Canola | Meridian Seeds | Med/Late |
| **4** | CS2100 | Canola | Meridian Seeds | Med/Late | **4** | CS2500 | Canola | Meridian Seeds | Med/Late |
| **5** | L140P | Canola | Invigor | Medium | **5** | CS2600 | Canola | Meridian Seeds | Early/Med |
| **6** | L233P | Canola | Invigor | Early | **6** | DKTF91SC | Canola | DEKALB Canola | Early |
| **7** | DKL7114BL | Canola | DEKALB Canola | - | **7** | DKTF92SC | Canola | DEKALB Canola | Early |
| **8** | DH140251 | Carinata | Agrisoma | - | **8** | DKTFLL21SC | Canola | DEKALB Canola | - |
| **9** | DH069485 | Carinata | Agrisoma | - | **9** | DKTF96SC | Canola | DEKALB Canola | - |
| **10** | A120 | Carinata | Agrisoma | - | **10** | DKLL82SC | Canola | DEKALB Canola | - |
| **11** | DKTF91SC | Canola | DEKALB Canola | - | **11** | L233P | Canola | Invigor | Early |
| **12** | DKTF92SC | Canola | DEKALB Canola | Early | **12** | L140P | Canola | Invigor | Med/Late |
| **13** | CS2600 | Canola | Meridian Seeds | Early/Med | | | | | |

Table 1.3. Monthly precipitation data (mm) collected throughout the growing seasons for 2019 and 2020 at Brookings and Pierre, SD.

| Months | P. Date | Apr | May | Jun | Jul | Aug | Total |
|---|---|---|---|---|---|---|---|
| | .....mm..... | | | | | | |
| Brookings 2019 | 5/18/ 2019 | 62.8 | 105.7 | 67.1 | 132.4 | 62.3 | 430.3 |
| Brookings 2020 | 5/8/ 2020 | 15.3 | 61.8 | 67.3 | 83.6 | 35.4 | 263.4 |
| **30-Year average** | | **63.0** | **90.0** | **111.0** | **84.0** | **84.0** | **432.0** |
| Pierre 2019 | 5/3/2019 | 17.4 | 67.0 | 12.9 | 69.7 | 79.1 | 246.1 |
| Pierre 2020 | 4/28/2020 | 6.5 | 48.7 | 88.6 | 61.4 | 29.6 | 234.7 |
| **30-year average** | | **54.1** | **81.0** | **93.0** | **65.0** | **56.0** | **349.0** |

Source: (Mesonet, 2020; http://www.noaa.gov/). 30-year average is the average rainfall from 1985-2015.

Table 1.4. Maximum and average monthly temperatures (ºC) during 2019 and 2020 growing seasons at Brookings and Pierre SD.

| Site-Year | Apr | | May | | Jun | | Jul | | Aug | |
|---|---|---|---|---|---|---|---|---|---|---|
| Temp. (°C) | Max. [a] | Avg [b]. | Max. | Avg. | Max. | Avg. | Max. | Avg. | Max | Avg. |
| | ..... °C ..... | | | | | | | | | |
| Brookings 2019 | 11.1 | 6.2 | 16.5 | 11.4 | 25.2 | 19.5 | 26.9 | 21.9 | 24.2 | 19.3 |
| Brookings 2020 | 12.5 | 5.6 | 17.5 | 12.4 | 27.8 | 21.8 | 28.6 | 22.8 | 28.5 | 22.0 |
| **30-Year average** | **13.3** | **6.9** | **20.0** | **13.9** | **25.0** | **19.7** | **28.3** | **22.2** | **27.2** | **20.8** |
| Pierre 2019 | 13.7 | 7.4 | 17.4 | 11.7 | 27.2 | 20.2 | 29.6 | 23.4 | 26.4 | 21.0 |
| Pierre 2020 | 14.6 | 6.5 | 19.1 | 13.1 | 28.3 | 22.2 | 31.0 | 24.3 | 31.6 | 23.8 |
| **30-year average** | **16.1** | **9.2** | **22.2** | **15.3** | **27.8** | **21.1** | **32.2** | **25.0** | **31.1** | **29.9** |

Source: (Mesonet, 2020; http://www.noaa.gov/) 30-year average is the average rainfall from 1985-2015.

Abbreviations:

***a*** Maximum temperatures.

***b*** Average temperatures.

Table 1.5. Number of days above 25, 28, and 30 °C in June and July at Brookings and Pierre SD in 2019 and 2020 growing seasons.

| | | 2019 | | | | 2020 | | |
|---|---|---|---|---|---|---|---|---|
| Month | Location | Periods month$^{-1}$ | Days >25 | Days >28 | Days >30 | Days >25 | Days >28 | Days >30 |
| June | Brookings | 1 to 10 | 6 | 5 | 4 | 8 | 4 | 3 |
| June | Brookings | 11 to 20 | 5 | 2 | 1 | 8 | 6 | 5 |
| June | Brookings | 21 to 30 | 7 | 5 | 5 | 8 | 6 | 4 |
| July | Pierre | 1 to 10 | 10 | 4 | 2 | 10 | 8 | 5 |
| July | Pierre | 11 to 20 | 9 | 9 | 9 | 9 | 9 | 4 |
| July | Pierre | 21 to 30 | 9 | 5 | 4 | 10 | 10 | 8 |

Table 1.6. Growth, yield, and yield traits of canola and carinata genotypes at Pierre 2019.

| Genotype | PH [a] | PD [b] | SW [c] | BY [d] | SY [e] | OC [f] | SR [g] | OY [h] | Rank |
|---|---|---|---|---|---|---|---|---|---|
| | cm | Count | G | g $m^{-2}$ | kg $ha^{-1}$ | g $kg^{-1}$ | % | kg $ha^{-1}$ | |
| Canola Genotypes | | | | | | | | | |
| CS2100 | 107.8 de | 67 | 3.8 abc | 1946 | 1687 a | 437 a | 2.8 c | 476 ab | 1 |
| CS2300 | 123.9 a | 70 | 2.3 c | 1843 | 1409 abcd | 415 a | 11.3 ab | 403 abc | 6 |
| CS2500 | 113.5 bc | 61 | 3.7 abc | 1771 | 1477 abc | 432 a | 8.8 ab | 240 bc | 5 |
| DKL7114BL | 108.2 de | 67 | 3.0 bc | 2002 | 1485 abc | 383 ab | 2.5 c | 504 ab | 4 |
| DKTF91SC | 106.8 e | 82 | 2.9 bc | 1824 | 1531 abc | 426 a | 0.5 c | 531 a | 3 |
| DKTF92SC | 110.9 cde | 58 | 2.1 c | 1601 | 1653 ab | 380 ab | 2.8 c | 542 a | 2 |
| L140P | 112.1 bcd | 57 | 2.8 bc | 1827 | 1237 bcd | 387 ab | 2.0 c | 409 abc | 9 |
| L233P | 114.8 bc | 80 | 3.3 bc | 2115 | 1400 abcd | 396 a | 0.5 c | 353 abc | 7 |
| NCC101S | 96.1 f | 71 | 3.5 bc | 2427 | 1352 abcd | 216 c | 2.8 c | 287 abc | 8 |
| Carinata genotypes | | | | | | | | | |
| A120 | 114.3 bc | 60 | 4.1 ab | 1824 | 1001 d | 241 c | 1.3 c | 150 c | 12 |
| DH069485 | 111.0 cde | 70 | 4.4 ab | 2322 | 1207 bcd | 302 bc | 7.5 b | 255 bc | 10 |
| DH140251 | 116.3 b | 67 | 5.2 a | 2026 | 1172 cd | 258 c | 12.5 a | 190 c | 11 |
| Mean | 111.3 | 67 | 3.4 | 1960 | 1384 | 356 | 4.6 | 361 | |
| P-value | <0.000 | 0.266 | 0.008 | 0.065 | 0.034 | <0.000 | <0.000 | 0.013 | |
| [i] SEM | 1.6 | 6.9 | 0.5 | 166.4 | 135.0 | 28.0 | 1.3 | 83.0 | |
| [j] SEd | 2.3 | 9.8 | 0.7 | 235.4 | 190.5 | 39.2 | 1.8 | 117.3 | |
| [k] CV | 2.9 | 20.6 | 30.3 | 17.0 | 19.5 | 16.0 | 57.5 | 46.0 | |

Mean values followed by same lower-case letters within the column are not significantly different at $P \leq 0.05$.

Abbreviations: [a] Plant height, [b] Pods $plant^{-1}$, [c] Weight of 1000 seeds, [d] Biomass yield, [e] Seed yield, [f] Oil concentration, [g] Shatter rates, [h] Oil yield, [i] Standard error of mean, [j] Standard error of difference, [k] Coefficient of variations.

Table 1.7. Growth, yield, and yield traits of canola genotypes at Brookings in 2020.

| Genotype | PH [a] | LR[b] | DFL[c] | DM [d] | PD [e] | SD [f] | SW [g] | BY [h] | SY [i] | OC [j] | OY [k] | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cm | 1-9 | Days | Days | Count | count | g | g $m^{-2}$ | kg $ha^{-1}$ | g $kg^{-1}$ | kg $ha^{-1}$ | |
| CS2100 | 89.4 b | 5.8 ab | 39 cd | 80 cd | 26 c | 10.0 bc | 2.7 abc | 604 bc | 1661 bc | 343 bc | 557 bc | 8 |
| CS2300 | 117.0 a | 0.3 b | 45 a | 93 a | 57 a | 15.9 a | 3.1 ab | 1237 a | 1797 bc | 416 abc | 764 b | 3 |
| CS2500 | 97.3 b | 3.8 b | 42 abc | 83 bcd | 44 abc | 15.4 ab | 3.3 a | 1014 ab | 1793 bc | 449 ab | 804 b | 4 |
| CS2600 | 92.7 b | 3.5 b | 40 bc | 85 bcd | 54 ab | 13.8 ab | 3.0 ab | 1231 a | 1600 bc | 312 c | 510 bc | 9 |
| DKLL82SC | 88.0 b | 5.3 b | 41 bc | 85 bc | 33 abc | 12.9 ab | 3.0 ab | 588 bc | 1407 bc | 423 abc | 603 bc | 11 |
| DKTF91SC | 88.1 b | 6.3 ab | 40 bc | 79 d | 18 c | 7.1 c | 2.7 abc | 329 c | 1509 bc | 468 a | 705 bc | 10 |
| DKTF92SC | 89.1 b | 4.3 b | 40 bc | 84 bcd | 40 abc | 13.1 ab | 3.0 ab | 675 abc | 1697 bc | 436 ab | 737 b | 7 |
| DKTF 96SC | 96.4 b | 4.5 b | 44 ab | 84 bcd | 30 bc | 11.9 abc | 2.6 abc | 717 abc | 1930 b | 438 ab | 849 b | 2 |
| DKTFLL21SC | 93.7 b | 8.5 a | 40 bc | 81 cd | 25 c | 10.4 abc | 2.3 bc | 679 abc | 1104 c | 305 c | 323 c | 12 |
| L140P | 94.3 b | 2.5 b | 43 ab | 88 ab | 40 abc | 12.8 ab | 2.6 abc | 1044 ab | 2964 a | 469 a | 1405 a | 1 |
| L233P | 87.8 b | 3.5 b | 41 bc | 83 bcd | 38 abc | 12.9 ab | 1.9 c | 847 abc | 1701 bc | 452 ab | 766 b | 6 |
| NCC101S | 85.7 b | 3.3 b | 36 d | 79 cd | 20 c | 11.6 abc | 2.9 ab | 546 bc | 1711 bc | 368 abc | 626 bc | 5 |
| Mean | 93.3 | 4.3 | 41 | 84 | 35 | 12.3 | 2.7 | 793 | 1740 | 406 | 720 | |
| P-value | 0.001 | 0.021 | 0.002 | 0.001 | 0.014 | 0.050 | 0.045 | 0.033 | 0.002 | 0.019 | <0.000 | |
| [l] SEM | 4.1 | 1.3 | 1.2 | 2.0 | 7.7 | 1.7 | 0.3 | 188.3 | 232.4 | 37.2 | 118.0 | |
| [m] SEd | 5.8 | 1.8 | 1.6 | 2.7 | 11.0 | 2.3 | 0.4 | 266.3 | 329.0 | 53.0 | 167.0 | |
| [n] CV | 8.8 | 61.0 | 5.7 | 4.5 | 43.3 | 27.0 | 19.0 | 47.5 | 27.0 | 18.3 | 33.0 | |

Mean values followed by same lower-case letters for each treatment within the column are not significantly different at P≤0.05. Abbreviations: [a] Plant height, [b] Lodging rates, [c] Days to flowering, [d] Days to maturity, [e] Pods $plant^{-1}$, [f] Seeds $pod^{-1}$, [g] 1000-seed weight, [h] Biomass yield, [j] Seed yield, [l] Oil concentration, [k] Oil yield, [l] Standard error of mean, [m] Standard error of difference, [n] Coefficient of variation.

Table 1.8. Growth, yield, and yield traits of canola genotypes at Pierre in 2020.

| Genotype | PH [a] | PD [b] | SD [c] | SW [d] | BY [e] | SR [f] | SY [g] | Rank |
|---|---|---|---|---|---|---|---|---|
| | cm | Plant$^{-1}$ | Pod$^{-1}$ | g | g m$^{-2}$ | % | kg ha$^{-1}$ | |
| CS2100 | 94.2 | 22 | 11.1 abc | 1.7 | 660 | 3.0 c | 576 b | 12 |
| CS2300 | 100.0 | 22 | 16.1 a | 2.1 | 980 | 10.3 bc | 1355 a | 1 |
| CS2500 | 100.0 | 32 | 14.5 ab | 1.9 | 823 | 22.5 a | 1345 a | 2 |
| CS2600 | 100.4 | 33 | 11.3 abc | 2.0 | 1035 | 6.5 c | 1375 a | 3 |
| DKLL82SC | 98.0 | 30 | 7.4 c | 1.5 | 914 | 1.0 c | 512 b | 10 |
| DKTF91SC | 90.0 | 23 | 10.0 bc | 2.2 | 986 | 3.3 c | 749 b | 8 |
| DKTF92SC | 103.0 | 24 | 9.7 bc | 1.7 | 1038 | 1.8 c | 607 b | 9 |
| DKTF 96SC | 72.3 | 19 | 10.0 bc | 2.0 | 722 | 1.7 c | 863 ab | 7 |
| DKTFLL21SC | 93.4 | 23 | 9.2 bc | 1.8 | 776 | 15.0 ab | 666 b | 6 |
| L140P | 102.4 | 23 | 14.4 ab | 1.7 | 880 | 3.8 c | 1084 ab | 4 |
| L233P | 94.1 | 21 | 8.3 c | 2.3 | 771 | 4.8 c | 504 b | 11 |
| NCC101S | 93.8 | 20 | 9.3 bc | 2.0 | 695 | 1.8 c | 667 b | 5 |
| Mean | 95.1 | 24 | 10.9 | 1.9 | 857 | 6.2 | 858 | |
| P value | 0.520 | 0.524 | 0.008 | 0.782 | 0.559 | <0.000 | 0.003 | |
| [h] SEM | 4.2 | 5.0 | 1.6 | 0.2 | 135.4 | 2.7 | 185.4 | |
| [I] Sed | 6.0 | 7.0 | 2.2 | 0.3 | 191.5 | 4.0 | 262.1 | |
| [j] CV | 8.7 | 37.0 | 28.6 | 21.0 | 31.0 | 86.4 | 43.2 | |

Mean values followed by same lower-case letters within the column are not significantly different at P≤ 0.05.

Abbreviations: [a] Plant height, [b] Pod plant$^{-1}$, [c] Seeds pod$^{-1}$, [d] 1000-seed weight, [e] Biomass yield, [f] Pod Shatter, [g] Seed yield, [h] Standard error of mean, [i] Standard error of difference, [j] Coefficient of variation.

Table 1.9. Growth, yield, and yield traits of canola and carinata genotypes at Brookings in 2019.

| Genotypes | LR [a] | DFL [b] | DM [c] | PD [d] | SW [e] | BY [f] | SY [g] | OC [h] | SR [i] | OY [j] | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-9 | Days | Days | Plant$^{-1}$ | g | g m$^{-2}$ | kg ha$^{-1}$ | g kg$^{-1}$ | % | kg ha$^{-1}$ | |
| Canola genotypes | | | | | | | | | | | |
| CS2100 | 4.8 abcd | 45 bcde | 95 cd | 95 | 3.4 cde | 2227 abc | 1907 abc | 429 bc | 3.8 | 803 bc | 6 |
| CS2300 | 5.0 abcd | 46 bcd | 94 d | 68 | 3.4 cde | 1770 cd | 1778 bcd | 420 c | 6.3 | 761 c | 7 |
| CS2500 | 1.8 d | 47 bcd | 96 bc | 69 | 3.5 cde | 1834 bcd | 1679 cde | 475 abc | 5.0 | 800 bc | 10 |
| CS2600 | 7.8 a | 46 bcd | 94 de | 87 | 3.5 cde | 1723 cd | 2012 abc | 516 a | 3.0 | 1038 ab | 3 |
| DKL7114BL | 6.8 ab | 44 de | 95 d | 78 | 3.9 bcd | 1673 d | 1811 bc | 467 abc | 5.5 | 843 bc | 8 |
| DKTF91SC | 5.5 abc | 43 e | 93 f | 91 | 3.5 cde | 1843 bcd | 2349 a | 501 ab | 4.8 | 1178 a | 1 |
| DKTF92SC | 4.5 abcd | 45 cde | 94 ef | 74 | 3.0 de | 2029 abcd | 1956 abc | 477 abc | 3.5 | 922 bc | 5 |
| L140P | 3.5 bcd | 48 bc | 95 cd | 64 | 2.8 e | 1692 cd | 1927 abc | 469 abc | 3.8 | 903 bc | 9 |
| L233P | 4.5 abcd | 46 bcd | 97 ab | 106 | 3.2 cde | 2499 a | 2178 ab | 467 abc | 1.8 | 1017 ab | 2 |
| NCC101S | 3.3 cd | 41 f | 87 g | 92 | 4.0 bc | 2031 abcd | 2106 abc | 342 d | 4.0 | 726 c | 4 |
| Carinata genotypes | | | | | | | | | | | |
| A120 | 4.8 abcd | 48 bc | 98 a | 69 | 4.0 bc | 1811 bcd | 1338 def | 263 e | 3.8 | 350 d | 11 |
| DH069485 | 2.5 cd | 48 b | 97 ab | 84 | 4.7 ab | 2334 ab | 1182 f | 276 de | 0.7 | 327 d | 13 |
| DH140251 | 4.8 abcd | 51 a | 96 bc | 81 | 5.3 a | 2107 abcd | 1298 ef | 281 de | 0.5 | 365 d | 12 |
| Mean | 4.5 | 46 | 95 | 82 | 3.7 | 1967 | 1809 | 414 | 3.6 | 771 | |
| P-value | 0.021 | <0.000 | <0.000 | 0.175 | <0.000 | 0.013 | <0.000 | <0.000 | 0.252 | <0.000 | |
| [k] SEM | 1.0 | 0.6 | 0.5 | 10.3 | 0.3 | 163.4 | 149.0 | 22.3 | 1.5 | 74.3 | |
| [l] SEd | 1.5 | 1.2 | 0.6 | 14.5 | 0.4 | 231.1 | 210.3 | 31.5 | 2.1 | 105.1 | |
| [m] CV | 46.0 | 4.0 | 1.0 | 25.1 | 14.4 | 17.0 | 16.4 | 11.0 | 82.0 | 19.2 | |

Mean values followed by same lower-case letters within the column are not significantly different at P≤ 0.05.

Abbreviations: [a] Lodging rate, [b] Days to flowering, [c] Days to maturity, [d] Pods plant$^{-1}$, [e] 1000-seed weight, [f] Biomass yield, [g] Seed yield, [h] Oil concentration, [i] Shatter rates, [j] Oil yield, [k] Standard error of mean, [l] Standard Error of Difference, [m] Coefficient of variation.

Table 1.10. Pearson correlations between traits of canola genotypes evaluated at Brookings and Pierre SD in 2019 and 2020.

| Trait | Units | df | DFL [a] Days | DM [b] Days | PD [c] Plant$^{-1}$ | SD [d] Pod$^{-1}$ | BY [e] g m$^{-2}$ | TSW [f] g | YLD [g] kg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| DFL [a] | Days | 94 | 1 | 0.69*** | 0.55*** | 0.00 | 0.61*** | 0.28* | 0.22* |
| DM [b] | Days | 94 | | 1 | 0.77*** | 0.07 | 0.80*** | 0.49*** | 0.14 |
| PD [c] | Plant$^{-1}$ | 190 | | | 1 | 0.06 | 0.84*** | 0.34** | 0.38*** |
| SD [d] | Pod$^{-1}$ | 94 | | | | 1 | 0.17 | -0.29* | 0.41*** |
| BY [e] | g m$^{-2}$ | 190 | | | | | 1 | 0.34*** | 0.26*** |
| TSW [f] | g | 190 | | | | | | 1 | -0.04 |
| YLD [g] | kg ha$^{-1}$ | 190 | | | | | | | 1 |

*=Significant at P≤0.05, ** Significant at p≤0.1, *** Significant at p<0.00.

[a] Days to flowering

[b] Days to maturity

[c] Pods plant

[d] Seeds pod$^{-1}$

[e] Biomass yield

[f] 1000-seed-weight

[g] Seed yield

df=Degrees of freedom

Note: Data for days to flowering and days to maturity were collected at only Brookings location.

Table 1.11. The additive main effects and multiplicative interaction (AMMI) analysis of variance (G by G*E (ANOVA) for yield and yield traits of eight canola genotypes in 2019 and 2020 at Pierre and Brookings SD.

| | Biomass yield | | | | Pods plant$^{-1}$ | | | 1000-seed weight. | | | Seed yield | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g m$^{-2}$ | | | | Count | | | g | | | kg ha$^{-1}$ | | |
| Source | df | ss (%)[a] | Fv | Pr(>F) | ss (%)[a] | Fv | Pr(>F) | ss (%)[a] | Fv | Pr(>F) | ss (%)[a] | Fv | Pr(>F) |
| Total | 127 | 100 | | | 100 | | | 100 | | | 100 | | |
| Env | 3 | 73.3 | 126.8 | <0.000 | 67.7 | 187.0 | <0.000 | 45.2 | 29.5 | <0.000 | 45.7 | 16.5 | <0.000 |
| Blocks (Env) | 12 | 2.3 | 1.3 | 0.221 | 1.4 | 0.6 | 0.806 | 6.2 | 1.2 | 0.304 | 11.1 | 3.5 | <0.000 |
| Gen | 7 | 1.7 | 1.7 | 0.124 | 2.3 | 1.6 | 0.188 | 5.6 | 2.0 | 0.088 | 2.9 | 1.6 | 0.151 |
| Env: Gen | 21 | 10.5 | 3.4 | <0.000 | 11.3 | 2.6 | <0.000 | 6.8 | 0.8 | 0.748 | 18.1 | 3.2 | <0.000 |
| PC1 | 9 | [61.6][b] | 4.9 | <0.000 | [83.2][b] | 5.1 | <0.000 | [57.1][b] | 1.0 | 0.423 | [61.2][b] | 4.6 | 0.000 |
| PC2 | 7 | [22] | 2.2 | 0.040 | [11] | 0.8 | 0.573 | [36] | 0.8 | 0.573 | [32] | 3.1 | 0.006 |
| PC3 | 5 | [17] | 2.4 | 0.042 | [7] | 0.7 | 0.610 | [7] | 0.2 | 0.944 | [7] | 1.0 | 0.429 |
| Residuals | 84 | 12.2 | | | 17.2 | | | 36.2 | | | 22.2 | | |

Significant level P≤ 0.05.

Abbreviations:

[a] Percentage of the sum of squares; [b][ ] Percentage of the sum of squares of Genotype-by-Environment Interaction

Env=Environment, Blocks (Env)=Replications, Gen=Genotypes, Env: Gen=Genotype by Environment interactions (GEi)

df= Degrees of freedom, Fv= F value, ss= Sum of squares.

The principal components are equal to the environment degrees of freedom, and the first two principal components explained >80% of the overall variations in the observed traits among genotypes.

Table 1.12. Genotype means and stability indices for yield and yield traits for eight canola genotypes evaluated at four environments in SD.

| | .....Biomass yield..... ($g\ m^{-2}$) | | | | .....Pods $plant^{-1}$..... Count | | | |
|---|---|---|---|---|---|---|---|---|
| Gen | ASV[a] | rASV[b] | Mean[c] | rY[d] | ASV[a] | rASV[b] | Mean[c] | rY[d] |
| CS2100 | 28.7 | 4 | 1359 | 6 | 13.3 | 3 | 53 | 4 |
| CS2300 | 45.4 | 8 | 1458 | 2 | 20.1 | 8 | 54 | 2 |
| CS2500 | 27.2 | 3 | 1360 | 5 | 15.2 | 5 | 51 | 5 |
| DKTF91SC | 18.7 | 2 | 1246 | 8 | 19.5 | 7 | 53 | 3 |
| DKTF92SC | 17.3 | 1 | 1336 | 7 | 9.0 | 1 | 49 | 7 |
| L140P | 35.1 | 6 | 1361 | 4 | 14.9 | 4 | 46 | 8 |
| L233P | 30.4 | 5 | 1558 | 1 | 10.1 | 2 | 64 | 1 |
| NCC101S | 43.5 | 7 | 1425 | 3 | 16.7 | 6 | 51 | 6 |

| | …. Thousand seed weight…. $g\ m^2$ | | | | ……...Seed yield……...…… $kg\ ha^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|
| Gen | ASV[a] | rASV[b] | Mean[c] | rY[d] | ASV[a] | rASV[b] | Mean[c] | rY[d] |
| CS2100 | 0.9 | 6 | 2.5 | 3 | 15.3 | 3 | 1458 | 7 |
| CS2300 | 1.1 | 7 | 2.3 | 6 | 20.7 | 5 | 1585 | 2 |
| CS2500 | 0.4 | 4 | 2.8 | 1 | 22.2 | 6 | 1574 | 3 |
| DKTF91SC | 0.4 | 3 | 2.4 | 5 | 26.5 | 7 | 1535 | 4 |
| DKTF92SC | 0.8 | 5 | 1.8 | 8 | 14.0 | 2 | 1478 | 5 |
| L140P | 0.2 | 1 | 2.2 | 7 | 54.4 | 8 | 1803 | 1 |
| L233P | 0.3 | 2 | 2.5 | 2 | 17.7 | 4 | 1446 | 8 |
| NCC101S | 1.1 | 8 | 2.4 | 4 | 10.0 | 1 | 1459 | 6 |

Abbreviations:

AMMI stability value, ***b*** Rank of AMMI stability value, ***c*** Mean yield for each trait,

***d*** Rank of yield.

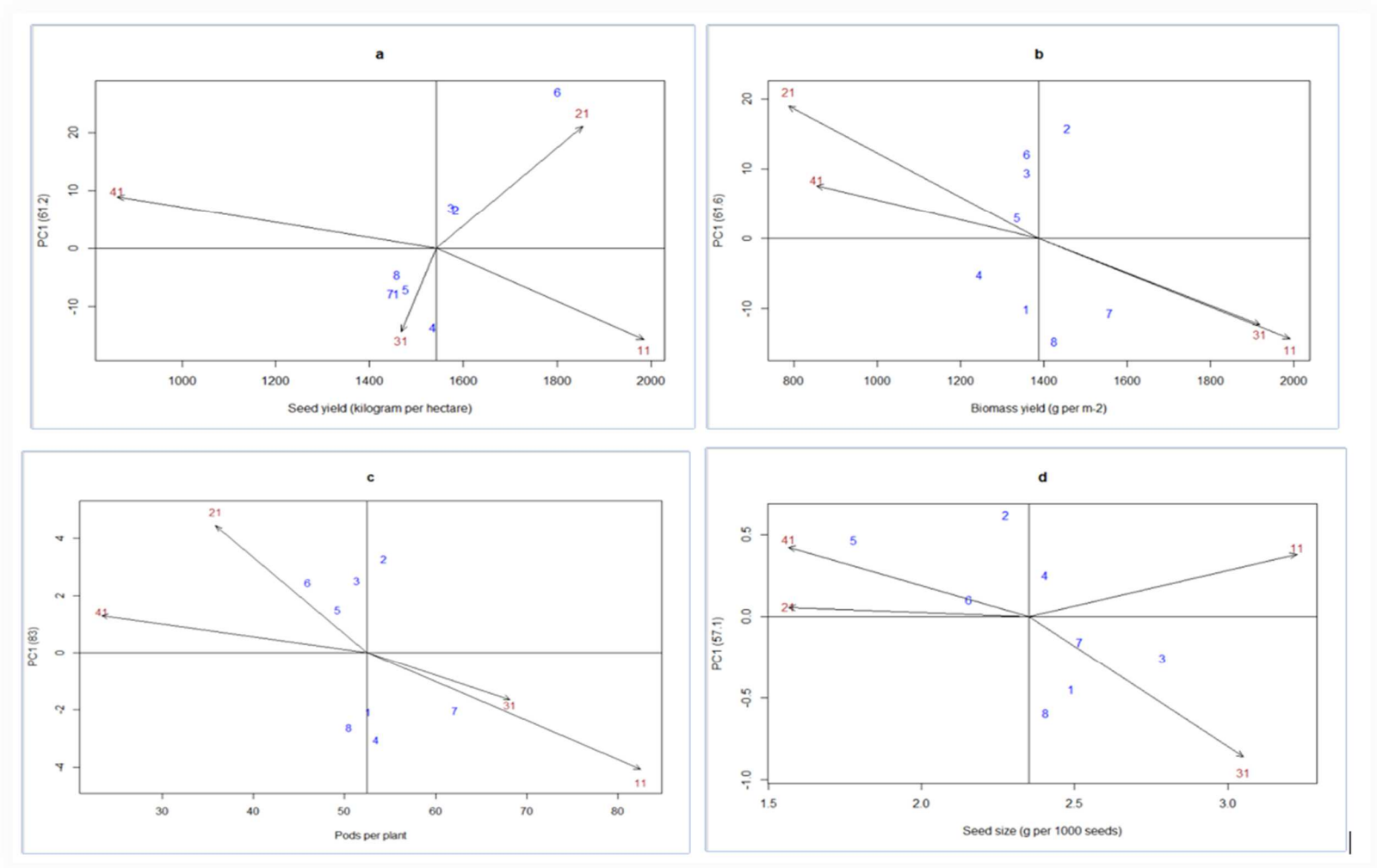


Fig 1.1. AMMI-1 biplot of seed yield (kg ha$^{-1}$) (a), biomass yield (b) pods plant$^{-1}$ (c) and 1000-seed weight (d) of eight canola genotypes (blue) across four environments (red) characterized with varied weather conditions (Tables 1.2 to 1.4) in 2019 and 2020. Environment Key: 11= Brookings 2019, 21= Pierre 2019, 31= Brookings 2020, and 41= Pierre 2020

Genotypes Key: [1] CS2100, [2] CS2300, [3] CS2500, [4] DKT F91SC, [5] DKTF92SC, [6] L140P, [7] L233P, [8] NCC101S.

# CHAPTER TWO
# EVALUATION OF CANOLA GENOTYPES FOR TOLERANCE TO SALINE/SODIC SOILS UNDER GREENHOUSE CONDITIONS

## LITERATURE REVIEW

The global demand for food, feed and fuel is increasing due to increasing population that is estimated to reach 9.1 billion by year 2050 (Shelley et al., 2015). To feed this population food and biofuels need to be increased by at least 70% (Shelley et al., 2015). Some of the past strategies to increase food production through input and crop intensification have resulted in soil degradation including sodicity and salinity which is reducing arable land for food production. Potentially, the soil is saline when its electrical conductivity (EC) of the saturated solution extract is greater than 5 mmho/cm (Shahid et al., 2018). However, this can be categorized into saline, sodic, and saline-sodic soil based on the ratio of concentration of soluble salts to amount of sodium salts in the soil solution (Franzen, 2003). Soil that has electrical conductivity (EC) (mmho/cm) >4.0, SAR<13 and pH >8.5 is saline, while soil with EC <4, SAR>13, and pH>8.5 are sodic. Soil with EC> 4, SAR> 13, and pH <8.5 is saline-sodic (Franzen, 2003; Bauder et al., 2008). Therefore, saline soil can be simply grouped as saline, sodic and saline sodic (Franzen, 2003; Bauder et al., 2008; Dahlawi et al., 2018).

Today, over one billion hectares are saline, which is over 30% of the world's arable soil (Drake et al., 2016; Ivushkin et al., 2019). Irrigated soil is becoming saline at a rate of 10 million ha $year^{-1}$ (Pimentel et al., 2004, Rengasamy, 2006). This is estimated to cause over $27 billion in financial loss annually on global basis at an average of $441 $acre^{-1}$ ($1,067 $ha^{-1}$) (Shelley et al., 2015). In SD, between 2008 and 2012, the surface soil electrical conductivity (EC) increased by at least 1 mmho/cm on over 1.4 million acres (566,560 ha) (13.4% of cropped acres) due to the rising water table where saline seeps are deposited on the soil surface.

The annual economic losses due to salinity in SD are estimated at about $26.2 million on over 113,312 ha) mostly in Beadle, Brown, and Spink counties (USDA NRCS, 2019). The commonly affected system is corn in eastern SD where 14% of the farmers are already affected (Birru et al., 2019). It has been reported that Montana alone has over 121406 ha off production due to salinity (USDA NRCS, 2019).

Despite the farmers efforts to remediate these saline soil, chemical and mechanical restoration methods used have additional non-economic costs and negative impact on untargeted salt tolerant microbial organisms, and ground water contaminations (Birru et al., 2019). Moreover, these methods are expensive for example, about $968-1,936 $ha^{-1}$ is needed to install tile drainage (Hadrich, 2012) therefore alternative salt tolerant crops may be a cheaper option (Flowers et al., 1997).

Previous studies have indicated the potential for Brassica species like canola, mustard and carinata to become alternative salt tolerant crops (Kumar, 1984; Chandler and Thorpe, 1987; Huang and Redmann, 1995; Flowers et al., 1997; Wright et al., 1997; Zheng et al., 1998; Bybordi et al., 2010; Zamani et al., 2010; Tunçtürk et al., 2011). But these crops need to be evaluated since salt tolerance vary by species. For example, Brassica amphidiploid are relatively more salt tolerant compared to Brassica diploid species (Ashraf and McNeilly, 1990; 2004). Seedling stages are more vulnerable to salt stress compared with vegetative and reproductive growth stages (Greenway and Munns, 1980; Sakamoto and Murata, 2000; Bybordi et al., 2010), but even biomass and yield were found to be negatively related with soil salts (Kumar, 1984; Francois, 1994 Zamani et al., 2010).

Soils high in salt content impede seed germination by reducing water availability for hydrolyzing endosperm seed tissue reserves to enhance radicle emergence (Kaymakanova,

2009), and reduce water and nutrient uptake by emerged seedling leading to reduced stomatal conductance and depression in carbon uptake (Seemann and Critchley, 1985). This means that photosynthesis can be used as nondestructive phenotyping technique for growth and stress in plants (Krause and Weis, 1991; Stirbet et al., 2018). More so, salt stress reduces shoot and root growth (Delfine et al., 1999; Sakamoto and Murata, 2000; Munns, 2002; Zamani et al., 2010; Shabani et al., 2013; Athar et al., 2015), basically due to ionic and osmotic stress (Munns and Tester, 2008). Salt stress also reduces growth specific leaf area index, number of leaves plant$^{-1}$, fresh and dry root biomass, and leaf longevity (Francois, 1994).

Recently, biochar (a solid carbonaceous residue, produced under oxygen-free or oxygen-limited conditions at temperatures ranging from 200 to 1000 °C) has attracted considerable attention as a soil amendment to remediate and improve physical, chemical, and biological properties of degraded soil (Dahlawi et al., 2018) and promote plant growth (Cárdenas-Aguiar et al., 2020). However, this depends on biochar source (biomass type) and pyrolysis temperature; either slow or carbonization pyrolysis (~500 °C), fast pyrolysis (>500 °C) or intermediate gasification (Resende, 2016).

Biochar is highly heterogeneous in nature and generally contains volatile compounds, labile and recalcitrant carbons, ash, and moisture (Antal and Gronli, 2003). Carbon yield may range from 400 g kg$^{-1}$ to up to 900 g kg$^{-1}$ depending on feedstock and pyrolysis conditions (Antal and Gronli, 2003; Van Zwieten et al., 2010b; Gaskin et al., 2010). Hardwood feedstock pyrolyzed at high temperature produces high carbon char, while soft and non-wood feedstocks such as crop residues, manures and straw biomass produces biochar with low carbon content. For example, Gaskin et al. (2010) reported 817 g kg$^{-1}$ carbon content of biochar produced from pinewood chips at 500 $^{o}$C under slow pyrolysis, whereas poultry manure pyrolyzed char had

only 399. 9 g $kg^{-1}$ carbon content. Biochar produced from herbaceous feedstock tends to contain lower proportions of mesopores and macropores and exhibit smaller surface area compared with biochar pyrolyzed from woody biomass (Van Zwieten et al., 2010a). Biochars properties vary greatly, even when produced from the same or related feedstock due to pyrolysis conditions like kiln temperature and pressure held on the biomass (Van Zwieten et al., 2010a; Van Zwieten et al., 2010b; Zimmerman, 2010; Mukherjee et al., 2011). Biochar produced at temperatures above 1000 °C tends to have lower surface area (Lua and Guo, 1998) compared to one produced at lower temperatures. These properties affect biochar functioning. For example, biochar prepared at 450 °C, and 600 °C with canola as a test crop increased uptake of arsenic, and soil bacteria enzyme activity (Cárdenas-Aguiar et al., 2020). In another study by Song et al. (2020) using camellia (*Camellia sinensis*) derived char at three pyrolysis temperatures (300, 500, and 700 ˚C), and two application rates (3% and 5%), biochar improved soil pH, total P, and available P at 700 ºC and 5% application rate. Similarly, greater beneficial properties of biochar were reported at pyrolysis temperature between 400 to 700 ºC (Kimetu and Lehmann, 2010; US-Biochar, 2016; Mohanty et al., 2018). So, all these variations in biochar properties influence its performance in different soil conditions (Nguyen et al., 2004).

Although so much good has been reported on biochar by various studies, a review of 17 studies on biochar by Jeffery et al. (2011) reported mixed results on crop yield ranging from -28 to +39 %, with an overall average yield increase of 10 %. Some studies reported completely negative effects of biochar on soil organic carbon and nutrient mineralization (Kuzyakov et al., 2009; Van Zwieten et al., 2010a; Wang et al., 2015) while others showed that biochar application negatively affected plant growth on case-by-case basis (Jones and Quilliam, 2014).

The problems associated with biochar application in agricultural systems could be alleviated by using a biochar-composted manure mixture but use of composted manure is limited by undisputed claims on antibiotic contaminants (Steiner et al., 2008; Qian et al., 2016), soil salt contaminants (Steiner et al., 2008; Qian et al., 2016; Zhou et al., 2020), and ground water contaminations (Hao and Chang, 2002; Gondek et al., 2020). However, when composted manure is used in combination with biochar it is a less expensive soil ameliorant (Zeng et al., 2015) that can improve soil organic-matter content, nutrients levels, soil water-storage capacity and other soil physical and biological properties (Schulz et al., 2013; Zeng et al., 2015; Abujabhah et al., 2016). For example, Rogovska et al. (2011) reported 17.6 to 68.8% increase in soil organic carbon by addition of biochar to the soil compared to untreated control and reduction in $N_2O$ emissions. The same study found that biochar-by-manure interaction for $CO_2$ flux indicated that biochar either helped stabilize manure carbon or the presence of manure reduced the effect of biochar on the mineralization of SOC.

Corn and soybean rotation systems dominate in SD especially in the Central and eastern regions (O'Brien et al., 2020). More so, long-term application of livestock manure in corn-based cropping systems in SD enhances soil physical and hydraulic properties (Ozlu et al., 2019). This is because manure improves soil bulk density, porosity and water holding capacity (Xin et al., 2016), although this varies depending on the manure application rates, source, chemical and biological properties (Asada et al., 2012; Bottinelli et al., 2013; Khalid et al., 2014). Manure improves soil structure by binding soil particles (Celik et al., 2010) and increasing N, P, and K mineralization and availability to plants (Vivekanandan and Fixen, 1990).

Most of the previous studies on plant tolerance to saline-sodic soils in SD evaluated mostly native plants with less attention given to traditional crops. Besides, most of the chemical and physical methods employed in remediation of salt impacted soil are expensive and less environmental and ecosystem friendly. Different biomass types for charring are available in SD, but these may behave differently in different soil types. Therefore, investigating the influence of different types of biochar and composted manure mix rates in degraded soil is necessary to supplement the available literature. The objectives of the study were to (i) evaluate different canola and carinata genotypes for tolerance to saline-sodic soils, and (ii) evaluate canola and mustard genotypes for emergence and growth in saline-sodic amended with biochar and composted manure.

## MATERIALS AND METHODS

### *Soil description*

Soils used in this study were collected from Clark (44.7$^{o}$ N, -97.8$^{o}$ S), SD on a site with historical saline seeps, eroded and degraded soil at a depth of up to 20 cm. The landscape was not in use for crop production before sampling of the soil. Soils were collected at three landscape positions; (i) a highly saline soil with no vegetation growing on it, (ii) transitioning zone (moderately saline) characterized by scattered vegetation, and (ii) good soil zone (non-saline) characterized by vigorous vegetation growth. A sub-sample from each soil zone was taken for chemical and biological analysis. The soil samples for biological analysis were sealed in Ziplock bags and held at <4 $^{o}$C, while soil samples for chemical analysis were air dried, ground and sieved to pass through a 2-mm sieve. These were then forwarded to Ward Laboratories Inc, Nebraska for analysis. Post termination, soil chemical analysis was also conducted by Ward laboratories Inc. for chemical changes in the soil.

*Biochar source and characterization*

The softwood and hardwood biochar used in experiment two were produced using carbon optimized gasification technique with reactor temperatures ranging from 90 to 200 °C and a residence time of 0.5 hour and 3 minutes by Wakefield biochar-particle technology labs, USA. Hardwood biochar was processed from maple wood chips, while pinewood chips was used for softwood biochar. Biochar used in the third experiment were produced under medium pyrolysis conditions using carbon optimized gasification technique up to 450 ºC by advanced renewable energy technology international (ARTi) http://www.arti.com/, a biomass incubation center located at Iowa State University. The biomass used was pine wood chips for softwood biochar, and maple wood chips for hardwood biochar. Biochar was dried to moisture free weight before mixing it with the soil. The biomass types used in this study were alkaline (pH=7.9-8.2), low in micro and macronutrients but with high ash content (820 g $kg^{-1}$ and 842 g $kg^{-1}$) for softwood and hardwood biochar. Composted manure used in this study was low in micro and macronutrients but higher cation exchange capacity (CEC) and organic matter content. The chemical properties of biochar and composted manure used in this study are included in Table 2.2. The two biochar types and composted manure were selected to represent common bioenergy feedstocks available for farmers in SD.

*Experiment 1*

The experiment was conducted at the Plant Science Greenhouse, South Dakota State University (44.3° N; 96.8° W). The experimental design was a randomized complete block design with treatment arranged in a factorial design, (13 genotypes x 3 soil type) for a total of 39 treatments replicated three times. Soils with salinity ranging from non-saline (0.62 mmho/cm), moderately saline (5.17 mmho/cm), and highly saline (8.47 mmho/cm)

corresponding to good, transitioning, and saline soils used in the study are detailed in Table 2.1. These soil samples were collected at a site near Clark, SD that is characterized with saline-sodic soil issues which is the current focus of SDSU saline/sodic soil remediation investigations. Soil samples were from topsoil (0-20 cm). A total of 117 pots, 500 ml each, were arranged on the table in the greenhouse chamber and filled with the soils. Ten canola (CS2100, CS2300, CS2500, CS2600, DKTF7114BL, DKTF91SC, DKTF92SC, L140P, L233P, and NCC101S) and three carinata (A120, DH069485, and DH140251) genotypes were evaluated for seedling emergence at each soil salinity level. Eight seeds of a genotype were hand-planted in each pot. The pots were watered to maintain sufficient soil moisture for emergence and growth. The greenhouse air temperature ranged between 22 and 27 degrees Celsius with a 14 to 16-hr photoperiod. Seedling emergence was observed on daily basis in each of the treatments for up to four weeks after planting after which the experiment was terminated.

*Experiment 2*

This experiment was like experiment one, only that biochar was introduced into each soil salinity level (soil type) at a rate of 5% by volume with untreated control (0% biochar/no biochar), and the three carinata genotypes (AC120, DH069485, DH140251) replaced with three mustard genotypes (Broadleaf mustard, African cabbage, and Brown mustard). Two biomass-based char types differing by wood type (softwood and hardwood biomass), and physical properties (large and small surface area) due to sources and pyrolysis conditions were introduced into each of the soil type at 5% dry weight basis. The soil salinity level, biochar and genotype treatments were arranged in a split-plot with soil type/salinity level as the main plot and biochar and genotypes factorially arranged in subplots within each soil salinity level and

replicated three times. This experiment was carried out in small pots of 500 ml each, arranged on greenhouse bench and laid in flat trays. The pots were watered and allowed to settle before planting. Eight seeds of a genotype were planted by hand into each pot and monitored for days to emergence. These were later thinned to uniformly one vigorous seedling $pot^{-1}$ at 21 DAP. Plants were watered to maintain sufficient soil moisture for emergence and growth. Measurements included seedling emergence 7DAP, shoot dry weight, and leaf chlorophyll content (SPAD values). Leaf chlorophyll content (SPAD values) was measured using SPAD meter version SPAD-502, Minolta, Japan. An average of four leaves $plant^{-1}$ were used to record the SPAD values. Fresh biomass weight was determined for all treatments at termination of the study at 59 DAP. After taking fresh weight (g) of plants, whole plants were oven dried at 105 $^{o}$C for 3 days to a constant moisture free weight, and dry weight determined using a high precision electrical balance.

*Experiment 3*

The best genotypes identified in the second experiment (NCC101S, DKTF91SC, African cabbage and Brown mustard) were evaluated in the third experiment. In experiment 3 composted manure rates of 0, 30 and 50 % by volume basis were introduced to each of the soil-biochar treatment (no biochar, softwood, and hardwood) within each soil salinity level (as illustrated in Figure 2A.1). Soil salinity levels were the main plots and biochar, composted manure and genotypes were arranged in a factorial design within each soil type/salinity level. Composted manure was mixed thoroughly by hand into each soil salinity level biochar treatment combinations before filling the pots. Pots were watered thoroughly and allowed to settle before seeding. Eight seeds of each genotype were planted in each pot and thinned to uniformly one plant $pot^{-1}$ at 21 DAP. Plants were watered based on visual observations on the

plants. Measurements included percentage emergence, number of leaves plant$^{-1}$, and leaf chlorophyll content (SPAD values) at 39 DAP using a soil plant analysis development (SPAD) version SPAD-502, Minolta, Japan. This was achieved using an average of four young fresh growing leaves plant$^{-1}$ (Uddling et al., 2007; Ehsanzadeh et al., 2009; Nauš et al., 2010; Ling et al., 2011). The whole plant was then harvested, and oven dried at 105 ºC for 3 days to constant moisture free weight and then plant dry biomass weight determined using a high precision electrical balance.

*Statistical analysis*

Analysis of variance (ANOVA) was conducted for each soil type using linear mixed model for RCBD experiments at alpha 0.05 in RStudio version 0.1.0 using the package "doe bioresearch" (De Mendiburu, 2017) and significant differences among means were separated using Fisher's least significant difference (LSD) at 95% confidence level.

## RESULTS AND DISCUSSION

*Experiment 1.*

Seedling emergence for each soil salinity level differed among genotypes (Table 2.3). In each salinity level, percentage of emerged seedlings were much lower at 7DAP than at 28DAP. The percentage of emerged seedlings declined with increasing soil salt content. In the good soil, DKTF91SC had greatest emergence (90%) at 28DAP whereas NCC101S had the lowest (49.2%). In the soil collected from the transitioning zone, the best emergence at 28DAP was observed for NCC101S and L140P (29.2 %) and DH140257 (27.5%). In the saline soil, seedling emergence was low ranging from 0% (DH140251, L233P and CS2600) to 29.2% (NCC101S) (Table 2.3). High soil salt content in the seed-soil contact zone lowers available moisture for hydrolyzing seed endosperm contents (Bybordi et al., 2010; Mousavi and Omidi,

2019) resulting in seedling desiccation due to reverse osmosis (Kaymakanova, 2009). The results in the current study are consistent with Francois (1994); Sakamoto and Murata, (2000); Bybordi et al., (2010) who observed a negative association of seedling emergence and high soil salt content.

*Experiment 2*

Seedling emergence at 7DAP was influenced by biochar in transitioning soil ($P<0.000$) and in saline soil ($P = 0.017$) but not in good soil (Table 2.4). In transitioning soil, seedling emergence was greater with addition of softwood biochar while hardwood biochar and no biochar had similar results based on percentage of emerged seedlings. In the saline soil however, addition of hardwood biochar resulted in the greatest percentage of emerged seedlings, significantly greater than no biochar but similar to that of soil amended with softwood biochar (Figure 2.1). Greater performance of softwood biochar relative to hardwood biochar is likely explained by the fact that softwood biochar has a larger surface area (376.0 $m^2/g$) compared to 18.0 $m^2/g$ for hardwood biochar (Table 2.2) which increases on water holding capacity and nutrient availability that enhance seedling emergence.

Genotypes differed in seedling emergence only in saline soil (Table 2.4). Florida broadleaf mustard had the greatest emergence (52.2%) under saline soil. The other canola genotypes (NCC101S and L233P) had 48.2% emerged seedlings. However, even the best seedling emergence in transition and saline soil was much lower than the mean emergence in good soil (73.6%). The variation in salt tolerance among genotypes in saline soil used in this study is likely related to the fact that genotypes such as Florida broadleaf mustard is an amphidiploid as Brassica amphidiploid species have been said to be relatively salt tolerant compared to Brassica diploid species (Kumar,1984; Ashraf and McNeilly, 2004). Even though

Francois (1994), reported the threshold for growth in saline soil to be 10 $dS/m^{-1}$ for most canola genotypes, in the current study, only 36.0% of the seeds emerged at EC 5.16 and pH 6.9.

Leaf chlorophyll content (SPAD values) varied by biochar type only in transitioning soil (P = 0.052) (Table 2.4, Fig 2.2). The highest SPAD values were recorded on transitioning soil with no biochar, although this was not significantly different from soil treated with hardwood biochar. Hardwood biochar used in this study has small surface area, but higher ash content compared with softwood biochar (Table 2.1). This small` surface area improves soil porosity, aeration and improves water and nutrient availability thereby reducing osmotic stress which results in greater growth and higher solute accumulation (Zhao et al., 2020). In addition, biochar is reported to improve soil physical and chemical properties of saline soil, thereby enhancing nutrient uptake by plants (Sajedi and Sajedi, 2019), and reduce salt stress (Naveed et al., 2020). However, this may vary based on the biochar (Cárdenas-Aguiar et al., 2020), and soil properties as well (Nguyen et al., 2004; Nobile et al., 2020). These may be reasons why there was no differences in SPAD values in saline and good soil when biochar was applied.

## Experiment 3

*Seedling emergence (%)*

In good soil, seedling emergence was influenced by the main effects of biochar, composted manure, and genotype (Table 2.6). The interaction between biochar and composted manure for percent seedling emergence was also significant (Table 2.6). The interaction between biochar and composted manure was because of increase in seedling emergence with increase in applied composted manure in good soil without biochar with the best seedling emergence obtained at the highest rate of composted manure applied (50%). In good soil with softwood biochar, however, the best seedling emergence was obtained at 30% composted

manure rate. In good soil with hardwood biochar, seedling emergence was not influenced by application of composted manure but was significantly greater than that in all other treatment combination except good soil without biochar at 50% composted manure rate. Genotypes also influenced seedling emergence in good soil with the best seedling emergence obtained in DKTF91SC (57.4%), but this emergence was similar to African cabbage and Brown mustard but significantly greater than seedling emergence for NCC101S (43.4%).

In transitioning soil, seedling emergence was also influenced by the main effects of biochar, composted manure, and genotype (Table 2.6). Seedling emergence was greater in transitioning soil with no biochar than in the soil with either softwood or hardwood biochar. Emerged seedlings increased with increasing rate of composted manure applied (Table 2.6). The interaction effects between biochar and genotype as well as composted manure and genotype for percentage of emerged seedlings was also significant. The interaction between biochar and genotype (Fig 2.3b) is explained by variations among genotypes in percent of emerged seedling in transitioning soil treated with different types of biochar in relation to the unamended soil. For example, Brown mustard had the best emergence in transitioning soils with no biochar or in transitioning soil with softwood biochar but not in soil with hardwood biochar (Fig 2.3 b). Seedling emergence was also influenced by the interaction between composted manure rate and genotypes (Fig 2.3c). This again was due to variations in performance of genotypes with changes in composted manure application rate. For example, African cabbage had the worst seedling emergence in transitioning soil with no composted manure but was among the best when composted manure at 50% was present (Fig 2.3 c).

In saline soil only composted manure had a significant effect on seedling emergence with seedlings emerging greatest in saline soil mixed with composted manure at the 50% rate

(Table 2.6). The interaction between biochar and genotypes was likely due to Brown mustard being among the best emerged genotype in saline soils with no biochar or saline soils with softwood biochar but the same genotype emerging worst in saline soils with hardwood biochar added (Fig 2.3 d)

The mixed effects of biochar on seedling emergence in salt impacted soils is explained by the fact that moisture stress is one of the challenges in saline soil (Wadleigh, 1946), yet biochar absorbs moisture to its surface (Tanure et al., 2019). This means that seeds in the soil-biochar contact zone may not get enough moisture for radicle emergence. However, this varies based on the soil condition, and biochar type. For example, saline soil used in this study (Table 2.1) were higher in EC (7.21), higher SAR (4.6) and very high ESP (44.9 cmol/kg$^{-1}$) which makes it very poorly drained but having high macro nutrients. Therefore, addition of biochar may have improved its physical structural properties as well as increasing moisture and nutrient/availability improving seedling emergence (Fig 2.3 d). Li et al. (2015) found that higher application rates of slow pyrolyzed residue char inhibited tomato germination than lower rates, but Silva et al. (2020) found no significant effect of similar biochars on seed germination but both studies related the problem to insufficient moisture. Improvement in seedling emergence in composted manure-amended soil is explained by the fact that composted manure application increased organic matter, which improved moisture holding capacity and available moisture in the seed-soil contact zone, resulting into higher seedling emergence an assertion that is consistent with Smith et al. (2001); Srivastava et al. (2016). In addition, composted manure can stabilize pH and increase phosphorous mineralization, which can enhance seedling emergence (Jin et al., 2019). Differences in genotypes emergence rates in different soil-biochar composted manure combinations is likely related to the differences in

genetic inherent traits in salt tolerance. For example, Brassica amphidiploids are said to be more salt tolerant compared to Brassica diploids (Ashraf et al., 2001). This means that mustard may be more salt tolerant than canola used in this study.

*Number of leaves plant$^{-1}$*

In good soil, leaves plant$^{-1}$ was influenced by the main effects of biochar, composted manure, and genotype with no interaction between them. The number of leaves plant$^{-1}$ were significantly greater in good soil with softwood biochar (5.0 leaves plant$^{-1}$) relative to good soil with no biochar and with hardwood biochar (4.1 leaves plant$^{-1}$). Number of leaves plant$^{-1}$ were greatest at 30% composted manure application rate (5.3 leaves plant$^{-1}$), but these were similar and significantly lower than at 0% and 50% composted manure rates. The number of leaves plant$^{-1}$ also varied by genotypes in good soil with African cabbage having the greatest number of leaves plant$^{-1}$ at 5.1 leaves plant$^{-1}$ although this was similar to DKTF91SC at 4.5 leaves plant$^{-1}$.

In transitioning soil, number of leaves plant$^{-1}$ was influenced by the main effects of composted manure and genotype (Table 2.6) with number of leaves plant$^{-1}$ greatest at 50% composted manure rate and African cabbage having the greatest number of leaves plant$^{-1}$. The interaction effects between biochar and composted manure, biochar and genotype, and composted manure and genotypes for leaves plant$^{-1}$ was also significant (Table 2.6). The interaction between biochar and composted manure for leaves plant$^{-1}$ is shown on Figure 2.4a and was due to the greater number of leaves for transitioning soils with a combination of biochar amendments (softwood and hardwood) and composted manure applied at a rate of 50% compared to soil with no biochar at the same composted manure rate (Figure 2.4a). In transitioning soil, leaves plant$^{-1}$ was also influenced by the interaction between biochar and

genotype (Table 2.6, Fig 2.4b) because Brown mustard had lower number of leaves in transitioning soil amended with hardwood biochar when compared to the same genotype in transitioning soil with no biochar or amended with softwood biochar. The significant interaction between composted manure and genotypes was because African cabbage had significantly greater leaves plant$^{-1}$ at 50% composted manure rate compared to the same genotypes at lower rates (Fig 2.4c).

In saline soils the interaction effects between biochar and composted manure, biochar and genotype and composted manure with genotype for the most part, mirrored what was observed in transitioning soil for the same interactions. For example, the greatest number of leaves plant$^{-1}$ were observed in the saline soils amended with a combination of softwood biochar and composted manure at a rate of 50%. The interactions of genotype with biochar or composted manure rates were as observed in saline soil, due change in genotype rank as biochar amendment changed or as composted manure rate changed.

Salts in the root zone lowers the water available in the salt-contaminated soil, inducing reduction in nutrients availability to plants, resulting in osmotic, and ionic stress (Munns and Tester, 2008; Siringam et al., 2011) which result in limited or no growth (Irshad et al., 2002). The increase in number of leaves with increasing composted manure rates, observed in this study was likely because composted manure improved soil moisture retention capacity which increased nutrient availability to plants resulting in an increase in growth of leaves an assertion that is consistent with Irshad et al. (2002). Brassica amphidiploid are relatively more salt tolerant compared to Brassica diploid species (Ashraf et al., 2001; Ashraf and McNeilly, 2004). This explains higher number of leaves plant$^{-1}$ in mustard genotypes relative to canola which is due to inherent salt tolerance traits. Even though Francois (1994) did not find a

negative effect of salt stress on vegetative growth of canola, in the current study, leaves plant$^{-1}$ reduced with increasing soil salt content especially in no amendments treatments which is consistent with (Kumar, 1984; Zamani et al., 2010).

*Leaf chlorophyll content (SPAD values)*

In good soil, SPAD values were influenced by the main effects of biochar, composted manure, and genotype (Table 2.6). Good soil amended with softwood biochar had similar SPAD values as the untreated control, but greater SPAD values compared to soil amended with hardwood biochar. Good soil with no composted manure amendments and soil amended with composted manure at 30% rate had greater SPAD values compared to soil amended with composted manure at 50% rate. The interaction effects between biochar and genotype and composted manure and genotype for SPAD values were also significant (Table 2.6). The genotype by biochar interaction was due to Brown mustard having a lower SPAD values compared to all other genotypes in all biochar treatments (Figure 2.5a). The genotype by composted manure interaction on the other hand was due to inconsistent SPAD values for NCC101S across composted manure rates.

The main effect of biochar, composted manure, and genotypes also influenced SPAD values in the transitioning soil (Table 2.6). In terms of main effects of biochar, softwood still maintained greater SPAD values compared to no biochar and soil amended with hardwood biochar. On the other hand, transitioning soil amended with composted manure at 50% rate had greater SPAD values than soil with no amendment or amended with composted manure at 30%. Interactions between main effects were all significant (Table 2.6). Figure 2.5c shows interaction effects between biochar and composted manure rates for SPAD values. While SPAD values increased with increasing composted manure rates in transitioning soil with no

biochar or transitioning soil with hardwood biochar, SPAD values for soil amended softwood biochar were greater and but not influenced by composted manure rate (Figure 2.5c). Biochar and genotypes interactions also influenced SPAD values in transitioning soil (Fig 2.5d). The interaction was due to Brown mustard having significantly lower SPAD values in soil amended with hardwood biochar compared to soil amended with softwood biochar or soils with no biochar amendment.

SPAD values in saline soil were influenced by the main effect of biochar and composted manure rates (Table 2.6). In saline soils, as in good soil, soil amended with softwood biochar had similar SPAD values as good soil with no biochar and this value greater than that for soil amended with hardwood biochar. However, the interactions between biochar and composted manure, biochar and genotypes were also significant for SPAD values (Table 2.6). These interactions are presented on Figures 2.5e and f. For biochar by composted manure rates, the interaction was due differential response to composted manure application observed in the biochar treatments. In saline soils with no biochar, SPAD values increased with increase in composted manure rate peaking at a rate of 30% whereas in the softwood amended soil, the SPAD values peaked at the highest composted manure rate of 50% but lower compared with that in no biochar. In the saline soils amended with hardwood biochar the response to composted manure application was poor irrespective of rate. Biochar and genotypes interaction was because in saline soil without biochar, genotypes performed similarly in SPAD values. However, in saline soil with softwood biochar, genotype NCC101S had higher SPAD values than DKTF91SC and African cabbage but similar to that in saline soil with no biochar. In saline soil with hardwood biochar, SPAD values were lower on all genotypes relative to saline soil with no biochar and softwood.

Softwood biochar in combination with composted manure increased leaf chlorophyll content (SPAD values). This elevated leaf chlorophyll content is explained by high phosphorous accumulation from composted manure (Eghball, 2002), and the fact that softwood biochar had a large surface area for moisture storage and increase on availability of other nutrients (Asai et al., 2009). The lower SPAD values in saline soil with no amendments is likely due to ionic and osmotic stresses that result in production of reactive oxygen species leading to degradation of photosystem 1 and 2 in thylakoid membrane (Wiencke, 1982) which resulted into lower solutes accumulation in the leaf due to depression in carbon assimilation. However, saline soils used in this study were high in nitrates. Therefore, composted manure and softwood biochar application may have increased availability of nitrates and moisture to plants which reduced the effects of sodium in the soil, relieving the plants of osmotic stress and increased on leaf tissue chlorophyll development (Akhtar et al., 2015; Marchand et al., 2016).

*Shoot dry weight*

In good soil, shoot dry weight was influenced by the main effects of biochar, composted manure rates and genotype (Table 2.6). However, the interactions between biochar and composted manure rates, biochar and genotypes and composted manure and genotypes were also significant. Shoot dry weight was greater in good soil with softwood biochar than in soil amended with hardwood biochar but this value was not different from untreated control/no biochar. Shoot dry weight was significantly greater at 30% composted manure application rate (Table 2.6). The interactions effects between biochar and composted manure, biochar and genotypes, and composted manure and genotypes for shoot dry weight were also significant in good soil (Table 2.6). The interaction between biochar and composted manure was a result of

a significantly lower shoot dry weight when 30% composted manure was applied in good soil amended with hardwood biochar as compared to the same composted manure rate in the soil with no amendment and soil amended with softwood biochar (Figure 2.6a). The significant interaction between biochar and genotypes was because of inconsistencies in ranking of genotypes for shoot dry weight in soils amended with biochar as compared to the soil with no biochar (Figure 2.6b). Composted manure and genotypes interactions were also significant for shoot dry weight in good soil due to higher variations in shoot dry weight among genotypes.

In transitioning soil, the interaction between biochar and composted manure for shoot dry weight was because in soil amended with hardwood biochar, shoot dry weight response to composted manure rate of 30% was significantly lower compared to the same rate in softwood biochar or in soils with no biochar (Figure 2.6d). Biochar and genotypes interaction was significant for shoot dry weight due to changes in genotype ranking for shoot dry weight from one biochar treatment to the other (Fig 2.6e).

In saline soil, shoot dry weight varied by biochar and composted manure, and the interaction between biochar and composted manure, as well as interaction between biochar and genotype were significant (Table 2.6). The interaction between biochar and composted manure for shoot dry weight was because application of biochar only influenced shoot dry weight in saline soil with no biochar (Figure 2.6f). In addition, saline soil amended with hardwood biochar had extremely low shoot dry weight irrespective of composted manure application rate as compared to same soil amended with softwood biochar or control (Figure 2.6f). Shoot dry weight also varied by biochar and genotypes interactions in saline soil. This was due to variable shoot dry weight among genotypes with Brown mustard reaching the highest in saline soil with

no biochar, while NCC101S had the highest shoot dry weight in saline soil with softwood biochar (Fig 2.6g).

The increase in shoot dry weight with increase in composted manure rate in saline soil was due to increased nutrients and moisture availability resulting in higher plant growth. However, the variation between the biochar types also influenced plants growth due to the biochars physical properties that influenced on the availability of moisture and nutrients to plants. When comparing application of biochar and composted manure combinations and their interactions on plant growth in saline soil, shoot dry weight was greater in treatments with no biochar, and increased with increasing composted manure rate applied and this was greater than when composted manure is added in either softwood or hardwood biochar treatment. This was because biochar can sequester nutrients and reduce availability of soil moisture leading to reduced growth (Qasim et al., 2002). However, biochar improves soil physical and biological properties of saline soil which can also support growth (Dahlawi et al., 2018). This therefore explains a slightly higher shoot dry weight at 0% composted manure with softwood biochar compared to 0% composted manure in no biochar in saline soil. Despite the mixed results of biochar on seedling emergence and growth of genotypes evaluated under different soil salt contents (salinity levels), softwood biochar produced better results in most cases compared to hardwood biochar and no biochar and addition of composted manure improved the overall performance in all treatments.

## CONCLUSIONS

Seedling emergence and growth decreased with increasing soil salt concentrations. The impact of biochar application on seedling emergence and growth varied depending on type of biochar and soil salinity level. In good soil (non-saline) biochar did not influence seedling

emergence. In transitioning soil (moderately saline) seedling emergence was best with softwood biochar application while in saline soil (highly saline), the two biochar types showed slightly improved seedling emergence compared to the control (no biochar). On average, application of composted manure improved seedling emergence and growth in all soil salinity levels. However, significant interactions were observed between biochar and composted manure in transitioning and saline soils for leaf chlorophyll content, number of leaves plant$^{-1}$ and shoot dry weight plant$^{-1}$. These interactions were mostly due to inconsistence of response of these genotypes to composted manure application in soil amended with softwood biochar when compared to those amended with hardwood biochar. In most cases, plant growth traits had a positive and more consistent response is transition and saline soil amended with softwood biochar than those amended with hardwood biochar. Four genotypes NCC101S, DKTF91SC, African cabbage and Brown mustard have salt tolerance traits and could be further investigated for their physiological salt tolerance mechanisms as well as their genetic characteristics under salt stress. Biochar use in remediating saline soils needs further investigation due to the mixed results produced under different soil salt content.

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Table 2.1. Chemical properties for good, transitioning, and saline soils used in Experiment 1, Experiment 2, and Experiment 3

| | Soil type | pH | EC | OM | $NO_3$ | P | K | Ca | Mg | Na | S | SAR | ESP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mmho/cm | % | $kg/ha^{-1}$ | .....$mg/kg^{-1}$..... | | | | | | .....$cmol/kg^{-1}$ | |
| Expt 1 | Good soil | 7.5 | 0.62 | 4.9 | 30 | 0.02 | 0.36 | 2.74 | 0.41 | 0.05 | 0.14 | 2.82 | 8.1 |
| | Transitioning soil | 6.9 | 5.17 | 4.4 | 67 | 0.03 | 0.20 | 2.07 | 1.16 | 2.35 | 2.74 | 4.25 | 45.5 |
| | Saline soil | 6.8 | 8.47 | 4.3 | 100 | 0.02 | 0.19 | 1.61 | 1.24 | 4.29 | 3.51 | 5.46 | 50.6 |
| Expt 2 | Good soil | 6.5 | 1.85 | 5.3 | 100 | 0.03 | 0.11 | 2.48 | 0.87 | 0.21 | 0.79 | 2.71 | 11.4 |
| | Transitioning soil | 7.0 | 4.53 | 4.4 | 128 | 0.03 | 0.11 | 2.01 | 1.25 | 1.56 | 2.08 | 3.41 | 34.4 |
| | Saline soil | 6.9 | 5.16 | 4.4 | 335 | 0.02 | 0.14 | 2.33 | 1.31 | 1.97 | 1.22 | 4.05 | 38.2 |
| Expt 3 | Good soil | 7.3 | 0.42 | 5.4 | 104 | 0.01 | 0.38 | 2.51 | 0.42 | 0.04 | 0.00 | 2.57 | 9.5 |
| | Transitioning soil | 7.1 | 5.54 | 4.6 | 6.7 | 0.02 | 0.23 | 2.20 | 1.36 | 1.94 | 2.96 | 3.86 | 35.0 |
| | Saline soil | 7.4 | 7.21 | 4.3 | 121 | 0.05 | 0.13 | 1.96 | 1.50 | 3.24 | 3.15 | 4.61 | 44.9 |

Abbreviations: Expt=Experiment, EC=Electrical conductivity, OM=Organic matter

**Note**: EC>4.0, SAR<13, pH >8.5 = saline soil, EC <4, SAR>13, and pH>8.5 = sodic soil, EC> 4, SAR> 13, and pH <8.5 =saline-sodic

(Franzen, 2003; Bauder et al., 2008; Dahlawi et al., 2018).

Table 2.2. Chemical properties of biochar and composted manure used in the Experiment 3

| Parameter | Unit | Softwood | Hardwood | Composted manure |
|---|---|---|---|---|
| SA | $m^2/g$ | 375.80 | 18.00 | nd |
| Ph | | 7.90 | 8.20 | 7.90 |
| EC | dS $m^{-1}$ | Nd | nd | 0.41 |
| OM | % | Nd | nd | 13.20 |
| N | kg N $ha^{-1}$ | 1.28 | 0.00 | 0.00 |
| P | g kg-1 | 0.13 | 0.00 | 0.08 |
| K | g $kg^{-1}$ | 1.20 | 0.00 | 5.99 |
| S | g $kg^{-1}$ | 0.27 | 0.00 | 0.17 |
| Ca | g $kg^{-1}$ | 1.20 | 0.00 | 19.20 |
| Mg | g $kg^{-1}$ | 0.57 | 0.00 | 4.00 |
| Zn | g $kg^{-1}$ | 0.03 | 0.00 | nd |
| Fe | g $kg^{-1}$ | 1.51 | 2.73 | nd |
| Mn | g $kg^{-1}$ | 0.09 | 0.29 | nd |
| Na | g $kg^{-1}$ | 0.01 | 0.00 | 0.13 |
| C | g $kg^{-1}$ | 820.00 | 842.00 | nd |

Abbreviations: SA= Surface area, nd=Undetermined

**Experiment 1**

Table 2.3. Percentage seedling emergence of canola and carinata genotypes at 7 DAP and 28 DAP in good, transitioning, and saline soils in greenhouse experiment 1.

| | Good soil | | Transitioning soil | | Saline soil | |
|---|---|---|---|---|---|---|
| | 7DAP [a] | 28DAP [b] | 7DAP [c] | 28DAP [d] | 7DAP [e] | 28DAP [f] |
| **Genotype** | .....Percentage seedling emergence at seven and twenty-eight days after planting..... | | | | | |
| **Canola** | | | | | | |
| CS2100 | 45.5 bcd | 59.2 d | - | 17.5 b | - | 10.0 b |
| CS2300 | 65.9 a | 57.5 d | - | 17.5 b | - | 9.2 b |
| CS2500 | 44.9 bcd | 67.5 c | 10.0 | 9.2 c | 3.3 | 7.5 b |
| CS2600 | 49.8 b | 80.0 b | - | 17.5 b | - | - |
| DKL14BL | 34.0 de | 59.2 d | - | 19.2 b | - | 10.0 b |
| DKTF91SC | 39.6 bcde | 90.0 a | 10.0 | 19.2 b | 3.3 | 9.2 b |
| DKTF92SC | 48.0 bc | 47.5 e | 10.0 | 20.0 b | - | 7.5 b |
| L140P | 47.7 bc | 70.0 c | - | 29.2 a | - | 10.0 b |
| L233P | 45.0 bcd | 68.3 c | 10.0 | 9.2 c | - | - |
| NCC101S | 30.0 e | 49.2 e | - | 29.2 a | 3.3 | 29.2 a |
| **Carinata** | | | | | | |
| AC120 | 37.5 bcde | 80.0 b | 10 | 7.5 c | - | 9.2 b |
| DH069485 | 34.8 cde | 60.0 d | 20.0 | 7.5 c | 7.5 | 10.0 b |
| DH140251 | 38.2 bcde | 58.3 d | 20.3 | 27.5 a | - | - |
| Mean | 43.0 | 65.1 | 13.4 | 17.7 | 1.4 | 11.2 |
| CV | 19 | 3 | 108 | 9 | 218 | 15 |
| P-value | 0.002 | <0.000 | <0.000 | <0.000 | 0.080 | <0.000 |

Different letters in each column indicate significant differences ($P \leq 0.05$) due to treatments.

Abbreviations: DAP= Days after planting. Seedling emergence recorded in percentage based on eight seeds planted in each pot.

**Experiment 2**

Table 2.4. Percentage of emerged seedlings at 7DAP based on eight seeds, and shoot dry weight plant$^{-1}$, (59DAP), and SPAD (59DAP) for canola and mustard genotypes, in good, transitioning, and saline soils at 59DAP in greenhouse experiment 2.

| | Good soil | | | Transitioning soil | | | Saline soil | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | 7DAP [a] % | SDW [b] g/plant | SPAD [c] nmol/mg$^{-1}$ | 7DAP [a] % | SDW [b] g/plant | SPAD [c] nmol/mg$^{-1}$ | 7DAP [a] % | SDW [b] g/plant | SPAD [c] nmol/mg$^{-1}$ |
| **Canola** | | | | | | | | | |
| NCC101S | 70.4 | 12.4 | 43.0 | 35.1 | 3.9 | 44.8 | 48.2 ab | 3.7 | 52.5 |
| DKTF91SC | 88.7 | 9.2 | 43.6 | 40.9 | 5.4 | 46.9 | 30.2 bcd | 2.6 | 44.1 |
| CS2500 | 65.9 | 8.7 | 40.4 | 19.0 | 2.9 | 40.8 | 20.2 d | 3.1 | 46.4 |
| L140P | 75.7 | 10.4 | 42.3 | 47.9 | 4.7 | 43.0 | 32.2 bcd | 5.0 | 56.2 |
| L233P | 71.4 | 8.7 | 39.9 | 45.0 | 1.6 | 39.0 | 48.2 ab | 3.5 | 44.6 |
| DKL7114BL | 73.6 | 8.3 | 43.5 | 42.6 | 4.7 | 47.0 | 25.2 cd | 2.5 | 45.5 |
| DKTF96SC | 77.5 | 7.6 | 40.4 | 33.8 | 2.6 | 45.6 | 34.2 abcd | 2.3 | 48.5 |
| DKLL82SC | 85.2 | 8.5 | 44.2 | 47.5 | 4.7 | 43.5 | 32.2 bcd | 2.4 | 42.9 |
| DKTFLL21SC | 67.1 | 6.0 | 39.9 | 46.9 | 4.0 | 45.4 | 38.2 abcd | 1.9 | 37.2 |
| DKTF92SC | 66.4 | 8.9 | 41.1 | 36.6 | 2.3 | 40.9 | 42.2 abc | 4.3 | 43.5 |
| **Mustard** | | | | | | | | | |
| A.Cabbage [d] | 55.6 | 12.4 | 45.7 | 38.6 | 4.5 | 46.4 | 29.2 bcd | 2.3 | 47.5 |
| B.Mustard [e] | 83.0 | 10.0 | 37.1 | 44.0 | 3.6 | 40.2 | 33.2 abcd | 5.5 | 43.4 |
| Fb.Mustard [f] | 75.9 | 8.3 | 38.2 | 41.8 | 3.8 | 37.3 | 52.2 a | 5.0 | 34.1 |
| Mean | 73.6 | 9.2 | 41.4 | 40.0 | 3.8 | 43.2 | 36.0 | 3.3 | 44.4 |
| SEM | 5.514 | 0.962 | 2.087 | 5.257 | 0.624 | 1.896 | 4.874 | 0.540 | 1.995 |
| | Analysis of variance (P-value) | | | | | | | | |
| Biochar type (a) | 0.729 | 0.733 | 0.244 | 0.000 | 0.216 | 0.052 | 0.017 | 0.501 | 0.170 |
| Genotypes (b) | 0.266 | 0.476 | 0.864 | 0.468 | 0.645 | 0.589 | 0.051 | 0.305 | 0.246 |
| Factor a*b | 0.705 | 0.608 | 0.995 | 0.161 | 0.720 | 0.849 | 0.433 | 0.786 | 0.916 |

Different letters within each column indicate significant differences (P≤ 0.05) among treatments.
Abbreviations: [a] Percentage seedling emergence at 7DAP, [b] Shoot dry weight plant$^{-1}$,(59DAP) [c] Leaf chlorophyll content (59DAP).
Genotype key: [d] African cabbage, [e] Broadleaf mustard, [f] Florida broadleaf mustard. DAP=Days after planting

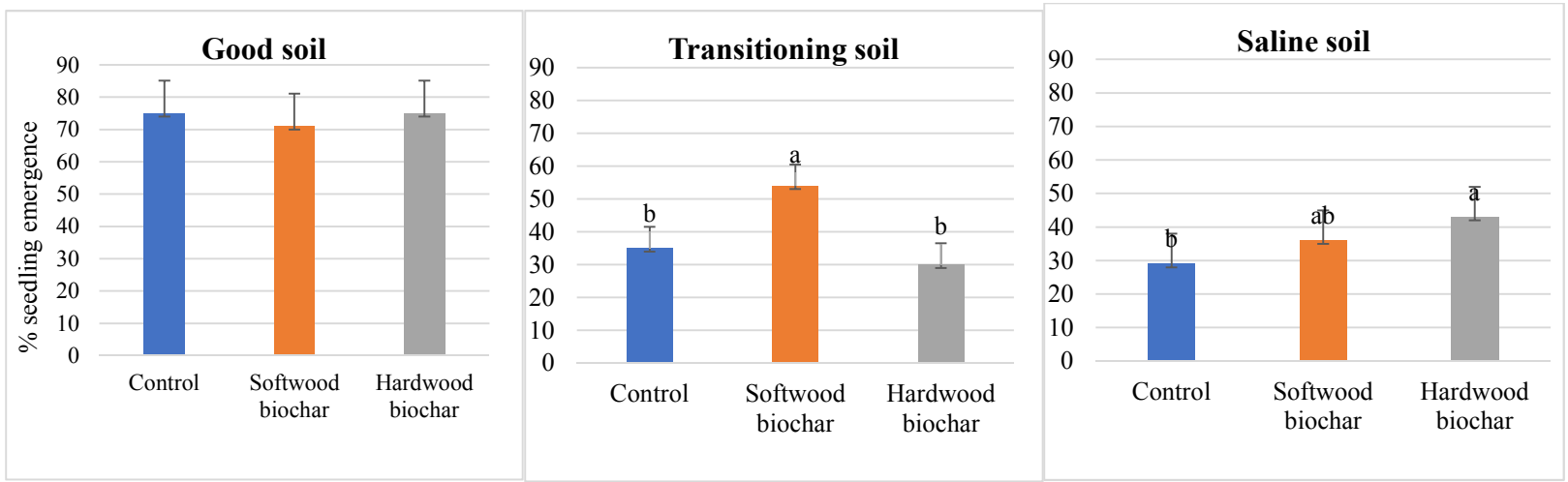


Figure 2.1. Percentage seedling emergence under different soil salt content at 7DAP in experiment 2

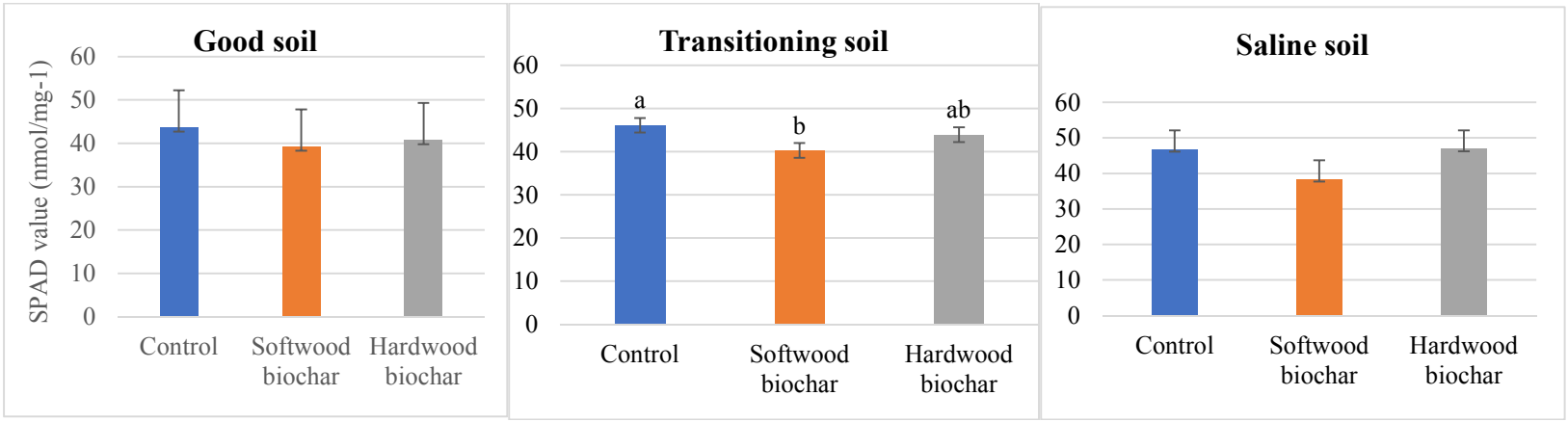


Figure 2.2. Influence of biochar soil amendments on SPAD values under different soil salt conntent at 59DAP in experiment 2

Abbreviations: DAP=Days after planting

**Experiment 3**

Table 2.5. Changes in chemical properties of soil used in experiment 3 after applying biochar in greenhouse experiment 3

| Treatment | pH | EC | OM | $NO_3$ | P | K | Ca | Mg | Na | S |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mmho/cm | % | kg $ha^{-1}$ | ..... g $kg^{-1}$ ..... | | | | | |
| ***Good soil (Control)*** | ***7.3*** | ***0.42*** | ***5.4*** | ***104*** | ***0.01*** | ***0.38*** | ***2.51*** | ***0.42*** | ***0.04*** | ***0.00*** |
| Good soil +Softwood biochar | 6.6 | 1.72 | 5.6 | 82 | 0.02 | 0.31 | 2.08 | 0.46 | 0.10 | 0.28 |
| ***Transitioning soil*** | ***7.1*** | ***5.54*** | ***4.6*** | ***6.7*** | ***0.02*** | ***0.23*** | ***2.20*** | ***1.36*** | ***1.94*** | ***2.96*** |
| Transitioning + Softwood biochar | 6.6 | 5.93 | 9.2 | 0.1 | 0.02 | 0.22 | 1.68 | 1.21 | 1.55 | 1.97 |
| ***Saline soil*** | ***7.4*** | ***7.21*** | ***4.3*** | ***121.0*** | ***0.05*** | ***0.13*** | ***1.96*** | ***1.50*** | ***3.24*** | ***3.15*** |
| Saline soil + Softwood biochar | 6.7 | 6.87 | 9.2 | 5.0 | 0.05 | 0.18 | 1.6 | 1.28 | 2.99 | 2.52 |
| Saline soil + Hardwood biochar | 7.3 | 5.77 | 3.5 | 0.7 | 0.04 | 0.19 | 1.6 | 1.19 | 4.34 | 4.22 |

Key: Italicized and colored values represent the original soil properties before adding either softwood or hardwood biochar

Table 2.6. Effect of biochar type, composted manure rates, and genotypes on seedling emergence (14DAP), number of leaves plant$^{-1}$ (39DAP), SPAD values (39DAP) and shoot dry weight plant$^{-1}$ (39DAP) in greenhouse experiment 3.

| | Good soil | | | | Transitioning soil | | | | Saline soil | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | 14DAP[a] % | NL[b] Plant$^{-1}$ | SPAD[c] nmol/mg$^{-1}$ | SDW[d] g plant$^{-1}$ | 14DAP[a] % | NL[b] Plant$^{-1}$ | SPAD[c] nmol/mg$^{-1}$ | SDW[d] g plant$^{-1}$ | 14DAP[a] % | NL[b] Plant$^{-1}$ | SPAD[c] nmol/mg$^{-1}$ | SDW[d] g plant$^{-1}$ |
| Biochar type (Factor a) | | | | | | | | | | | | |
| Control | 46.4 b | 4.1 b | 41.0 ab | 1.4 a | 37.0 a | 2.1 | 22.1 b | 0.4 | 39.1 | 4.0 a | 33.0 a | 0.8 a |
| Softwood | 34.4 c | 5.0 a | 44.0 a | 1.6 a | 13.1 b | 3.0 | 32.2 a | 0.5 | 32.0 | 3.4 a | 30.0 a | 0.8 a |
| Hardwood | 71.0 a | 4.1 b | 36.1 b | 1.1 b | 19.0 b | 2.1 | 22.1 b | 0.6 | 36.1 | 1.1 b | 8.1 b | 0.2 b |
| Composted manure rates (Factor b) | | | | | | | | | | | | |
| 0 | 41.1 b | 3.9 b | 40.3 a | 1.2 b | 7.0 c | 0.4 c | 8.4 c | 0.1 c | 9.6 c | 0.7 b | 8.1 b | 0.9 a |
| 30 | 55.0 a | 5.3 a | 45.2 a | 1.7 a | 21.0 b | 2.2 b | 26.0 b | 0.5 b | 43.1 b | 3.4 a | 31.0 a | 0.8 a |
| 50 | 57.1 a | 3.7 b | 34.1 b | 1.1 b | 41.0 a | 4.1 a | 33.5 a | 0.9 a | 55.1 a | 3.9 a | 29.2 a | 0.2 b |
| Genotypes (Factor c) | | | | | | | | | | | | |
| A.cabbage | 52.0 ab | 5.1 a | 40.4 ab | 1.4 b | 24.8 a | 3.2 a | 28.7 a | 0.6 a | 35.0 | 2.8 | 24.8 | 0.5 |
| B.mustard | 50.9 ab | 3.7 b | 35.1 b | 1.1 b | 30.3 a | 2.2 b | 26.9 a | 0.6 a | 35.7 | 2.5 | 21.7 | 0.6 |
| DKTF91SC | 57.4 a | 4.5 ab | 44.6 a | 1.7 a | 26.0 a | 2.0 b | 24.0 ab | 0.5 a | 35.2 | 2.7 | 23.7 | 0.5 |
| NCC101S | 43.4 b | 4.0 b | 38.7 ab | 1.3 b | 10.4 b | 1.4 b | 18.0 b | 0.3 b | 37.2 | 3.0 | 23.4 | 0.7 |
| Analysis of variance (P-value) for main effects | | | | | | | | | | | | |
| Factor a | <0.000 | 0.052 | 0.022 | 0.000 | <0.000 | 0.087 | 0.007 | 0.155 | 0.263 | <0.000 | <0.000 | <0.000 |
| Factor b | 0.000 | <0.000 | 0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 |
| Factor c | 0.023 | 0.015 | 0.040 | 0.002 | <0.000 | 0.000 | 0.031 | 0.004 | 0.973 | 0.751 | 0.877 | 0.102 |
| Analysis of variance (P-value) for interactions | | | | | | | | | | | | |
| Factor a * b | 0.002 | 0.082 | 0.257 | 0.051 | 0.189 | <0.000 | <0.000 | <0.000 | 0.171 | <0.000 | <0.000 | <0.000 |
| Factor a * c | 0.223 | 0.167 | 0.023 | 0.029 | 0.003 | 0.007 | 0.041 | 0.004 | 0.002 | 0.018 | 0.026 | <0.000 |
| Factor b * c | 0.722 | 0.478 | 0.000 | <0.000 | 0.003 | 0.000 | 0.312 | 0.121 | 0.527 | 0.035 | 0.092 | 0.233 |
| Factor a * b *c | 0.211 | 0.408 | 0.058 | 0.202 | 0.225 | 0151 | 0.328 | 0.555 | 0.916 | 0.035 | 0.035 | 0.084 |

Mean values followed by different lower-case letters within the column represent significant differences at P<0.05. No letters are shown where there are no significant differences.

Abbreviations: [a] Percentage of emerged seedlings (14DAP) based on eight seeds, [b] Number of leaves plant$^{-1}$, [c] Leaf chlorophyll content (SPAD values), and [d] Shoot dry weight plant$^{-1}$ at 39 DAP

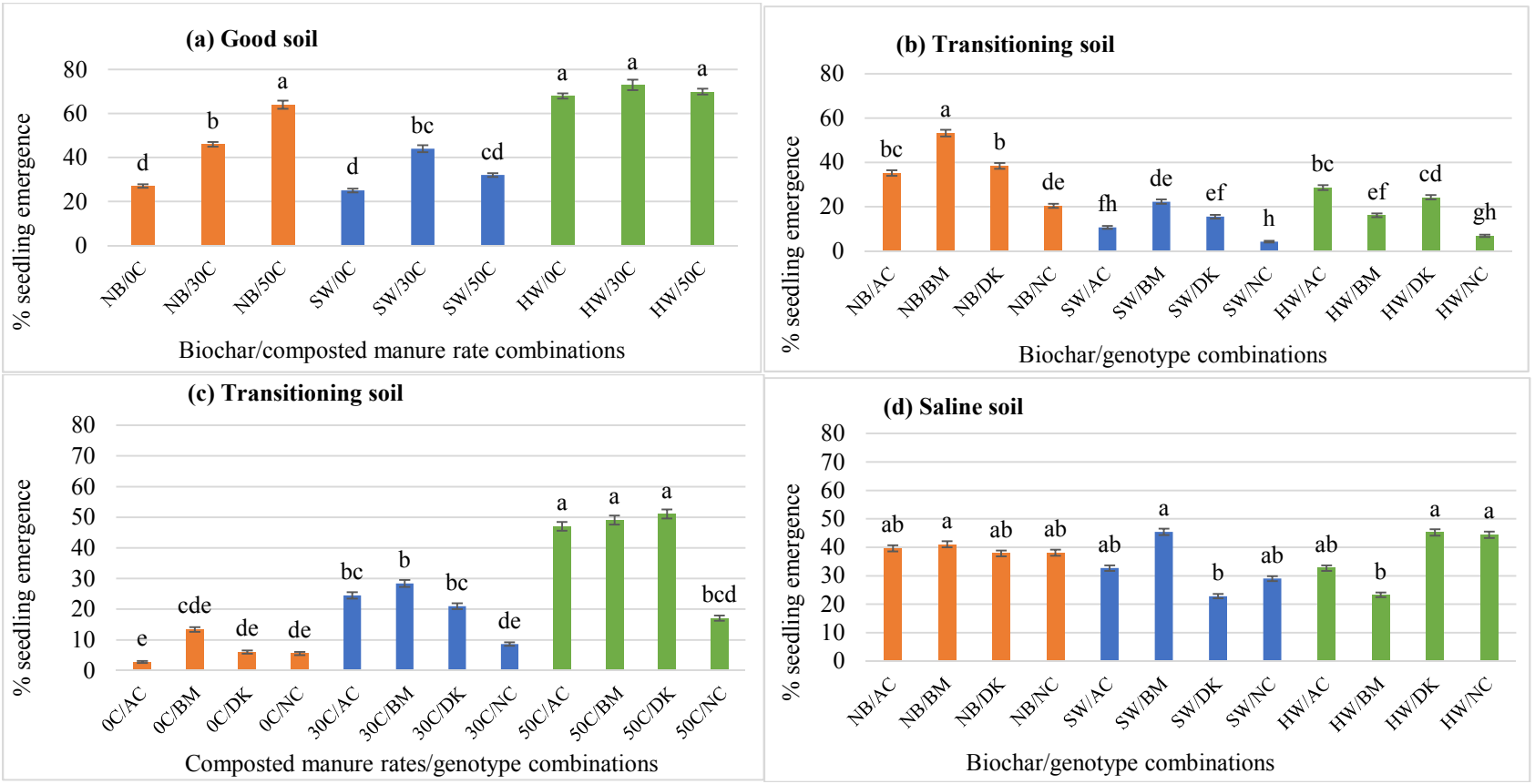


Figure 2.3. Effect of biochar, composted manure rates and genotypes interactions on percentage of emerged seedlings of canola and mustard genotypes at 14DAP in good (a), transitioning (b-c), and saline soils (d) in experiment 3

Means are averaged over each treatment. Bars sharing different lower-case letters represent a significant difference at P≤0.05
Treatment key: Biochar levels: NB= no biochar, SW= softwood biochar, HW = hardwood biochar. Composted manure levels: 0C, 30C, and 50C = 0%, 30%, and 50% composted manure application rates. Genotypes: AC= African cabbage, BM= Brown mustard, DK= DKTF91SC, NC= NCC101S.

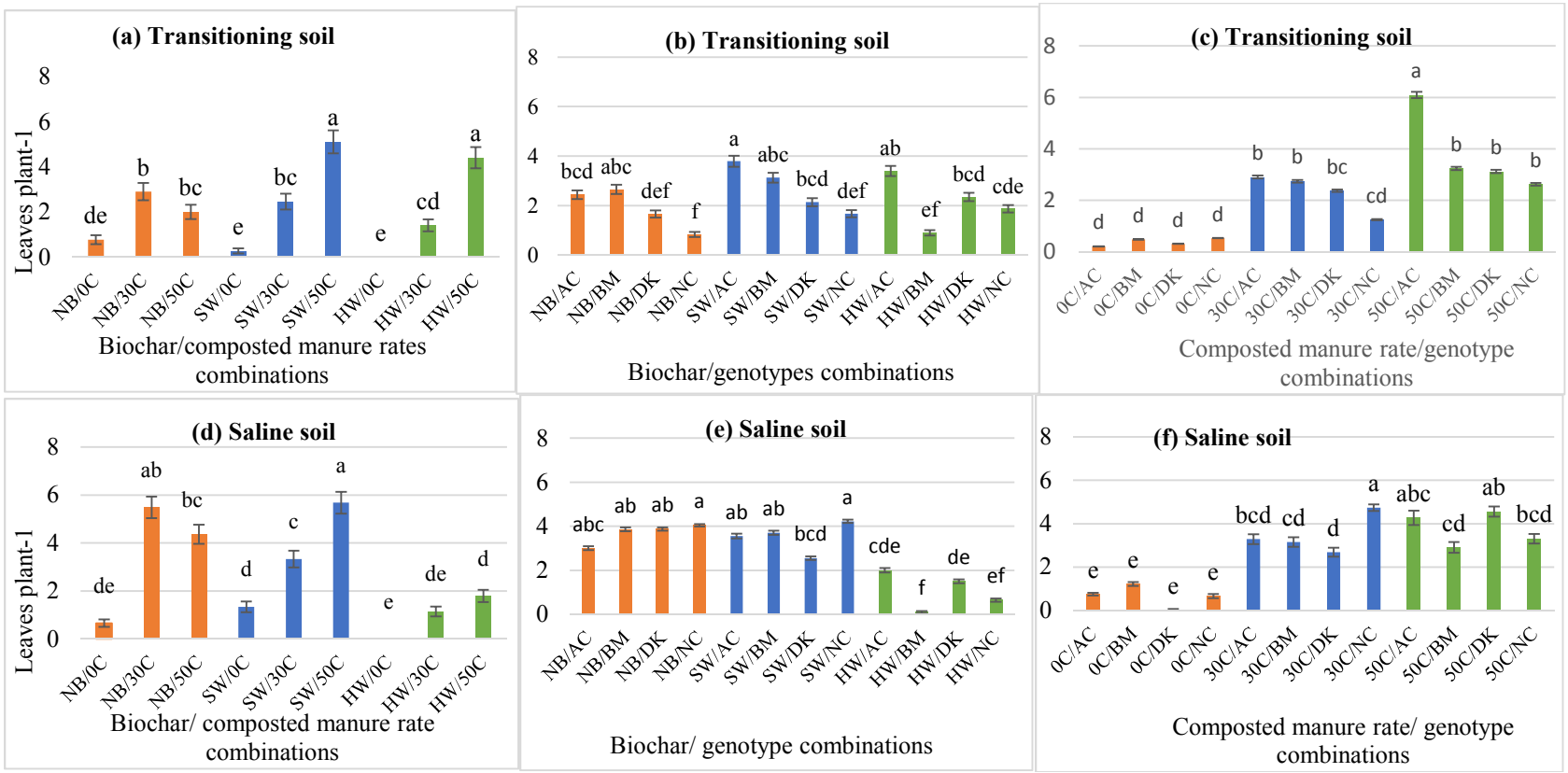


Figure 2.4. Effects of biochar, composted manure rates and genotypes interactions on number of leaves plant$^{-1}$ of canola and mustard genotypes in transitioning soil (a-c) and saline soil (d-f) at 39 DAP in greenhouse experiment 3.

Bars sharing different lower-case letters represent a significant difference at P≤0.05
Treatment key: Biochar levels: NB= no biochar, SW= softwood biochar, HW = hardwood biochar. Composted manure levels: 0C, 30C, and 50C = 0%, 30%, and 50% composted manure application rates. Genotypes: AC= African cabbage, BM= Brown mustard, DK= DKTF91SC, NC= NCC101S

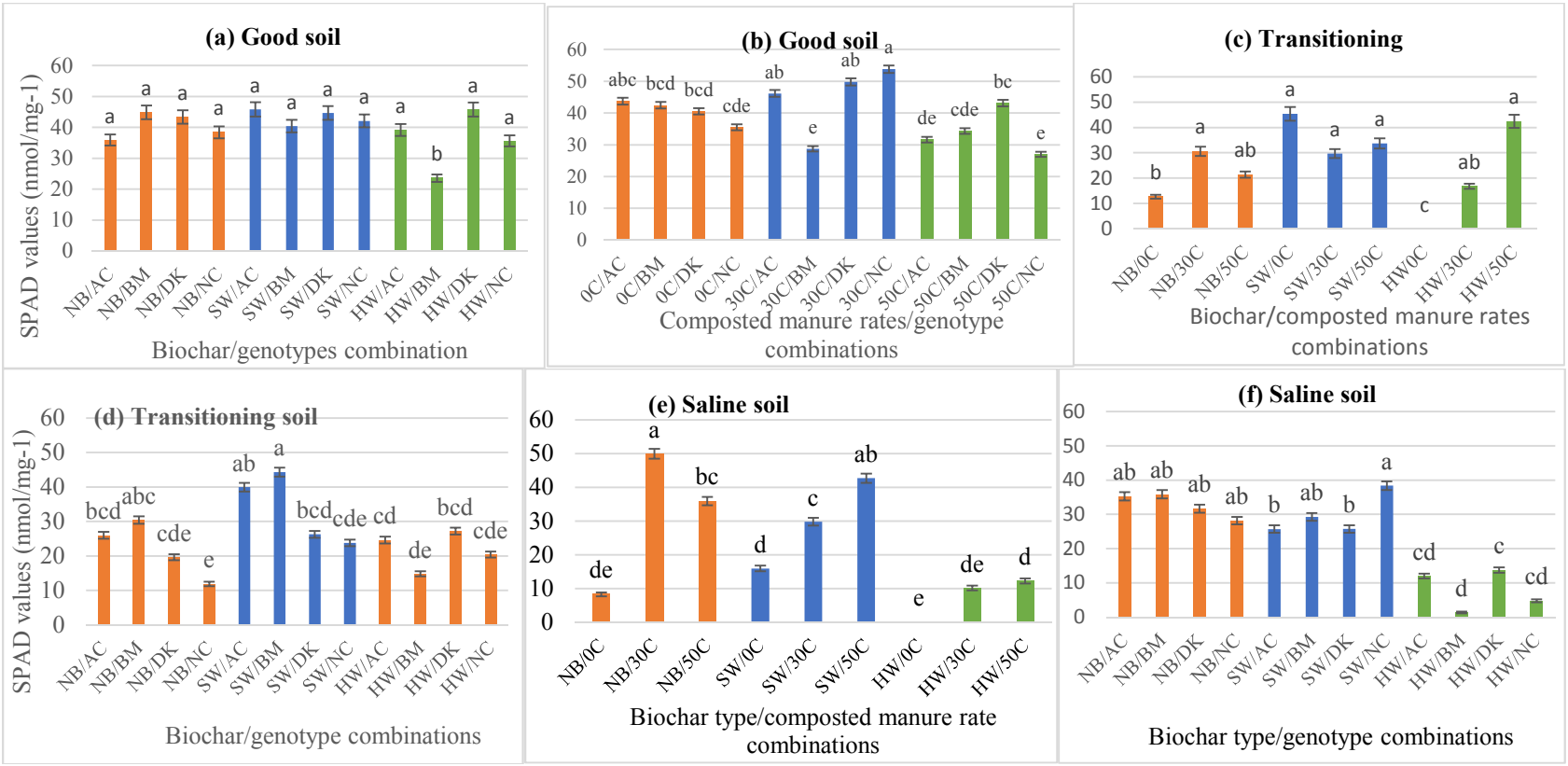


Figure 2.5. Effects of biochar, composted manure rates and genotypes on leaf chlorophyll content (SPAD values) of canola and mustard genotypes in good (a-b), transitioning soils (c-d) and saline soil (e-f) at 39 DAP in greenhouse experiment 3.

Bars sharing different lower-case letters represent a significant difference at P≤0.05
Treatment key: Biochar levels: NB= no biochar, SW= softwood biochar, HW = hardwood biochar. Composted manure levels: 0C, 30C, and 50C = 0%, 30%, and 50% composted manure application rates. Genotypes: AC= African cabbage, BM= Brown mustard, DK= DKTF91SC, NC= NCC101S

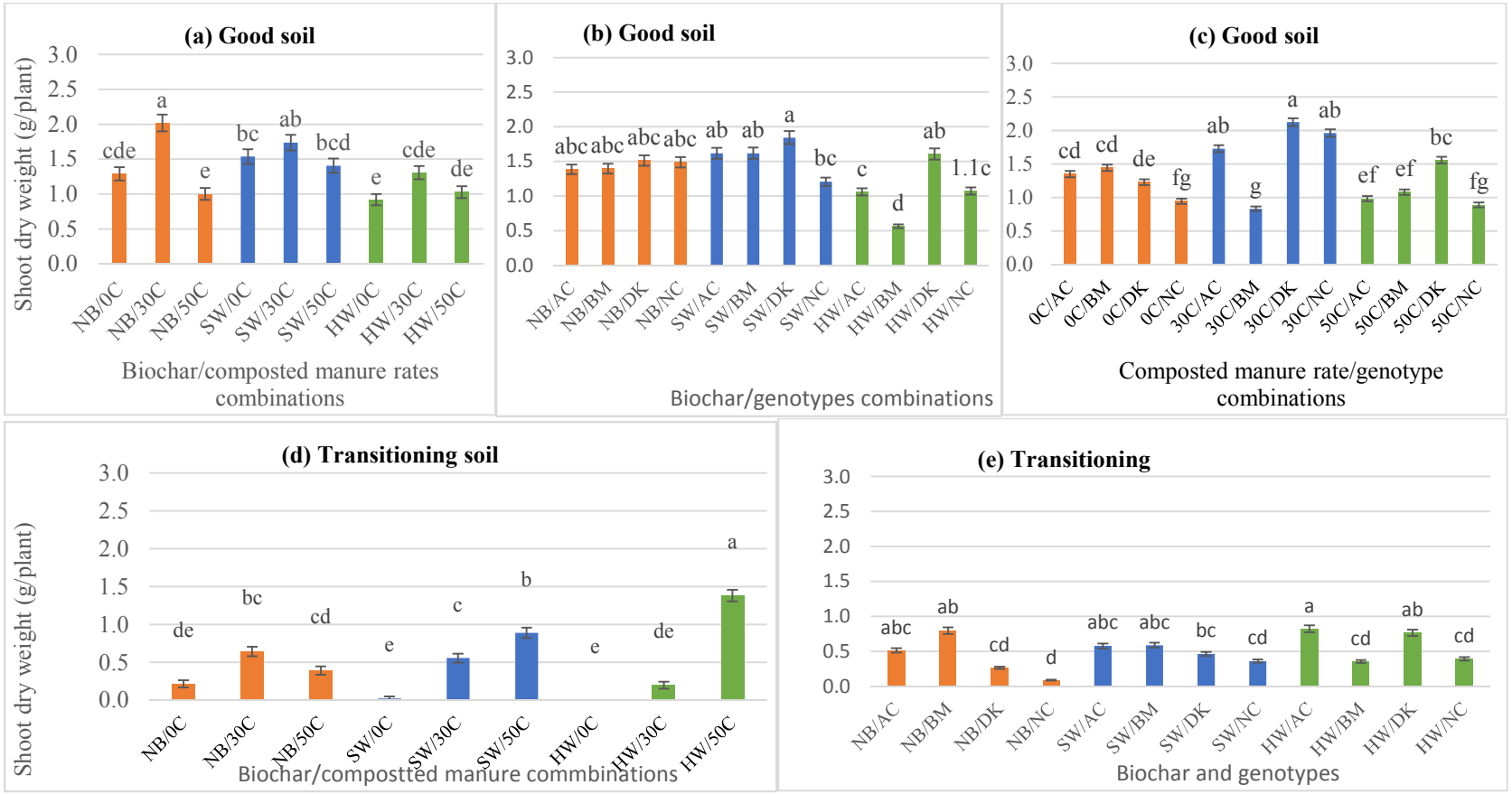


Figure 2.6. Effect of biochar, composted manure rate, and genotypes on shoot dry weight plant$^{-1}$ of canola and mustard genotypes in good (a-c) and transitioning soil (d-e) measured at 39 DAP in greenhouse experiment 3

Bars sharing different lower-case letters represent a significant difference at $P \leq 0.05$
Treatment key: Biochar levels: NB= no biochar, SW= softwood biochar, HW = hardwood biochar. Composted manure levels: 0C, 30C, and 50C = 0%, 30%, and 50% composted manure application rates. Genotypes: AC= African cabbage, BM= Brown mustard, DK= DKTF91SC, NC= NCC101S

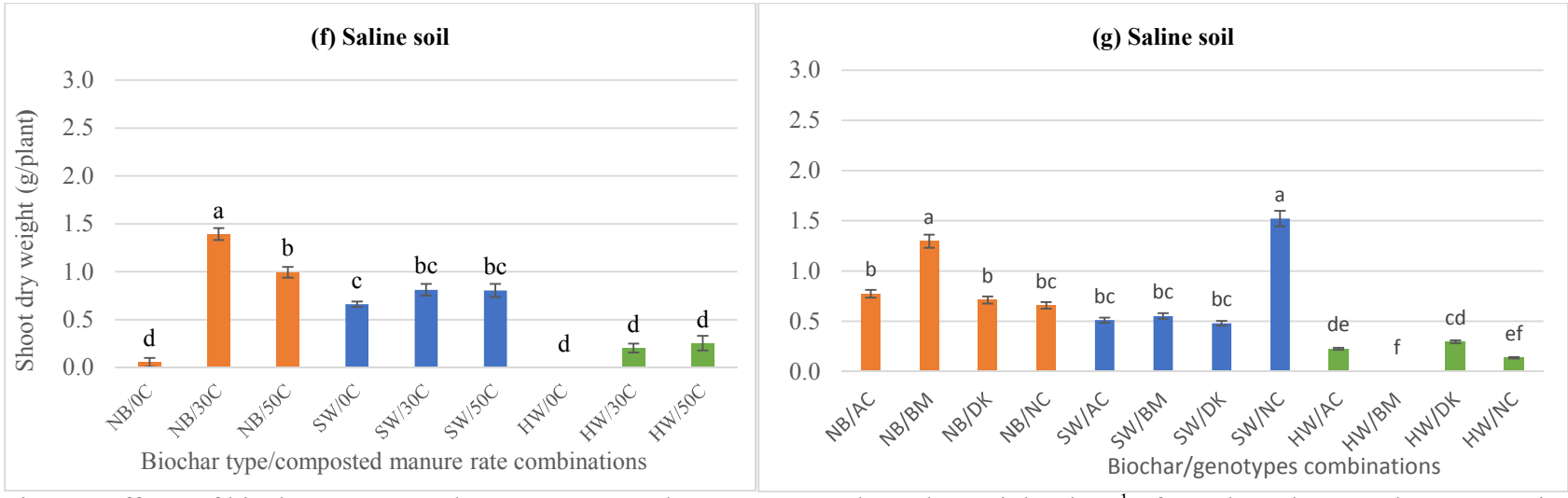


Fig 2.6. Effects of biochar, composted manure rates and genotypes on shoot dry weight plant$^{-1}$ of canola and mustard genotypes in saline soil (a-b) at 39 DAP in greenhouse experiment 3.

Bars sharing different lower-case letters represent a significant difference at P≤0.05
Treatment key: Biochar levels: NB= no biochar, SW= softwood biochar, HW = hardwood biochar. Composted manure levels: 0C, 30C, and 50C = 0%, 30%, and 50% composted manure application rates. Genotypes: AC= African cabbage, BM= Brown mustard, DK= DKTF91SC, NC= NCC101S.

# APPENDICES

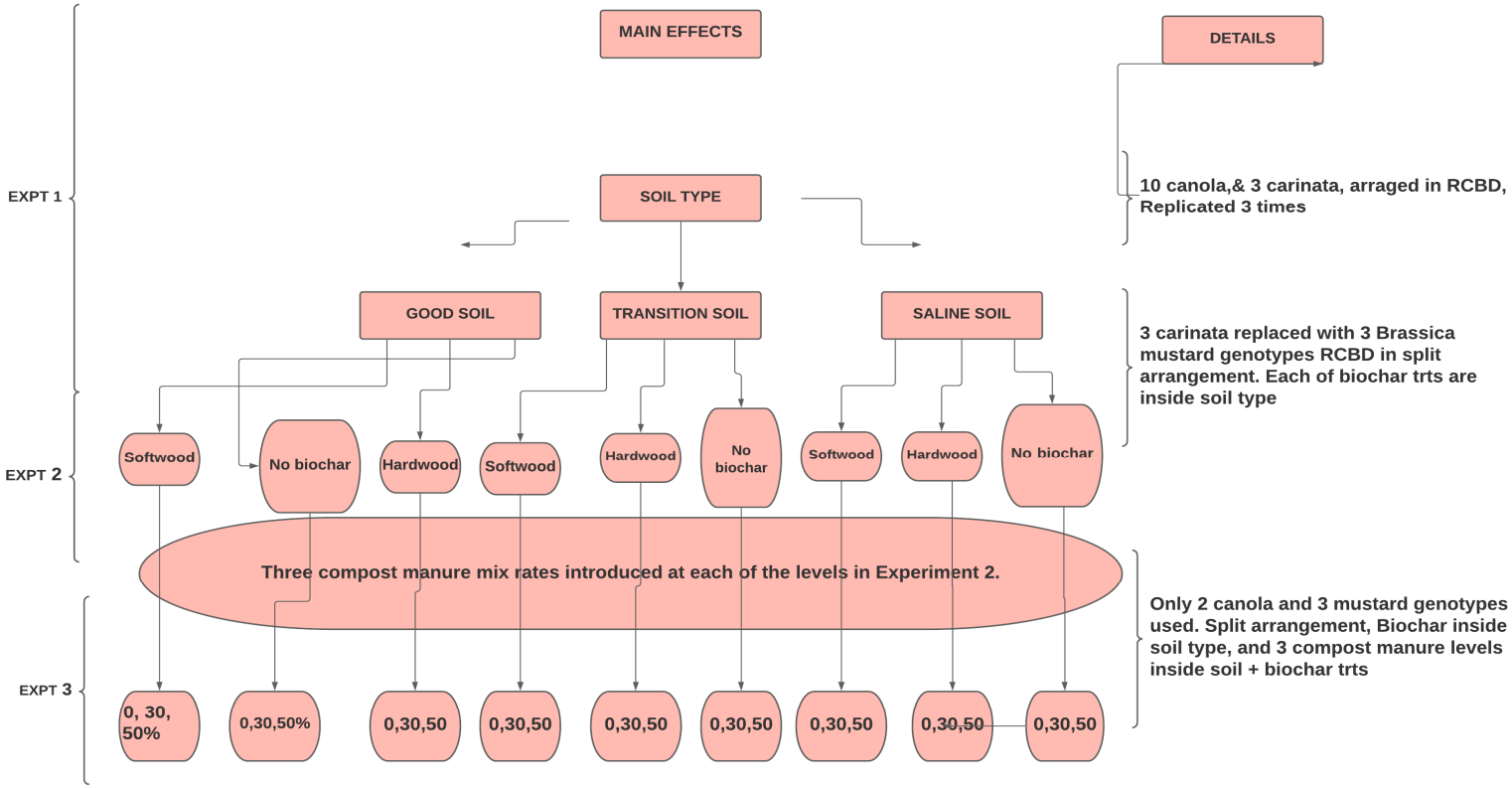


Fig 2A.1. The diagrammatical flow of the greenhouse experiments 1, 2, and 3.

Table 2A.1. ANOVA for effect of composted manure, biochar, and genotypes on canola and mustard seedling emergence (7 and 14DAP), leaf plant$^{-1}$ SPAD and shoot dry weight 39 DAP in greenhouse experiment 3

| Source | | 7DAP [a] | 14DAP [b] | NL [c] | SPAD [d] | SDW [e] |
|---|---|---|---|---|---|---|
| Unit | | % | % | count | nmol/mg$^{-1}$ | g |
| | Df | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) | Pr(>F) |
| Block | 2 | 0.935 | 0.863 | 0.731 | 0.843 | 0.652 |
| Soil type | 2 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 |
| Composted manure | 2 | <0.000 | <0.000 | <0.000 | <0.000 | 0.000 |
| Genotype | 3 | 0.121 | 0.003 | 0.001 | 0.120 | 0.279 |
| Soil type: Composted manure | 4 | 0.000 | 0.000 | 0.000 | <0.000 | 0.000 |
| Soil type: Genotype | 6 | 0.005 | 0.031 | 0.114 | 0.111 | 0.000 |
| Composted manure: Genotype | 6 | 0.576 | 0.250 | 0.066 | 0.010 | 0.047 |
| Soil type: Composted manure: Genotype | 12 | 0.710 | 0.331 | 0.028 | 0.051 | 0.001 |
| $R^2$ | | 0.321 | 0.298 | 0.299 | 0.266 | 0.329 |
| Residuals | 934 | 934 | 932 | 759 | 729 | 759 |
| Block | 2 | 0.936 | 0.873 | 0.759 | 0.849 | 0.662 |
| Soil type | 2 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 |
| Biochar | 2 | <0.000 | 0.000 | 0.000 | 0.000 | <0.000 |
| Genotype | 3 | 0.099 | 0.007 | 0.004 | 0.137 | 0.271 |
| Soil type: Biochar | 4 | <.0000 | 0.000 | 0.000 | 0.000 | <0.000 |
| Soil type: Genotype | 6 | 0.003 | 0.038 | 0.151 | 0.166 | 0.001 |
| Biochar : Genotype | 6 | 0.002 | 0.011 | 0.008 | 0.001 | 0.000 |
| Soil type: Biochar: Genotype | 12 | 0.795 | 0.274 | 0.690 | 0.691 | 0.016 |
| $R^2$ | | 0.313 | 0.237 | 0.202 | 0.231 | 0.303 |
| Residuals | 934 | 934 | 932 | 959 | 729 | 758 |

Abbreviations: [a, b] Percentage seedling emergence at 7 and 14DAP, [c] Number of leaves plant$^{-1}$, [d] leaf chlorophyll content (SPAD values), [e] Shoot dry weight plant$^{-1}$ at 39DAP. DAP=Days after planting.

Table 2A.2. Effect of biochar (softwood, hardwood, and no biochar) and composted manure mix rates (0,30,50%) on seedling emergence of canola and mustard genotypes in good, transitioning, and saline soil at 14DAP in greenhouse experiment 3.

| | Good soil | | | Transitioning soil | | | Saline soil | | |
|---|---|---|---|---|---|---|---|---|---|
| Composted manure rates | 0% | 30% | 50% | 0% | 30% | 50% | 0% | 30% | 50% |
| **Factor a** | | | | | | | | | |
| No biochar (control) | 29.0 b | 46.8 b | 64.4 a | 18.2 a | 39.1 a | 53.5 a | 13.4 a | 47.3 ab | 57.1 |
| Softwood | 26.0 b | 44.3 c | 33.1 b | 2.1 b | 10.3 b | 27.5 b | 10.2 ab | 32.1 b | 57 |
| Hardwood | 69.1 a | 73.3 a | 71.1 a | 0.7 b | 13.1 b | 41.1 ab | 5.1 b | 50.1 a | 52 |
| **Factor b** | | | | | | | | | |
| A.cabbage | 39.1 | 56.2 ab | 61.1 | 2.8 b | 24.5 a | 47.1 a | 6.5 bc | 43.2 | 62.5 |
| B.mustard | 46.1 | 48.9 b | 58.2 | 13.4 a | 28.4 a | 49.1 a | 15.0 a | 45.7 | 55.4 |
| DKTF91SC | 45.1 | 66.0 a | 61.6 | 6.0 ab | 21.0 a | 51.1 a | 4.3 c | 46.6 | 54.9 |
| NCC101S | 34.4 | 48.1 b | 47.5 | 5.6 b | 8.6 b | 17.1 b | 12.5 ab | 36.6 | 46.8 |
| Mean | 41 | 55.3 | 57.1 | 0.41 | 21.1 | 41.1 | 9.6 | 43.1 | 55.1 |
| | Analysis of variance (P-value) | | | | | | | | |
| Factor a | <0.000 | 0.001 | <0.000 | <0.000 | <0.000 | 0.004 | 0.035 | 0.073 | 0.842 |
| Tactor b | 0.399 | 0.119 | 0.278 | 0.036 | 0.002 | 0.003 | 0.015 | 0.709 | 0.583 |
| Factor a * b | 0.337 | 0.969 | 0.065 | 0.018 | <0.000 | 0.183 | 0.023 | <0.000 | 0.349 |

Genotypes Key: A.cabbage = African cabbage, B.mustard = Brown mustard.

Table 2A.3. Effect of biochar (softwood, hardwood, and no biochar), and composted manure mix rates (0,30,50%) on number of leaves plant$^{-1}$ of canola and mustard genotypes in good, transitioning, and saline soil measured at 39 DAP in greenhouse experiment 3.

| | Good soil | | | Transitioning soil | | | Saline soil | | |
|---|---|---|---|---|---|---|---|---|---|
| Composted manure rates | 0% | 30% | 50% | 0% | 30% | 50% | 0% | 30% | 50% |
| **Factor (a)** | | | | | | | | | |
| No biochar (control) | 4.1 | 5.6 a | 2.8 b | 0.8 a | 2.9 a | 2.0 b | 0.6 ab | 5.5 a | 4.4 a |
| Softwood | 3.9 | 5.8 a | 4.6 a | 0.3 ab | 2.5 ab | 5.1 a | 1.3 a | 3.3 b | 5.7 a |
| Hardwood | 3.8 | 4.5 b | 3.9 ab | 0.0 b | 1.4 b | 4.4 a | 0.0 b | 1.1 c | 1.8 b |
| **Factor (b)** | | | | | | | | | |
| A.cabbage | 5.0 a | 6.1 a | 4.1 | 0.2 | 2.9 a | 6.1 a | 0.7 ab | 3.3 ab | 4.3 ab |
| B.mustard | 3.9 ab | 4.4 b | 2.7 | 0.5 | 2.7 a | 3.2 b | 1.2 a | 3.2 b | 2.9 b |
| DKTF91SC | 3.8 ab | 5.3 ab | 2.7 | 0.3 | 2.4 ab | 3.1 b | 0.0 b | 2.7 b | 4.6 a |
| NCC101S | 3.2 b | 5.3 ab | 3.4 | 0.5 | 1.2 b | 2.6 b | 0.7 ab | 4.7 a | 3.3 ab |
| Mean | 3.9 | 5.3 | 3.7 | 0.4 | 2.2 | 3.7 | 0.7 | 3.4 | 3.8 |
| | | | | Analysis of variance (P-value) | | | | | |
| Factor a | 0.889 | 0.025 | 0.073 | 0.024 | 0.020 | <0.000 | 0.008 | <0.000 | <0.000 |
| Tactor b | 0.018 | 0.050 | 0.343 | 0.666 | 0.039 | 0.000 | 0.096 | 0.064 | 0.270 |
| Factor a * b | 0.411 | 0.066 | 0.098 | 0.522 | 0.048 | 0.075 | 0.239 | 0.002 | 0.290 |

Genotypes Key: A.cabbage = African cabbage, B.mustard = Brown mustard

Different letters in each column indicate significant differences at P≤ 0.05 due to treatments.

Table 2A.4. Effect of biochar (softwood, hardwood, and no biochar), and composted manure mix rates (0,30,50%) on shoot dry weight (g plant$^{-1}$) of canola and mustard genotypes in good, transitioning, and saline soil measured at 39 DAP in greenhouse experiment 3.

| | Good soil | | | Transitioning soil | | | Saline soil | | |
|---|---|---|---|---|---|---|---|---|---|
| Composted manure rates | 0% | 30% | 50% | 0% | 30% | 50% | 0% | 30% | 50% |
| Factor a | | | | | | | | | |
| Control | 1.9 a | 2.0 a | 1.0 | 0.2 a | 0.64 a | 0.4 c | 0.1 b | 1.4 a | 1.1 a |
| Softwood | 1.5 a | 1.7 a | 1.4 | 0.1 b | 0.5 a | 0.8 b | 0.7 a | 0.8 b | 0.8 a |
| Hardwood | 0.9 b | 1.3 b | 1.0 | 0.0 b | 0.2 b | 1.4 a | 0.0 b | 0.2 c | 0.2 b |
| Factor b | | | | | | | | | |
| A.cabbage | 1.3 a | 1.7 a | 1.0 b | 0.0 b | 0.7 a | 1.2 a | 0.1 b | 0.9 | 0.6 |
| B.mustard | 1.4 a | 0.8 b | 1.1 ab | 0.3 a | 0.7 a | 0.8 ab | 0.1 b | 0.9 | 0.7 |
| DKTF91SC | 1.2 ab | 2.1 a | 1.5 a | 0.1 b | 0.4 ab | 0.9 ab | 0.0 b | 0.6 | 0.7 |
| NCC101S | 0.9 b | 1.9 a | 0.9 b | 0.1 b | 0.2 b | 0.6 b | 0.6 a | 0.9 | 0.7 |
| Mean | | | 1.1 | | 0.5 | | 0.2 | 0.8 | 0.7 |
| | Analysis of variance (P-value) | | | | | | | | |
| Factor a | 0.001 | 0.000 | 0.181 | 0.047 | 0.000 | <0.000 | <0.000 | <0.000 | 0.000 |
| Tactor b | 0.050 | <0.000 | 0.075 | 0.057 | 0.001 | 0.119 | <0.000 | 0.465 | 0.902 |
| Factor a * b | 0.012 | 0.053 | 0.005 | 0.031 | 0.022 | 0.277 | <0.000 | 0.001 | 0.048 |

Genotypes Key: A.cabbage = African cabbage, B.mustard = Brown mustard
Different letters in each column indicate significant differences at P≤ 0.05 due to treatments.

Table 2A.5. Effect of biochar (softwood, hardwood, and no biochar) and composted manure mix rates (0,30,50%) on leaf chlorophyll content (SPAD values) in good, transitioning, and saline soils measured at 39 DAP in greenhouse experiment 3

| | .....Good soil..... | | | ..... Transitioning soil..... | | | .....Saline soil..... | | |
|---|---|---|---|---|---|---|---|---|---|
| CM | Control | Softwood | Hardwood | control | softwood | Hardwood | Control | Softwood | Hardwood |
| 0 | 40.1 ab | 48.3 a | 34.1 | 13.1 b | 45.4 | 0.0 a | 8.3 c | 16.1 c | 0.0 b |
| 30 | 48.5 a | 45.2 a | 42.1 | 31.0 a | 33.7 | 16.8 b | 50.1 a | 30.1 b | 10.1 a |
| 50 | 33.1 b | 37.1 b | 32.7 | 21.4 ab | 29.7 | 42.4 a | 36.1 b | 43.1 a | 12.2 a |
| mean | 41.0 | 43.5 | 36.1 | 21.6 | 32.2 | 21.7 | 33.0 | 30.0 | 8.1 |
| SEM | 5.314 | 4.238 | 6.420 | 6.523 | 6.339 | 5.140 | 5.487 | 5.488 | 4.922 |
| P-value | 0.003 | 0.013 | 0.197 | 0.012 | 0.639 | <0.000 | <0.000 | <0.000 | 0.024 |

CM= Composted manure rates

Table 2A.6. Effect of biochar (softwood, hardwood, and no biochar) and genotypes on leaf chlorophyll content in good, transitioning, and saline soils measured at 39 DAP in greenhouse experiment 3

| | .....Good soil..... | | | ..... Transitioning soil..... | | | .....Saline soil..... | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Control | Softwood | Hardwood | Control | Softwood | Hardwood | Control | Softwood | Hardwood |
| A.CABBAGE | 35.9 | 45.8 | 39.1 a | 26.1 a | 40.1 ab | 24.6 | 35.0 | 26.0 | 12.1 |
| B.MUSTARD | 44.9 | 40.4 | 23.5 b | 30.4 a | 44.3 a | 14.9 | 35.0 | 29.2 | 1.3 |
| DKTF91SC | 43.3 | 44.6 | 45.7 a | 19.6 ab | 26.3 b | 27.2 | 32.0 | 26.1 | 14.0 |
| NCC101S | 38.3 | 42.1 | 35.6 ab | 11.8 b | 23.8 b | 20.4 | 28.1 | 38.1 | 4.8 |
| SEM | 6.102 | 4.893 | 7.413 | 7.532 | 7.319 | 6.235 | 6.336 | 6.337 | 5.684 |
| P-value | 0.268 | 0.492 | 0.005 | 0.036 | 0.033 | 0.219 | 0.445 | 0.127 | 0.078 |