### GENETIC DIFFERENCES OF SALINITY TOLERANCE IN DRY PEA

A Dissertation Submitted to the Graduate Faculty of the North Dakota State University of Agriculture and Applied Science

By

Emmanuella Bredu

In Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE

> Major Department: Plant Science

> > June 2024

Fargo, North Dakota

## North Dakota State University Graduate School

#### Title

#### GENETIC DIFFERENCES OF SALINITY TOLERANCE IN DRY PEA

By

Emmanuella Bredu

The Supervisory Committee certifies that this *disquisition* complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Qi Zhang Ph.D

Chair

Nonoy Bandillo Ph.D

Thomas DeSutter Ph.D.

Approved:

06/13/2024

Date

Richard Horsley Ph.D

Department Chair

#### ABSTRACT

This study aimed to establish a sulfate-salinity screening protocol for dry pea (*Pisum sativum* L.) and assess salinity responses from two North American pea germplasms. 'Agassiz' grown in cone-containers (410 or 556 ml) was irrigated with a sulfate salt mixture at 0 - 20 dS m<sup>-1</sup> for four weeks with phenotypic data sampled once weekly. The results showed that a 4-week saline exposure at 10 dS m<sup>-1</sup> with plants grown in the 556 ml containers can be used for salinity screening. When plants from two pea germplasms (North Dakota State University and United States Department of Agriculture) were exposed to the aforementioned saline condition, salinity reduced genetic diversity in both germplasms. Thirty-one genotypes with high performance under either growing condition were identified in the study, in which NDP080169, PI\_117998 PSP and PI\_270536 PSP showed good growth under both the stressed and non-stressed conditions.

Keywords: *Pisum sativum*, salt, sodium sulfate, magnesium sulfate, heritability, principal component analysis

#### ACKNOWLEDGMENTS

I would thank N.D. Specialty Crop Block Grant Project (Award no. 18-265) and the USDA Hatch project (no. ND01509) for funding this research. My profound gratitude goes to Dr. Qi Zhang, my primary adviser, who gave me the privilege of pursuing my master's in Horticulture. I am thankful for her patience, advice, guidance, and support, which have brought me this far. The knowledge and experience I have earned are invaluable. I would also like to thank the committee members, Dr. Nonoy Bandillo and Dr. Thomas DeSutter, for their immense contributions and feedback throughout the experiment and preparation of this thesis. Dr. Sikiru A. Atanda and Dr. Bright Adu helped me greatly in data analysis. I am also grateful to my course instructors, friends, and colleagues in this institution who taught, trained, and motivated me in various ways. Many thanks to my parents and family for their love and support.

## DEDICATION

I dedicated this to the Almighty God and my family.

ABSTRACT	iii
ACKNOWLEDGMENTS	iv
DEDICATION	v
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	xii
LIST OF APPENDIX TABLES	xiii
LITERATURE REVIEW	1
Pea	1
Pea Production	2
Soil Salinity	2
Salinity and its causes	2
Salinity in North Dakota	3
Salinity effects on plants	3
Salinity Management	5
Cultural practices	5
Use of tolerant plants	6
References	8
DRY PEA GROWTH IN RESPONSE TO SULFATE SALINITY AND CONTAINER SIZE UNDER THE CONTROLLED ENVIRONMENT	19
Abstract	19
Introduction	19
Materials and Methods	21
Result and Discussions	23
Soil electrical conductivity (EC <sub>e</sub> ) and soil pH as affected by container size (CS) and salt irrigation concentration (SI) and duration of salt irrigation	23

## TABLE OF CONTENTS

Plant phenotypic responses as affected by CS, SI, W, and their interactions	25
Correlations	35
Conclusions	39
References	39
GENOTYPE DIFFERENCES IN TOLERANCE TO SULFATE-SALINITY IN DRY PEA	48
Abstract	48
Introduction	48
Materials and Methods	50
Results and Discussions	53
Soil analysis	53
Heritability $(H^2)$ and genetic variation of phenotypic traits under non-saline and saline conditions	53
Principal component analysis (PCA) and cluster analysis of phenotypic traits under non-saline and saline conditions	58
Conclusions	75
References	75
APPENDIX	82

## LIST OF TABLES

<u>Table</u>		Page 1
1.	Description of plant visual damage using a 1 – 5 scale score	23
2.	Soil ECe and pH as affected by container size, salt irrigation concentration, duration of saline exposure, and their interactions.	25
3.	Root growth of pea seedlings as affected by container size, salt concentration, duration of saline exposure, and their interactions.	29
4.	Above ground growth and visual damage rating of pea seedlings as affected by container size, salt concentration, duration of saline exposure, and their interactions.	30
5.	Pearson correlation coefficient analyses between soil electrical conductivity (ECe), soil pH, and phenotypic traits in pea seedlings.	38
6.	Description of plant visual damage using a 1 – 5 scale score	52
7.	The variation (mean and ranges estimated based on the best linear unbiased predictor) of phenotypic traits and heritability of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.	55
8.	The variation (mean and ranges estimated based on the best linear unbiased predictor) of phenotypic traits and heritability of a USDA pea germplasm [including 199 collection and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.	56
9.	Recommended genotypes from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)], selected based on high performance from phenotypic traits highly associated with the first two principal components under non-saline and saline conditions.	67
10.	Recommended genotypes from a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)], selected based on high performance from phenotypic traits highly associated with the first two principal components under non-saline and saline conditions.	67
11.	Mean values of phenotypic traits of each cluster group of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.	73
12.	Mean values of phenotypic traits of each cluster group of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.	74

## LIST OF FIGURES

## <u>Figure</u>

1.	Total above-ground fresh weight (g) (A), absolute water content (g) (B), and visual damage (1 – 5 scale, 1 = healthy plants and 5 = dead plants) (C) as affected by container size (big and small) and duration of saline exposure (week). Uppercase letters indicate differences between container sizes in the same week at $P \le 0.05$ . Lowercase letters indicate differences among weeks in the same container size at $P \le 0.05$ . Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long)
2.	Total above-ground dry weight (g) as affected by container size (big and small) and salt irrigation concentration (0 – 20 dS m-1). Uppercase letters indicate differences among salt irrigation concentrations in the same container size at P $\leq$ 0.05. Lowercase letters indicate differences between container sizes at the same salt irrigation concentration at P $\leq$ 0.05. Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long)
3.	Total above ground fresh (A) and dry (B) weight (g) as affected by salt irrigation concentration $(0 - 20 \text{ dS m-1})$ and duration of saline exposure (week). Uppercase letters indicate differences among salt irrigation concentrations in the same week at P $\leq 0.05$ . Lowercase letters indicate differences among weeks at the same salt irrigation concentration at P $\leq 0.05$
4.	Absolute water content (g) (A) and visual damage $(1 - 5 \text{ scale}, 1 = \text{healthy plants})$ and $5 = \text{dead plants}$ (B) as affected by salt irrigation concentration $(0 - 20 \text{ dS m-}1)$ and duration of saline exposure (week). Uppercase letters indicate differences among salt irrigation concentrations within the same week at P $\leq 0.05$ . Lowercase letters indicate differences among weeks at the same salt irrigation concentration at P $\leq 0.05$ .
5.	Scree plot of principal components (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline condition. HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total above-ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, VD = visual damage rating (1 – 5 scale, 1 = healthy plants and 5 = dead plants)

- 6. Scree plot of principal components (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under saline condition. HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total above-ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, VD = visual damage rating (1 5 scale, 1 = healthy plants and 5 = dead plants).

10.	Principal component analysis-biplot depicting the classification phenotypic traits of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline (A) and saline condition (B). The graded color scale, contribution, is used to estimate the quality of the representation. A high contribution value indicates a good representation of a trait on the principal component. HT = plant height, RL = root length, SFW = shoot fresh weight, PFW = pod fresh weight, SDW = shoot dry weight, PDW = pod dry weight, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating $(1 - 5 \text{ scale}, 1 = \text{healthy plants and 5})$	
	= dead plants)	65
11.	Cluster analysis of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline condition. The cluster analysis revealed five major clusters $(1 - 5)$ . Accessions of the germplasm (numbered $1 - 294$ ) and 'Agassiz' are listed on the bottom.	69
12.	Cluster analysis of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under saline conditions. The cluster analysis revealed five major clusters $(1 - 5)$ . Accessions of the germplasm (numbered $1 - 294$ ) and 'Agassiz' are listed at the bottom.	70
13.	Cluster analysis of a USDA pea germplasm [including 199 inbred lines and 'Agassiz' (the check, a commercial pea variety)] under non-saline conditions. The cluster analysis revealed five major clusters $(1 - 5)$ . Accessions of the germplasm (numbered $1 - 199$ ) and 'Agassiz' are listed at the bottom.	71
14.	Cluster analysis of a USDA pea germplasm [including 199 inbred lines and 'Agassiz' (the check, a commercial pea variety)] under saline conditions. The cluster analysis revealed five major clusters $(1 - 5)$ . Accessions of the germplasm (numbered $1 - 199$ ) and 'Agassiz' are listed at the bottom.	72

## LIST OF ABBREVIATIONS

Abbreviation	Explanation of the Abbreviation.
ha	Hectare
FAOSTAT	Food and agriculture statistics department
EC	Electrical conductivity
pH	potential of hydrogen
SAR	Sodium Absorption Ratio
USDA	United States Department of Agriculture
NDSU	North Dakota State University
ANOVA	Analysis of variance
ASREML- R	Average Information Restricted Maximum Likelihood in R

## LIST OF APPENDIX TABLES

<u>Table</u>		Page 1
A1.	List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition	82
A2.	List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing conditions.	97
A3.	Top performers (10% of the population) from a NDSU germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)], selected based on the performance from major phenotypic traits (i.e. contribution $\geq 10\%$ ) associated with the first two principal components	104
A4.	Top performers (10% of the population) from a USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)], selected based on the performance from major phenotypic traits (i.e. contribution $\geq 10\%$ ) associated with the first two principal components).	109

#### LITERATURE REVIEW

#### Pea

*Pisum sativum* (L.), commonly known as dry pea or field pea, belongs to the kingdom *Plantae* and the family *Fabaceae*. Pea is native to southwestern Asia and later spread throughout the continent and other parts of the world (Makasheva, 1984). Pea can grow in many soil types ranging from sandy to clay. High organic matter-containing and well-drained soils are more suitable for optimum pea growth (Mahler *et al.*, 1988). Pea tolerates soil pH ranging from 5.5 - 9.0 but performs the best at 5.9 - 7.5. Pea plants require a relatively humid climate for high yield with the optimal germination temperature of 12 - 18 °C (Khan and Croser, 2004). Dry pea is a valuable crop because of its high protein, vitamin, mineral, and prebiotic carbohydrate contents which are essential for human health, yet affordable to consumers (Behera *et al.*, 2022). Pea is mainly grown for its green pods consumed fresh as vegetables (Duke, 2012) or canned and frozen for processing and marketed as a dry-shelled product for human or livestock food (Tassoni *et al.*, 2020).

Dry pea is beneficial for soil health. Pea can enhance soil microbial biodiversity, increase soil organic carbon levels, and improve water retention (Foyer *et al.*, 2016; Stagnari *et al.*, 2017). Like other leguminous crops, pea can fix nitrogen (N) from the atmosphere through a symbiotic relationship with rhizobia, resulting in 50 - 150 kg N ha<sup>-1</sup> added to the soil each year (Kakraliya *et al.*, 2018). The process of N fixing also improves soil nutrient cycling. It enhances phosphorus availability to plants by modifying soil properties, such as soil texture and structure, water-holding capacity, and organic matter content (Piotrowska-Długosz, 2020). When incorporated into crop rotation, pea plants can help improve soil fertility and break disease cycles (Evans *et* 

1

*al.*, 1995; O'Connor *et al.*, 1993; White, 1987). Thus, pea is a valuable crop for its potential to improve soil health and crop yield, making it a sustainable choice for agricultural practices.

#### **Pea Production**

Dry pea is the most-grown grain legume worldwide, with a global production exceeding 16.2 million metric ton in 2017 (Gurusamy *et al.*, 2022). The highest producers include Canada, Russia, France, China, India, and the United States (Janzen *et al.*, 2006; Janzen *et al.*, 2014). In the U.S., the Northern Great Plains (Wisconsin, North Dakota, South Dakota, Montana, Minnesota) and the Palouse Region of Washington and Idaho have become significant producers of peas in recent years (McPhee, 2004; Vandemark *et al.*, 2014). Approximately 0.5 million metric ton of dry pea is produced annually in North Dakota (~ 1/3 of the total pea production in the U.S.), followed by Montana (0.2 million metric ton) and Washington (0.08 million metric ton) (Janzen *et al.*, 2006; Vandemark *et al.*, 2014). The annual economic value of dry pea in North Dakota is more than \$90 million (Jantzi *et al.*, 2018).

#### **Soil Salinity**

#### Salinity and its causes

Except for Antarctica, every continent experiences a salinity problem (International Technical Panel on Soils, 2015). Salinity affects roughly 833 million ha of agricultural land (about 20% of cultivated land and 33% of the irrigated land), reducing crop yield (quantity and quality) [Food and Agriculture Organization Statistics Division (FAOSD), 2020; Machado and Serralheiro, 2017]. The annual global economic loss from salinity-related agricultural reduction is approximately \$27.3 billion (Qadir *et al.*, 2014).

There are two possible origins of salinity: primary salinity, which is the consequence of natural causes, and secondary salinity, caused by human activity (Parihar *et al.*, 2015). The

accumulation of salts in the soil profile of primary salinity is caused by prolonged weathering of primary minerals in the soil profile (Rengasamy and Olsson, 1993). These minerals are transported in groundwater to lower elevations, causing salt to accumulate in the soil profile. The salt deposition transported by rain, wind, or saltwater infiltration by ocean tides can also contribute to primary salinization. The intensity of salt deposition in a soil profile is affected by climate and other variables, such as lengthy droughts and times of abundant precipitation (Archer and Predick, 2008). Human activities like irrigated agriculture and crop-fallow dryland cropping techniques result in secondary salinity (Sharma and Singh, 2015). In dry and semiarid areas where freshwater is scarce, irrigation can cause soil salinization (Cuevas *et al.*, 2019). The use of low-quality, salty water can also lead to the accumulation of salt.

#### Salinity in North Dakota

Salinity is one of the major obstacles to agricultural production in North Dakota. Approximately 2.4 million ha of land in North Dakota has been affected by salinity by 2010, with more expansion expected (Franzen *et al.*, 2014). "Over 90% of producers in North Dakota are experiencing some sort of reduced productivity due to salinity" (NDSU AgHub, 2023). In most saline regions, chloride salts, such as NaCl, MgCl<sub>2</sub>, and CaCl<sub>2</sub> are the most prevalent (Parihar *et al.*, 2015; Szabolcs and Pessarakli, 2010). However, sulfate salts, such as Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>, are commonly seen in salt-affected areas in the North Dakota (Franzen, 2003).

#### Salinity effects on plants

Salinity adversely affects plant growth and development, leading to plant death in extreme cases (Safdar *et al.*, 2019). High salt concentration lowers water potential in soil, resulting in reduced water availability to plants, i.e., osmotic stress or physiological drought (Cuartero and Fernández-Muñoz, 1998). Osmotic stress triggers long-distance signals such as an

3

increase in abscisic acid and cellular  $Ca^{2+}$  imbalance, resulting in stomata closure, thus reducing water uptake (Hartung *et al.*, 2018). Symptoms induced by osmotic stress are primarily on new growth, including lack of emergence and slow expansion of new leaves (Munns and Tester, 2008).

Plants in salt-affected areas absorb and accumulate large amounts of ions, such as Na<sup>+</sup>, Cl<sup>-</sup> and SO4<sup>2-</sup>, resulting in ion toxicity and nutrient imbalance (Hailu and Mehari, 2021). For example, strawberry (*Fragaria ananassa* L.) roots had 2 – 4 times higher levels of Na when irrigated with a mixture of NaCl and CaCl<sub>2</sub> than the non-treated ones (Ferreira *et al.*, 2019). Furthermore, the salt-treated strawberries also had a lower level of P (0.7 vs. 1.4 g kg<sup>-1</sup>) and K (6.1 vs. 9.8 g kg<sup>-1</sup>) but a higher Ca (17.5 vs. 12.3 g kg<sup>-1</sup>) compared to the control plants (Ferreira *et al.*, 2019). Similarly, NaCl salt-treated chickpeas (*Cicer arietinum* L.) had a higher Na<sup>+</sup>/K<sup>+</sup> ratio than the non-stressed plants (Turner *et al.*, 2013). Sodium can cause membrane disorganization in plants, inhibiting the passive uptake of many essential nutrients like K<sup>+</sup>, a common co-factor of cytoplasmic enzymes, as Na and K have similar physiochemical properties (Munns and Tester, 2008; Shabala and Munns, 2017). Ion homeostasis and plant metabolic activities are known to be highly negatively impacted by Na<sup>+</sup> concentration greater than 50 mM in irrigation (Marschner, 1995; Munns and Tester, 2008).

Chloride is a micro essential nutrient that higher plants require for enzyme regulation and photosynthesis (Jing *et al.*, 1992). Too much Cl<sup>-</sup> prevents NO<sub>3</sub><sup>-</sup> uptake and degrades chlorophyll, resulting in decreased photosynthetic capacity (Grattan and Grieve, 1998; Tavakkoli *et al.*, 2010). According to Maas *et al.* (1983), every dS m<sup>-1</sup> increase in saline irrigation (a mixture of NaCl, MgCl<sub>2</sub>, and CaCl<sub>2</sub>) beyond the threshold level, 5.5 dS m<sup>-1</sup>, would result in an average

4

10.1% reduction in corn (*Zea mays* L.) grain yield. When Cl<sup>-</sup> content exceeds 800 mg soil kg<sup>-1</sup>, corn yield could be reduced up to 95% (Jing *et al.*, 1992).

Sulfate is a macro essential nutrient that is reduced and incorporated into two amino acids, cysteine and methionine, which play central roles in protein biosynthesis and biochemical reactions in plant cells (Leustek and Saito, 1999). High sulfate content lowers the osmotic potential in the soil, causing a physiological drought that leads to structural degradation of soil particles like compaction (Tölgyessy *et al.*, 1993). In sulfate salt-affected soils,  $Ca^{2+}$  and  $Mg^{2+}$  can intensify the effects of soil dispersion compared to chloride salt-affected ones (Springer *et al.*, 1999).

Reactive oxygen species (ROS), such as hydrogen peroxide ( $H_2O_2$ ), superoxide, hydroxyl radicals, and singlet oxygen, are highly reactive molecules containing oxygen. They are produced as byproducts of normal cellular metabolism in living organisms, including plants (Brobbey *et al.*, 2020). In small amounts, ROS plays a vital role in plant growth and development, including germination, photosynthesis, and reproduction (Bhattacharjee, 2005; Quan *et al.*, 2008). However, excessive ROS commonly seen under stress conditions (e.g., salinity) can cause oxidative stress, causing excessive damage to deoxyribonucleic acid , proteins, and lipids (Wu and Cederbaum, 2003). Nxele *et al.* (2017) reported a higher level of  $H_2O_2$  and lipid damage and lower tissue biomass in the leaves and roots of the salt-treated sorghum *(Sorghum bicolor L. Moench)* than the non-treated ones.

#### Salinity Management

#### **Cultural practices**

Leaching, drainage, and crop-based management systems may help manage salinity (Goyal *et al.*, 1999). Leaching can reduce soluble salt through the downward movement of water out of the root zone (Fipps, 2003). Leaching is best achieved when the applied water has low salt, and the soil has good drainage. Appropriate leaching (i.e. good water quality, sufficient amount of water, and good drainage) not only helps alleviate salinity but also improves nutrient cycling and increases microbial activities (Singh *et al.*, 2007; Wilson *et al.*, 2000).

The spread of dryland salinity has led to the re-adoption of a crop-based management system. According to Munn *et al.* (2002), a farming system can be a sustainable way of managing salinity stress by incorporating perennials in rotation with annual crop (phase farming), in mixed planting (intercropping, alley farming), or site-specific planting (precision farming). Deep-rooted, salt-tolerant perennial plants can absorb and use water from the lower soil profile (Lambers, 2003). The absorbed water causes the translocation of salt from the root zones into the perennial plants through osmosis, decreasing soil salinity; thus, improving the survival and growth of annual plants. Additionally, by limiting the rise of water tables and the movement of salt to the soil surface, these intercropping techniques cause equilibrium between rainfall and water use (Manchanda and Garg, 2008).

#### **Use of tolerant plants**

Using salt-tolerant plants is one of the most economical and sustainable methods for salinity management (Shannon, 1985). There are interspecific and intraspecific variations in salinity tolerance. For example, halophytes such as Suaeda (*Suaeda vermiculata* L.) and Atriplex (*Atriplex halimus* L.) are capable of surviving and growing under salt concentrations as high as 50 dS m<sup>-1</sup> (~ 50% seawater) (Joshi *et al.*, 2015). Among the common field crops, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are salt tolerant or moderately tolerant, followed by corn (moderately sensitive), and white rice (*Oryza sativa* L.) being the most sensitive (Grieve *et al.*, 2012). Page *et al.* (2021) reported the EC<sub>e</sub> (electrical conductivity from

saturated soil paste) causing 50% of yield reduction was 3.4 dS m<sup>-1</sup> for lentil (*Lens culinaris* L.), 6.3 dS m<sup>-1</sup> for chickpea, 6.9 dS m<sup>-1</sup> for pea, and 8.2 dS m<sup>-1</sup> for soybean (*Glycine max* L.). Ashraf and McNeilly (1990) compared salinity tolerance among four *Brassica* species. The results showed that *B. napus* had the highest seed yield of 1.74 g plant<sup>-1</sup>, while *B. campestris* had the lowest seed yield (0.61 g plant<sup>-1</sup>). Similarly, Nakamura *et al.* (2002) reported genotype differences in tolerance to NaCl in wild and cultivated *Oryza* species.

For field pea, Shahid *et al.* (2012) determined the salinity tolerance of 30 pea genotypes collected from Pakistan during the germination and early growth stage. Their results showed that the germination rate was reduced by 4% to 48%, and the leaf Na<sup>+</sup> level was increased by 15% to 57% among the genotypes. Leonforte *et al.* (2013) evaluated the visual symptoms and growth habits of 780 pea accessions, primarily from Asia (428 accessions) and Western Europe (113 accessions), under saline conditions (0 – 18 dS m<sup>-1</sup>). In their study, over 80% of the accessions were susceptible to salinity, with a symptom score equal to or above 7 (1 = healthy plant and 10 = dead plant). They also discovered that the pea varieties from China and Greece had the lowest symptom scores compared to those from Afghanistan, Ethiopia, Finland, Sweden, and the United States. Currently, information on the salinity tolerance in the North American pea germplasm is scarce.

Furthermore, previous research has primarily used chloride salts like NaCl to study plant responses to salinity. However, in regions such as North Dakota, sulfate salts are the dominant salt type in most saline areas (Franzen, 2003). Plant responses to sulfate- and chloride-salinity are different. For instance, chloride salts reduced soybean yield by 24% at 6.88 dS m<sup>-1</sup>, while sulfate salts did not influence soybean growth at the same concentration (Gupta and Gupta, 1984). Similarly, Kentucky bluegrass (*Poa pratensis* L.) and rice are more sensitive to NaCl than Na<sub>2</sub>SO<sub>4</sub> (Irakoze, 2021; Yang and Zhang, 2019). In contrast, barley showed a higher tissue biomass and photosynthetic rate under the NaCl treatment than the Na<sub>2</sub>SO<sub>4</sub> treatment at 8.1 dS  $m^{-1}$  (Datta *et al.*, 1994). Jing *et al.* (1992) also reported that corn is more sensitive to SO<sub>4</sub><sup>2-</sup> than Cl<sup>-</sup>. Limited information is available on dry pea responses to sulfate salts-induced salinity stress. There is great need to determine salinity tolerance, especially sulfate-salinity which is dominant in the Norther Great Plains (including North Dakota), in the North American pea germplasms. Such information will help identify potential dry pea materials to breed for sulfate-salt tolerant plants to help dry pea growers and breeders address salinity issues, improving production and economic profit.

#### References

- Archer, S. R., & Predick, K. I. (2008). Climate change and ecosystems of the southwestern United States. *Rangelands*, 30(3), 23-28. https://doi.org/10.2111/1551-501X(2008)30[23:CCAEOT]2.0.CO;2.
- Ashraf, M., & McNeilly, T. (1990). Responses of four *Brassica* species to sodium chloride. *Environmental and Experimental Botany*, 30(4), 475-487. https://doi.org/10.1016/0098-8472(90)90028-3.
- Behera, S., Jyotirmayee, B., Mandal, U., Mishra, A., Mohanty, P., & Mahalik, G. (2022). Effect of organic fertilizer on growth, yield and quality of *Pisum sativum* L.: A Review. *Ecology, Environment and Conservation, 28*, S233-S241. http://doi.org/10.53550/EEC.2022.v28i02s.039.
- Bhattacharjee, S. (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transducation in plants. *Current Science*, 89(7), 1113-1121. http://www.jstor.org/stable/24110963.

- Brobbey, A. A., Jibira, Y., Fuseini, B., Nii-Lamptey, R., & Adu, J. K. (2020). Antioxidant and hepatoprotective properties of *Helianthus annuus* seed extract against paracetamolinduced liver toxicity. *Journal of Phytopharmacology*, 9(5), 361-366. https://doi.org/10.31254/phyto.2020.9512.
- Cuartero, J., & Fernández-Muñoz, R. (1998). Tomato and salinity. *Scientia Horticulturae*, 78(1-4), 83-125. https://doi.org/10.1016/s0304-4238(98)00191-5
- Cuevas, J., Daliakopoulos, I. N., del Moral, F., Hueso, J. J., & Tsanis, I. K. (2019). A review of soil-improving cropping systems for soil salinization. *Agronomy*, 9(6), 295. https://doi.org/10.3390/agronomy9060295.
- Datta, K. S., Kumar, A., & Verma, S. K. (1994). Variations in growth and physiology of barley under chloride and sulphate salinity. *Annals of Arid Zone, 33*, 302-307.
- Duke, J. (2012). *Handbook of legumes of world economic importance*. Springer Science and Business Media. https://doi.org/10.1007/978-1-4684-8151-8.
- Evans, J., Chalk, P. M., & O'Connor, G. E. (1995). Potential for increasing N<sub>2</sub> fixation of field pea through soil management and genotype. *Biological Agriculture and Horticulture*, *12*(2), 97-112. https://doi.org/10.1080/01448765.1995.9754730
- FAO, ITPS. (2015). Status of the world's soil resources (Main Report), No. 608. FAO, Rome, Italy.
- Ferreira, J. F., Liu, X., & Suarez, D. L. (2019). Fruit yield and survival of five commercial strawberry cultivars under field cultivation and salinity stress. *Scientia Horticulturae*, 243, 401-410. https://doi.org/10.1016/j.scienta.2018.07.016.
- Fipps, G. 2003. Irrigation water quality standards and salinity management strategies. Texas Farmer Collection, USA.

FAOSTAT, (2020). *Land use in agriculture by the numbers*. Food and Agriculture Organization of the United Nations.

https://www.fao.org/sustainability/news/detail/en/c/1274219/#:~:text=Globally%20agricu ltural%20land%20area%20is,and%20pastures)%20for%20grazing%20livestock

- Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T.A., Hodgson, M. J., Cooper, J.W, Miller, A.J., Kunert, K., Vorster, Juan., Cullis, C., Ozga J. A., Wahlqvist, M.L., Liang, Y., Shou, Huixia., Shi, K., Yu, J.,. ... & Considine, M. J. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants, 2*, 16112. https://doi.org/10.1038/nplants.2016.112
- Franzen, D. (2003). Managing saline soils in North Dakota. North Dakota State University, Fargo, ND 58105, SF-1087 (revised).
- Franzen, D., Wick, A., Augustin, C., & Kalwar, N. (2014). Saline and Sodic Soils. North Dakota State University Extension Service: Fargo, North Dakota, 58105. https://www.ag.ndsu.edu/langdonrec/soil-health/saline-sodic-soils
- Goyal, S. S., Sharma, S. K., Rains, D. W., & Läuchli, A. (2000). Long-term reuse of drainage waters of varying salinities for crop irrigation in a cotton-safflower rotation system in the San Joaquin Valley of California—a nine-year study: I. Cotton (*Gossypium hirsu*tum L.). *Journal of Crop Production*, 2(2), 181-213. https://doi.org/10.1300/J144v02n02\_07.
- Grattan, S. R., & Grieve, C. M. (1998). Salinity–mineral nutrient relations in horticultural crops. *Scientia Horticulturae*, 78(1-4), 127-157. https://doi.org/10.1016/S0304-4238(98)00192-7.

- Grieve, C. M., Grattan, S. R., & Maas, E. V. (2012). Plant salt tolerance. In W.W. Wallender & K.K. Tannji, (Eds.), ASCE Manual and Reports on Engineering Practice, No. 71
  Agricultural Salinity Assessment and Management (2<sup>nd</sup> ed., pp. 405-459). ASCE.
- Gupta, V. K., & Gupta, S. P. (1984). Effect of zinc sources and levels on the growth and Zn nutrition of soybean (*Glycine max*. L.) in the presence of chloride and sulphate salinity. In H. Lambers (Ed,), *Plant and Soil* (pp. 299-304). Springer. https://doi.org/10.1007/bf02197164.
- Gurusamy, S., Vidhya, C. S., Khasherao, B. Y., & Shanmugam, A. (2022). Pulses for health and their varied ways of processing and consumption in India-A review. *Applied Food Research*, 2(2), 100171. https://doi.org/10.1016/j.afres.2022.100171.
- Hailu, B., & Mehari, H. (2021). Impacts of soil salinity/sodicity on soil-water relations and plant growth in dry land areas: a review. *Journal of Natural Science Research*, *12(3)*, 1-10.
- Hartung, W., & Jeschke, W. D. (2018). Abscisic acid: a long-distance stress signal in saltstressed plants. In H.R. Lerner (Ed.), *Plant Responses to Environmental Stresses*, (pp. 333-348). Routledge. https://doi.org/10.1201/9780203743157.
- Irakoze, W., Prodjinoto, H., Nijimbere, S., Bizimana, J. B., Bigirimana, J., Rufyikiri, G., & Lutts, S. (2021 NaCl-and Na<sub>2</sub>SO<sub>4</sub>- induced salinity differentially affect clay soil chemical properties and yield components of two rice cultivars (*Oryza sativa* L.) in Burundi. *Agronomy*, 11(3), 571. https://doi.org/10.3390/agronomy11030571.
- Jantzi, D., K. Hagemeister, and B. Krupich. 2018. North Dakota agricultural statistics 2018 Ag Statistics No. 87 August 2018. US Department of Agriculture National Agriculture.
   Statistics Service. Northern Plains Regions ND Field Office: Fargo, ND, USA.

https://www.nass.usda.gov/Statistics\_by\_State/North\_Dakota/Publications/Annual\_Statis tical\_Bulletin/2018/ND-Annual-Bulletin18.pdf

- Janzen, E., Flaskerud, G., & Fisher, J. (2006). *Pulse crop marketing guide*. North Dakota State University Extension Service, Fargo, ND 58105.
- Janzen, J. P., Brester, G. W., Smith, V. H., Hall, L., & Box, P. O. (2014). *Dry peas: trends in production, trade, and price*. Agricultural Marketing Policy Center, Briefing No. 57.
- Jing, A. S., Guo, B. C., & Zhang, X. Y. (1992). Chloride tolerance and its effects on yield and quality of crops. *China Journal of Soil Science*, 33(6), 257-259.
- Joshi, R., Mangu, V. R., Bedre, R., Sanchez, L., Pilcher, W., Zandkarimi, H., & Baisakh, N. (2015). Salt adaptation mechanisms of halophytes: improvement of salt tolerance in crop plants. In G. K. Pandey (Ed.), *Elucidation of Abiotic Stress Signaling in Plants, Functional Genomics Perspectives* (pp. 243-279). Springer. https://doi.org/10.1007/978-1-4939-2540-7\_9.
- Kakraliya, S. K., Singh, U., Bohra, A., Choudhary, K. K., Kumar, S., Meena, R. S., & Jat, M. L.
  (2018). Nitrogen and legumes: a meta-analysis, In R.S. Meena et al. (Eds.), *Legumes for Soil Health and Sustainable Management* (pp. 277-314). Springer. https://doi.org/10.1007/978-981-13-0253-4\_9.
- Khan, T., & Croser, J. (2004). Pea overview. *Encyclopedia of Grain Science*, *1*, 418-427. https://doi.org/10.1016/b0-12-765490-9/00126-9.
- Lambers, H. (2003). Introduction: dryland salinity: a key environmental issue in southern Australia. *Plant and Soil*, 257(2), 5-7.

https://doi.org/10.1023/b:plso.0000003909.80658.d8.

- Leonforte, A., Forster, J. W., Redden, R. J., Nicolas, M. E., & Salisbury, P. A. (2013). Sources of high tolerance to salinity in pea (*Pisum sativum* L.). *Euphytica*, 189(2), 203-216. https://doi.org/10.1007/s10681-012-0771-4.
- Leustek, T., & Saito, K. (1999). Sulfate transport and assimilation in plants. *Plant Physiology*, *120(3)*, 637-644. https://doi.org/10.1104/pp.120.3.637.

Maas, E. V., Hoffman, G. J., Chaba, G. D., Poss, J. A., & Shannon, M. C. (1983). Salt sensitivity of corn at various growth stages. *Irrigation Science*, *4*, 45-57. https://doi.org/10.1007/bf00285556.

- Machado, R. M. A., & Serralheiro, R. P. (2017). Soil salinity: effect on vegetable crop growth.
  Management practices to prevent and mitigate soil salinization. *Horticulturae*, 3(2), 30.
  https://doi.org/10.3390/horticulturae3020030
- Makasheva, R. K. (1984). *The pea*. The US Department of Agriculture and the National Science Foundation., Oxonian Press.
- Mahler, R.L., Saxena, M.C., & Aeschlimann, J. (1988). Soil fertility requirements of pea, lentil, chickpea and faba bean. In R.J. Summerfield (Ed.) *World crops: Cool season food legumes. Current Plant Science and Biotechnology in Agriculture* (vol 5, pp. 279-289).
  Springer. https://doi.org/10.1007/978-94-009-2764-3\_27.
- Manchanda, G., & Garg, N. (2008). Salinity and its effects on the functional biology of legumes. *Acta Physiologiae Plantarum, 30*, 595-618. https://doi.org/10.1007/s11738-008-0173-3.

Marschner, H. (1995). Functions of mineral nutrients: micronutrients. In H. Marschner (Ed.), *Mineral Nutrition in Higher Plants* (pp. 313-404), Academic Press. https://doi.org/10.1016/b978-0-08-057187-4.50015-1.

- Mcphee, K., (2003). Dry pea production and breeding-a mini-review. Journal of Food, *Agriculture and Environment, 1(1),* 64-69.
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
- Munns, R., Husain, S., Rivelli, A. R., James, R. A., Condon, A. T., Lindsay, M. P., ... & Hare, R. A. (2002). Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. In W. J. Horst et al. (Eds.), *Progress in Plant Nutrition: Plenary Lectures of the XIV International Plant Nutrition Colloquium, Food Security and Sustainability of Agro-ecosystems through Basic and Applied Research* (pp. 93-105). Springer Science and Business Media. https://doi.org/10.1007/978-94-017-2789-1\_7.
- Nakamura, I., Murayama, S., Tobita, S., Bong, B. B., Yanagihara, S., Ishimine, Y., & Kawamitsu, Y. (2002). Effect of NaCl on the photosynthesis, water relations and free proline accumulation in the wild *Oryza* species. *Plant Production Science*, *5(4)*, 305-310. https://doi.org/10.1626/pps.5.305.
- North Dakota Agricultural Statistics (2018). North Dakota Agriculture Ag Statistics No.87. https://www.nass.usda.gov/Statistics\_by\_State/North\_Dakota/Publications/Annual\_Statistical\_Bulletin/2018/ND-Annual-Bulletin18.pdf

NDSU AgHub on soil salinity. (2023). Saline and sodic soil.

- https://www.ndsu.edu/agriculture/ag-hub/ag-topics/crop-production/soil-health/saline-and-sodicsoils/soil-salinity
- Nxele, X., Klein, A., & Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. South African *Journal of Botany*, 108, 261-266. https://doi.org/10.1016/j.sajb.2016.11.003.

- O'Connor, G. E., Evans, J., Fettell, N. A., Bamforth, I., Stuchberry, J., Heenan, D. P., & Chalk,
  P. M. (1993). Sowing date and varietal effects on the N<sub>2</sub> fixation of field pea and implications for improvement of soil nitrogen. *Australian Journal of Agricultural Research*, 44(1), 151-163. https://doi.org/10.1071/ar9930151.
- Page, K. L., Dang, Y. P., Martinez, C., Dalal, R. C., Wehr, J. B., Kopittke, P. M., Orton, T.G., & Menzies, N. W. (2021). Review of crop-specific tolerance limits to acidity, salinity, and sodicity for seventeen cereal, pulse, and oilseed crops common to rainfed subtropical cropping systems. *Land Degradation and Development*, *32*(8), 2459-2480. https://doi.org/10.1002/ldr.3915.
- Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*, 22, 4056-4075. https://doi.org/10.1007/s11356-014-3739-1.
- Piotrowska-Długosz, A. (2020). Significance of the enzymes associated with soil C and N transformation. In R. Datta et al. (Eds.), *Carbon and Nitrogen Cycling in Soil* (pp. 399-437). Springer. https://doi.org/10.1007/978-981-13-7264-3\_12.
- Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R. J., Drechsel, P., & Noble, A. D. (2014). Economics of salt-induced land degradation and restoration. *Natural Resources Forum*, 38(4), 282-295. https://doi.org/10.1111/1477-8947.12054.
- Quan, L. J., Zhang, B., Shi, W. W., & Li, H. Y. (2008). Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology*, 50(1), 2-18. https://doi.org/10.1111/j.1744-7909.2007.00599.x.
- Rengasamy, P., & Olsson, K. A. (1993). Irrigation and sodicity. *Soil Research*, *31*(6), 821-837. https://doi.org/10.1071/sr9930821.

- Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., Ul Hussan ,M., & Sarwar, M. I. (2019). A review: Impact of salinity on plant growth. *Nature and Science*, 17(1), 34-40. https://doi.org/10.7537/marsnsj170119.06.
- Shabala, S., & Munns, R. (2017). Salinity stress: physiological constraints and adaptive mechanisms, In S. Shabala (Ed), *Plant Stress Physiology* (2<sup>nd</sup> ed, pp. 24-63). CABI https://doi.org/10.1079/9781780647296.002
- Shahid, M. A., Pervez, M. A., Balal, R. M., Abbas, T., Ayyub, C. M., Mattson, N. S., Riaz, A., & Iqbal, Z. (2012). Screening of pea ('*Pisum sativum*' L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. *Australian Journal of Crop Science*, 6(9), 1324-1331.
- Shannon, M. C. (1985). Principles and strategies in breeding for higher salt tolerance. In D.
  Pasternak & A. San Pietro (Eds.), *Biosalinity in Action: Bioproduction with Saline Water* (Volume 17, pp. 227-241). Springer. https://doi.org/10.1007/978-94-009-5111-2\_15.
- Sharma, D. K., & Singh, A. (2015). Salinity research in India-achievements, challenges and future prospects. *Water and Energy International*, 58(6), 35-45.
- Singh, M., Pabbi, S., Bhattacharya, A. K., & Singh, A. K. (2007). Nitrite accumulation in coastal clay soil of India under inadequate subsurface drainage. *Agricultural Water Management*, 91(1-3), 78-85. https://doi.org/10.1016/j.agwat.2007.04.010.
- Springer, G., Wienhold, B. J., Richardson, J. L., & Disrud, L. A. (1999). Salinity and sodicity induced changes in dispersible clay and saturated hydraulic conductivity in sulfatic soils. *Communications in Soil Science and Plant Analysis, 30(15-16),* 2211-2220. https://doi.org/10.1080/00103629909370366.

- Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture*, 4(2), 1-13. https://doi.org/10.1186/s40538-016-0085-1.
- Szabolcs, I., & Pessarakli, M. (2010). Soil salinity and sodicity as particular plant/crop stress factors. In M. Pessarakli (Ed), Handbook of Plant and Crop Stress (pp. 3-10.), CRC Press. https://doi.org/10.1201/9781351104609-1.
- Tassoni, A., Tedeschi, T., Zurlini, C., Cigognini, I. M., Petrusan, J. I., Rodríguez, Ó, Neri, S.,
  Celli, A., Siisti, L., Cinelli, P., Signori F., Tsatsos, G., Bondi, M., Verstringe, S.,
  Bruggerman, G., & Corvini, P. F. (2020). State-of-the-art production chains for peas,
  beans and chickpeas—valorization of agro-industrial residues and applications of derived
  extracts. *Molecules*, 25(6), 1383. https://doi.org/10.3390/molecules25061383.
- Tavakkoli, E., Rengasamy, P., & McDonald, G. K. (2010). High concentrations of Na<sup>+</sup> and Cl<sup>-</sup> ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of Experimental Botany*, *61(15)*, 4449-4459. https://doi.org/10.1093/jxb/erq251.
- Tölgyessy, J. (1993). Water, air and soil-fundamental sources of the biosphere. In J. Tölgyessy (Ed), *Chemistry and Biology of Water, Air and Oil Environmental Aspects* (pp. 3-13), Elsevier.
- Turner, N. C., Colmer, T. D., Quealy, J., Pushpavalli, R., Krishnamurthy, L., Kaur, J., Singh, G., Siddique, K.H.M., & Vadez, V. (2013). Salinity tolerance and ion accumulation in chickpea (*Cicer arietinum* L.) subjected to salt stress. *Plant and Soil, 365*, 347-361. https://doi.org/10.1007/s11104-012-1387-0.

- Vandemark, G. J., Brick, M. A., Osorno, J. M., Kelly, J. D., & Urrea, C. A. (2014). Edible grain legumes. In S. Smith et al. (Eds.), *Yield gains in major US field crops* (Volume 33, pp. 87-123), Crop Science Society of America Special Publication. https://doi.org/10.2135/cssaspecpub33.c5.
- White, J. G. H. (1987). The importance of pea in New Zealand arable agriculture. Peas: management for quality. *Agronomy Society of New Zealand special*, *6*, 7-11.
- Wilson, G. W. (1990). *The evaluation of evaporative fluxes from unsaturated soil surfaces*.[Doctoral dissertation, , University of Saskatchewan, Saskatoon].
- Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. Alcohol Research and Health, 27(4), 277-284.
- Yang, L., & Zhang, Q. (2019) Kentucky bluegrass growth and quality as affected by salt type and concentration. *Agronomy Journal*, *111(1)*, 233-241.

## DRY PEA GROWTH IN RESPONSE TO SULFATE SALINITY AND CONTAINER SIZE UNDER THE CONTROLLED ENVIRONMENT

#### Abstract

Dry pea (*Pisum sativum* L.) is an important cool-season legume species with a high nutrient profile; however, it is salt sensitive. Previous research has been mostly focusing on chloride salts-induced salinity (i.e. chloride-salinity); however, sulfate salts are dominant in the Northern Great Plains, the major production area of dry pea in the U.S. The objective of this research was to determine dry pea responses to sulfate-salinity and container size. 'Agassiz' was seeded in big (556 ml) or small (410 ml) cone-containers and exposed to sulfate-salinity (0 - 20 dS m<sup>-1</sup>) through irrigation for four weeks at the seedling stage. Plants were sampled once weekly and phenotypic traits were quantified. Plants treated at 5 dS m<sup>-1</sup> performed similarly as the non-stressed plants, 10 dS m<sup>-1</sup> caused moderate damage (50% - 60% in tissue biomass), while plants under 15 and 20 dS m<sup>-1</sup> were severely damaged or dead by week 4. Absolute water content, above ground fresh weight, and visual damage were the most sensitive to salinity damage among the phenotypic traits evaluated. Plants grown in the big containers performed better than those in the small ones, especially those under 0 – 10 dS m<sup>-1</sup> treatments. The results may help develop a screening protocol for the tolerance to sulfate-salinity in dry pea.

#### Introduction

Dry pea (*Pisum sativum* L.) is one of the most important leguminous crops, with high nutritional values in protein, minerals, and vitamins (Kumari and Deka, 2021). It is the second most cultivated grain legume in the world, following dry beans (*Phaseolus vulgaris* L.), with production exceeding 16.2 million metric tons globally (FAOSTAT, 2017). It is mostly consumed fresh as vegetables or processed as dry-shelled products for human and livestock

(Taasoni *et al.*, 2020). Dry pea also contributes to sustainable agriculture. Approximately 50 – 150 kg nitrogen ha<sup>-1</sup> year<sup>-1</sup> is added to the soil through symbiotic nitrogen fixation by pea (Kakraliya *et al.*, 2018). Furthermore, pea improves soil microbial biodiversity, increases soil organic carbon levels, and improves water retention (Foyer *et al.*, 2016; Stagnari *et al.*, 2017). When incorporated into crop rotation, pea plants can help improve soil fertility and break disease cycles (Evans *et al.*, 1995; O'Connor *et al.*, 1993; White, 1987).

Pea is sensitive to soil salinity, a common problem in the Northern Great Plains (including North Dakota, South Dakota, Montana and Minnesota), a leading area of pea production in the U.S. (Derner et al., 2015; Tracy, 2020). Salinity causes osmotic stress (inhibition of the capacity of water absorption), ionic toxicity (toxic effect of high concentration of ions such as Na<sup>+</sup>) and nutrient imbalance in plants, led to reduced crop growth and yield (Ouerghi et al., 2016). A few studies have reported reduced germination (final germination and germination speed), inhibited growth (e.g. tissue biomass and leaf number and size), and increased Na content of field pea under saline conditions (Ehtaiwwesh and Munira, 2020; Leonforte et al., 2013; Shahid et al., 2012, 2013). The aforementioned research used chloride salts (e.g. NaCl) to induce salinity stress; however, sulfate salts (e.g.  $Na_2SO_4$ ) are dominant in salt-affected areas in the Northern Great Plains (Franzen, 2003; Tracy, 2020). Plant responses to sulfate- and chloride-salinity are different. For instance, chloride salts are more detrimental to soybean (Glycine max L.), Kentucky bluegrass (Poa pratensis L.) and rice (Oryza sativa L.) (Gupta and Gupta, 1984; Irakoze, 2021; Yang and Zhang, 2019). In contrast, barley (Hordeum vulgare L.) and corn are less affected by NaCl than Na<sub>2</sub>SO<sub>4</sub> (Datta et al., 1994; Jing et al., 1992). Limited information is available on dry pea responses to sulfate salts-induced salinity stress.

20

Plant growth, especially roots, is highly influenced by container size (Elwan *et al.*, 2017). When a large number of plants are studied, small containers can help reduce cost in labor, time and space. However, reduced container size may restrict root volume, resulting in reduced water and nutrient uptake in roots. It, in turn, impacts the above ground characteristics including leaf number and size, biomass, chlorophyll content, and photosynthetic ability. Impaired photosynthesis leads to reduced root and shoot growth and crop yield. Thus, it is important to select appropriate size containers when evaluating plant performance to minimize its influence on treatments.

The objective of this research was to determine dry pea growth as affected by the sulfatesalt irrigation (intensity and duration) and container size. This research will help expand the knowledge on the effects of sulfate-salinity on dry pea. It will also provide useful information for developing a screening protocol for sulfate-salinity tolerance in pea.

#### **Materials and Methods**

Cone-containers in two different sizes, big (556 ml, 6.4 cm-diam. x 25.4 cm long) and small (410 ml, 3.8 cm-diam. x 20.3 cm long), were filled with 850 g and 220 g of coarse sand, respectively. Each container was seeded with two untreated seeds of a commercially available pea variety, 'Agassiz'. The containers were then placed in the tubs filled with half-strength Hoagland solution (Li and Cheng, 2015) (~ 2.5 cm above the bottom of the cone-containers) overnight and then transferred and maintained in tubs filled with tap water to the depth as described above during germination.

Germinated plants were thinned to one plant per container 7-10 days after seeding. Subsequently, plants were subjected to saline conditions at 0 (i.e., control, tap water), 5, 10, 15, or 20 dS m<sup>-1</sup>. Salinity was induced using a mixture of Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>·7H<sub>2</sub>O at 2:1, M: M.

21

To prevent salinity shock, salt-treated plants were gradually acclimated at a rate of 2.5 dS m<sup>-1</sup> per day. Non-stressed plants (i.e., 0 dS m<sup>-1</sup>) were watered with tap water when the salt-treated ones were acclimated with salt solutions. pH of the salt solutions and tap water was adjusted to ~ 6.0 to facilitate nutrient uptake. Upon reaching the final concentrations, plants were kept in tubs filled with designated saline solutions as described previously. Plants were exposed to saline conditions for four weeks. All solutions in the tubs were refreshed once weekly. Plants were fertilized with full-strength Hoagland solution by hand on the first day of salt acclimation and once weekly during the saline exposure. Electrical conductivity (EC) and pH of the solutions in the tubs were measured using an EC/pH meter (Oakton PC 450, Oakton Instruments, Vernon Hills, IL) before and after refreshment to monitor the saline conditions.

The experiment was conducted in a split-plot design. The main plot was the salt solutions in the tubs, arranged in RCBD with 4 replicates, and the subplot was the container size. Plants were sampled once weekly for 4 weeks. Phenotypic data were collected on root length (RL), total above-ground fresh and dry weight (TAGFW, TAGDW), root dry weight (RDW), absolute water content (AWC), root dry weight to total above-ground dry weight ratio (RTTDW), specific root length (SRL), and plant visual damage (VD, rated visually with a 1 - 5 score described in Table 1). AWC and SRL were derived from the equations below.

$${}^{1}[AWC = TAGFW - TAGDW]$$
(1)

$$^{2}[SRL = RL/RDW]$$
(2)

TAGDW and RDW were recorded after oven-drying at 65°C for 48 hr. EC and pH were measured from the saturated sand media following the USDA method (1954) using the EC/pH

<sup>&</sup>lt;sup>1</sup>Source: Hughes *et al.*, 1970

<sup>&</sup>lt;sup>2</sup>Source: Ostonen et al., 2007

meter. Data were subjected to ANOVA using PROC MIXED (SAS, V9.4, SAS Institute Inc.,

Cary, NC, USA). Least squares means (LSMEANS) were separated using the pdiff option at the

0.05 probability level. Correlations among the phenotypic traits were determined using PROC

CORR (SAS, V 9.4, SAS Institute, Inc., Cary, NC, USA).

Table 1. Description of plant visual damage using a 1-5 scale score.

Score	Description of plant visual damage
1	Healthy, green
2	No more than 25% of leaves are chlorotic/wilted, but no necrosis
3	Chlorosis, wilting, or necrosis on 50% of leaves
4	About 75% of the plants have chlorotic, wilted, or necrotic stem necrosis beginning
5	Plant is dead

#### **Result and Discussions**

# Soil electrical conductivity (EC<sub>e</sub>) and soil pH as affected by container size (CS) and salt irrigation concentration (SI) and duration of salt irrigation

No two-way or three-way interactions were observed in soil salinity and pH (Table 2). Soil salinity was not affected by CS, averaging 7.2 dS m<sup>-1</sup>. Soil salinity increased from 2.37 dS m<sup>-1</sup> in the non-salt treated containers to 12.53 dS m<sup>-1</sup> in the containers treated with the 20 dS m<sup>-1</sup> solution when data were pooled across CS and duration of exposure. Soil salinity was steady from week (W) 1 to W3, averaging 6.51 dS m<sup>-1</sup>. At W4, soil EC<sub>e</sub> increased up to 9.35 dS m<sup>-1</sup>. In contrast, soil pH was only affected by the duration of saline exposure. The highest soil pH was observed at W4 (7.45), 9.8% higher than that of W1 and W3 (averaged 6.79). Over the 4-week saline exposure, soil pH was in the optimal range (5.9 - 7.5) for pea growth (Mahler *et al.*, 1988). Various research has shown that soil salinity increases with increasing salt concentrations in irrigation and along the duration of salt treatment (Chen *et al.*, 2010; Kim *et al.*, 2016; Malash *et al.*, 2008; Tedeschi and Dell'Aquila, 2005). Soil EC<sub>e</sub> was 7.04 dS m<sup>-1</sup> when the present
experiment was terminated at W4 (Table 2), similar to the concentration (6.9 dS m<sup>-1</sup>) that caused 50% yield in pea (Page *et al.*, 2021). Similar to our observations, Kim *et al.* (2016) reported no significant difference in soil pH from lettuce (*Lactuca sativa* L.) and Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*) irrigated with NaCl (0.3 - 1.9 dS m<sup>-1</sup>) for 1 - 1.5 month. The previous and current research results suggest that soil salinity is more sensitive to saline conditions than soil pH.

Treatment	$EC_e (dS m^{-1})$	pH						
Container size (CS)								
<sup>a</sup> Big	<sup>b</sup> 7.41±3.76a	7.02±1.34a						
Small	7.04±3.76a	7.07±1.34a						
P values	<sup>c</sup> ns	ns						
Salt irrigation (SI, dS m <sup>-1</sup> )								
0	$2.37 \pm 3.05e$	6.98±0.62a						
5	$4.59 \pm 3.00 d$	7.01±0.62a						
10	$7.04 \pm 3.05c$	7.06±0.62a						
15	$9.58 \pm 3.05b$	7.03±0.62a						
20	$12.53 \pm 3.00a$	7.14±0.62a						
P values	*	ns						
Duration of saline exposure (W, week)								
1	6.44±3.16b	6.73±0.63c						
2	6.24±3.16b	7.16±0.63b						
3	6.86±3.23b	6.84±0.63c						
4	9.35±3.16a	7.45±0.63a						
P values	*	*						
$CS \times SI$	ns	ns						
$\mathrm{SI}  imes \mathrm{W}$	ns	ns						
$\mathrm{CS}  imes \mathrm{W}$	ns	ns						
CS  imes SI  imes W	ns	ns						

Table 2. Soil electrical conductivity (EC<sub>e</sub>) and pH as affected by container size, salt irrigation concentration, duration of saline exposure, and their interactions.

<sup>a</sup>Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long).

<sup>b</sup>Values represent mean±standard deviation. Values followed by a common letter within each column are not significantly different at  $P \le 0.05$ .

<sup>c</sup>ns and \* indicate no significant differences and significant differences at  $P \le 0.05$ , respectively.

## Plant phenotypic responses as affected by CS, SI, W, and their interactions

The main effects and their interactions on phenotypic responses are shown in Tables 3

and 4. There was no three-way interaction observed in any traits. Plants grown in small cone-

containers had lower values for all growth indices than those in the big containers when data

were pooled across SI and W, except RTTDW, SRL, and VD (Tables 3 and 4). The highest and

lowest growth reduction induced by container size was observed in AWC (34.0%) and RL

(14.9%), respectively. Higher SRL in the small containers (210.31 cm g<sup>-1</sup>) compared to that in the big containers (156.35 cm g<sup>-1</sup>) was due to a higher reduction of RDW (30.0%) than RL (14.1%) in the small containers (Table 3). Plants grown in the small containers had more leaf yellowing/chlorosis on the bottom of stems, resulting in a higher VD than those in the big containers (Table 4). Similar trends were observed in TAGFW, AWC, and VD when the CS x W interactions were detected and in TAGDW from the CS x SI interaction that the plants in the big containers performed better than those in the small ones (Figures 1 and 2). The aforementioned interactions were mostly due to the magnitude of changes at different SI or W. The differences in TAGDW and RDW between the two containers were similar (32.7% and 30.0%, respectively); thus, RTTDW did not show responses to CS (Table 3).

There is other research showing that plant growth is affected by container size. For instance, Ruff *et al.* (1987) reported fewer leaves, lower dry matter, and less yield in tomato (*Solanum lycopersicum* L.) plants grown in the 1.0 L containers than those in the 1.5 L. Similarly, cucumber (*Cucumis sativus* L.) plants showed reduced leaf area, fewer lateral stems and lower shoot and root biomass as container size reduced from 5.9 to 0.4 L (Robbin and Pharr, 1988). Zhou *et al.* (2021) observed higher plant height, stem diameter, and root length of tomato plants grown in the 200 L container than those in the 8 – 48 L containers under both saline and non-saline conditions. Broccoli (*Rassica oleracea* var. *italica*) grown in the small containers (2 L) had lower curd fresh and dry weight than those in the big containers (4 L), and the differences were more pronounced under salinity stress (Elwan *et al.*, 2017). The below and above-ground biomass ratio (i.e. RTTDW) was not affected by CS in the present study (Table 3), consistent with the results from Ruff *et al.* (1987) and Robbin and Pharr (1988). Small containers restrict rooting volume, resulting in reduced water and nutrient uptake and low demand for

photoassimilates. It, in return, causes physiological and morphological changes in the aboveground tissue, including small leaf size, reduced photosynthesis, and low biomass accumulation (Elwan *et al.*, 2017; Ruff *et al.*, 1987). Thus, a stable RTTDW ratio (i.e. an equilibrium between above and below ground growth) was observed (Robbin and Pharr, 1988). In the present study, the plants grown in the small cone-containers (410 ml) at 10 dS m<sup>-1</sup> showed more rapid senescence of old leaves (VD = 2.7) by W4 compared to those in the big cone-containers (556 ml) (VD = 1.3), suggested that the big-containers (556 ml) were able to support 4-week normal growth of pea seedlings with no adverse effects from container size. Salt concentrations at 15 and 20 dS m<sup>-1</sup> caused severe plant damage grown in both container sizes with an averaged VD of 4.3 at W4.

Plant responses to harsh environments are related to stress intensity and duration of exposure (Hessini *et al.*, 2014). Among the growth indices related to root status, RDW and RL were negatively affected by SI when data were pooled across CS and W, while RTTDW and SRL showed no response to SI (Table 3). Compared to the non-stressed plants (0 dS m<sup>-1</sup>), RDW was reduced starting at 10 dS m<sup>-1</sup> compared to RL at 15 dS m<sup>-1</sup>. Plants watered at 0 and 5 dS m<sup>-1</sup> performed similarly in the above-ground growth, better than those at 10 and 15 dS m<sup>-1</sup>, and the plants under the 20 dS m<sup>-1</sup> treatment performed the worst (Table 4). Similar trends were observed when the two-way interactions, CS x SI and SI x W, were detected (Figures 2 – 4). Root length did not change from W1 to W4, averaging 10.8 cm when data were pooled across CS and SI (Table 3). The SRL was steady from W1 to W3 (averaged 156.8 cm g<sup>-1</sup>), 41.4% less than that of W4. The highest and lowest RDW was detected in W2 and W4, respectively. The above ground growth was higher in W3 and W4 compared to W1 and W2, which was mostly due to the growth under the low saline conditions ( $\leq 10$  dS m<sup>-1</sup>) (Table 4, Figures 3 and 4A). Plants

under the 15 and 20 dS m<sup>-1</sup> treatments generally showed limited changes in the above-ground tissue biomass and AWC from W2 to W4. The VD was 2.4 in the plants at 20 dS m<sup>-1</sup> at W2, significantly higher than the other treatments (Figure 4B). By W4, plants at 15 and 20 dS m<sup>-1</sup> were almost all dead (averaged VD = 4.3).

	<sup>a</sup> RDW	RL	RTTDW	SRL			
Treatment	(g)	(cm)	(%)	$(cm g^{-1})$			
Container size (CS)							
Big <sup>b</sup>	0.10±0.09a <sup>c</sup>	11.61±4.47a	22.88±13.42a	156.35±130.94b			
Small	$0.07 \pm 0.09 b$	9.97±5.10b	21.82±15.12a	210.31±152.59a			
P values	*	*	ns	*			
Salt irrigation (	SI, dS $m^{-1}$ )						
0	0.11±0.06a	11.83±0.85ab	22.29±12.84a	124.25±164.95a			
5	0.11±0.06ab	12.58±0.85a	23.03±12.78a	147.55±162.58a			
10	0.08±0.06bc	10.84±0.85ab	20.08±11.65a	195.38±147.53a			
15	0.07±0.06cd	10.31±0.85b	22.49±11.03a	209.53±139.22a			
20	0.05±0.06d	8.37±0.85c	23.86±11.31a	239.96±143.23a			
P values	*	*	ns	ns			
Duration of saline exposure (W, week)							
1	0.09±0.06b	10.75±4.05a	35.79±12.65a	146.02±136.42b			
2	0.11±0.06a	11.52±4.24a	29.48±12.65b	146.31±136.42b			
3	0.08±0.06b	10.86±4.36a	14.83±12.27c	178.11±134.46b			
4	0.06±0.06c	10.02±4.24a	9.29±12.14d	262.89±131.55a			
P values	*	ns	*	*			
$CS \times SI$	<sup>d</sup> ns	ns	ns	ns			
$\mathrm{SI}  imes \mathrm{W}$	ns	ns	ns	ns			
$\mathbf{CS}  imes \mathbf{W}$	ns	ns	ns	ns			
$CS \times SI \times W$	ns	ns	ns	ns			

Table 3. Root growth of pea seedlings as affected by container size, salt concentration, duration of saline exposure, and their interactions.

<sup>a</sup>RDW, root dry weight; RL, root length; RTTDW, root dry weight to total above-ground dry weight ratio; SRL, specific root length.

<sup>b</sup>Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long).

<sup>c</sup>Values represent mean±standard deviation. Values followed by a common letter within each column are not significantly different at  $P \le 0.05$ .

<sup>d</sup>ns and \* indicate no significant differences and significant differences at  $P \le 0.05$ , respectively.

		TAGFW	TAGDW	AWC		
Treatment	<sup>a</sup> VD	(g)	(g)	(g)		
Container size (CS)						
<sup>b</sup> Big	<sup>c</sup> 1.69±1.07b	3.01±1.61a	0.55±0.45a	2.47±1.16a		
Small	2.07±1.25a	1.99±1.70b	0.37±0.45b	1.63±1.34b		
P values	*	*	*	*		
Salt irrigation (SI, dS m	1 <sup>-1</sup> )					
0	1.28±1.02c	3.87±1.41a	0.65±0.34a	3.23±1.13a		
5	1.36±1.02c	3.53±1.41a	0.59±0.34a	2.95±1.13a		
10	$1.87{\pm}1.02b$	2.24±1.36b	0.45±0.34b	$1.80{\pm}1.07b$		
15	2.14±0.96b	1.79±1.36b	0.36±0.34bc	$1.44{\pm}1.07b$		
20	2.76±1.02a	1.06±1.36c	0.25±0.34c	0.82±1.07c		
P values	*	*	*	*		
Duration of saline exposure (W, week)						
1	1.04±0.95c	1.69±1.33c	0.25±0.32c	1.45±1.08c		
2	1.35±1.01c	2.36±1.39b	0.36±0.38b	$2.01{\pm}1.14b$		
3	1.95±1.01b	3.02±1.39a	0.58±0.38a	2.45±1.14a		
4	3.19±1.01a	2.93±1.39a	0.65±0.38a	2.28±1.08ab		
P values	*	*	*	*		
$CS \times SI$	<sup>d</sup> ns	ns	*	ns		
$\mathrm{SI}  imes \mathrm{W}$	*	*	*	*		
$\mathbf{CS}  imes \mathbf{W}$	*	*	ns	*		
$CS \times SI \times W$	ns	ns	ns	ns		

Table 4. Above ground growth and visual damage rating of pea seedlings as affected by container size, salt concentration, duration of saline exposure, and their interactions.

<sup>a</sup>VD, visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants); TAGFW, total above-ground fresh weight; TAGDW, total above-ground dry weight; AWC, absolute water content.

<sup>b</sup>Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long).

<sup>c</sup>Values represent mean±standard deviation. Values followed by a common letter within each column are not significantly different at  $P \le 0.05$ .

<sup>d</sup>ns and \* indicate no significant differences and significant differences at  $P \le 0.05$ , respectively.



Figure 1. Total above-ground fresh weight (g) (A), absolute water content (g) (B), and visual damage (1 - 5 scale, 1 = healthy plants and 5 = dead plants) (C) as affected by container size (big and small) and duration of saline exposure (week). Uppercase letters indicate differences between container sizes in the same week at  $P \le 0.05$ . Lowercase letters indicate differences among weeks in the same container size at  $P \le 0.05$ . Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long).



Figure 2. Total above-ground dry weight (g) as affected by container size (big and small) and salt irrigation concentration  $(0 - 20 \text{ dS m}^{-1})$ . Uppercase letters indicate differences among salt irrigation concentrations in the same container size at  $P \le 0.05$ . Lowercase letters indicate differences between container sizes at the same salt irrigation concentration at  $P \le 0.05$ . Big container = 556 ml (6.4 cm-diam. x 25.4 cm long); small container = 410 ml (3.8-cm diam. x 20.3 cm long).



Figure 3. Total above ground fresh (A) and dry (B) weight (g) as affected by salt irrigation concentration  $(0 - 20 \text{ dS m}^{-1})$  and duration of saline exposure (week). Uppercase letters indicate differences among salt irrigation concentrations in the same week at  $P \le 0.05$ . Lowercase letters indicate differences among weeks at the same salt irrigation concentration at  $P \le 0.05$ .



Figure 4. Absolute water content (g) (A) and visual damage (1 - 5 scale, 1 = healthy plants and 5 = dead plants) (B) as affected by salt irrigation concentration  $(0 - 20 \text{ dS m}^{-1})$  and duration of saline exposure (week). Uppercase letters indicate differences among salt irrigation concentrations within the same week at  $P \le 0.05$ . Lowercase letters indicate differences among weeks at the same salt irrigation concentration at  $P \le 0.05$ .

## Correlations

A positive correlation (r = 0.22,  $P \le 0.05$ ) between soil salinity and pH was detected in the present study (Table 5). Rahman *et al.* (1993) reported that water soluble Na was a better indicator of soil salinity (r = 0.99) than soil pH (r = 0.27). Among the eight phenotypic traits that were correlated with soil EC<sub>e</sub> ( $P \le 0.05$ ), VD (r = 0.49) and SRL (r = 0.34) were positively correlated with soil salinity. In contrast, AWC, TAGFW, TAGDW, and RDW (-0.49  $\le r \le -0.26$ ) were negatively associated with soil salinity (Table 5). Only four phenotypic measurements were correlated with soil pH ( $P \le 0.05$ ), with r ranging from -0.20 to 0.27 (Table 5). The findings suggest that plant performance was more affected by soil salinity than soil pH.

Salinity inhibits plant growth and development and induces leaf discoloration and defoliation. Ljubojević *et al.* (2016) reported that VD and growth indices (e.g. plant height, fresh weight, and root system volume) are reliable criteria in evaluating the salinity tolerance of three *Salvia* species. In the present study, the correlation coefficients between VD, RDW, RL, AWC, and TAGFW to soil salinity were above |0.40| (Table 5), close to the threshold level  $[0.50 \le |\mathbf{r}| \le 1.00)$ ] for a strong correlation (Statistic Solution, 2024). Severe damage induced by salt irrigation at 15 and 20 dS m<sup>-1</sup>, especially at W3 and W4, may contribute to the relatively low correlation coefficients observed in the current study compared to Ljubojević *et al.* (2016). For example, AWC of the plants under the high salt treatments (i.e. 15 and 20 dS m<sup>-1</sup>) at W4 was 0.60 g, 84.1% lower than the control treatment (0 dS m<sup>-1</sup>) in the present study (Figure 4A). Root volume (the index showed the highest sensitive to salinity) was reduced by 54.5% in *S. splendens*, the most salt-sensitive species among the three *Salvia* species, when salinity increased from 0 to 100 mM NaCl (~ 10 dS m<sup>-1</sup>) after a 10-week treatment (Ljubojević *et al.*, 2016).

The AWC, an indicator of leaf size, was calculated as the difference between tissue fresh and dry weight (Hughes *et al.*, 1970). Thus, it was strongly associated with TAGFW (r = 0.99, P  $\leq 0.05$ ) and TAGDW (r = 0.86, P  $\leq 0.05$ ) (Table 5). The AWC was also highly correlated with soil salinity (r = -0.49,  $P \le 0.05$ ), RDW (r = 0.61,  $P \le 0.05$ ), and RL (r = 0.56,  $P \le 0.05$ ) (Table 5). Furthermore, AWC showed the earliest responses and highest reduction to saline conditions (Table 4; Figure 4A). The TAGFW was reduced by 72.6% as salinity increased from 0 to 20 dS m<sup>-1</sup>, the 2<sup>nd</sup> highest reduction in the growth indices (Table 4). As saline conditions induce osmotic stress (or physiological drought) at the early stage of salinity stress, initial plant responses to salinity and drought are similar. Cell expansion (i.e. size) is more sensitive to internal water content than other growth and physiological indices, such as shoot and root dry weight, chlorophyll content, and photosynthetic rate (Pugnaire et al., 1999; Zhang et al., 2018). Frank and McNaughton (1990) reported plants with big leaf sizes have high light capture efficiency, leading to high biomass accumulation. The VD had the same level of correlation as AWC to soil salinity (r = 0.49,  $P \le 0.05$ ), although it was not as closely related to other growth indices as AWC. The VD changes, such as leaf chlorosis and defoliation, are more closely related to nutrient imbalance/toxicity, especially the Na/K ratio, occurring at the late stage of salinity stress (Munns and Tester, 2008). Therefore, AWC, TAGFW, and VD have the potential to be used as key criteria to evaluate plant responses to salinity.

The SRL, calculated as RL divided by RDW, reflects the energy distribution in roots between water/nutrient uptake (i.e. RL) and carbohydrate storage (i.e. RDW) (Pérez-Harguindeguy *et al.*, 2013). Ostonen *et al.* (2007) suggest that SRL is a good indicator of root responses to environmental changes. The SRL was positively related to soil salinity in the present study (r = 0.34,  $P \le 0.05$ ), consistent with the previous findings of Abbas *et al.* (2018) and Rue and Zhang (2019). Chen *et al.* (2022) and the current study both showed that RL was less affected by salinity than RDW, resulting in increased SRL under saline conditions. Furthermore, the current study showed a stronger correlation between SRL and RDW (r = -0.71,  $P \le 0.05$ ) than SRL and RL (r = -0.33,  $P \le 0.05$ ) (Table 5). However, the correlation between SRL and soil EC<sub>e</sub> was not as strong as that between RDW, RL, and soil EC<sub>e</sub> (Table 5), suggesting that all three root characteristics must be taken into consideration to have a good understanding of the influence of salinity on root morphology (Pérez-Harguindeguy *et al.*, 2013; Zhang *et al.*, 2018).

	ECe	pН	RDW	RL	RTTDW	SRL	AWC	TAGDW	TAGFW	VD
<sup>a</sup> EC <sub>e</sub>										
лIJ	0.22									
рп	0.22 *									
RDW	-0.45	-0.2								
	*	*								
RL	-0.42	-0.08	0.64							
	*	<sup>b</sup> ns	*							
RTTDW	-0.15	-0.26	0.36	0.10						
	ns	*	*	ns						
SRL	0.34	0.24	-0.71	-0.33	-0.46					
	*	*	*	*	*					
AWC	-0.49	0.03	0.61	0.56	-0.24	-0.44				
	*	ns	*	*	*	*				
TAGDW	-0.26	0.16	0.48	0.46	-0.47	-0.29	0.86			
	*	ns	*	*	*	*	*			
TAGFW	-0.46	0.06	0.60	0.55	-0.29	-0.42	0.99	0.90		
	*	ns	*	*	*	*	*	*		
VD	0.49	0.27	-0.4	-0.3	-0.38	0.44	-0.37	-0.02	-0.31	
	*	*	*	*	*	*	*	ns	*	

Table 5. Pearson correlation coefficient analyses between soil electrical conductivity (EC<sub>e</sub>), soil pH, and phenotypic traits in pea seedlings.

<sup>a</sup>VD = visual damage rating (1 – 5 scale, 1 = healthy plants and 5 = dead plants), TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, RL = root length, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, EC<sub>e</sub> = electrical conductivity from saturated paste. <sup>b</sup>ns and \* indicate no significant differences and significant differences at  $P \le 0.05$ , respectively.

38

### Conclusions

Dry pea is a nutritive leguminous crop mainly produced in the Northern Great Plains, where sulfate-salinity is a major obstacle for agricultural production. Therefore, it is critical to understand the growth and development of dry pea as affected by sulfate salinity (intensity and duration) and container size in order to develop a screening protocol for salt tolerance. The present study showed that the 556 ml cone-container was sufficient to support the growth of a single pea plant for up to 4 weeks. Use of such cone-containers can largely help reduce cost of labor, time and space in dry pea evaluations under the controlled environment. This study also demonstrated that a 4-week saline irrigation at 10 dS m<sup>-1</sup> of a Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub> mixture (2:1, M:M), can be used to explore pea tolerance to sulfate-salinity, with TAGFW, AWC, and VD being the best indicators of salinity responses. Further research should explore the underlying physiological and molecular mechanisms of sulfate-salinity on dry pea.

#### References

- Abbas, G., Chen, Y., Khan, F. Y., Feng, Y., Palta, J. A., & Siddique, K. H. (2018). Salinity and low phosphorus differentially affect shoot and root traits in two wheat cultivars with contrasting tolerance to salt. *Agronomy*, 8(8), 155. https://doi.org/10.3390/agronomy8080155.
- Arzani, A., & Ashraf, M. (2016). Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. *Critical Reviews in Plant Sciences*, 35(3), 146-189. https://doi.org/10.1080/07352689.2016.1245056.
- Chen, W., Hou, Z., Wu, L., Liang, Y., & Wei, C. (2010). Evaluating salinity distribution in soil irrigated with saline water in arid regions of Northwest China. *Agricultural Water Management*, 97(12), 2001-2008. https://doi.org/10.1016/j.agwat.2010.03.008.

- Chen, Y., Liu, Y., Ge, J., Li, R., Zhang, R., Zhang, Y., Huo, Z., Xu, K., Wei, H., & Dai, Q. (2022). Improved physiological and morphological traits of root synergistically enhanced salinity tolerance in rice under appropriate nitrogen application rate. *Frontiers in Plant Science*, *13*, 982637. https://doi.org/10.3389/fpls.2022.982637.
- Datta, K. S., Kumar, A., & Verma, S. K. (1994). Variations in growth and physiology of barley under chloride and sulphate salinity. *Annals of Arid Zone, 33*, 303–307.
- Derner, J., Joyce, L., Guerrero, R., Steele, R., & Anderson, T. (2015). Northern plains regional climate hub assessment of climate change vulnerability and adaptation and mitigation strategies. United States Department of Agriculture.
  http://climatehubs.oce.usda.gov/sites/default/files/Northern%20Plains%20Vulnerability%20Assessment%205\_1\_2015\_Compressed.pdf
- Duke, J. (2012). *Handbook of legumes of world economic importance*. Springer Science and Business Media.
- Egamberdieva, D., & Lugtenberg, B. (2014). Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants. In M. Miransari., (Ed.), *Use of Microbes for the Alleviation of Soil Stresses* (Volume 1, pp. 73-96). Springer.
- Ehtaiwwesh, A. F., & Emsahel, M. J. (2020). Impact of salinity stress on germination and growth of pea (*Pisum sativum* L) plants. *Al-Mukhtar Journal of Science*, 35(2), 146-159. https://doi.org/10.54172/mjsc.v35i2.319.
- Elwan, M., El-Hamed, A., & Khalil, M. (2017). Influence of genotype, salinity, sulfur treatments and planting container size on growth, yield and incidence of gray mold in broccoli plants with propolis extract as disease control treatment. *Hortscience Journal of Suez Canal University*, 6(1), 51-63.

- Evans, J., Chalk, P. M., & O'Connor, G. E. (1995). Potential for increasing N<sub>2</sub> fixation of field pea through soil management and genotype. *Biological Agriculture and Horticulture*, *12(2)*, 97-112. https://doi.org/10.1080/01448765.1995.9754730.
- FAOSTAT. (2017). *Production of Crops*. http://www.fao.org/faostat/en/#data/QC (accessed on March 9, 2024).
- FAOSTAT. (2020). *Land use in agriculture by the numbers*. Food and Agriculture Organization of the United Nations.

https://www.fao.org/sustainability/news/detail/en/c/1274219/#:~:text=Globally%20agricu ltural%20land%20area%20is,and%20pastures)%20for%20grazing%20livestock

- Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T.A., Hodgson, M. J., Cooper, J.W, Miller, A.J., Kunert, K., Vorster, Juan., Cullis, C., Ozga J. A., Wahlqvist, M.L., Liang, Y., Shou, Huixia., Shi, K., Yu, J.,. ... & Considine, M. J. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants*, *2*, 16112. https://doi.org/10.1038/nplants.2016.112
- Frank, D. A., & McNaughton, S. J. (1990). Aboveground biomass estimation with the canopy intercept method: a plant growth form caveat. *Oikos*, 57(1), 57-60. https://doi.org/10.2307/3565736
- Franzen, D. (2003). Managing saline soils in North Dakota. North Dakota State University, Fargo, ND 58105, SF-1087 (revised).
- Gupta, V. K., & Gupta, S. P. (1984). Effect of zinc sources and levels on the growth and Zn nutrition of soybean (*Glycine max*. L.) in the presence of chloride and sulphate salinity.

In H. Lambers (Ed.), *Plant and Soil* (pp. 299-304). Springer. https://doi.org/10.1007/bf02197164.

- Hessini, K., Ferchichi, S., Ben Youssef, S., Werner, K. H., Cruz, C., & Gandour, M. (2015).
  How does salinity duration affect growth and productivity of cultivated barley? *Agronomy Journal, 107*, 174-180. https://doi.org/10.213/agronj14.0281.
- Hughes, A. P., Cockshull, K. E., & Heath, O. V. S. (1970). Leaf area and absolute leaf water content. *Annals of Botany*, 34(2), 259-266. https://doi.org/10.1093/oxfordjournals.aob.a084366.
- Irakoze, W., Prodjinoto, H., Nijimbere, S., Bizimana, J. B., Bigirimana, J., Rufyikiri, G., & Lutts, S. (2021). NaCl<sup>-</sup>and Na<sub>2</sub>SO<sub>4</sub><sup>-</sup>induced salinity differentially affect clay soil chemical properties and yield components of two rice cultivars (*Oryza sativa* L.) in *Burundi Agronomy*, 11(3), 571. https://doi.org/10.3390/agronomy11030571
- Jing, A. S., Guo, B. C., & Zhang, X. Y. (1992). Chloride tolerance and its effects on yield and quality of crops. *China Journal of Soil Science*, *33*(6), 257-259.
- Kakraliya, S., U. Singh, A. Bohra, K. Choudhary, S. Kumar, R. S. Meena, and M. Jat. 2018.
  Nitrogen and legumes: a meta-analysis. In R.S. Meena et al. (Eds.) Legumes for Soil
  Health and Sustainable Management (pp. 277-314). Springer.
  https://doi.org/10.1007/978-981-13-0253-4\_9
- Kim, H., Jeong, H., Jeon, J., & Bae, S. (2016). Effects of irrigation with saline water on crop growth and yield in greenhouse cultivation. *Water*, 8(4), 127. https://doi.org/10.3390/w8040127.
- Kumari, T., & Deka, S. C. (2021). Potential health benefits of garden pea seeds and pods: A review. *Legume Science*, 3(2), e82. https://doi.org/10.1002/leg3.82.

- Leonforte, A., Forster, J. W., Redden, R. J., Nicolas, M. E., & Salisbury, P. A. (2013). Sources of high tolerance to salinity in pea (*Pisum sativum* L.). *Euphytica*, 189(2), 203-216. https://doi.org/10.1007/s10681-012-0771-4.
- Li, H., & Cheng, Z. (2015). Hoagland nutrient solution promotes the growth of cucumber seedlings under light-emitting diode light. *Acta Agriculturae Scandinavica, Section B—Soil and Plant Science*, 65(1), 74-82. https://doi.org/10.1080/09064710.2014.967285.
- Ljubojević, M., Ognjanov, V., Maksimović, I., Čukanović, J., Dulić, J., Szabò, Z., & Szabò, E.
  (2017). Effects of hydrogel on growth and visual damage of ornamental salvia species exposed to salinity. *Clean Soil Air Water*, 45, 1600128.
  https://doi.org/10.1002/clen.201600128.
- Maas, E. V., 1990. Crop Salt Tolerance. In K.K. Tanji (Ed.), Agricultural Salinity Assessment and Management (pp. 262-304). ASCE Publications.
- Mahler, R.L., Saxena, M.C., & Aeschlimann, J. (1988). Soil fertility requirements of pea, lentil, chickpea and faba bean. In R.J. Summerfield (Ed.) World crops: Cool season food legumes. Current Plant Science and Biotechnology in Agriculture (vol 5, pp. 279-289). Springer. https://doi.org/10.1007/978-94-009-2764-3\_27
- Malash, N. M., Flowers, T. J., & Ragab, R. (2008). Effect of irrigation methods, management and salinity of irrigation water on tomato yield, soil moisture and salinity distribution. *Irrigation Science*, 26, 313-323.
- Montanarella, L., & Panagos, P. (2015). Policy relevance of critical zone science. *Land Use Policy*, 49, 86-91.
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651-681.

- O'Connor, G. E., Evans, J., Fettell, N. A., Bamforth, I., Stuchberry, J., Heenan, D. P., & Chalk,
  P. M. (1993). Sowing date and varietal effects on the N<sub>2</sub> fixation of field pea and implications for improvement of soil nitrogen. *Australian Journal of Agricultural Research*, 44(1), 151-163. https://doi.org/10.1071/AR9930151.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lõhmus, K., Majdi, H., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M., & Brunner, I. (2007). Specific root length as an indicator of environmental change. *Plant Biosystems*, 141(3), 426-442. https://doi.org/10.1080/11263500701626069.
- Ouerghi, K., Abdi, N., Maazaoui, H., Hmissi, I., Bouraoui, M., & Sifi, B. (2016). Physiological and morphological characteristics of pea (*Pisum sativum* L.) seeds under salt stress. *Journal of New Sciences*, 28.
- Page, K. L., Dang, Y. P., Martinez, C., Dalal, R. C., Wehr, J. B., Kopittke, P. M., Orton, T.G. & Menzies, N. W. (2021). Review of crop-specific tolerance limits to acidity, salinity, and sodicity for seventeen cereal, pulse, and oilseed crops common to rainfed subtropical cropping systems. *Land Degradation and Development*, *32*(*8*), 2459-2480. https://doi.org/10.1002/ldr.3915.
- Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P. Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S., & Cornelissen, J. H. C. (2016). Corrigendum to: new handbook for

standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, *64*(8), 715-716.

- Pugnaire, F. I., Serrano, L. & Pardos, J. (1999). Constraints by water stress on plant growth. In
  M. Pessarakli (Ed.) *Handbook of plant and crop stress* (2<sup>nd</sup> Ed, pp. 271–313), Marcel Dekker.
- Rahman, S., Vance, G. F., & Munn, L. C. (1993). Salinity induced effects on the nutrient status of soil, corn leaves and kernels. *Communications in Soil Science and Plant Analysis*, 24(17-18), 2251-2269. https://doi.org/10.1080/00103629309368953.
- Robbins, N. S., & Pharr, D. M. (1988). Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. *Plant Physiology*, 87(2), 409-413. https://doi.org/10.1104/pp.87.2.409.
- Rue, K., & Zhang, Q. (2020). Kentucky bluegrass growth under saline, waterlogging, and salinewaterlogging conditions during germination and seedling growth. *Crop, Forage and Turfgrass Management*, 6(1), e20002. https://doi.org/10.1002/cft2.20002.
- Ruff, M. S., Krizek, D. T., Mirecki, R. M., & Inouye, D. W. (1987). Restricted root zone volume: influence on growth and development of tomato. *Journal American Society for Horticultural Science*, *112*(5), 763-769. https://doi.org/10.21273/JASHS.112.5.763.
- Shahid, M. A., Pervez, M. A., Balal, R. M., Abbas, T., Ayyub, C. M., Mattson, N. S. & Iqbal, Z. (2012). Screening of pea (*Pisum sativum* L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. *Australian Journal of Crop Science*, 6(9), 1324-1331.

- Shahid, M. A., Ashraf, M. Y., Pervez, M. A., Ahmad, R., Balal, R. M., & Garcia-Sanchez, F. (2013). Impact of salt stress on concentrations of Na<sup>+</sup>, Cl<sup>-</sup> and organic solutes concentration in pea cultivars. *Pakistan Journal of Botany*, 45(3), 755-761.
- Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture*, 4(2), 1-13.
- Statistics Solutions. (2024). *Pearson's Correlation Coefficient*. Statistics Solutions. https://www.statisticssolutions.com/free-resources/directory-of-statisticalanalyses/pearsonscorrelationcoefficient/#:~:text=High%20degree%3A%20If%20the%20 coefficient,to%20be%20a%20small%20correlation.
- Tassoni, A., Tedeschi, T., Zurlini, C., Cigognini, I. M., Petrusan, J. I., Rodríguez, Ó, Neri, S.,
  Celli, A., Siisti, L., Cinelli, P., Signori F., Tsatsos, G., Bondi, M., Verstringe S.,
  Bruggerman, G., & Corvini, P. F. (2020). State-of-the-art production chains for peas,
  beans and chickpeas—valorization of agro-industrial residues and applications of derived
  extracts. *Molecules*, 25(6), 1383. https://doi.org/10.3390/molecules25061383.
- Tedeschi, A., & Dell'Aquila, R. (2005). Effects of irrigation with saline waters, at different concentrations, on soil physical and chemical characteristics. *Agricultural Water Management*, 77(1-3), 308-322. https://doi.org/10.1016/j.agwat.2004.09.036
- Toker, C., & Mutlu, N. (2011). Breeding for abiotic stresses. In A. Pratap and J. Kumar (Eds.), Biology and Breeding of Food Legumes (pp. 241-261), CABI https://doi.org/10.1079/9781845937669.0241.

- Tracy, J. D. (2020). Screening field pea (Pisum sativum L.) for tolerance to high salinity conditions [Master's thesis, Montana State University-Bozeman, College of Agriculture]. https://scholarworks.montana.edu/handle/1/16611
- White, J. G. H. (1987). The importance of pea in New Zealand arable agriculture. Peas: management for quality. *Agronomy Society of New Zealand special*, *6*, 7-11.

Yang, L., & Zhang, Q. (2019). Kentucky bluegrass growth and quality as affected by salt type and concentration. *Agronomy Journal*, 111(1), 233-241. https://doi.org/10.2134/agronj2018.04.0264

- Zhang, Q., Yang, L., & Rue, K. (2018). Differences in seedling growth of 23 creeping bentgrass cultivars under polyethylene glycol-induced drought conditions. *HortTechnology*, 28, 327-331. https://doi.org/10.21273/HORTTECH03990-18.
- Zhou, K., Lazarovitch, N., & Ephrath, J. (2021). Effects of container size and fruit load intensity on tomato under salt stress. In EGU General Assembly Conference Abstracts, pp. EGU21-1960. http://doi.org/10.5194/egusphere-egu21-1960.

# GENOTYPE DIFFERENCES IN TOLERANCE TO SULFATE-SALINITY IN DRY PEA Abstract

The objective of this research was to determine genotype differences in tolerance to sulfate-salinity in dry pea. Plants from two germplasms [(including 294 accessions from the North Dakota State University Pulse Breeding Program (NDSU) and 199 collections from the United States Department of Agriculture Western Regional Plant Introduction Program (USDA)] and 'Agassiz' (a commercial variety) were exposed to non-saline (i.e. tap water) or saline irrigation (10 dS m<sup>-1</sup> induced by a mixture of Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>) for four weeks. Data were collected on above and below ground growth (shoot, pod, and root) in height, weight and visual damage. Salinity adversely affected pea growth and appearance. Broad-sense heritability  $(H^2)$ was reduced by salinity in both populations. Principle component analysis (PCA) showed that total above ground fresh weight and absolute water content (an indicator of leaf size) were highly associated with principal component 1 (PC1), while root dry weight/total above ground dry weight ratio and root dry weight were highly correlated to PC2 in both germplasms and growing conditions. Twelve and 19 genotypes were identified from the NDSU germplasm and the USDA germplasm, respectively, based on their high performance in either condition from the PCA and cluster analysis. NDP080169 (NDSU), PI\_117998 PSP and PI\_270536 PSP (USDA) showed good growth under both stressed and non-stressed conditions. The aforementioned genotypes may help improve sulfate-salinity tolerance in dry pea.

#### Introduction

Dry pea, also known as field pea (*Pisum sativum* L.), is high in nutritional and agricultural value (McPhee, 2003). Pea is a significant economic and nutritive crop due to its high protein, vitamin, mineral and prebiotic carbohydrates yet affordable to the poor consumer

(Behera *et al.*, 2022). It improves soil health by increasing microbial biodiversity in the soil and enhances soil water retention and soil organic carbon (Foyer *et al.*, 2016). Pea is the second most cultivated grain legume in the world, following dry beans (*Phaseolus vulgaris* L.), with production exceeding 16.2 million metric tons globally (Bekhit et al 2022). Pea production and yield have increased globally in the last ten years (Uskutoğlu and İdikut, 2023). Approximately 7 million ha of land was planted for pea production in 2023, with 0.4 million ha in the U.S. (Uskutoğlu and İdikut, 2023). About 1/3 of the total pea produced in the U.S. is from North Dakota (Janzen *et al.*, 2006; Vandemark *et al.*, 2014).

Salinity is a major obstacle to agricultural production worldwide. Approximately \$27.3 billion is lost globally each year as a result of reduced agricultural productivity caused by soil salinity (Qadir et al, 2014). "Ninety percent of the producers in North Dakota are challenged with salinity" (NDSU AgHub, 2023). One of the most economically effective methods to reduce stress damage is use of tolerant plants. Shahid et al. (2012) determined the salinity tolerance of 30 pea genotypes collected from Pakistan during the germination and early growth stage. Their results showed that the reduction of germination rate ranged from 4% to 48% and the accumulation of the leaf Na<sup>+</sup> level ranged from 15% to 57% among the genotypes. Leonforte etal. (2013) evaluated the visual symptoms and growth habits of 780 pea accessions, primarily from Asia (428 accessions) and Western Europe (113 accessions), under saline conditions (0 -18 dS m<sup>-1</sup>). In their study, over 80% of the accessions were susceptible to salinity, with a symptom score equal to or above 7 (1 = healthy plant and 10 = dead plant). However, limited information is available on salt tolerance in the Northern American germplasms. Furthermore, sulfate salts such as Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub> are dominant salts in the salt-affected areas in the Northern Great Plains (including North Dakota, South Dakota, Montana and Minnesota), a

leading area of pea production in the U.S. (Derner *et al.*, 2015; Tracy, 2020). Plant responses to chloride salts (such as NaCl) induced salinity have been extensively studied; however, the effects of sulfate-salinity on plant growth and development is not well documented. Plant sensitivity to chloride- and sulfate-salinity can be largely different (Jing *et al.*, 1992). The objective of this study was to determine genotype differences in tolerance to sulfate-salinity in the Northern American germplasms.

#### **Materials and Methods**

A total of 493 genotypes, including 294 accessions from the North Dakota State University Pulse Breeding Program (NDSU) and 199 collections from the United State Department of Agriculture Western Regional Plant Introduction Program (USDA), were included in this study (Tables A1 and A2). The NDSU and USDA germplasms were studied and analyzed separately because of the large variation in genetic background. 'Agassiz', a common commercial variety, was included in each germplasm as the check.

Untreated seeds were initially germinated in a potting mixture (Pro-mix Bx, Premier Horticulture Inc., Quakertown, PA) for five days and then transplanted to cone-containers (556 ml) filled with a mix of perlite and sand (2:1, v: v). Containers were soaked in the tubs filled with half-strength Hoagland solution (Li and Cheng, 2015) (~ 2.5 cm above the bottom of the cone-containers) overnight and moved and maintained in the tubs filled with tap water as described previously for 1 week. Then, the plants were split into two groups, one grown under non-stress conditions (i.e., tap water) and the other under salinity. Salinity stress was induced by setting the containers in the tubs filled with a Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>·7H<sub>2</sub>O mixture (2:1, M: M) at 10 dS m<sup>-1</sup> about ~2.5 cm deep. The tubs were filled with tap water (~2.5 cm deep) for the nonstressed plants. To prevent salinity shock, salt-treated plants were gradually acclimated at a rate

50

of 2.5 dS m<sup>-1</sup> per day. Non-stressed plants were watered with tap water when the salt-treated ones were acclimated with salt solution. Upon reaching the final concentrations, plants were kept in tubs filled with designated saline solutions in tubs as described previously. Plants were exposed to saline conditions for four weeks. All solutions were refreshed once weekly. Plants were fertilized with full-strength Hoagland solution by hand on the first day of salt acclimation and once weekly during the saline exposure. The EC and pH of the solutions in the tubs were measured using an EC/pH meter (Oakton PC 450, Oakton Instruments, Vernon Hills, IL) before and after refreshment to monitor the saline conditions.

The experiment was set up in a split-plot design, with the whole-plot treatment being the growing condition (saline vs. non-saline irrigation in the tubs) and the sub-plot treatment being genotype. Due to the large number of genotypes included in each germplasm, the experiment was conducted one replication at a time. Data were collected on plant height (HT), root length (RL), total above-ground fresh and dry weight (TAGFW, TAGDW), root dry weight (RDW), absolute water content (AWC), root dry weight to total above-ground dry weight ratio (RTTDW), specific root length (SRL), and plant visual damage (VD, rated visually with a 1 - 5 score described in Table 6. A repeat of equation on AWC and SRL were shown below.

$$^{3}[AWC = TAGFW - TAGDW]$$
(1)

$${}^{4}[SRL = RL/RDW]$$
(2)

TAGDW and RDW were recorded after oven-drying at 65°C for 48 hr. EC and pH were measured following the 1:2 dilution method (USDA, 1954) method using the EC/pH meter.

<sup>&</sup>lt;sup>3</sup>Source: Hughes *et al.*, 1970

<sup>&</sup>lt;sup>4</sup>Source: Ostonen et al., 2007

When the USDA germplasm was evaluated, TAGFW and TAGDW were further separated into pod fresh and dry weight (PFW, PDW) and shoot fresh and dry weight (SFW and SDW).

Table 6. Description of plant visual damage using a 1-5 scale score.

Score	Description of plant visual damage
1	Healthy, green
2	No more than 25% of leaves are chlorotic/wilted, but no necrosis
3	Chlorosis, wilting, or necrosis on 50% of leaves
4	About 75% of the plants have chlorotic, wilted, or necrotic stem necrosis beginning
5	Plant is dead

Best Linear Unbiased Prediction (BLUP) was generated for all phenotypic traits using the following equation,

$$trait = genotype + replicate + error$$
(3)

which genotype was treated as a random effect and the replicate as the fixed effect.

Broad-sense heritability  $(H^2)$  was analyzed in a mixed model using the following formula,

$$H^{2} = \sigma^{2} g / (\sigma^{2} g + \sigma^{2} \varepsilon / er)$$
(4)

in which  $\sigma^2 g$  is genotypic variance,  $\sigma^2 \varepsilon$  is error variance, and er is the number of replicates, in the R Statistical Software (v4.1.2; R Core Team 2021, Vienna, Austria). Principal component analysis (PCA) was computed using the PCA function and implemented in the "Facto-MineR" package in the R Software, with the graphical outputs visualized through the "Facto extra" package using the same software (Lê *et al.* 2008). The PCA shows the contribution of each trait to the total phenotypic variations observed among genotypes, indicating trait(s) with the most selective ability based on the magnitude of loadings. Hierarchical cluster analysis was performed using the elbow method and plots were generated using the 'Dend extend' package in the R Software. Cluster analysis groups similar genotypes which show a level of association.

### **Results and Discussions**

# Soil analysis

Soil EC<sub>1:2</sub> was 1.0 and 4.4 dS m<sup>-1</sup> for the non-saline and saline conditions, respectively, when the NDSU germplasm was evaluated ( $P \le 0.05$ ). Soil EC<sub>1:2</sub> was in a similar range, 0.8 and 4.4 dS m<sup>-1</sup>, for the USDA germplasm study ( $P \le 0.05$ ). Soil pH was not significantly different between the two growing conditions in either germplasm evaluation (averaged 7.75 and 7.50 for the NDSU and USDA germplasms, respectively). This result suggested that soil salinity was more affected by salt level in irrigation than soil pH. Johansen *et al.* (1990) reported a 50% shoot mass reduction in chickpea (*Cicer arietinum* L.) at EC<sub>1:2</sub> of 5 to 6 dS m<sup>-1</sup>, similar to the soil salinity level observed in the current study.

# Heritability $(H^2)$ and genetic variation of phenotypic traits under non-saline and saline conditions

Heritability indicates the proportion of phenotypic variation in a population due to genetic values that may include effects due to dominance and epistasis (Wray and Visscher, 2008). A high  $H^2$  value indicates that genetic variations influence a character more than environmental effects (Torche *et al.*, 2018). Within each germplasm, no consistent trends of  $H^2$  of phenotypic traits in response to salinity were observed (Tables 7 and 8). For example,  $H^2$  of traits like RL, RDW, and AWC was lower under the saline conditions than the non-saline conditions in the NDSU germplasm, while  $H^2$  of traits like TAGDW, RTTDW, and VD was higher (Table 7).  $H^2$  of HT, TAGFW, and SRL showed limited differences between the two growing conditions. Similar results were observed in the USDA population. Our findings were consistent with those of Torche *et al.* (2018), in which  $H^2$  showed positive, negative, or neutral responses to salt stress in different phenotypic traits of a common bean (*Phaseolus vulgaris* L.)

population. In addition,  $H^2$  of a phenotypic trait can show different trends depending on stress level. For instance,  $H^2$  of RDW was 83.6%, 53.5%, and 60.6%, respectively, in the common bean lines treated with NaCl at 0, 50, and 100 mM (Torche *et al.*, 2018). In contrast,  $H^2$  of SDW decreased from 81.6% to 75.2% as salinity increased from 0 to 50 mM NaCl, then increased to 97.9% as salinity further increased to 100 mM (Torche *et al.*, 2018). The results indicate the complexity of plant responses to stresses. In both germplasms of the current study, both saline and non-saline conditions, HT and RL had the highest and lowest  $H^2$ , respectively, among the phenotypic traits evaluated (Tables 7 and 8).

Salinity inhibited root and shoot growth and increased leaf wilting, chlorosis, and defoliation (i.e. VD) ( $P \le 0.05$ ) in both populations (Tables 7 and 8). The magnitude of reduction induced by salinity ranged from 11.6% in RL to 56.3% in VD in the NDSU population and from 7.8% in RL to 47.3% in PFW in the USDA germplasm. The salt-treated plants in both populations showed a narrower range of the mean of each phenotypic response than the non-treated ones, consistent with the findings of Manna *et al.* (2010), which indicates reduced population diversity under saline conditions.

Table 7. The variation (mean and ranges estimated based on the best linear unbiased predictor) of phenotypic traits and heritability of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.

Phenotypic Trait <sup>a</sup>	Mean±Standard Deviation	tandard Deviation Range		Heritability ( $H^2$ , %)
Non-saline				
HT (cm)	$45.88 \pm 3.56^{b}$	36.92-57.43	49.47	54.78
RL (cm)	$27.71 \pm 0.29^{b}$	26.90-28.62	27.25	10.72
TAGFW (g)	$7.58 \pm 0.02^{b}$	7.17-8.12	7.85	13.89
TAGDW (g)	$1.59 \pm 0.20^{b}$	1.39–1.81	1.59	23.98
RDW (g)	$0.27 \pm 0.07^{b}$	0.22-0.36	0.29	45.34
AWC (g)	$5.99 \pm 0.17^{b}$	5.67-6.49	6.10	15.09
RTTDW (%)	$19.62 \pm 0.83^{b}$	18.12-24.00	7.97	20.21
SRL (cm $g^{-1}$ )	$108.39 \pm 7.78^{b}$	90.70-137.91	102.93	33.20
VD $(1-5 \text{ scale}, 1 = \text{health and } 5 = \text{dead})$	$1.58 \pm 0.04^{b}$	1.50-1.72	1.75	13.73
Saline				
HT (cm)	$36.89 \pm 2.57^{b}$	30.08-48.40	39.21	52.58
RL (cm)	$24.95 \pm 6.32^{b}$	24.95-24.95	24.95	1.66x10 <sup>-7</sup>
TAGFW (g)	$4.64 \pm 0.02^{b}$	4.29-4.93	4.52	11.32
TAGDW (g)	$0.98{\pm}0.10^{ m b}$	0.85-1.12	0.98	28.47
RDW (g)	$0.20 \pm 0.05^{b}$	0.16-0.27	0.20	39.23
AWC (g)	$3.66 \pm 0.07^{b}$	3.42-3.84	3.54	8.35
RTTDW (%)	$23.26 \pm 1.57^{b}$	19.97–28.62	21.40	33.73
SRL (cm $g^{-1}$ )	133.82±9.68 <sup>b</sup>	114.96–184.87	137.68	32.51
VD $(1 - 5 \text{ scale}, 1 = \text{health and } 5 = \text{dead})$	$2.48 \pm 0.12^{b}$	2.15-2.84	2.83	21.90

55

<sup>a</sup>Phenotypic traits: HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating.

<sup>b</sup>Means are significantly different between the non-saline and saline conditions in the same phenotypic trait at  $P \le 0.05$ .

Table 8. The variation (mean and ranges estimated based on the best linear unbiased predictor) of phenotypic traits and heritability of a USDA pea germplasm [including 199 collection and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions (continued).

Phenotypic Trait <sup>a</sup>	Mean±Standard Deviation	Range	Check	Heritability ( $H^2$ , %)
Non-saline				
HT (cm)	$116.17 \pm 24.59^{b}$	40.05–158.13	31.31	83.87
RL (cm)	31.99±0.61 <sup>b</sup>	30.33-33.77	36.46	22.62
SFW (g)	$7.08{\pm}1.94^{b}$	2.20-1.97	1.53	78.17
PFW (g)	$1.66 \pm 0.98^{b}$	0.64-2.85	2.11	82.28
SDW (g)	$1.88{\pm}0.42^{b}$	0.33-4.49	0.44	67.76
PDW (g)	$0.44 \pm 0.22^{b}$	0.14–1.39	8.57	77.02
TAGFW (g)	$8.96 \pm 1.74^{b}$	3.69-12.91	1.98	59.35
TAGDW (g)	2.10±0.31 <sup>b</sup>	1.43-3.21	0.28	78.00
RDW (g)	$0.41 \pm 0.10^{b}$	0.14-0.70	6.59	77.36
AWC (g)	$6.86 \pm 1.45^{b}$	2.25-9.87	14.35	75.04
RTTDW (%)	$20.81 \pm 6.70^{b}$	8.57-43.39	123.79	68.89
SRL (cm $g^{-1}$ )	$95.57{\pm}33.08^{b}$	60.53-280.76	1.80	76.35
VD $(1-5 \text{ scale}, 1 = \text{health and } 5 = \text{dead})$	$2.06 \pm 0.62^{b}$	1.24-4.32	63.75	88.40
Saline				
HT (cm)	$97.86 \pm 20.54^{b}$	39.51-137.11	54.38	87.19
RL (cm)	$29.50 \pm 0.09^{b}$	29.29–29.70	29.50	3.98
SFW (g)	$4.77 \pm 1.28^{b}$	1.70-7.95	4.63	78.74
PFW (g)	$1.15 \pm 0.52^{b}$	0.58–1.69	1.14	74.16
SDW (g)	$0.99 \pm 0.23^{b}$	0.26–2.74	0.76	72.77
PDW (g)	$0.24 \pm 0.16^{b}$	0.04-0.85	0.13	72.52
TAGFW (g)	$5.75 \pm 1.19^{b}$	2.66-8.18	5.40	72.98

Table 8. The variation (mean and ranges estimated based on the best linear unbiased predictor) of phenotypic traits and heritability of a USDA pea germplasm [including 199 collection and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions (continued).

Phenotypic Trait <sup>a</sup>	Mean±Standard Deviation	Range	Check	Heritability $(H^2, \%)$
TAGDW (g)	1.38±0.19 <sup>b</sup>	0.95–1.88	1.28	60.82
AWC (g)	$4.37 \pm 1.05^{b}$	1.48-6.55	4.12	73.83
RTTDW (%)	$22.77 \pm 6.12^{b}$	9.76-41.75	18.45	77.91
SRL (cm $g^{-1}$ )	121.76±35.39 <sup>b</sup>	83.03-290.94	141.47	44.41
VD $(1-5 \text{ scale}, 1 = \text{health and } 5 = \text{dead})$	$2.60 \pm 0.60^{b}$	1.50-4.25	2.48	68.88

<sup>a</sup>Phenotypic traits: HT = plant height, RL = root length, SFW = shoot fresh weight, PFW = pod fresh weight, SDW = shoot dry weight, PDW = pod dry weight, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating.

<sup>b</sup>Means are significantly different between the non-saline and saline conditions in the same phenotypic trait at  $P \le 0.05$ .

57

# Principal component analysis (PCA) and cluster analysis of phenotypic traits under nonsaline and saline conditions.

Principal component analysis groups variables into principal components (PC), demonstrating the relationships among different variables (Wold *et al.*, 1987). The contribution of the traits to the total variation observed in the NDSU population under the non-saline and saline conditions are represented in Figures 5 and 6. The first four PCs cumulatively explained 85.4% of the variations in the NDSU population under the non-saline conditions, with each explained more than 10% of the variations (i.e. major PC) (Figure 5A). Under the saline conditions, there were three major PCs that counted for 76.5% of the total variations (Figure 6A). Major phenotypic traits ( $\geq$  10% contribution to a PC) associated to PC1 were AWC, RDW, TAGDW, and TAGFW (Figures 5B and 6B), while RDW, RTTDW, and SRL were major contributors to PC2 (Figures 5C and 6C) under both conditions. The RL (69.1% of contrition) and VD (68.7% of contribution) were the main contributors to PC3 and PC4, respectively, under the control treatment. The HT (24.0% of contribution) and VD (55.6% of contribution) were highly associated with PC3 for the plants treated with salt.

The first two PCs (i.e. PC1 and PC2) together explained over 70% of the variances in the USDA population under either growing condition (Figures 7A and 8A). Six phenotypic traits that made a high contribution to PC1 under the non-stress condition were AWC, RDW, SDW, SFW, SRL, and TAGFW, with contributions ranging from 10.3% (SRL) to SFW (13.6%) (Figure 7B). SRL was not a major contributor to PC1 under salinity stress (Figure 8B). Averaged contribution from PFW, PDW, TAGDW, and RTTDW for PC2 was 19.9% and 21.0% for the non-saline and saline condition, respectively (Figures 7C and 8C).



Figure 5. Scree plot of principal components (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline condition. HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total above-ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, VD = visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants).


Figure 6. Scree plot of principal components (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under saline condition. HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total above-ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, VD = visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants).



Figure 7. Scree plot of each principal component (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline condition. HT = plant height, RL = root length, SFW = shoot fresh weight, PFW = pod fresh weight, SDW = shoot dry weight, PDW = pod dry weight, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants).



Figure 8. Scree plot of each principal component (PC) (A) and contribution of phenotypic traits to PC1 (B) and PC2 (C) of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under saline condition. HT = plant height, RL = root length, SFW = shoot fresh weight, PFW = pod fresh weight, SDW = shoot dry weight, PDW = pod dry weight, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating (1 – 5 scale, 1 = healthy plants and 5 = dead plants).

The biplots, Figures 9 and 10 showed the relationships among phenotypic traits. The dimension of the angle between two traits determines if the traits are positively (i.e. an acute angle), negatively (i.e. an obtuse angle), or not associated (i.e. a right angle) with each other (Yan and Rajcan, 2002). The correlation biplot showed that aboveground traits like TAGFW, TAGDW, and AWC were closely positively related to each other under both growing conditions in both germplasms (Figures 9 and 10). The results from both germplasms showed that SRL was more highly negatively associated with RDW than RL regardless of the growing conditions (Figures 9 and 10). The TAGDW was closely related to TAGFW and AWC in the NDSU population (Figure 9), but not in the USDA population (Figure 10). The SDW and SFW were closely related in the USDA germplasm. Furthermore, pod biomass (i.e. PFW and PDW) was not related to shoot or total above ground biomass when the genotypes from the USDA germplasm were evaluated (Figure 10).



Figure 9. Principal component analysis-biplot depicting the classification phenotypic traits of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline (A) and saline condition (B). The graded color scale, contribution, is used to estimate the quality of the representation. A high contribution value indicates a good representation of a trait on the principal component. HT = plant height, RL = root length, TAGFW = total above-ground fresh weight, TAGDW = total above-ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, VD = visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants).



Figure 10. Principal component analysis-biplot depicting the classification phenotypic traits of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline (A) and saline condition (B). The graded color scale, contribution, is used to estimate the quality of the representation. A high contribution value indicates a good representation of a trait on the principal component. HT = plant height, RL = root length, SFW = shoot fresh weight, PFW = pod fresh weight, SDW = shoot dry weight, PDW = pod dry weight, TAGFW = total above-ground fresh weight, TAGDW = total-above ground dry weight, RDW = root dry weight, AWC = absolute water content, RTTDW = root dry weight to total above-ground dry weight ratio, SRL = specific root length, and VD = visual damage rating (1 - 5 scale, 1 = healthy plants and 5 = dead plants).

To screen for high stress tolerance, plants should be evaluated on the traits associated with the major PCs that explain most of the variance in a population (Bairwa *et al.*, 2023; Joshi *et al.*, 2023; Mannan *et al.*, 2010; Salsman *et al.*, 2021). The top performers (10% of the population) selected based on each major phenotypic trait highly associated with PC1 and PC2 ( $\geq 10\%$  contribution) under either condition are presented in Tables A3 and A4. Genotypes that showed overall good performance are presented in Tables 9 and 10. One genotype, NDP080169, from the NDSU germplasm and two genotypes, PI\_117998 PSP and PI\_270536 PSP, from the USDA germplasm showed good growth under both the stressed and non-stressed conditions.

In the present study, tissue biomass was highly associated with the first two PCs, in which the above ground traits (e.g. TFW, AWC) were more associated with PC1, and the root characteristics (e.g. RDW) were more associated with PC2, especially for the NDSU germplasm. Tracy (2020) suggested that shoot and root biomass were significant contributors to the quantification of crop responses to salinity, including dry pea, which is similar to our results. However, HT was not associated with major PCs in the present study [except NDSU germplasm under salinity), contradicting the findings of Tracy (2020). Mannan *et al.* (2010) observed high contributions of total dry weight, shoot dry weight, and petiole dry weight to PC1 (97.0% of the variance) and root dry weight to PC2 (2.5% of the variance) in soybean plants under salinity (NaCl = 15 dS m<sup>-1</sup>). In contrast, Joshi *et al.* (2023) reported root traits, such as RL, RFW, and RDW, as the best descriptors (PC1 = 86.9%) in chickpea at 8 dS m<sup>-1</sup>, while the aboveground traits, such as total fresh weight, total dry weight, shoot dry weight, shoot fresh weight and shoot length contributed to PC 2 (5.3%). The discrepancy in the aforementioned research might be due to the differences in growth characteristics of the genotypes (species/accessions) included in each

study. For instance, chickpea has the strongest root system among the cool-season pulse crops,

followed by field pea, and lentil has the weakest roots.

Table 9. Recommended genotypes from a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)], selected based on high performance from phenotypic traits highly associated with the first two principal components under non-saline and saline conditions.

Non-saline			Saline		
No. of Genotype Recommended	Genotype Name	Cluster Group	No. of Genotype Recommended	Genotype Name	Cluster Group
5	NDP080169	1	7	NDP080169	2
	NDP121638	4		NDP130010	2
	NDP150001	4		NDP130085	2
	NDP150206	4		NDP150047	2
	PS07100995	1		NDP150232	2
				NDP170004G	2
				NDP170182Y	2

Table 10. Recommended genotypes from a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)], selected based on high performance from phenotypic traits highly associated with the first two principal components under non-saline and saline conditions.

Non-saline			Saline		
No. of Genotype	Genotype Name	Cluster	No. of Genotype	Genotype	Cluster
Recommended		Group	Recommended	Name	Group
9	PI_117264_PSP	4	10	PI_116056_PSP	1
	PI_117998_PSP	4		PI_117998_PSP	1
	PI_179459_PSP	1		PI_142775_PSP	1
	PI_180693_PSP	1		PI_155109_PSP	1
	PI_206006_PSP	1		PI_169603_PSP	1
	PI_249646	1		PI_171814	1
	PI_270536_PSP	1		PI_210571_PSP	1
	PI_280619_PSP	1		PI_269543_PSP	1
	PI_285710_PSP	1		PI_270536_PSP	1
				PI_340126	1

Based on the variation, the NDSU and USDA germplasms were grouped into five clusters using the hierarchical method (Figures 11 - 14). The mean value of phenotypic traits of each cluster group is presented in Tables 11 and 12. The number of NDSU accessions in each cluster group ranged from 2 (Cluster 5, saline) to 185 (Cluster 1, non-saline) (Table 11). Cluster 4 and 2 exhibited the highest TFW, TDW, AWC, and RDW under the non-saline and saline conditions, respectively. In the USDA population, Cluster 1 had the maximum genotypes for both non-saline (158) and saline conditions (131), and the plants in Cluster 1 performed better (e.g. higher SFW and SDW) than the plants in other clusters (Table 12). The recommended genotypes in both germplasms were primarily located in the clusters with the best phenotypic performance, especially under saline conditions (Tables 11 - 12).



Figure 11. Cluster analysis of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline condition. The cluster analysis revealed five major clusters (1 - 5). Accessions of the germplasm (numbered 1 - 294) and 'Agassiz' are listed on the bottom.



Figure 12. Cluster analysis of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under saline conditions. The cluster analysis revealed five major clusters (1-5). Accessions of the germplasm (numbered 1-294) and 'Agassiz' are listed at the bottom.

Cluster Dendrogram







Figure 14. Cluster analysis of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under saline conditions. The cluster analysis revealed five major clusters (1 - 5). Accessions of the germplasm (numbered 1 - 199) and 'Agassiz' are listed at the bottom.

Traits	Mean±Standard Deviation of Cluster (no. of genotype in a cluster)				
Non-	Cluster 1 (40)	Cluster 2 (99)	Cluster 3 (90)	Cluster 4 (56) <sup>a</sup>	Cluster 5 (10)
saline					
$HT^{b}$	$44.90 \pm 2.65$	$44.90 \pm 2.77$	44.50±3.13	$50.60 \pm 2.08$	$45.40 \pm 1.96$
RL	27.56±0.29	27.69±0.29	$27.75 \pm 0.30$	27.72±0.25	$27.93 \pm 0.29$
RDW	$0.30 \pm 0.02$	$0.27 \pm 0.01$	$0.25 \pm 0.01$	$0.29 \pm 0.02$	$0.24 \pm 0.01$
TFW	$7.64 \pm 0.20$	7.54±0.19	7.51±0.17	7.73±0.16	$7.46 \pm 0.21$
TDW	$1.61 \pm 0.06$	$1.58\pm0.06$	$1.56 \pm 0.06$	$1.66 \pm 0.06$	$1.55 \pm 0.05$
AWC	$6.04 \pm 0.18$	5.96±0.17	$5.94 \pm 0.14$	6.11±0.14	5.90±0.19
RTTDW	$20.23 \pm 0.84$	19.72±0.72	$19.38 \pm 0.85$	19.53±0.74	$18.87 \pm 0.57$
SRL	$99.06 \pm 2.76$	$106.55 \pm 2.52$	116.01±3.43	102.58±4.21	127.87±3.94
VD	$1.58 \pm 0.04$	$1.58\pm0.04$	$1.57 \pm 0.03$	$1.59\pm0.04$	$1.57 \pm 0.04$
Saline	Cluster 1 (185) <sup>a</sup>	Cluster 2 (48)	Cluster 3 (48)	Cluster 4 (12)	Cluster 5 (2)
HT	$37.05 \pm 2.34$	37.89±3.34	$36.05 \pm 1.88$	34.18±2.09	$34.54 \pm 0.58$
RL	$24.95 \pm 0.00$	$24.95 \pm 0.00$	$24.95 \pm 0.00$	$24.95 \pm 0.00$	$24.95 \pm 0.00$
RDW	$0.20\pm0.01$	$0.22 \pm 0.01$	$0.19 \pm 0.01$	$0.18 \pm 0.01$	$0.18 \pm 0.02$
TFW	4.65±0.10	4.68±0.10	4.61±0.08	4.58±0.12	4.36±0.10
TDW	$0.98 \pm 0.06$	$0.99 \pm 0.06$	$0.96 \pm 0.04$	$0.93 \pm 0.05$	$0.90 \pm 0.01$
AWC	$3.67 \pm 0.07$	$3.68 \pm 0.06$	$3.64 \pm 0.05$	$3.63 \pm 0.08$	$3.47 \pm 0.07$
RTTDW	23.19±1.37	$24.95 \pm 1.43$	22.29±0.94	21.75±1.39	$21.69 \pm 1.61$
SRL	132.36±4.35	121.62±2.85	$144.07 \pm 2.58$	156.63±5.66	179.38±7.76
VD	2.48±0.13	$2.47 \pm 0.11$	$2.45 \pm 0.12$	2.46±0.13	$2.62 \pm 0.20$

Table 11. Mean values of phenotypic traits of each cluster group of a NDSU pea germplasm [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.

<sup>a</sup>Cluster in which Agassiz (check) was located.

<sup>b</sup>Plant height (HT, cm), root length (RL, cm), shoot fresh weight (SFW, g), shoot dry weight (SDW, g), pod fresh weight (PFW, g), pod dry weight (PDW, g), total above-ground fresh weight (TAGFW, g), total above-ground dry weight (TAGDW, g), root dry weight (RDW, g), absolute water content (AWC, g), root dry weight to total above-ground dry weight ratio (RTTDW, %), specific root length (SRL, cm g<sup>-1</sup>), and visual rating of plant damage (VD, rated with a 1 - 5 scale in which 1 = healthy plants and 5 = dead plants).

Condition	n Mean±Standard Deviation of Cluster (no. of genotype in a cluster)				
Non-saline	Cluster 1 (158)	Cluster 2 (24)	Cluster 3 (5) <sup>a</sup>	Cluster 4 (12)	Cluster 5 (1)
HT	$124.94{\pm}15.32$	88.85±24.01	56.79±12.47	84.24±25.69	67.01
RL	32.10±0.55	31.81±0.60	31.34±0.23	31.20±0.62	30.73
SFW	$7.70{\pm}1.44$	$5.49 \pm 1.67$	4.50±1.23	$3.56 \pm 1.50$	2.22
PFW	$1.85 \pm 0.99$	2.13±1.16	$2.46 \pm 0.34$	$1.64 \pm 0.42$	1.20
SDW	$1.79 \pm 0.34$	$1.29 \pm 0.27$	$1.04 \pm 0.33$	$0.98 \pm 0.38$	0.72
PDW	$0.42 \pm 0.23$	$0.51 \pm 0.22$	$0.54 \pm 0.11$	$0.55 \pm 0.10$	0.62
RDW	9.50±1.19	$7.73 \pm 1.85$	$7.14 \pm 0.89$	5.50±1.73	3.87
TFW	$2.18 \pm 0.28$	$1.89\pm0.20$	$1.81 \pm 0.18$	$1.69\pm0.24$	1.55
TDW	$0.44 \pm 0.08$	$0.33 \pm 0.08$	$0.26 \pm 0.02$	$0.22 \pm 0.08$	0.14
AWC	$7.30 \pm 0.94$	$5.90{\pm}1.72$	5.40±0.83	$3.91{\pm}1.49$	2.45
RTTDW	21.77±6.46	19.84±6.81	$15.04{\pm}1.12$	$12.95 \pm 2.67$	8.57
SRL	83.66±11.21	110.81±18.59	132.43±13.28	193.51±21.82	280.76
VD	$1.91 \pm 0.45$	$2.36 \pm 0.80$	$2.09 \pm 0.46$	3.16±0.69	4.03
Saline	Cluster 1 (131)	Cluster 2 (25)	Cluster 3 (33) <sup>a</sup>	Cluster 4 (8)	Cluster 5 (3)
HT	107.11±12.64	93.21±14.54	$72.00 \pm 20.17$	$72.84{\pm}19.88$	69.11±22.49
RL	$29.52 \pm 0.08$	29.46±0.09	$29.48 \pm 0.07$	$29.46 \pm 0.08$	$29.34 \pm 0.05$
SFW	$5.37 \pm 0.96$	$3.72 \pm 0.86$	3.89±1.03	$2.78 \pm 0.88$	$2.42 \pm 0.80$
PFW	$0.92 \pm 0.54$	$1.10\pm0.45$	$1.15 \pm 0.49$	1.15±0.26	$0.88 \pm 0.24$
SDW	$1.24\pm0.18$	$0.97 \pm 0.15$	$0.99 \pm 0.20$	$0.84 \pm 0.14$	$0.75 \pm 0.14$
PDW	0.19±0.13	$0.33 \pm 0.20$	0.30±0.15	$0.41 \pm 0.12$	$0.39 \pm 0.14$
RDW	$6.24 \pm 0.96$	$4.89 \pm 0.80$	$5.10 \pm 1.10$	$4.06 \pm 0.96$	$3.48 \pm 0.96$
TFW	$1.43 \pm 0.19$	1.31±0.17	$1.30\pm0.19$	$1.26\pm0.12$	$1.17 \pm 0.02$
TDW	$0.33 \pm 0.04$	$0.25 \pm 0.03$	$0.27 \pm 0.04$	0.21±0.03	$0.21 \pm 0.05$
AWC	4.81±0.79	$3.58 \pm 0.74$	3.81±1.01	$2.81 \pm 0.92$	2.33±0.97
RTTDW	$24.46 \pm 5.58$	$18.51 \pm 4.62$	21.86±6.15	$14.27 \pm 2.54$	$15.72 \pm 5.47$
SRL	$104.68 \pm 10.66$	153.43±12.76	127.71±17.37	$221.08 \pm 16.72$	$280.17 \pm 12.08$
VD	$2.38\pm0.44$	3.03±0.61	$2.81 \pm 0.59$	3.48±0.64	$3.76\pm0.70$

Table 12. Mean values of phenotypic traits of each cluster group of a USDA pea germplasm [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions.

<sup>a</sup>Cluster in which Agassiz (check) was located. <sup>b</sup>Plant height (HT, cm), root length (RL, cm), shoot fresh weight (SFW, g), shoot dry weight (SDW, g), pod fresh weight (PFW, g), pod dry weight (PDW, g), total above-ground fresh weight (TAGFW, g), total above-ground dry weight (TAGDW, g), root dry weight (RDW, g), absolute water content (AWC, g), root dry weight to total above-ground dry weight ratio (RTTDW, %), specific root length (SRL, cm g<sup>-1</sup>), and visual rating of plant damage (VD, rated with a 1 - 5 scale in which 1 = healthy plants and 5 = dead plants

## Conclusions

Dry pea is mainly produced in the Northern Great Plains, where sulfate-salinity is a major obstacle to its production. One of the most economically effective methods to reduce salinity damage is use of tolerant plants. The current research suggested there were genotype differences in the tolerance to sulfate-salinity. Twelve genotypes (5 from non-saline condition and 7 from saline) and 19 genotypes (9 from non-saline and 10 from saline) were identified from the NDSU germplasm and the USDA germplasm, respectively, based on their high performance from PCA and cluster analysis. Among them, NDP080169 (NDSU), PI\_117998 PSP and PI\_270536 PSP (USDA) showed good growth under both stressed and non-stressed conditions. Further field investigation is needed to validate the results of the present study before incorporating the aforementioned genotypes into dry pea breeding program for sulfate-salinity tolerance.

## References

- Ashraf, M., & McNeilly.T. (1987). Salinity effects five cultivars/lines of pearl millet (*Pennisetum americanum* L. Leeke). *Plant and soil*, 103,13-19. https://doi.org/10.1007/bf02370662.
- Ashraf, M., McNeilly, T., & Bradshaw. A. D. (1987). Selection and heritability of tolerance to sodium chloride in four forage species L. *Crop Science*, 27(2), 232-234. https://doi.org/10.2135/cropsci1987.0011183X002700020021x.
- Bairwa, R. K., Yadav, M. C., Tiwari, S., Mahendran, A., Ellur, R. K., & Krishnan, S. G. (2024). Aromatic rice (*Oryza sativa* L.) landraces of Ido-Gangetic Plains of India harbor rice genetic diversity. *Crop Science*, 64(3), 1711-1728. https://doi.org/10.1002/csc2.21211.

Barba-Espín, G., Diaz-Vivancos P., Job, D., Belghazi, M., Job, C., & Hernández, J. A. (2011).
Understanding the role of H<sub>2</sub>O<sub>2</sub> during pea seed germination: a combined proteomic and hormone profiling approach. *Plant, Cell and Environment, 34(11),* 1907-1919.
https://doi.org/10.1111/j.1365-3040.2011.02386.x.

Behera, S., Jyotirmayee, B., Mandal, U., Mishra, A., Mohanty, P., & Mahalik, G. (2022). Effect of organic fertilizer on growth, yield and quality of *Pisum sativum* L.: A Review.
Ecology, *Environment and Conservation*, 28, S233-S241.
http://doi.org/10.53550/EEC.2022.v28i02s.039

- Bekhit, A. E. D. A., Riley, W. W., & Hussain, M. A. (2022). *Alternative Proteins: Safety and Food Security Considerations*. CRC Press.
- Butler, D., Cullis, B. R., Gilmour, A. R., & Gogel, B. J. (2009). ASReml-R reference manual: ASREML estimates variance components under a general linear mixed model by residual maximum likelihood (REML). The State of Queensland, Department of Primary Industries and Fisheries, Brisbane.
- Cerda, A., Caro, M., & Fernandez, F. G. (1982). Salt tolerance of two pea cultivars L. *Agronomy Journal*, *74*(*5*), 796-798. https://doi.org/10.2134/agronj1982.00021962007400050007x.
- Derner, J., Joyce, L., Guerrero, R., Steele, R., & Anderson, T. (2015). Northern plains regional climate hub assessment of climate change vulnerability and adaptation and mitigation strategies. In T. Anderson (Ed.), United States Department of Agriculture (pp. 14) http://climatehubs.oce.usda.gov/sites/default/files/Northern%20Plains%20Vulnerability% 20Assessment% 205\_1\_2015\_Compressed.pdf
- Dewey, D. R. (1962). Germination of crested wheatgrass in salinized soil. *Agronomy Journal*, *54*(*4*), 353-355. https://doi.org/10.2134/agronj1962.00021962005400040024x.

- Ehtaiwwesh, A. F., & Emsahel, M. J. (2020). Impact of salinity stress on germination and growth of pea (*Pisum sativum* L) plants. *Al-Mukhtar Journal of Sciences*, 35(2), 146-159. https://doi.org/10.54172/mjsc.v35i2.319.
- Epstein, E. 1983. Crops tolerant of salinity and other mineral stresses. In J. Nugent & M.
  O'Connor (Eds.), *Ciba Foundation Symposium 97-Better Crops for Food* (pp. 69-82),
  John Wiley and Sons, Ltd. https://doi.org/10.1002/9780470720783.ch6.
- Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T.A., Hodgson, M. J., Cooper, J.W, Miller, A.J., Kunert, K., Vorster, Juan., Cullis, C., Ozga J. A., Wahlqvist, M.L., Liang, Y., Shou, Huixia., Shi, K., Yu, J.,. ... & Considine, M. J. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants, 2*, 16112. https://doi.org/10.1038/nplants.2016.112.
- Hughes, A. P., Cockshull, K. E., & Heath, O. V. S. (1970). Leaf area and absolute leaf water content. *Annals of Botany*, *34*(2), 259-266.

https://doi.org/10.1093/oxfordjournals.aob.a084366.

- Janzen, E., Flaskerud, G., & Fisher, J. (2006). *Pulse crop marketing guide*. North Dakota State University Extension Service, Fargo, ND 58105.
- Jing, A. S., Guo, B. C., & Zhang, X. Y. (1992). Chloride tolerance and its effects on yield and quality of crops. *China Journal of Soil Science*, 33(6), 257-259.
- Johansen, C., Saxena, N. P., Chauhan, Y. S., Rao, G. S., Pundir, R. P. S., Rao, J. V. D. K. K., & Jana, M. K. (1990). Genotypic variation in salinity response of chickpea and pigeonpea.In S. Bhargava & M. Agrawal (Eds.), *Proceedings of the International Congress of Plant*

*Physiology* (2<sup>nd</sup> ed, pp. 977–983), Sinha, Sane, Society of Plant Physiology and Biochemistry. http://oar.icrisat.org/id/eprint/3934.

- Joshi, N., Reddy, S. P. P., Kumar, N., Bharadwaj, C., Tapan, K., Patil, B. S., Jain, P.K., Nimmy, M.S., Roorkiwal, M., Verma, P., Varshney, R. K., Siddique, K.H.M., & Sudhir, K. (2023). Siphoning novel sources of seedling salinity tolerance from the diverse chickpea landraces. *Crop and Pasture Science*, *74*(*11*), 1080-1093. https://doi.org/10.1071/CP22319.
- Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, 25, 1-18.
- Leonforte, A., Forster, J. W., Redden, R. J., Nicolas, M. E., & Salisbury, P. A. (2013). Sources of high tolerance to salinity in pea (*Pisum sativum* L.). *Euphytica*, 189(2), 203-216.
- Li, H., & Cheng, Z. (2015). Hoagland nutrient solution promotes the growth of cucumber seedlings under light-emitting diode light. *Acta Agriculturae Scandinavica, Section B—Soil and Plant Science*, 65(1), 74-82. https://doi.org/10.1080/09064710.2014.967285.
- Mannan, M. A., Karim, M. A., Khaliq, Q. A., Haque, M. M., Mian, M. A. K., & Ahmed, J. U.
  (2010). Assessment of genetic divergence in salt tolerance of soybean (*Glycine max* L.) genotypes. *Journal of Crop Science and Biotechnology*, *13*, 33-37.
- Mcphee, K., (2003). Dry pea production and breeding-a mini-review. *Journal of Food, Agriculture and Environment, 1(1),* 64-69.
- Misra, N., & Dwivedi, U. N. (2004). Genotypic difference in salinity tolerance of green gram cultivars. *Plant Science*, *166*(*5*), 1135-1142.
- Negrão, S., Schmöckel, S. M., & Tester, M. J. (2017) Evaluating physiological responses of plants to salinity stress. *Annals of botany*, *119*(1), 1-11.

NDSU AgHub on soil salinity. (2023). Saline and sodic soil.

- https://www.ndsu.edu/agriculture/ag-hub/ag-topics/crop-production/soil-health/saline-and-sodicsoils/soil-salinity
- North Dakota Agriculture Statistics. (2023). Crops Planted, harvested, yield, production, price (MYA), values of production sorted by value of production in dollars. 2023 State agriculture overview.

https://www.nass.usda.gov/Quick\_Stats/Ag\_Overview/stateOverview.php?state=NORTH %20DAKOTA

- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lõhmus, K., Majdi, H., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M., & Brunner, I. (2007). Specific root length as an indicator of environmental change. *Plant Biosystems*, 141(3), 426-442. https://doi.org/10.1080/11263500701626069.
- Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R. J., Drechsel, P., & Noble, A. D. (2014). Economics of salt-induced land degradation and restoration. *Natural Resources Forum*, 38(4), 282-295. https://doi.org/10.1111/1477-8947.12054.
- Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., Ul Hussan, M., & Sarwar, M. I. (2019). A review: Impact of salinity on plant growth. *Nature and Science*, 17(1), 34-40. https://doi.org/10.7537/marsnsj170119.06.
- Salsman, E., Liu, Y., Hosseinirad, S. A., Kumar, A., Manthey, F., Elias, E., & Li, X. (2021). Assessment of genetic diversity and agronomic traits of durum wheat germplasm under drought environment of the Northern Great Plains. *Crop Science*, 61(2), 1194-1206. https://doi.org/10.1002/csc2.20449.

- Seelig, B.D. (2000). *Salinity and sodicity in North Dakota soils*. North Dakota State University, Fargo, ND 58105, EB-57.
- Shahid, M. A., Pervez, M. A., Balal, R. M., Abbas, T., Ayyub, C. M., Mattson, N. S., Riaz, A., & Iqbal, Z. (2012). Screening of pea (*Pisum sativum* L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. *Australian Journal of Crop Science*, 6(9), 1324-1331.
- Shannon, M. C. (1985). Principles and strategies in breeding for higher salt tolerance. In D.
  Pasternak & A. San Pietro (Eds.), *Biosalinity in Action: Bioproduction with Saline Water*(pp. 227-241). Springer. https://doi.org/10.1007/978-94-009-5111-2\_15.
- Toker, C., Lluch, C., Tejera, N. A., Serraj, R., & Siddique, K. H. M. (2007). Abiotic stresses. In S.S. Yadav et al. (Eds.), *Chickpea Breeding and Management* (pp. 474-496). CABI. https://doi.org/10.1079/9781845932138.000
- Torche, Y., Blair, M., & Saida, C. (2018). Biochemical, physiological and phenological genetic analysis in common bean (*Phaseolus vulgaris* L.) under salt stress. *Annals of Agricultural Sciences*, 63(2), 153-161. https://doi.org/10.1016/j.aoas.2018.10.002.
- Tracy, J. D. (2020). Screening field pea (Pisum sativum L.) for tolerance to high salinity conditions [Master's thesis, Montana State University-Bozeman, College of Agriculture]. https://scholarworks.montana.edu/handle/1/16611
- USDA. (1954). *Diagnosis and improvement of saline and alkali soils*. Agric. Handbook. no. 60. United States Salinity Laboratory. https://doi.org/10.1097/00010694-195408000-00012.
- Uskutoğlu, D., & İdikut. L. (2023). Pea Production Statistics in the World and in Turkey. In A.
  B. Uçak and S. Güneş Sen (Eds.), *Innovative Research in Agriculture, Forest and Water issues* (pp. 25-38). Duvar. https://doi.org/10.59287/irafwi.473.

- Vandemark, G. J., Brick, M. A., Osorno, J. M., Kelly, J. D., & Urrea, C. A. (2014). Edible grain legumes. In S. Smith et al. (Eds.), *Yield gains in major US field crops* (Volume 33, pp. 87-123), Crop Science Society of America Special Publication. https://doi.org/10.2135/cssaspecpub33.c5.
- Wei, Z., Julkowska, M. M., Laloë, J. O., Hartman, Y., de Boer, G. J., Michelmore, R. W., van Tienderen, P.H., Testerinik, C., & Schranz, M. E. (2014). A mixed-model QTL analysis for salt tolerance in seedlings of crop-wild hybrids of lettuce. *Molecular Breeding, 34*, 1389-1400. https://doi.org/10.1007/s11032-014-0123-2,
- Wold, S., Esbensen, K. & Geladi, P. (1987). Principal component analysis. Chemometrics and Intelligent Laboratory Systems, 2(1-3), 37-52. https://doi.org/10.1016/0169-7439(87)80084-9.
- Wray, N., & Visscher, P. (2008). Estimating trait heritability. *Nature Education*, 1(1), 29. https://doi.org/10.1038/nrg2322.
- Yan, W., & Rajcan, I. (2002). Biplot analysis of test sites and trait relations of soybean in Ontario. *Crop Science*, 42(1), 11-20. https://doi.org/10.2135/cropsci2002.1100.
- Yoshida, H., Tomiyama, Y., Tanaka, M., & Mizushina, Y. (2007). Characteristic profiles of lipid classes, fatty acids and triacylglycerol molecular species of peas (*Pisum sativum* L.). *European Journal of Lipid Science and Technology*, *109*(6), 600-607. https://doi.org/10.1002/ejlt.200600219.
- Zörb, C., Geilfus, C. M., & Dietz, K. J. (2019). Salinity and crop yield. *Plant Biology*, 21, 31-38. https://doi.org/10.1111/plb.12884.

## APPENDIX

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition.

			Cluster Group	
GENOTYPE	PEDIGREE	Accession number	Saline	Non-saline
DAG	Not available	1	1	1
	BIG-			
NDP080169	DADDY//STO_4031_AM2_160_8321/PS310150	2	2	1
NDP080173	Not available	3	1	1
NDP080175	PS01101184/SUPRA	4	2	2
NDP080176	PS01101184/SUPRA	5	1	2
NDP101185	SUPRA/PS01102929	6	1	2
NDP120018	CDC MEADOW/PS05ND310	7	1	3
NDP120057	COOPER/PS05ND430	8	3	2
NDP120071	THUNDERBIRD/PS05ND310	9	1	1
NDP120078	THUNDERBIRD/PS05ND325	10	1	2
NDP120080	THUNDERBIRD/PS05ND325	11	1	2
NDP120083Y	THUNDERBIRD/PS05ND325	12	2	1
NDP120084Y	THUNDERBIRD/PS05ND325	13	1	2
NDP120099	PS05ND218/STIRLING	14	2	2
NDP120143G	PS05ND327/CDC GOLDEN	15	1	1
NDP120150	PS05ND327/CDC MEADOW	16	3	3
NDP120157	PS05ND327/CDC MEADOW	17	2	2
NDP120176	PS05ND327/THUNDERBIRD	18	1	2
NDP120180	PS05ND330/THUNDERBIRD	19	1	2

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

			Cluster Group	
GENOTYPE	PEDIGREE	Accession number	Saline	Non-saline
NDP120181	PS05ND330/THUNDERBIRD	20	1	4
NDP121166	STIRLING/PS05ND310	21	1	1
NDP121556	PS02100151/STIRLING	22	3	3
NDP121608	DS ADMIRAL/PS03100278	23	1	2
NDP121638	PS03100278/DS ADMIRAL	24	1	4
NDP121688	UNIVERSAL/PS01102958	25	1	4
NDP121711	STIRLING/PS05100914	26	1	1
NDP130001	DS ADMIRAL/PS05ND218	27	2	4
NDP130002	DS ADMIRAL/PS05ND218	28	1	1
NDP130010	DS ADMIRAL/PS05ND310	29	2	2
NDP130013	DS ADMIRAL/PS05ND310	30	3	2
NDP130046	MEDORA/PS05ND327	31	1	4
NDP130059	LIFTER/PS05ND310	32	3	2
NDP130079	STIRLING/PS05ND330	33	1	2
NDP130085	CDC MOZART/PS05ND218	34	2	4
NDP130110	CDC GOLDEN/PS05ND310	35	1	4
NDP130134	CDC MEADOW/PS05ND310	36	1	2
NDP130152	COOPER/PS05ND227	37	1	1
NDP130158	COOPER/PS05ND310	38	1	2
NDP130167	COOPER/PS05ND430	39	1	2
NDP130212	PS05ND227/DS ADMIRAL	40	1	3
NDP130302	STIRLING/PS03100546	41	2	2

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

			Cluster Group	
GENOTYPE	PEDIGREE	Accession number	Saline	Non-saline
NDP130337	DS ADMIRAL/PS01102958	42	3	3
NDP130340	DS ADMIRAL/PS01102958	43	1	4
NDP140005	DS ADMIRAL/PS05ND218	44	2	1
NDP140006	DS ADMIRAL/PS05ND218	45	3	2
NDP140295G	THUNDERBIRD/PS05ND310	46	1	3
NDP140366	PS05ND327/CDC GOLDEN	47	2	2
NDP140390	PS05ND327/THUNDERBIRD	48	1	3
NDP140510Y	DS ADMIRAL/PS05ND310	49	1	3
NDP140852	PS05ND325/CDC MOZART	50	3	2
NDP150001	GSP-Ae-D9904-17/MEDORA	51	2	4
NDP150013	DS ADMIRAL/PS05ND218	52	4	2
NDP150025Y	DS ADMIRAL/PS05ND310	53	1	2
NDP150037	DS ADMIRAL/PS05ND310	54	1	3
NDP150038	DS ADMIRAL/PS05ND310	55	1	4
NDP150042	DS ADMIRAL/PS05ND310	56	5	2
NDP150045	MEDORA/PS05ND218	57	1	2
NDP150046	MEDORA/PS05ND218	58	2	1
NDP150047	MEDORA/PS05ND218	59	2	4
NDP150049	MEDORA/PS05ND218	60	1	4
NDP150051	MEDORA/PS05ND218	61	3	2
NDP150052	MEDORA/PS05ND218	62	1	3

GENOTYPE	PEDIGREE		Cluster group	
		Accession number	Saline	Non-saline
NDP150053	MEDORA/PS05ND218	63	1	4
NDP150054	MEDORA/PS05ND218	64	1	3
NDP150055	MEDORA/PS05ND218	65	1	3
NDP150058	MEDORA/PS05ND218	66	1	2
NDP150059	MEDORA/PS05ND218	67	1	2
NDP150060	MEDORA/PS05ND218	68	1	1
NDP150062	MEDORA/PS05ND218	69	1	4
NDP150063	MEDORA/PS05ND218	70	2	1
NDP150066	MEDORA/PS05ND227	71	1	2
NDP150068	MEDORA/PS05ND227	72	3	2
NDP150069	MEDORA/PS05ND227	73	1	3
NDP150070G	MEDORA/PS05ND227	74	3	3
NDP150073	MEDORA/PS05ND227	75	1	5
NDP150075	MEDORA/PS05ND227	76	1	2
NDP150076	MEDORA/PS05ND227	77	1	5
NDP150077	MEDORA/PS05ND227	78	3	3
NDP150079	MEDORA/PS05ND227	79	3	3
NDP150080	MEDORA/PS05ND227	80	1	3
NDP150081	MEDORA/PS05ND227	81	3	3
NDP150082	MEDORA/PS05ND227	82	3	3
NDP150084	MEDORA/PS05ND227	83	1	2

GENOTYPE	PEDIGREE		Clus	ter Group
		Accession number	Saline	Non-saline
NDP150085	MEDORA/PS05ND310	84	3	3
NDP150087	MEDORA/PS05ND310	85	1	1
NDP150089	MEDORA/PS05ND310	86	2	2
NDP150090	MEDORA/PS05ND310	87	3	3
NDP150091	MEDORA/PS05ND310	88	3	3
NDP150094	MEDORA/PS05ND310	89	1	3
NDP150097	MEDORA/PS05ND310	90	3	3
NDP150099	MEDORA/PS05ND310	91	1	2
NDP150100	MEDORA/PS05ND310	92	1	3
NDP150105	STIRLING/PS05ND430	93	1	2
NDP150106	STIRLING/PS05ND430	94	3	3
NDP150108	STIRLING/PS05ND430	95	4	2
NDP150109	STIRLING/PS05ND430	96	1	3
NDP150110	STIRLING/PS05ND430	97	4	3
NDP150112	STIRLING/PS05ND430	98	1	2
NDP150113	STIRLING/PS05ND430	99	3	5
NDP150114	STIRLING/PS05ND430	100	1	3
NDP150117	STIRLING/PS05ND430	101	1	3
NDP150119	STIRLING/PS05ND430	102	4	3
NDP150121	CDC GOLDEN/PS05ND227	103	1	2
NDP150125	CDC GOLDEN/PS05ND227	104	1	2

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

GENOTYPE	PEDIGREE		Clus	ter Group
		Accession number	Saline	Non-saline
NDP150126	CDC GOLDEN/PS05ND227	105	1	2
NDP150127	CDC GOLDEN/PS05ND227	106	3	3
NDP150128	CDC GOLDEN/PS05ND227	107	4	3
NDP150129	CDC GOLDEN/PS05ND227	108	1	3
NDP150130	CDC GOLDEN/PS05ND227	109	5	2
NDP150131	CDC GOLDEN/PS05ND227	110	3	3
NDP150140	CDC GOLDEN/PS05ND310	111	1	1
NDP150142	CDC GOLDEN/PS05ND310	112	2	2
NDP150151	CDC GOLDEN/PS05ND310	113	1	3
NDP150160	CDC MEADOW/PS05ND227	114	1	2
NDP150162	CDC MEADOW/PS05ND227	115	1	4
NDP150168	CDC MEADOW/PS05ND227	116	1	3
NDP150169	CDC MEADOW/PS05ND227	117	1	3
NDP150176	CDC MEADOW/PS05ND227	118	4	1
NDP150178	CDC MEADOW/PS05ND310	119	1	3
NDP150179	CDC MEADOW/PS05ND310	120	1	1
NDP150184	CDC MEADOW/PS05ND310	121	1	3
NDP150187	CDC MEADOW/PS05ND310	122	1	3
NDP150191	CDC MEADOW/PS05ND310	123	1	2
NDP150192	CDC MEADOW/PS05ND310	124	1	4
NDP150193	CDC MEADOW/PS05ND310	125	2	2

GENOTYPE	PEDIGREE		Clus	ter Group
		Accession number	Saline	Non-saline
NDP150197	THUNDERBIRD/PS05ND310	126	1	2
NDP150198G	THUNDERBIRD/PS05ND310	127	2	2
NDP150199	THUNDERBIRD/PS05ND310	128	1	4
NDP150200	THUNDERBIRD/PS05ND310	129	1	2
NDP150201	THUNDERBIRD/PS05ND310	130	1	4
NDP150178	CDC MEADOW/PS05ND310	119	1	3
NDP150179	CDC MEADOW/PS05ND310	120	1	1
NDP150184	CDC MEADOW/PS05ND310	121	1	3
NDP150187	CDC MEADOW/PS05ND310	122	1	3
NDP150191	CDC MEADOW/PS05ND310	123	1	2
NDP150192	CDC MEADOW/PS05ND310	124	1	4
NDP150193	CDC MEADOW/PS05ND310	125	2	2
NDP150197	THUNDERBIRD/PS05ND310	126	1	4
NDP150198G	THUNDERBIRD/PS05ND310	127	2	4
NDP150199	THUNDERBIRD/PS05ND310	128	1	4
NDP150200	THUNDERBIRD/PS05ND310	129	1	2
NDP150201	THUNDERBIRD/PS05ND310	130	1	4
NDP150203	THUNDERBIRD/PS05ND310	131	2	1
NDP150206	THUNDERBIRD/PS05ND310	132	1	4
NDP150210	THUNDERBIRD/PS05ND310	133	1	4
NDP150213	THUNDERBIRD/PS05ND430	134	3	3

GENOTYPE	PEDIGREE		Clus	ter Group
		Accession number	Saline	Non-saline
NDP150214	THUNDERBIRD/PS05ND430	135	1	1
NDP150215	THUNDERBIRD/PS05ND430	136	4	3
NDP150216	THUNDERBIRD/PS05ND430	137	3	5
NDP150217	THUNDERBIRD/PS05ND430	138	3	3
NDP150218	THUNDERBIRD/PS05ND430	139	1	2
NDP150220	THUNDERBIRD/PS05ND430	140	2	1
NDP150221	THUNDERBIRD/PS05ND430	141	1	2
NDP150222	THUNDERBIRD/PS05ND430	142	1	4
NDP150223G	THUNDERBIRD/PS05ND430	143	1	2
NDP150224	THUNDERBIRD/PS05ND430	144	1	2
NDP150225	THUNDERBIRD/PS05ND430	145	1	5
NDP150226Y	THUNDERBIRD/PS05ND430	146	1	3
NDP150227	THUNDERBIRD/PS05ND430	147	1	3
NDP150228	THUNDERBIRD/PS05ND430	148	3	2
NDP150229	THUNDERBIRD/PS05ND430	149	1	3
NDP150230	THUNDERBIRD/PS05ND430	150	3	3
NDP150231Y	THUNDERBIRD/PS05ND430	151	1	3
NDP150232	PS05ND218/STIRLING	152	2	4
NDP150235	PS05ND218/STIRLING	153	1	2
NDP150237	PS05ND218/STIRLING	154	1	2
NDP150242	PS05ND218/STIRLING	155	3	5

GENOTYPE	PEDIGREE		Cluster Group	
		Accession number	Saline	Non-saline
NDP150250	Not available	156	1	1
NDP150258	PS05ND227/DS ADMIRAL	157	1	2
NDP150269	PS05ND325/DS ADMIRAL	158	3	3
NDP150288	MEDORA/PS05ND327	159	1	2
NDP150289	MEDORA/PS05ND327	160	1	3
NDP150317	CDC MOZART/PS05ND430	161	1	3
NDP150318	CDC MOZART/PS05ND430	162	1	2
NDP150321	CDC MEADOW/PS05ND327	163	1	3
NDP150326	CDC MEADOW/PS05ND327	164	1	4
NDP150338	PS05ND218/THUNDERBIRD	165	1	1
NDP150344	PS05ND218/THUNDERBIRD	166	1	3
NDP150378	PS05ND430/DS ADMIRAL	167	1	4
NDP150380	PS05ND430/DS ADMIRAL	168	1	3
NDP150382	PS05ND430/DS ADMIRAL	169	1	3
NDP150386	PS05ND430/DS ADMIRAL	170	1	5
NDP150387	PS05ND430/DS ADMIRAL	171	1	3
NDP150392	PS05ND430/MEDORA	172	1	2
NDP150397G	PS05ND430/MEDORA	173	1	3
NDP150401	PS05ND430/CDC GOLDEN	174	1	2
NDP150407	PS05ND430/CDC MEADOW	175	1	2
NDP150410	PS05ND430/CDC MEADOW	176	1	3

	GENOTYPE	PEDIGREE		Clus	ter Group
			Accession number	Saline	Non-saline
	NDP150412G	PS05ND430/CDC MEADOW	177	1	3
	NDP150416	PS05ND430/CDC MEADOW	178	4	3
	NDP150417G	PS05ND430/CDC MEADOW	179	2	3
	NDP150418	PS05ND430/CDC MEADOW	180	1	2
	NDP150419	PS05ND430/CDC MEADOW	181	1	4
	NDP150456	NDP080174/NDP080169	182	2	2
	NDP150459	NDP080174/NDP080169	183	2	1
	NDP150476	CDC GOLDEN/PS05ND310	184	3	2
91	NDP150495	CDC MEADOW/PS05ND227	185	3	2
	NDP150501	CDC MEADOW/PS05ND227	186	1	4
	NDP150513	CDC MEADOW/PS05ND310	187	1	2
	NDP150528	PS05ND325/DS ADMIRAL	188	3	3
	NDP160010	CDC GOLDEN/PS05ND227	189	3	2
	NDP160022	CDC MEADOW/PS05ND227	190	3	1
	NDP160028G	CDC MEADOW/PS05ND310	191	2	2
	NDP160034	CDC MEADOW/PS05ND310	192	1	3
	NDP160049	THUNDERBIRD/PS05ND430	193	4	3
	NDP160051	THUNDERBIRD/PS05ND430	194	1	1
	NDP160055	THUNDERBIRD/PS05ND430	195	1	3
	NDP160057Y	PS05ND218/STIRLING	196	1	3
	NDP160060	PS05ND218/STIRLING	197	4	3

GENOTYPE	PEDIGREE		Cluster Group	
		Accession number	Saline	Non-saline
NDP160062	PS05ND218/STIRLING	198	2	3
NDP160066	PS05ND218/STIRLING	199	1	1
NDP160069G	PS05ND227/DS ADMIRAL	200	1	2
NDP160070Y	PS05ND227/DS ADMIRAL	201	2	1
NDP160071	PS05ND227/DS ADMIRAL	202	1	3
NDP160075	PS05ND227/DS ADMIRAL	203	1	2
NDP160076Y	PS05ND227/DS ADMIRAL	204	1	2
NDP160129G	CDC MEADOW/PS05ND310	205	1	4
NDP160153Y	PS05ND218/THUNDERBIRD	206	1	3
NDP160168	PS05ND325/DS ADMIRAL	207	3	1
NDP160169	PS05ND325/DS ADMIRAL	208	4	2
NDP160176	PS05ND325/MEDORA	209	1	2
NDP160177G	PS05ND325/MEDORA	210	1	4
NDP160180	PS05ND325/MEDORA	211	4	5
NDP160183	PS05ND325/MEDORA	212	1	2
NDP160188	PS05ND330/CDC MEADOW	213	1	4
NDP160193	PS05ND330/CDC MEADOW	214	3	3
NDP160195	PS05ND430/DS ADMIRAL	215	3	4
NDP160196	PS05ND430/DS ADMIRAL	216	1	2
NDP160197	PS05ND430/DS ADMIRAL	217	1	2
NDP160199Y	PS05ND430/DS ADMIRAL	218	1	3

	GENOTYPE	PEDIGREE		Clust	ter Group
			Accession number	Saline	Non-saline
	NDP160200Y	PS05ND430/DS ADMIRAL	219	1	3
	NDP160201	PS05ND430/DS ADMIRAL	220	3	3
	NDP160200Y	PS05ND430/DS ADMIRAL	219	1	3
	NDP160201	PS05ND430/DS ADMIRAL	220	3	3
	NDP160204	PS05ND430/DS ADMIRAL	221	1	3
	NDP160208Y	PS05ND430/DS ADMIRAL	222	3	3
	NDP160210Y	PS05ND430/DS ADMIRAL	223	1	3
	NDP160216	PS05ND430/MEDORA	224	1	2
93	NDP160217G	PS05ND430/MEDORA	225	1	3
	NDP160218	PS05ND430/MEDORA	226	1	2
	NDP160231	PS05ND430/CDC MEADOW	227	3	3
	NDP160278	PS07ND0102/STIRLING	228	1	3
	NDP160279	PS07ND0102/STIRLING	229	1	1
	NDP160281	NDP080138/STIRLING	230	1	3
	NDP160305	NDP080142/LIFTER	231	2	1
	NDP170001G	N16P098/PS07ND0190	232	3	3
	NDP170004G	N16P097/PS07ND0190	233	2	2
	NDP170006G	NDP121166/N16P105	234	2	2
	NDP170008G	N16P106/NDP121166	235	1	2
	NDP170011G	N16P106/NDP121166	236	1	3
	NDP170012G	NDP121166/N16P105	237	2	2
	NDP170017G	N16P098/PS07ND0190	238	1	2

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

GENOTYPE	PEDIGREE		Cluster Group	
		Accession number	Saline	Non-saline
NDP170018G	N16P098/PS07ND0190	239	1	3
NDP170022G	N16P105/NDP121166	240	1	2
NDP170027G	N16P106/NDP121166	241	3	2
NDP170028G	N16P097/PS07ND0190	242	1	2
NDP170031G	N16P098/PS07ND0190	243	1	5
NDP170037G	NDP121166/N16P106	244	1	4
NDP170043G	N16P097/PS07ND0190	245	1	1
NDP170052G	N16P098/PS07ND0190	246	1	4
NDP170054G	NDP121166/N16P106	247	1	2
NDP170056G	NDP121166/N16P106	248	1	2
NDP170057G	N16P098/PS07ND0190	249	1	2
NDP170062G	N16P099/PS07ND0190	250	2	1
NDP170075Y	N16P108/NDP121221	251	1	4
NDP170081Y	N16P132/NDP121361	252	1	2
NDP170084Y	N16P108/NDP121221	253	1	4
NDP170088Y	N16P108/NDP121221	254	2	4
NDP170089G	N16P106/NDP121166	255	1	1
NDP170093Y	N16P116/NDP121322	256	2	1
NDP170094Y	N16P108/NDP121221	257	2	4
NDP170099G	N16P132/NDP121361	258	2	4
NDP170101G	N16P132/NDP121361	259	1	3
NDP170104Y	N16P108/NDP121221	260	1	1

Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

GENOTYPE	PEDIGREE		Cluster Group	
		Accession number	Saline	Non-saline
NDP170110Y	N16P108/NDP121221	261	1	4
NDP170111G	N16P106/NDP121166	262	1	2
NDP170133G	N16P132/NDP121361	263	1	4
NDP170151Y	N16P134/NDP121361	264	1	4
NDP170153Y	N16P134/NDP121361	265	2	4
NDP170155Y	NDP121361/N16P134	266	3	4
NDP170156Y	NDP121361/N16P134	267	1	2
NDP170161Y	N16P134/NDP121361	268	1	2
NDP170175Y	NDP121361/N16P134	269	1	3
NDP170176Y	NDP121361/N16P134	270	1	2
NDP170177Y	N16P134/NDP121361	271	1	4
NDP170181Y	N16P134/NDP121361	272	2	4
NDP170182Y	N16P133/NDP121361	273	2	4
NDP170183Y	N16P134/NDP121361	274	1	4
NDP170185Y	NDP121361/N16P134	275	2	4
NDP170190Y	N16P134/NDP121361	276	3	5
NDP170197Y	NDP121361/N16P134	277	3	2
NDP170200Y	N16P134/NDP121361	278	2	2
NDP170202Y	N16P134/NDP121361	279	3	2
NDP170242Y	N16P136/NDP121361	280	1	4
NDP170245Y	N16P136/NDP121361	281	1	3
NDP170247Y	N16P136/NDP121361	282	1	4
Table A1. List of pea accessions included in the NDSU pea germplasm evaluation [including 294 accessions and 'Agassiz' (the check, a commercial pea variety)] across non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition(continued).

GENOTYPE	PEDIGREE		Clus	ter Group
		Accession number	Saline	Non-saline
NDP170249Y	N16P136/NDP121361	283	1	4
NDP170252Y	N16P136/NDP121361	284	2	4
NDP170253Y	N16P136/NDP121361	285	2	2
NDP170273Y	N16P136/NDP121361	286	1	2
NDP170278Y	N16P136/NDP121361	287	2	4
NDP170322Y	NDP121334/N16P154	288	1	3
NDP170328G	N16P140/NDP121548	289	3	3
NDP170336G	N16P140/NDP121548	290	2	4
NDP170350Y	N16P154/NDP121334	291	1	1
PS07100972	BIG- DADDY/MARO/PS310148	292	2	1
PS07100995	PS01101184/SUPRA	293	2	1
(PS07101014/NDP150258)	(MARROWFAT/WV135C*6A F/2/PS210713/3/CEB_1221/4/ MARO/PS310148)	294	1	1
Agassiz	A commercial variety		1	4

96

			Clust	er Group
GENOTYPE	Description	Accession number	Saline	Non-saline
PI_102888_PSP	Collected	1	1	1
PI_116056_PSP	Collected	2	1	1
PI_116944_PSP	Collected	3	2	1
PI_117264_PSP	Collected	4	1	1
PI_117998_PSP	Collected	5	1	1
PI_118501_PSP	Collected	6	1	2
PI_121352_PSP	Collected	7	1	1
PI_123246	No information	8	1	1
PI_124478_PSP	Collected	9	1	1
PI_125839_PSP	Collected	10	3	1
PI_134271_PSP	Collected	11	1	1
PI_137118	Collected	12	3	2
PI_137120	No information	13	3	3
PI_138945	No information	14	2	2
PI_140295	No information	15	1	1
PI_140296	No information	16	4	1
PI_140298_PSP	No information	17	1	1
PI_142774	No information	18	1	1
PI_142775_PSP	Collected	19	1	1
PI_142777	No information	20	2	1
PI_143485_PSP	Collected	21	1	1
PI_155109_PSP	Donated	22	1	1
PI_156720_PSP	Donated	23	3	3
PI_162909_PSP	Collected	24	1	1
PI_163125	No information	25	2	1
PI_163126_PSP	Collected	26	2	1
PI_163127	No information	27	1	1
PI_163129_PSP	Collected	28	1	1
PI_164285	No information	29	1	1
PI_164346	No information	30	1	1
PI_164396	No information	31	2	1
PI_164417	No information	32	2	1

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition.

			Clust	ter Group
GENOTYPE	Description	Accession number	Saline	Non-saline
PI_164548_PSP	Collected	33	1	1
PI_164612_PSP	Collected	34	1	1
PI_164614	No information	35	1	1
PI_164669	No information	36	1	1
PI_164779_PSP	Collected	37	2	4
PI_164836	No information	38	1	1
PI_164838	No information	39	1	1
PI_164972_PSP	Collected	40	1	1
PI_165949_PSP	Collected	41	5	4
PI_166142	No information	42	2	2
PI_166159_PSP	Collected	43	3	4
PI_167250	No information	44	3	4
PI_167253	No information	45	1	1
PI_169603_PSP	No information	46	1	1
PI_171810_PSP	No information	47	1	1
PI_171814	No information	48	1	1
PI_173930	No information	49	2	1
PI_174321_PSP	No information	50	3	1
PI_174921	Collected	51	5	5
PI_174922	No information	52	4	4
PI_174925	No information	53	1	1
PI_175231_PSP	Collected	54	4	4
PI_175232	No information	55	4	4
PI_179019	No information	56	1	1
PI_179449	Collected	57	3	1
PI_179450_PSP	Collected	58	1	1
PI_179451_PSP	Collected	59	1	1
PI_179459_PSP	Collected	60	1	1
PI_179722_PSP	Collected	61	4	1
PI_180329_PSP	Collected	62	2	1
PI_180693_PSP	Donated	63	1	1
PI_180702_PSP	No information	64	1	1

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition (continued).

			Clust	ter Group
GENOTYPE	Description	Accession number	Saline	Non-saline
PI_181800	No information	65	1	1
PI_183467_PSP	Collected	66	3	1
PI_184130_PSP	Collected	67	1	1
PI_184784_PSP	Collected	68	1	1
PI_193578_PSP	Collected	69	2	1
PI_193586	No information	70	1	1
PI_193588	No information	71	1	1
PI_193590_PSP	Collected	72	1	1
PI_193836	No information	73	1	1
PI_193837	No information	74	1	1
PI_193838	No information	75	3	1
PI_194339	No information	76	1	1
PI_194340	No information	77	1	1
PI_194349	No information	78	1	1
PI_195020_PSP	No information	79	1	1
PI_195404_PSP	No information	80	1	1
PI_195631_PSP	No information	81	1	1
PI_196017	No information	82	1	1
PI_196026	No information	83	2	1
PI_196027	No information	84	1	2
PI_196031	No information	85	1	1
PI_197990_PSP	No information	86	1	1
PI_198072_PSP	No information	87	1	1
PI_198074_PSP	No information	88	5	1
PI_201390_PSP	Collected	89	3	2
PI_203069_PSP	Collected	90	1	1
PI_204306_PSP	Donated	91	1	1
PI_206006_PSP	Donated	92	1	1
PI_207508_PSP	Collected	93	3	2
PI_209507_PSP	Collected	94	1	1
PI 210558 PSP	Collected	95	2	3

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition (continued).

			Clus	ter Group
GENOTYPE	Description	Accession number	Saline	Non-saline
PI_210569_PSP	Collected	Collected 96		1
PI_210571_PSP	Collected	97	1	1
PI_212031_PSP	Donated	98	1	1
PI_212112	No information	99	4	2
PI_220174_PSP	Collected	100	3	1
PI_220175	No information	101	1	2
PI_220189_PSP	No information	102	3	1
PI_222117_PSP	Collected	103	1	1
PI_223527_PSP	No information	104	1	1
PI_226561	No information	105	1	1
PI_226562	No information	106	1	1
PI_227258_PSP	Collected	107	3	2
PI_236492_PSP	Donated	108	1	1
PI_240516_PSP	No information	109	3	3
PI_241593_PSP	Donated	110	1	1
PI_242027_PSP	Donated	111	1	1
PI_244093_PSP	Donated	112	1	1
PI_244191_PSP	Donated	113	4	1
PI_244262	No information	114	3	2
PI_248181_PSP	Collected	115	2	1
PI_249645_PSP	Developed	116	1	1
PI_249646	No information	117	1	1
PI_250438_PSP	Donated	118	3	1
PI_250440_PSP	Donated	119	2	2
PI_253968_PSP	Collected	120	1	2
PI_257592_PSP	Donated	121	3	2
PI_261666	No information	122	3	1
PI_263011	No information	123	3	2
PI_263031_PSP	No information	124	3	2
PI_266070_PSP	Donated	125	2	1
PI 269543 PSP	No information	126	1	1

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition (continued).

			Clust	ter Group	
GENOTYPE	Description	Accession number	Saline	Non-saline	
PI_269761_PSP	Collected	Collected 127			
PI_269762_PSP	Donated	128	3	4	
PI_269763	No information	129	1	1	
PI_269771	No information	130	1	1	
PI_269774	No information	131	1	1	
PI_269775	No information	132	1	1	
PI_269776	No information	133	1	1	
PI_269777_PSP	Donated	134	2	1	
PI_269802_PSP	Donated	135	3	2	
PI_269804_PSP	Donated	136	1	1	
PI_269818_PSP	Donated	137	2	4	
PI_269825_PSP	Donated	138	1	1	
PI_270536_PSP	Donated	139	1	1	
PI_271116_PSP	Collected	140	1	1	
PI_271511_PSP	Collected	141	1	1	
PI_272148_PSP	Collected	Collected 142		1	
PI_272161	No information	No information 143		4	
PI_272171_PSP	Donated	Donated 144		1	
PI_272184_PSP	Collected	Collected 145		1	
PI_272194_PSP	Donated	Donated 146		1	
PI_272204_PSP	No information	147	1	1	
PI_272215_PSP	Donated	148	3	1	
PI_272216_PSP	Collected	149	1	1	
PI_272218_PSP	Collected	150	1	1	
PI_273605_PSP	Collected	Collected 151		1	
PI_273676	No information	152	1	1	
PI_274307_PSP	Collected	Collected 153			
PI_274308_PSP	Collected	154	3	1	
PI_274584_PSP	Collected	155	3	1	
PI_275821_PSP	Donated	156	3	2	
PI 277851	No information	157	1	1	

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition (continued).

			Clus	ter Group
GENOTYPE	Description	Accession number	Saline	Non-saline
PI_277852_PSP	Collected	158	1	1
PI_279823_PSP	Developed	159	3	2
PI_280252_PSP	Collected	160	1	1
PI_280607	No information	161	1	1
PI_280609_PSP	Donated	162	2	2
PI_280613_PSP	Collected	163	1	1
PI_280617_PSP	Collected	164	3	1
PI_280619_PSP	Collected	165	1	1
PI_280621	No information	166	1	1
PI_285708	No information	167	1	1
PI_285710_PSP	Donated	168	1	1
PI_285718_PSP	Donated	169	1	1
PI_285739	No information	170	1	1
PI_286430_PSP	Collected	171	2	4
PI_286607_PSP	Collected	172	1	1
PI_299023	No information	173	1	1
PI_306590	No information	174	2	2
PI_306591_PSP	Donated	175	1	1
PI_307666_PSP	Collected	176	1	1
PI_311112	No information	177	1	1
PI_314794_PSP	Donated	178	1	1
PI_314800	No information	179	1	1
PI_314803	No information	180	1	1
PI_319374_PSP	No information	181	1	2
PI_320972_PSP	No information	182	4	2
PI_314795_PSP	Donated	183	1	1
PI_324697_PSP	Donated	184	1	1
PI_324699	No information	185	1	1
PI_324702_PSP	Donated	186	1	1
PI_324703_PSP	Donated	187	1	1
PI_324706_PSP	Collected	188	1	1

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including 199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline conditions. Accessions were grouped into five clusters under either growing condition (continued).

Table A2. List of pea accessions included in the USDA pea germplasm evaluation [including
199 collections and 'Agassiz' (the check, a commercial pea variety)] under non-saline and saline
conditions. Accessions were grouped into five clusters under either growing condition
(continued).

			Cluster Group	
GENOTYPE Description		Accession number	Saline	Non-saline
PI_331413_PSP	Collected	189	1	1
PI_331414_PSP	Collected	190	1	1
PI_340126	No information	191	1	1
PI_340128_PSP	Donated	192	1	1
PI_340130_PSP	Donated	193	1	1
PI_343263	No information	194	1	1
PI_343267	No information	195	2	1
PI_343268	No information	No information 196		1
PI_343277	No information	No information 197		1
PI_343286	No information	No information 198		4
PI_343292_PSP	Donated	199	1	1
	A commercial			
Agassiz	variety		3	3

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30 genotypes = 10% of the tested NDSU population)
Non-saline		
PC1 (40.5%)	TFW (22.4%)	PS07100995, NDP160076Y, NDP080175, NDP121638, NDP150162, NDP160177G, NDP080169, PS07100972, NDP150228, NDP130340, NDP130046, NDP160197, NDP150227, NDP130085, NDP170037G, NDP150001, NDP160305, NDP150206, NDP170197Y, NDP130302, NDP150077, NDP160066, NDP170133G, NDP170004G, NDP150222, NDP170111G, NDP160176, NDP150201, Agassiz, NDP150318
	TDW (22.4%)	NDP121638, NDP150206, NDP150199, NDP150228, NDP150222, Agassiz, NDP160177G, NDP080169, NDP170133G, NDP150201, NDP160176, NDP160076Y, NDP160153Y, NDP130340, NDP150223G, NDP150232, NDP150062, NDP150162, NDP160197, NDP150001, NDP130046, NDP160022, NDP130110, NDP080175, NDP130085, NDP170155Y, PS07100995, NDP150326, NDP150231Y, NDP150227
	AWC (19.7%)	PS07100995, NDP080175, NDP160076Y, NDP150162, PS07100972, NDP121638, NDP170037G, NDP130046, NDP160177G, NDP080169, NDP150227, NDP160197, NDP130085, NDP130340, NDP130302, NDP160305, NDP150001, NDP150228, NDP170111G, NDP170004G, NDP160066, NDP150077, NDP170197Y, NDP150318, NDP150206, NDP121166, NDP160129G, NDP170075Y, NDP080173, NDP160071

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30  genotypes = 10%  of the tested NDSU population)
Non-saline		
PC1 (40.5%)	RDW (13.1%)	PS07100995, NDP121638, NDP170133G, NDP150047, NDP150001, NDP150459, NDP170182Y, NDP170043G, NDP170336G, NDP150206, NDP150232, NDP150198G, NDP170089G, NDP080169, NDP150419,
		NDP150062, NDP170099G, NDP170242Y, NDP170062G, NDP121711, NDP170350Y, NDP150201, NDP150326, NDP160051, NDP150140, NDP170022G, NDP120083Y, NDP170084Y, NDP150214, NDP150063
	SRL (11.0%)	NDP150459, PS07100995, NDP150047, NDP080169, NDP121638, NDP150206, NDP170182Y, PS07100972, NDP170350Y, NDP170242Y, NDP120143G, NDP170089G, NDP150060, NDP150201, NDP150176, NDP150001, NDP170336G, NDP150232, NDP170133G, NDP150338, NDP160051, DAG, NDP080173, NDP150250, NDP130002, NDP160022, NDP150214, NDP160279, NDP150140, NDP150087
PC2 (20.1%)	RTTDW (42.5%)	NDP150169, NDP150140, NDP170089G, NDP150178, NDP170350Y, NDP150214, NDP150129, NDP170182Y, NDP150160, NDP150176, NDP170022G, NDP170012G, NDP170336G, NDP140852, NDP150131, NDP150210, NDP101185, NDP150200, NDP150338, NDP130134, NDP120099, NDP150401, NDP150047, NDP150084, NDP150220, NDP170043G, DAG, NDP170081Y, NDP140510Y, NDP150418
	RDW (23.8%)	See results under PC1
	SRL (20.5%)	See results under PC1

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30  genotypes = 10%  of the tested NDSU population)
Saline		
PC1 (43.0%)	TFW (22.5%)	NDP150094, NDP121166, NDP130046, NDP150099, NDP170052G
		NDP170004G, NDP130010, NDP160071, NDP130167, NDP130152
		NDP160177G, NDP080169, NDP150053, NDP130085, NDP150001
		NDP150047, NDP170185Y, NDP130002, NDP150109, NDP150232,
		NDP170182Y, NDP150401, NDP170110Y, NDP120083Y, NDP150084
		NDP160049, NDP150113, NDP160075, NDP160028G, NDP130134
	AWC (20.8%)	NDP121166, NDP150094, NDP130046, NDP150099, NDP130152,
		NDP170004G, NDP160071, NDP150001, NDP130085, NDP130010,
		NDP150053, NDP130167, NDP160177G, NDP150047, NDP150401,
		NDP160049, NDP130134, NDP150084, NDP150113, NDP150109,
		NDP160075, NDP080169, NDP150232, NDP170182Y, NDP130002,
		NDP160028G, NDP170110Y, NDP160066, NDP170031G, NDP150225

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30 genotypes = 10% of the tested NDSU population)
Saline		
PC1 (43.0%)	TDW (19.7%)	NDP150094, NDP080169, NDP170151Y, NDP170185Y, NDP130046, NDP130010, NDP130167, NDP140390, NDP170018G, NDP150069, NDP160177G, NDP120083Y, NDP121166, NDP170004G, NDP150213, NDP120071, NDP150099, NDP150235, NDP150053, NDP170104Y, NDP130002, NDP150125, NDP150047, NDP140510Y, NDP160071, NDP150045, NDP150228, NDP170110Y, NDP150214, NDP150232,
	RDW (13.4%)	NDP170182Y, NDP150047, NDP170004G, NDP150046, NDP150232, NDP120083Y, NDP130010, PS07100995, NDP170185Y, NDP170099G, NDP080169, NDP170018G, NDP170133G, NDP150001, NDP080175, NDP120099, NDP130085, NDP130002, NDP150087, NDP150203, NDP150214, NDP150089, NDP170252Y, NDP150058, NDP150062, NDP130152, NDP150099, NDP160197, NDP140005, NDP130001
PC2 (22.8%)	RTTDW (37.5%)	NDP170006G, NDP170350Y, NDP170336G, NDP150191, NDP150160, NDP150199, NDP150203, NDP160305, NDP170182Y, PS07100972, PS07100995, NDP130302, NDP130152, NDP130085, NDP150169, NDP160188, NDP150179, NDP170012G, NDP150142, NDP150089, NDP150417-G, NDP150046, NDP150258, NDP160216, NDP120099, NDP150193, NDP121638, NDP170111G, NDP130212, NDP120157

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(20 genotypes = $10\%$ of the tested USDA population)
Saline		
PC2 (22.8%)		
	SRL (27.0%)	PS07100995, NDP150046, NDP130085, NDP150001, NDP170004G, NDP130010, NDP170182Y, NDP160028G, NDP150047, PS07100972, NDP080169, NDP150232, NDP170099G, NDP160305, NDP130302, NDP170185Y, NDP170006G, NDP170336G, NDP170200Y, NDP150203, NDP150089, NDP150193, NDP170253Y, NDP170093Y, NDP120083Y, NDP080175, NDP140005, NDP160070Y, NDP150220, NDP150063
	RDW (16.2%)	See results under PC1

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(20 genotypes = $10\%$ of the tested USDA population)
Non-saline		
PC1 (51.6%)	SFW (13.6%)	PI_179459, PI_280619, PI_206006, PI_285710, PI_270536, PI_117998,
		PI_117264, PI_204306, PI_269825, PI_155109, PI_272148, PI_271116,
		PI_331414, PI_209507, PI_269763, PI_196026, PI_196017, PI_249646,
		PI_269543, PI_180693
	AWC (11.8%)	PI_179459, PI_117998, PI_269802, PI_124478, PI_193578, PI_280619,
		PI_206006, PI_285710, PI_270536, PI_117264, PI_198072, PI_269825,
		PI_169603, PI_179450, PI_140295, PI_340130, PI_249646, PI_210571,
		PI_269763, PI_164972
	SDW (11.5%)	PI_179459, PI_142774, PI_206006, PI_117264, PI_209507, PI_142775,
		PI_285710, PI_210571, PI_180693, PI_194339, PI_169603, PI_204306,
		PI_280619, PI_179451, PI_269775, PI_171810, PI_155109, PI_249646,
		PI_193836, PI_196017
	TFW (11.5%)	PI_179459, PI_117998, PI_124478, PI_206006, PI_193578, PI_117264,
		PI_248181, PI_285710, PI_280619, PI_269802, PI_198072, PI_169603,
		PI_210571, PI_179450, PI_171810, PI_249646, PI_194339, PI_209507,
		PI_140295, PI_270536
	RDW (10.5%)	PI_179019, PI_285710, PI_324697, PI_280617, PI_280619, PI_324706,
		PI_270536, PI_143485, PI_275821, PI_269804, PI_117998, PI_340128,
		PI_269543, PI_180693, PI_271116, PI_203069, PI_241593, PI_269776,
		PI_195631, PI_196031
	SRL (10.3%)	PI_280619, PI_280617, PI_179019, PI_324706, PI_285710, PI_324697,
		PI_143485, PI_275821, PI_340128, PI_270536, PI_269804, PI_280613,
		PI_272218, PI_241593, PI_117264, PI_180693, PI_269776, PI_285739,
		PI_269543, PI_271116

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30  genotypes = 10%  of the tested NDSU population)
Non-saline		
PC2 (22.7%)	PFW (22.8%)	PI_193578, PI_320972, PI_124478, PI_198074, PI_198072, PI_279823, PI_249645, PI_269802, PI_263011, PI_244191, PI_250440, PI_164346, PI_248181, PI_137118, PI_140295, PI_343277, PI_311112, PI_261666, PI_164972, PI_285739
	RTTDW (20.4%)	PI_179019, PI_280617, PI_269804, PI_340128, PI_203069, PI_275821, PI_324706, PI_269761, PI_266070, PI_183467, PI_272184, PI_270536, PI_269777, PI_324703, PI_167253, PI_324702, PI_324697, PI_306591, PI_134271, PI_118501
	PDW (18.3%)	PI_248181, PI_164612, PI_343267, PI_194340, PI_242027, PI_250440, PI_249645, PI_180702, PI_227258, PI_198074, PI_193578, PI_164669, PI_261666, PI_320972, PI_343277, PI_244262, PI_179449, PI_124478, PI_164346, PI_244191
	TDW (18.1%)	PI_248181, PI_142774, PI_179459, PI_269775, PI_164612, PI_180702, PI_124478, PI_117264, PI_206006, PI_164669, PI_193836, PI_209507, PI_210571, PI_116056, PI_273676, PI_142775, PI_171810, PI_343267, PI_179451, PI_194340
Saline		
PC1 (51.8%)	SFW (13.8%)	PI_270536, PI_155109, PI_117998, PI_169603, PI_280619, PI_171814, PI_269543, PI_184130, PI_324697, PI_271116, PI_272218, PI_142775, PI_272148, PI_314803, PI_280621, PI_340126, PI_116056, PI_210571, PI_179459, PI_340130

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30  genotypes = 10%  of the tested NDSU population)
saline		
PC1 (51.8%)	AWC (13.0%)	PI_270536, PI_155109, PI_142775, PI_117998, PI_171814, PI_269802, PI_169603, PI_184130, PI_340130, PI_280619, PI_210571, PI_198072, PI_271116, PI_212031, PI_143485, PI_272194, PI_164972, PI_340126, PI_272218, PI_324697
	TFW (12.6%)	PI_142775, PI_155109, PI_171814, PI_270536, PI_117998, PI_169603, PI_210571, PI_184130, PI_340130, PI_198072, PI_269802, PI_164972, PI_340126, PI_143485, PI_272194, PI_212031, PI_269543, PI_116056, PI_193586, PI_271116
	SDW (11.3%)	PI_116056, PI_169603, PI_117264, PI_171814, PI_269543, PI_193586, PI_142775, PI_210569, PI_210571, PI_155109, PI_184130, PI_209507, PI_179451, PI_261666, PI_272218, PI_271511, PI_117998, PI_340126, PI_269762, PI_340130
	RDW (10.9%)	PI_270536, PI_269804, PI_285710, PI_272218, PI_143485, PI_324697, PI_269825, PI_236492, PI_280619, PI_117998, PI_269543, PI_155109, PI_171814, PI_204306, PI_203069, PI_198072, PI_272148, PI_116056, PI_210569, PI_196031
PC2 (23.7%)	PFW (23.6%)	PI_285739, PI_280607, PI_250440, PI_164972, PI_134271, PI_102888, PI- _143485, PI_263011, PI_193578, PI_198072, PI 212031, PI_249645, PI_274584, PI_279823, PI_142775, PI_164346, PI_331413, PI_257592, PI_274307, PI_193836

Principal Component number	Associated trait	Top Performers
(% of explained variance)	(% of contribution)	(30  genotypes = 10%  of the tested NDSU population)
saline		
PC2 (23.7%)	RTTDW (22.5%)	PI_183467, PI_269804, PI_275821, PI_179019, PI_203069, PI_285710, PI_299023, PI_324702, PI_272184, PI_167253, PI_307666, PI_269825, PI_324699, PI_343292, PI_236492, PI_269774, PI_204306, PI_270536, PI_266070, PI_324703
	PDW (19.1%)	PI_163125, PI_212112, PI_343286, PI_210558, PI_134271, PI_244262, PI_250440, PI_164838, PI_138945, PI_269818, PI_207508, PI_124478, PI_280607, PI_102888, PI_250438, PI_285739, PI_174922, PI_249645, PI_140296, PI_164346
	TDW (18.7%)	PI_163125, PI_116056, PI_261666, PI_193586, PI_117264, PI_142775, PI_285739, PI_193836, PI_210571, PI_171814, PI_169603, PI_164346, PI_210569, PI_197990, PI_269762, PI_124478, PI_340126, PI_269543, PI_164972, PI_209507