SCREENING FIELD PEA (*PISUM SATIVUM* L.) FOR TOLERANCE TO HIGH SALINITY CONDITIONS

by

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DEDICATION

To my momma bear Amy, for always being my rock, and my father Chris, for pushing me to play with plants and not letting me go into environmental law.

To Marilyn Larkin and in memory of John Larkin, for their long-standing support of my education.

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ABSTRACT

Field pea (*Pisum sativum* L.) is an important salt-sensitive crop utilized in rotation with cereals in semi-arid cropping systems in the Northern Great Plains (NGP). Saline soils (EC > 4 dS m^{-1}) negatively impact over 10.8 million acres in Montana, the second largest producer of field pea in the US. Despite its global importance, few studies have explored field pea response to high salinity conditions outside of germination testing and even fewer have looked at tolerance to sodium sulfate (Na_2SO_4), the dominant salt affecting plant growth in the NGP. In this study, 311 accessions comprising the genetically diverse USDA *Pisum* single plant (PSP) core collection were screened under high Na_2SO_4 conditions in germination and seedling experiments. Germination screening was conducted in petri dishes within a dark growth chamber. Accessions received H_2O (control) or 16 dS m^{-1} Na_2SO_4 (highly saline) solution for 8 days. The mean percent germination compared to the control was used as the indicator for tolerance. A preliminary greenhouse concentration series experiment using 7 levels of Na_2SO_4 (0, 3, 6, 9, 12, 15, and 18 dS m^{-1}), supported screening seedlings at 9 dS m^{-1} Na_2SO_4. Greenhouse screening was conducted in plastic pots of coarse sand media. Accessions received a nutrient solution (control) or 9 dS m^{-1} Na_2SO_4 and nutrient solution daily. Salinity symptom scores were assessed on days 21, 28, 35, and 38 post-sowing using a visual growth response scale of 1-9 (healthy-dead). Phenotypic measurements and the Area Under the Injury Curve (AUIC) were used as indicators for tolerance. A Genome Wide Association Study (GWAS) was conducted using the phenotypic data collected and a large dataset of 68,222 Single Nucleotide Polymorphisms developed from the USDA PSP plus core collection. Potential candidate breeding germplasm conferring high salinity tolerance at the germination and seedling growth stages was identified. Significant marker-trait associations were discovered for all traits measured, providing potential Marker-Assisted Selection (MAS) opportunities.

CHAPTER ONE

LITERATURE REVIEW

Field Pea Overview

Field pea (*Pisum sativum* L.) is an herbaceous cool-season annual grain legume widely grown throughout the world for human consumption, livestock feed, and as a green manure cover crop (Coyne et al., 2011; Smýkal et al., 2012). Field peas are classified as a pulse crop, differing from fresh ('garden' or 'succulent') peas in that they are intentionally cultivated to be marketed as a dry, shelled product for the human, livestock, and fractionated product markets (Endres et al., 2016). Pea is a self-pollinated diploid (2n = 14) belonging to the Leguminosae family, Faboideae subfamily, Fabeae tribe, and *Pisum* genus (Smýkal et al., 2012). Pea can be grown as a spring or winter crop and are well-adapted to growing conditions in semi-arid to sub-humid environments of the mid-latitudes (Coyne et al., 2011). The center of origin for *P. sativum* lies in the fertile crescent region of the Middle East where archeological remains suggest that domestication may have occurred as early as 10,000 B.C.E. (Smýkal et al., 2012).

Field pea provides an inexpensive source of protein (23-25%), slowly digestible starch (50%), complex carbohydrates, fiber, vitamins, and minerals (Bastianelli et al., 1998; Javid et al., 2015). The calcium, potassium, and iron mineral content in pea is particularly important in developing nations. Field peas also impose an important ecological and economic advantage to the development of low-input farming systems because of their symbiotic relationship with atmospheric nitrogen-fixing soil bacteria

(Smýkal et al., 2012). This relationship minimizes the need for external agronomic inputs, reducing the economic and environmental costs of production. The wide growing condition adaptability, high nutritional content, and economic and agronomic advantages of producing field peas has resulted in the crop being the second most grown grain legume in the world behind dry bean, with worldwide production exceeding 16.2 million metric tons in 2017 (FAOSTAT, 2017).

Morphology & Physiology

Peas are dicotyledonous, with the mature seed containing an embryo and two cotyledons encased in a seed coat called a testa (Muehlbauer & McPhee, 1997). A hilum (scar) representing the funiculus, a former vascular connection between the developing seed and the ovary wall of the pod, is visible on the testa (Smýkal et al., 2014). The hilum is flanked on the line of separation between the cotyledons by a micropyle and a raphe, two structures often associated with the exchange and sensing of water and gas (Muehlbauer & McPhee, 1997; Smýkal et al., 2014).

Upon exposure to appropriate exogenous factors, primarily moisture and temperature, mature pea seeds will initiate an imbibition phase, rehydrating important metabolic molecules and increasing vacuolar water content (Muehlbauer & McPhee, 1997; Smýkal et al., 2014). The seed will then enter an activation phase, beginning with the degradation of protein, starch, and phytic acid stored in the cotyledons (Muehlbauer & McPhee, 1997). Increasing concentrations of carbohydrates, inorganic phosphorus, and amides increase the rate of respiration within the seed, resulting in dramatic

ultrastructural changes that ultimately conclude germination with the radicle extending downward from the hypocotyl (Muehlbauer & McPhee, 1997).

Following germination, the radicle forms a taproot, producing lateral roots and root hairs as it elongates (Muehlbauer & McPhee, 1997). Simultaneously, the plumule will extend upward from the epicotyl, utilizing a hook structure to protect the young shoot meristem as it pushes through the soil to the surface (Muehlbauer & McPhee, 1997). The plumule hook will straighten upon contact with sunlight, exposing the first leaves, initiating photosynthesis, and accelerating vegetative growth (Muehlbauer & McPhee, 1997). Throughout the vegetative growth stage of a pea plant, 20 to 25 nodes will develop on a green hollow stem, two of which are small and remain below the soil surface; these belowground nodes may produce basal branches following stress events or may develop naturally in some cultivars (Muehlbauer & McPhee, 1997). Each aboveground node is comprised of a compound leaf of two fleshy stipules at the base of a petiole; each petiole will contain two to three leaflets and terminate with three to five tendrils (Muehlbauer & McPhee, 1997). Dependent on growing conditions and variety, the length of the stem will extend between 25 and 300 cm (Makasheva, 1983).

Flowering (anthesis) is initiated under adequate moisture conditions and the accumulation of required growing degree days. The growth habit of pea is indeterminate, with peduncles bearing one or two flowers emerging from the axis of the stipule (Makasheva, 1983; Muehlbauer & McPhee, 1997). Pea has a perfect flower and is described as papilionaceous, containing a calyx of five basally united sepals and a corolla consisting of a large standard (flag) petal, two wing petals, and a two-petal fusion

structure called a keel (Makasheva, 1983; Muehlbauer & McPhee, 1997). The keel encases the reproductive organs, comprised of a pistil and ten stamens, nine of which have fused filaments and one that is free but closely adjoined to the ovary (Makasheva, 1983). Upon maturation of the anthers, substantial amounts of pollen will deposit on the stigma, resulting in double fertilization of up to 12 ovules within the ovary and the subsequent initiation of pod and seed development (Makasheva, 1983; Muehlbauer & McPhee, 1997).

Pod and seed development begin with the 'flat pod' phase, characterized by pod elongation, wall thickening, and the downward curving of the peduncle as the pod increases in weight (Muehlbauer & McPhee, 1997). Pod shape is typically convex or concave to a certain degree and alongside pod length, will vary significantly across cultivars (Makasheva, 1983). Shelling and sweet pea pod types differ in the presence of a hard parchment layer of lignified cells that develop on the pod wall; this structure promotes pod shattering upon desiccation in shelling types and is absent in the edible sweet pea types (Makasheva, 1983). The end of the 'flat pod' phase, typically 20 days post-anthesis, is defined as the point at which seed development has exceeded a threshold where abortion is evaded and maturity is imminent (Muehlbauer & McPhee, 1997). The 'round pod' phase in seed development is characterized by the dramatic enlargement of the cotyledons and the formation of the embryonic structures and testa (Deunff & Rachidian, 1988; Muehlbauer & McPhee, 1997). Photoassimilate derived primarily from the leaf structures and the pod itself support the accumulation of starch, protein, and phosphate in the enlarging cotyledons (Muehlbauer & McPhee, 1997). Severance of the

vascular connection between the pod and the mother plant marks seed physiological maturity and initiates the drying of seed down to a final moisture content of less than 18 percent (Deunff & Rachidian, 1988). Seed size, shape, coloring, and coat pattern varies widely across cultivars, with measurements ranging from 3.5 to 10.5 mm, shapes ranging from round to angular, seed coat patterns ranging from mottled to speckled, and colors ranging from green to purple (Makasheva, 1983). Environmental variation at the reproductive and maturation stage can significantly influence the elongation rate and dry matter content of the pod, the seed abortion rate, and the final weight, size, and physical characteristics of the mature seed (Makasheva, 1983; Muehlbauer & McPhee, 1997).

Cultural Characteristics

The wide adaptability of field pea has led to an impressive amount of variation across cultivars throughout the world and a large assortment of cultural characteristics that must be selected from when choosing a line to grow in a particular environment and for a specific end-use (Endres et al., 2016). The primary factors to be considered when selecting cultivars include market class, biotic and abiotic stress tolerance, yield potential, leaf type, and internode length (Endres et al., 2016). The USDA Federal Grain Inspection Service (FGIS) recognizes four market classes of whole dry peas that can be easily grown and marketed in the US. These classes are comprised of smooth green, smooth yellow, wrinkled, and mottled whole dry peas (USDA/FGIS, 2014).

Smooth yellow market class peas are grown primarily for the fractionated food product market and are valued for their high protein content and their beneficial agronomic traits that favor plant robustness and higher yields. The smooth green market

class peas are grown primarily for human consumption, where they are sold whole or decorticated and split for use in soups; visual appearance is extremely important for marketability and can be the subject of severe dockages (Muehlbauer & McPhee, 1997). The wrinkled dry pea market class is grown primarily for a source of seed to be used in fresh pea production including freezer, canner, and edible pod type varieties (Muehlbauer & McPhee, 1997). The mottled market class is composed primarily of Austrian winter peas that are grown for animal feed and less often as a cover crop or filler for Asian food products; these peas have winter hardiness comparable to that of winter wheat and often exhibit greater biomass (Muehlbauer & McPhee, 1997). Dry field pea lots that do not meet the strict standards put forth by the FGIS for any of the four aforementioned market classes will be graded into the "miscellaneous" or "mixed" market classes, and will subsequently be used exclusively as animal feed (USDA/FGIS, 2014).

In field pea production, leaf type and internode length (height) are important phenotypic traits that differ across cultivars and importance is stressed on selecting appropriate lines for different regions. The most commonly seeded leaf type in commercial field pea production is the semi-leafless type that is characterized by numerous tendrils located at the terminal end of the petiole; these tendrils "grab" neighboring plants, increasing standability and lodging resistance (McVay et al., 2013; Endres et al., 2016). Semi-leafless types also permit increased light penetration and air flow through the stand canopy, reducing the relative humidity and incidence of foliar diseases (Muehlbauer & McPhee, 1997). Short internode length cultivars are generally utilized in regions with adequate precipitation and long internode cultivars are preferred

in water-limited production areas (Muehlbauer & McPhee, 1997). The primary goal is to select a cultivar that will remain standing and be tall enough to maintain seed quality and allow straight combining at harvest; seeding semi-leafless long-internode cultivars in water-limited areas will safeguard these requirements (Muehlbauer & McPhee, 1997). It is possible to swath pea prior to harvest if weed pressure is prevalent or uneven maturation has occurred, but this practice is not recommended as field pea is prone to shattering, bleaching, and being transported by wind (McVay et al., 2013).

Field pea seeding rates target a population of eight to ten plants per square foot and therefore typically range from 168 to 224 kg per hectare depending on the seed size of a selected cultivar (McVay et al., 2013). Row spacing is subject to the anticipated weed competition and water availability of a field, but typically ranges from 15 to 30 centimeters (McVay et al., 2013). Inoculation of field pea seed with specific soil bacteria is strongly encouraged as pea has the unique ability to form a symbiotic relationship with rhizobia that can result in the fulfillment of the plants total N requirement (McVay et al., 2013). Rhizobia are able to convert the abundant nitrogen gas (N_2) present in the atmosphere into plant-available ammonium (NH_{4+}); pea roots will produce nodules upon infection by certain *Rhizobium* species, forming a symbiotic relationship that supplies the bacteria with energy and the crop with ‘fixed’ plant-available N (GRDC, 2018a). *Rhizobium leguminosarum* sv. *viciae* is exclusively used in commercial field pea inoculant, as it is the primary colonizer of pea roots (Muehlbauer & McPhee, 1997; Endres et al., 2016) Recent discoveries have also found *R. fabae* and *R. pisi* to be important root colonizers and biological N fixers in field pea, and the species are

suspected to play an important future role in the genetic diversification of commercial inoculant (Wielbo et al., 2015). The near eradication of external fertilizer inputs coupled with remnant soil total N concentrations from roots and stubble potentially exceeding 112 kg/ha, renders the significance of the symbiotic relationship between pea and *Rhizobia* in relation to low-input farming systems difficult to overemphasize (GRDC, 2018a).

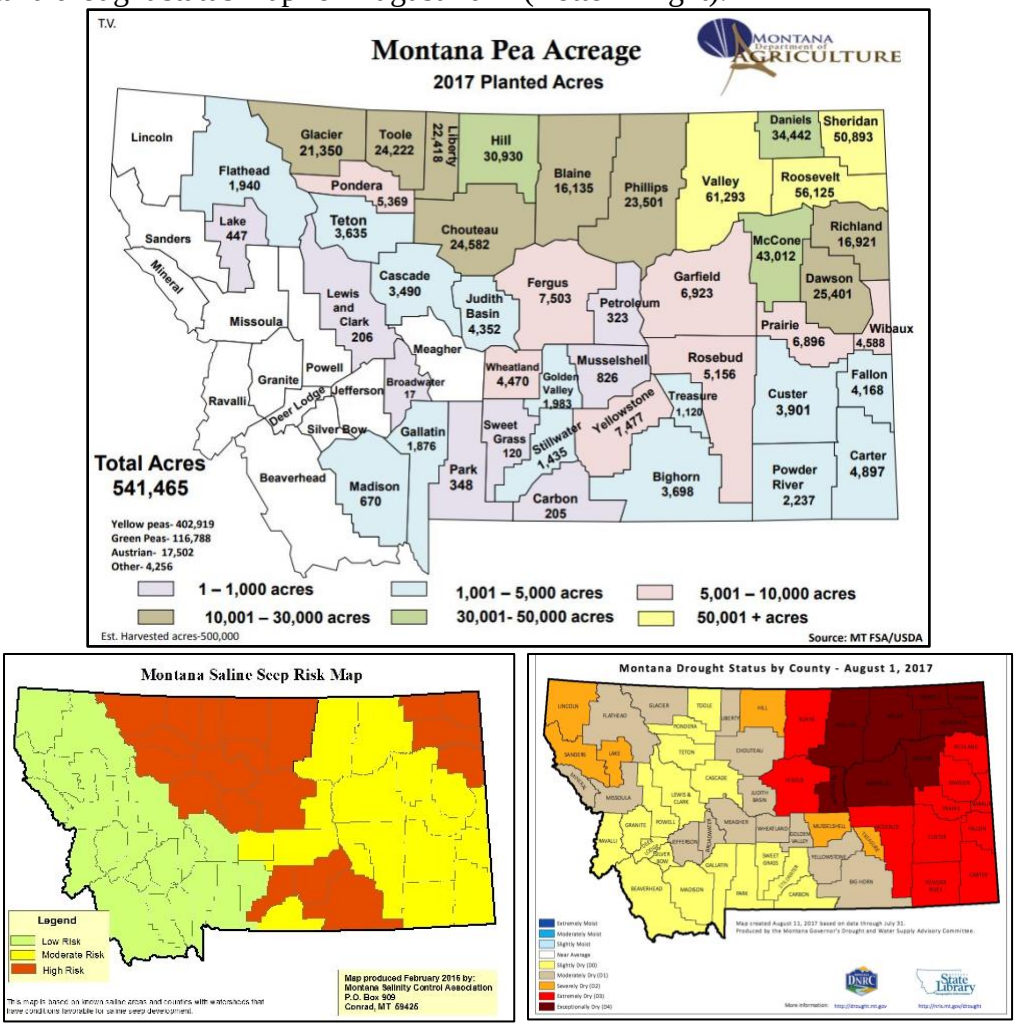
Field Pea in Montana

In Montana, field pea is an important dryland grain crop grown in rotation with cereals. Its relatively shallow root system, nitrogen-fixing potential, and high water use efficiency make pea an excellent rotational crop with small grains in the semi-arid climate of Montana, where soil fertility and moisture conservation is critical (Endres et al., 2016). Consequently, Montana is the second largest producer of dry pea in the United States behind North Dakota and accounted for nearly one third of all dry pea production in the country at 255,129 metric tons in 2018 (USDA-NASS, 2019). Farmers in Montana seeded and harvested 135,569 and 125,452 hectares of dry peas in 2018, respectively (USDA-NASS, 2019). The value of dry pea production in the state that same year exceeded 48.8 million dollars (USDA-NASS, 2019). The majority of field peas grown in Montana are of the smooth yellow market class, with grower decision based on demand from the fractionated product market and the robustness (e.g. tolerance to bleaching) associated with yellow peas over the green (McVay et al. 2013). Yellow field peas out-yield green field peas by about 10% in Montana; this yield bump is subject to specific varieties and environmental conditions, but generally remains constant (McVay et al.,

2013). Consequently, Montana field pea producers tend to seed yellow peas and green peas at a 3 to 1 ratio (USDA-FSA, 2018).

In Montana, field pea is typically inoculated with N fixating *Rhizobia* and seeded using the same equipment utilized in small grain production (McVay et al., 2013). Field pea is sown into no-till cereal stubble from the previous season in early spring when the soil moisture content is at its highest. Indeterminate semi-leafless varieties with long internode lengths are commonly chosen by Montana producers for their collective standability and their adaptability to moisture and heat stress (McVay et al., 2013; Endres et al., 2016). In late summer, field pea is straight combined when seed moisture content falls below 18 % (McVay et al., 2013). Yields in Montana will vary significantly depending on the environmental conditions and severity of stresses suffered throughout the growing season. For example, the average yield of dry peas in Montana varied from 919 kg/ha to 1,815 kg/ha in 2017 and 2018, respectively (USDA-NASS, 2019). The amount of acres seeded to field pea in Montana fluctuates from year to year based on market price, market demand for other pulse crops, and the presence of favorable spring conditions, but a general trend suggesting a leveling out of field pea acreage can be observed (USDA-FSA, 2018; USDA-NASS, 2019). Field pea production in Montana is concentrated in the northeast and Golden Triangle (northcentral) regions of the state (Figure 1) where favorable growing conditions are most prevalent (USDA-FSA, 2018).

Figure 1. Distribution of planted pea acres across Montana counties in 2017 (Top), the Montana Salinity Control Association saline seep risk map (2016) (Bottom Left), and the Montana drought status map for August 2017 (Bottom Right).



Abiotic Stresses

In Montana field pea production, abiotic stress can be a substantial limiting factor in overall plant vigor and yield potential. Despite adaptations to cool-season semi-arid environments, abiotic stresses such as drought, heat, cold, frost, soil pH, soil mineral deficiency and toxicity, and soil salinity can have substantial negative impacts on field pea productivity (GRDC, 2018b). The impact that an abiotic stress will impose on pea is

difficult to determine as they depend on unpredictable environmental factors and the growth stage of the plant; the spatial heterogeneity of soil stresses can also further complicate effects.

Heat and drought are the two interrelated abiotic stresses considered to be the most detrimental to field pea production, as they both have harmful impacts on plant physiological mechanisms and plant water relations (Stoddard et al., 2006). Heat stress will negatively impact grain yield in field pea at temperatures exceeding 25°C (Jiang et al., 2015). The flowering stage of pea development is extremely sensitive to heat and drought. At temperatures in excess of 33°C, reproductive organs will abort, and water deficits will force stomata to close, further initiating a cascade of detrimental intracellular reactions (Jiang et al., 2015). In Montana, the regions in which field pea production is concentrated (Figure 1) also reflect the areas in which drought is most severe (Figure 1), making it a very important limiting factor in overall field pea productivity in the state. The primary management tactic for heat and drought stress is to seed short-season, early maturing cultivars in the spring, avoiding the late-summer window of the greatest heat and water stress when the peas are flowering (McVay et al., 2013). Frost and cold stress are mostly associated with winter pea production but can have significant negative impacts if stress is put on spring-sown field pea at the reproductive stage (GRDC, 2018b). The management of frost and cold stress on field pea involves cultural practices that limit plant exposure at important growth stages and the development of cultivars exhibiting increased tolerance (McVay et al., 2013).

Field pea sensitivity to soil abiotic stresses is less often discussed but can have severe implications for plant vigor and overall yield. Field pea will exhibit optimum growth under soil pH values ranging from neutral to slightly alkaline; when the soil pH becomes acidic, nutrient availability (e.g. phosphorus) may decline and mineral toxicity (e.g. aluminum, manganese) will increase (Brady & Weil, 2010; GRDC, 2018b). Additionally, pH can have a major impact on soil *Rhizobium* populations and consequently, on total field pea nodulation and N fixation (Rice et al., 2000). The most widely used inoculant in field pea production, *Rhizobium leguminosarum* sv. *Viciae,* performs optimally in soils with pH values ranging from slightly acidic to slightly alkaline (pH 6-8) and dramatic declines in % N fixed from the atmosphere will be observed below a pH of 5.5 (Rice et al., 2000; Drew et al., 2014).

Salt-affected soils can also severely inhibit growth through mechanisms that will be discussed in detail in a later section. Unfortunately, the regions in Montana at the highest risk for soil salinization (Figure 1) are also the areas in which field pea production is centered (Figure 1). Unlike biotic stresses and nutrient deficiencies that can be remedied through chemical applications, abiotic stresses impose huge limitations on field pea production in Montana due to the limited practical options available for control and/or tolerance. Effective control of abiotic soil stresses relies primarily on breeding improvements in tolerance and the implementation of long-term cultural practices, such as fallow replacement. These limitations are abundantly clear when observing salt-affected soil remediation and control as salt-tolerant legume varieties are not available and producers cannot simply apply a product to manage salt toxicity, making it one of the

most detrimental and most difficult stresses that a pulse grower will encounter in Montana.

Biotic Stresses

Pathogens and insects that infect and feed on peas can have significant negative impacts on Montana field pea production and thus are the focus of most resistance improvement efforts and alternative cultural practice developments in the crop. The infectious diseases affecting seeds, seedlings, foliage, and/or reproductive tissues of field pea can be caused by fungi, bacteria, viruses, or nematode pathogens (APS, 2008). The fungal diseases negatively impacting field pea performance are generally separated into two categories, the soilborne seed and seedling fungal diseases and the foliar fungal diseases.

The important soilborne pathogens in field pea include Pythium seed and seedling rot (*Pythium spp*.), Rhizoctonia seed, seedling, and root rot (*Rhizoctonia solani*), Aphanomyces root rot (*Aphanomyces euteiches*), and Fusarium root rot (*Fusarium spp*.) (APS, 2008; Markell et al., 2018). The symptoms associated with the soilborne fungal diseases will vary substantially but typically involve necrosis of the root system, stunted growth, and chlorosis of the leaves (Markell et al., 2018). Control measures for soilborne fungal pathogens rely mostly on fungicidal seed treatments and cultural practices that reduce favorable soil conditions and prevent the accumulation of pathogen populations; resistant cultivars are also available for some soilborne diseases (APS, 2008). Important airborne fungal pathogens impacting field pea production in the Northern Great Plains include Ascochyta blight (*Ascochyta pisi* and *A.pinodes*), powdery mildew (*Erysiphe pisi*

and E. trifolii), white mold (*Sclerotinia sclerotiorum*), Septoria blight (*Septoria pisi*), Fusarium wilt (*Fusarium oxysporum* f. sp. *pisi*), and rust (*Uromyces vicia-fabae*) (Markell et al., 2018). The symptoms associated with the foliar fungal diseases of pea vary dramatically, but many are characterized by necrotic lesions and discoloration on leaves, stems, and pods and to a lesser extent by the loss of plant turgor (wilting) (Markell et al., 2018). Resistant cultivars, foliar fungicides, and the implementation of cultural practices that disrupt disease cycles and avoid favorable pathogen conditions are utilized in control programs for airborne fungal pathogens in field pea (APS, 2008; McVay et al., 2013).

The bacterial pathogens negatively affecting field pea production include bacterial blight (*Pseudomonas syringae* pv. *pisi*) and brown spot (*P.syringae* pv. *syringae*) (APS, 2008; Markell et al., 2018). The symptoms of these pathogens will affect all above-ground plant parts, typically being observed as water-soaked or necrotic lesions (Markell et al., 2018). Field pea bacterial pathogen transmission is typically seedborne or mechanically induced by hail events; control efforts focus on the use of clean seed and the development of resistant cultivars (Markell et al., 2018).

The most important viral pathogens affecting field pea include alfalfa mosaic virus, bean leaf roll virus, pea enation mosaic virus, pea seedborne mosaic virus (PSbMV), and pea streak virus (APS, 2008). Most of these viruses are vectored primarily by pea aphids (*Acyrthosiphon pisum*) or green peach aphids (*Myzus persicae*) and disease outbreaks usually correlate with increased aphid populations (Markell et al., 2018). Symptoms vary across viral diseases but generally involve the aboveground tissue where

chlorosis, stunted growth, leaf rolling, and pod deformity or lack of pod filling is observed (McVay et al., 2013). The control of field pea viral pathogens focuses primarily on resistant cultivar development, alternative host and aphid control practices, and the use of clean seed (Markell et al., 2018). Viral diseases are not presently a major concern in Montana, but incidences are anticipated to increase as total pulse crop acreage continues to grow in the state (McVay et al., 2013).

Plant-parasitic nematodes are also capable of negatively impacting field pea production, causing direct feeding damage and often being involved in soilborne disease complexes (APS, 2008). The pea cyst (*Heterodera goettingiana*), root-knot (*Meloidogyne incognita*), and root-lesion (*Pratylenchus spp*.) nematodes are species of primary concern in field pea (APS, 2008). The control of plant-parasitic nematodes relies primarily on the use of nematicides and cropping practices, with focus beginning to turn to increasing crop resistance to other root pathogens involved in disease complexes favoring nematode infection (APS, 2008).

Insect pests of field pea can also contribute to substantial crop damage and yield losses. In Montana, pea aphids (*Acyrthosiphon pisum*), grasshoppers (Melanoplus spp.), cutworms (*Euxoa auxiliary, Agrostis orthogonia*, and *Feltia jaculifera*), pea leaf weevils (*Sitona lineatus*), wireworms (Elateridae family), and lygus bugs (Lygus family) cause the most significant damage to field peas (APS, 2008; McVay et al., 2013). The symptoms associated with insect feeding damage on pea are extremely variable across taxonomic classifications, ranging from reduced emergence rates, the scalloping of leaf margins, and complete destruction of vegetative tissues (McVay et al., 2013). The

morphological stage and population size of the insect pest, along with the growth stage of the pea plant, will determine the severity of damage and yield losses. The control of insect pests in field pea is typically reliant on insecticides applied at specific growth stages of the plant and insect, but concerns surrounding insecticide resistance have been increasing in recent years (APS, 2008; McVay et al., 2013). This has resulted in a push for the development of tolerant cultivars and cultural practices that promote plant competition and inhibit insect population growth.

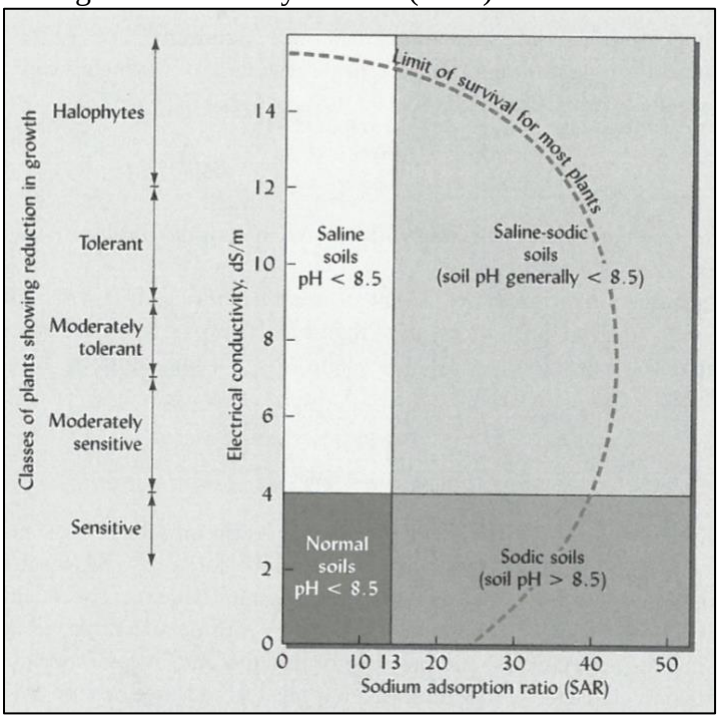
Field pea can also suffer substantial losses from weed pressure, as peas are notoriously uncompetitive at the seedling stage. The control of weeds in field pea generally involves an integrative approach that utilizes cultural, chemical, and mechanical strategies. Common and effective control methods may include altering plant spacing to increase canopy cover and competitiveness, applying herbicide pre or post-emergence, harrowing or rotary hoeing, and implementing a crop rotation to discourage weed seed bank accumulation (McVay et al., 2013).

Saline Soils

Salts are water-soluble ionic compounds with a neutral charge that are naturally found in low concentrations in all soils. Saline soils are characterized by excessive concentrations of soluble salts in the soil profile that negatively impact plant growth and soil structure (FAO and ITPS, 2015; Brady & Weil, 2010). These salts are primarily sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) or to a lesser extent, combinations of magnesium (Mg^{2+}), calcium (Ca^{2+}), Cl^-, and SO_4^- (Parihar et al., 2015). Soils are

generally sorted into one of four classes based on the Electrical Conductivity (EC), the concentration of hydrogen ions (pH), and the Sodium Absorption Ratio (SAR) of the soil solution (Brady & Weil, 2010). Based on the values of these measurements, the four classes are saline soils, saline-sodic soils, sodic soils, and normal soils (Figure 2) (Brady & Weil, 2010).

Figure 2. Diagram illustrating the salt-affected soil classifications based on EC, SAR, and pH values. Plant survival limit in each class and salt-sensitivity classifications are also depicted. Diagram from Brady & Weil (2010).



In relation to crop production, sodic soils are generally associated with adversely impacting plant growth through the deterioration of soil physical properties; primarily through the decreased movement of air and the infiltration rate throughout the soil profile, but may also lead to specific ion toxicity of Na^+ in plant tissues (Brady & Weil,

2010). Saline soils are associated with directly impacting plant growth through osmotic potential disruption and excess ion toxicity (Parihar et al., 2015).

The most common salinity test is to indirectly determine the amount of soluble salts in the soil solution by measuring its EC. The EC of a solution will increase in tandem with the amount of salts present, as pure water is a poor conductor of electricity (Brady & Weil, 2010). This test can be conducted with a hand-held EC meter and the results are commonly expressed in deciSiemens per meter (dS m^{-1}). The USDA Natural Resources Conservation Service (NRCS) has developed a salinity classification system composed of five classes of salinity severity based on soil solution EC values (NRCS Soil Science Division Staff, 2017) (Table 1).

Table 1. Soil salinity classes as defined in the USDA-NRCS Soil Survey Manual (2017)

Salinity Class	Electrical Conductivity (dS m^{-1})
Nonsaline	< 2
Very Slightly Saline	2 to < 4
Slightly Saline	4 to < 8
Moderately Saline	8 to < 16
Strongly Saline	≥ 16

The SAR of a soil solution provides information on the comparative concentrations of Mg^{2+} and Ca^{2+} in relation to Na^{+} (Scianna, 2002). Mg^{2+} and Ca^{2+} play an important role in moderating the adverse effects of sodium on physical soil properties and plant health; as the relative amount of Na^{+} to Mg^{2+} and Ca^{2+} (SAR) increases, the detrimental effects will be more severe (Brady & Weil, 2010). SAR is commonly used

for measuring sodicity in irrigated crop production where excess Na^+ in irrigation water is of great specific concern. The SAR of a soil is essential for classifying it as normal (SAR < 13; EC < 4 dS m^{-1}), saline-sodic (SAR > 13; EC > 4 dS m^{-1}), or sodic (SAR > 13; EC < 4 dS m^{-1}) (Figure 2) (Brady & Weil, 2010).

Measuring the pH of a soil solution is less common for determining the severity of salinity in a soil profile but can be an important indicator of its chemical status. The pH of a soil can be quantified using a hand-held pH electrode and is reported as the concentration of H^+ relative to OH^-. When excess exchangeable Na^+ in the soil profile begins to hydrolyze it will increase the concentration of OH^- ions and raise the pH, further dispersing soil colloids and creating an impervious soil structure that is not conducive to plant growth (Scianna, 2002; Brady & Weil, 2010). In relation to salinity, a high pH value can indicate excess Na^+, low plant nutrient solubility, and an unfavorable soil structure (Seelig, 2000; Scianna, 2002). The pH value of a soil can be a valuable measurement for classifying it as saline, saline-sodic (pH < 8.5), or sodic (pH > 8.5) (Figure 2) (Brady & Weil, 2010).

Despite their importance in classifying salt-affected soils, SAR and pH values are rarely discussed when looking at global salinity statistics because they are directly or indirectly measuring Na^+ salts exclusively. Therefore, salt-affected soils are generally reported using their EC values, taking into consideration all combinations of ionic compounds that constitute salts and classifying the soil more broadly as salt-affected or normal.

Global Extent of Salt-affected Soils

Salt-affected soils are globally widespread and can be found at varying levels of severity in all climates and on every continent except for Antarctica (Rengasamy, 2010). The world's highest concentrations of salt-affected soils occur in arid and semi-arid regions where evapotranspiration exceeds precipitation, preventing the leaching of excess ions out of the soil profile (Shahid et al., 2018). In 2015, the FAO estimated that 0.3-1.5 million ha of farmland are removed from production each year and an additional 20-46 million ha experience decreased production potential due to increasing soil salinity (FAO & ITPS, 2015). Current estimates put the amount of cultivated lands affected by salinity at one billion hectares or 23 % of the earth's arable land (Shahid et al., 2018). The global scope and increasing severity of salt-affected soils has garnered the interest of researchers for decades, with focus centered around the causes of soil salinization in an attempt to make adequate recommendations for control and prevention practices.

Development

The development of saline soils can be attributed to two main processes, primary salinity resulting from natural causes and secondary salinity due to certain agricultural practices and land use (FAO & ITPS, 2015). Primary (natural) salinity results from the accumulation of salts in the soil profile over extended periods of time; this commonly results from the natural weathering of primary minerals from parent material and the subsequent movement of soluble salts in groundwater to lower elevations in the landscape where they will accumulate in the soil profile (Brady & Weil, 2010). Primary

salinity also occurs as a result of the deposition of oceanic salt carried by the wind or in rain or by the intrusion of saltwater through ocean tides or contaminated groundwater (Brady & Weil, 2010; FAO & ITPS, 2015). The severity of salt accumulation in a soil profile will often be determined by climatic factors that influence the rate of evaporation and deposition of salts at the soil surface; these may include prolonged droughts, excess periods of precipitation, and the destruction of deep-rooted perennial vegetation.

Secondary (human-induced) salinity is generally attributed to irrigated agriculture and crop-fallow dryland cropping practices. Although proportionally much less of a contributor to global salt-affected land, human-induced salinity has become an increasing concern in important agricultural regions as more land is degraded each year than is newly brought under irrigation (Brady & Weil, 2010; FAO & ITPS, 2015). The irrigation of crops in arid and semi-arid regions is of particular concern, having huge impacts on soil water budgets, salt accumulation, and overall land productivity. The practice of using low quality salt-heavy and brackish water in regions where freshwater is scarce amplifies soil salinity; even the use of clean irrigation water with negligible concentrations of soluble salts can lead to the substantial accumulation of salts in the soil profile over time (Brady & Weil, 2010; Shahid et al., 2018). The primary concern with irrigated agriculture is not the introduction of salts specifically but the addition of excessive quantities of water to the soil profile that will disrupt the soil water balance under conditions of inadequate drainage. Excess water in a poorly drained soil profile will raise the groundwater table and as it approaches the soil surface through capillary rise, evaporative rates will increase, pulling more and more salts into the root zone as salinized water

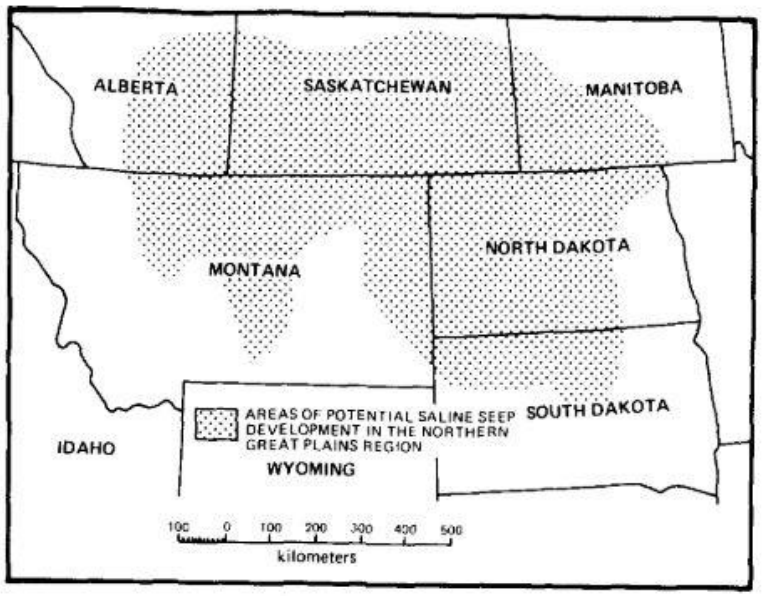
evaporates (Yadav et al., 2011; Shahid et al., 2018). Crop-fallow cropping practices in dryland agriculture will similarly introduce excessive water into the soil profile and therefore are of great concern in dryland production regions. The accumulation of salts in the soil profile will form saline seeps in these areas; the formation of saline seeps in dryland agriculture will be further discussed in detail, as they are of specific concern to production in the Northern Great Plains region of North America.

Saline Soils in Montana

The adverse effects of soil salinity on plant growth have been observed by Montana agricultural producers for over a century and of great concern since the 1970s (Miller et al., 1981). The primary source of soluble salts in Montana soil is the weathering of parent material rock high in concentration of the cations Na^{+}, Ca^{2+}, Mg^{2+}, and potassium (K^{+}), and the anions Cl^{-}, SO_4^{2-}, nitrate (NO_3^{-}), carbonate (CO_3^{2-}), and bicarbonate (HCO_3^{-}) (Scianna, 2002). Saline soils primarily dominated by sodium sulfate are distributed across much of the Northern Great Plains and are concentrated in the north central and north eastern regions of Montana (Figure 3) (Miller et al., 1981; Keller et al., 1986). In 1981, it was reported that the mean concentration of SO_4^{2-} in Montana groundwater was 6 to 42 times higher than that of Cl^{-} and that Na^{+} was 1.5 to 9 times higher than any other cation in the regions overlying shale formations (Miller et al., 1981). This indicates that the soils of Montana field pea growing regions are dominated by Na_2SO_4 and are generally saline with the potential to become saline-sodic where high pH values greater than 8.5 are present.

The Montana Salinity Control Association (MSCA) estimates that 80,937 ha of Montana cropland are currently negatively affected by soil salinity (MSCA, personal communication, May 22, 2018). It is estimated that over 4,370,604 ha of Montana land are impacted by saline soil conditions (EC > 4 dS m^{-1}) and that over 121,405 ha have been removed from production due to increasing soil salinity (Kharel, 2016; Scianna, 2002). The exact extent of soil salinity in Montana is difficult to determine as limiting funding and the fluctuating nature of salinized soil zones impede monitoring capabilities.

Figure 3. Potential saline seep development areas across the Northern Great Plains based on geological formations (Miller et al., 1981).



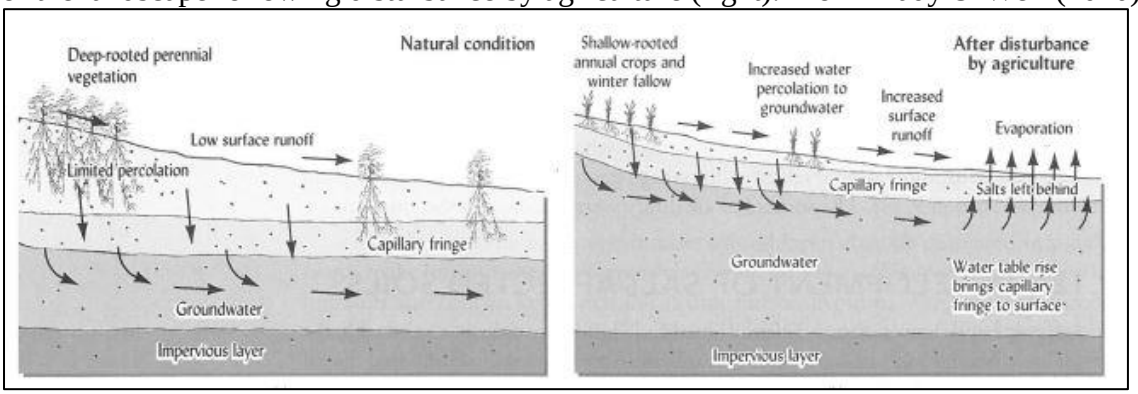
Saline Seeps

When saline water is discharged at or near the soil surface of a dryland landscape depression and accumulates to levels that adversely affect plant growth and soil structure, it is termed a saline seep. This phenomenon can be differentiated from other saline soil conditions by its shallow water table, local origin, saturated root zone, and sensitivity to

agronomic practices and fluctuations in precipitation (Brown et al., 1982). In Montana, saline seeps are observed as the iconic white incrustation of salts on the soil surface and the complete absence of vegetation (Scianna, 2002). The cause of a saline seep can vary dramatically between areas, as it is the result of a combination of geological, cultural, and climatic factors specific to that area (Miller et al., 1981).

In general, the development of a saline seep (Figure 4 right) begins when precipitation (or irrigation) exceeds the field capacity of a soil profile; this is often accelerated when the land is left unvegetated in a dryland crop-fallow system where evapotranspiration is greatly decreased (Brady & Weil, 2010). Excess water will percolate through the soil profile, collecting abundant soluble salts and raising the water table above an impermeable bedrock layer (Brown et al., 1982). Water movement is then forced laterally across the impermeable layer to a lower elevation depression in the landscape called the discharge area. In this localized area, the water table may rise to within 3 feet (1 m) of the soil surface where the highly saline water is subject to evaporation. As the water evaporates, salts will accumulate in the root zone and on the soil surface, forming a white crust and negatively affecting the hydrological and structural properties of the soil (Brown et al., 1982).

Figure 4. Diagram illustrating the natural condition of a landscape (left) and the condition of the landscape following disturbance by agriculture (right). From Brady & Weil (2010).



The soluble salts typically found in saline seeps are primarily sulfates of Ca^{+}, Na^{+}, or Mg^{+} (Brown et al., 1982). As discussed previously, the saline soils in Montana are dominated by Na_2SO_4, as opposed to the majority of global saline soils dominated by NaCl. This distinction presents a unique challenge to reclamation efforts and cropping options for Montana producers dealing with salinized soils, as varying plant species will respond differently to specific salt ions.

Reclamation of Salt-Affected Soils

The reclamation of salt-affected soils is often a long and expensive task, subject to the severity of the soluble salt concentrations in the soil profile and the resources available for leaching them out. The primary goal of any program directed at reclaiming salt-affected area or preventing the accumulation of soluble salts in the soil profile is to utilize water before it percolates beneath the root zone (Miller et al., 1981). Most reclamation programs rely on a combination of mechanical, chemical, and cultural approaches that may include the direct leaching of excess salts through irrigation, the construction or modification of drainage systems, the application of chemical or organic

amendments, the utilization of phytoremediation, the implementation of various cropping systems, or the planting of salt tolerant cultivars (Miller et al., 1981; FAO & ITPS, 2015).

The direct leaching of excess soluble salts out of the soil profile through irrigation is the method most commonly utilized in regions where adequate water is available (FAO & ITPS, 2015). This method must be implemented with caution using a deep understanding of the hydrological and geological properties of that particular soil profile. It is important to ensure that the precise amount of irrigation is applied to leach salts out and not affect the water balance of the soil profile. Where it is economically feasible, the labor-intensive practice of installing or manipulating drainage systems to lower the water table in the landscape is also a common practice.

The application of soil amendments to ameliorate saline-sodic and sodic soils may be effective in certain areas where there is adequate soil profile drainage and there is affordable product readily available. The primary objective of an ameliorate application to salt-affected soils is to replace the excess Na^{+} on the cation exchange complex of soil colloids with a different cation, primarily Ca^{2+} (Brady & Weil, 2010). The most commonly utilized soil amendment for reducing sodicity in soils is gypsum ($CaSO_4 \cdot 2H_2O$), which upon dissolution will produce Ca^{2+}, which displaces Na^{+} on mineral surfaces, allowing it (and SO_4^{-}) to leach (Qadir et al., 2007). This is not a practical amendment for the Northern Great Plains where many soils in the region already contain high concentrations of naturally occurring gypsum and water limitations inhibit the leaching abilities of the soil profile (Seelig, 2000). Other commonly used amendments

include calcium chloride, calcium carbonate, ferrous sulfate, aluminum sulfate, and sulfuric acid (Qadir et al., 2007).

Salt-affected soil reclamation research is now focusing on a plant-assisted approach called phytoremediation, of which the goal is to use plant roots to aid the replacement of Na^+ with Ca^+ dissolved from calcite ($CaCO_3$) naturally found in the soil (Qadir et al., 2007). This approach is economically and environmentally superior to chemical and organic amendments as it provides a source of crop income while improving the chemical and structural quality of the soil in a more uniform manner (Qadir et al., 2007). A similar biotic-based approach to salt-affected soil reclamation involves the inoculation of crops with Plant Growth-Promoting Rhizobacteria (PGPR). The efficacy of utilizing PGPR in salt-affected soil crop production has been supported for many crop species and the practice is the subject of increasing interest among abiotic crop stress researchers (Tank & Saraf, 2010). The primary disadvantage of these two plant-based approaches is that they require moderately salt tolerant crop species; therefore, it is anticipated that phytoremediation and PGPR reclamation practices will increase proportionally with breeding successes in salt tolerant crop development.

In arid and semi-arid dryland production regions that typically grow low-value crops on extensive acreage, the planting of salt-tolerant cultivars and forages that replace fallow in the recharge area is preferred. In salt-affected areas, deep-rooted perennial crops like alfalfa can be planted in the recharge area, utilizing seasonal precipitation while also lowering the water table and reducing the lateral movement of salts to the discharge area (Seelig, 2000). The seeding of halophytes (salt-tolerant species) that mirror the native

vegetation prior to agricultural disturbance, may be the only option for areas of extreme soil salinity that no longer support the growth of glycophytes (salt-sensitive), or even moderately salt tolerant species.

In salt-affected soil reclamation, the most suitable approach will vary significantly from region to region and implementation of a combination of approaches may be necessary to fully reclaim the land. Miller et al. (1981) reported that even severely salinized soils can be reclaimed by up to 70% using cropping practices alone and up to 100% using a combination of mechanical, chemical, and cultural practices. This indicates that the development of salt tolerant cultivars would undoubtedly provide substantial economic benefits to producers, as soils could be reclaimed primarily using high-value annual cropping practices. This is particularly important in the Northern Great Plains, where the climatic conditions, cultural practices, and geological and hydrological soil properties common to the region are not generally conducive to saline soil reclamation practices aside from flexible cropping approaches that utilize salt tolerant cultivars.

Salinity Stress and Tolerance in Plants

Worldwide, soil salinity is one of the most significant limiting factors affecting crop productivity as nearly all important crops for human consumption are sensitive to elevated salt levels (Parihar et al., 2015; Liang et al., 2017). In general, salinity stress occurs when excessive soluble salts dominate the soil profile, resulting in plants competing against an osmotic potential gradient for the uptake of water and mineral nutrients essential to their metabolic processes (Munns et al., 2002; Brady & Weil, 2010;

Isayenkov & Maathuis, 2019). In addition, excessive salt anions and cations will accumulate over time in various plant tissues, potentially reaching toxic levels (Munns & Tester, 2008). Therefore, plant response to salinity stress is observed in a biphasic manner; a rapid osmotic adjustment phase followed by an ionic exclusion and compartmentalization phase (Munns & Tester, 2008; Rajendran et al., 2009). It is important to note that although scientific advancements have increased our understanding of plant responses to salinity, it is still extremely difficult to distinguish between specific ion and water deficit effects on plant growth, leaving researchers to speculate on the responsible mechanisms and the potential methods utilized to tolerate them (Greenway & Munns, 1980).

Plants have evolved a suite of physiological and molecular adaptations to tolerate high soil salinity, with certain species utilizing multiple mechanisms and others relying on just a few (Roy et al., 2014). Research supporting mutual exclusivity or independence of these mechanisms is inconclusive (Roy et al., 2014). The primary mechanisms implemented by plants in the presence of saline conditions include osmotic stress tolerance, ion homeostasis and exclusion from root cells, and tissue tolerance via compartmentalization of excess ions; antioxidant regulation and hormonal regulation are also suspected to be important but are much less studied and understood (Munns & Tester, 2008; Rajendran et al., 2009; Nadeem et al., 2019). The ability of a plant to utilize multiple mechanisms will determine its fitness and overall salt tolerance, as rising concentrations of soluble salts will overload initial defenses and require the initiation of alternative tolerance strategies (Roy et al., 2014). For example, plants will respond to low

external osmotic potentials caused by the presence of excess soluble salts in the rhizosphere by increasing the uptake of solutes into their root cells, eventually leading to toxic ion concentrations within plant tissues that will require an additional ion compartmentalization defense response (Greenway & Munns, 1980; Roy et al., 2014).

Most dicotyledonous crops (e.g. legumes) will die before maturity in soils containing soluble salt concentrations exceeding 10 dS m^{-1} and will experience substantially reduced biomass production and yield at much lower salt levels (Munns et al., 2002). Plant species that are capable of survival and reproduction in extremely saline environments, even those with salt concentrations exceeding 20 dS m^{-1}, are called halophytes (Flowers & Colmer, 2008). Some halophyte species have evolved such complex tolerance mechanisms that they are able to cope with the excessive salt concentrations in the center of saline seeps, which can exhibit EC values exceeding those of seawater (Munns et al., 2002). Therefore, it is abundantly clear that extreme variability in salt tolerance response exists among plant species and that it is likely the result of the evolution of multiple tolerance mechanisms.

Symptoms

The manner in which a plant will respond to excessive soluble salts in the rhizosphere is extremely variable and will be dependent on the plant species and cultivar, the growth stage of the plant, specific ions present, soil characteristics, and environmental conditions (Brady & Weil, 2010; Maksimovic et al., 2010). The growth stage specifically is of major significance, with some cultivars being more sensitive to high salinity at

germination, during the vegetative pre-anthesis stage, at flowering, or at the pod filling stage (Stoddard et al., 2006).

The initial osmotic stress phase is considered the most detrimental to plants exposed to high salinity and is characterized by severely stunted growth; it is often quantified through reductions in biomass production, internode length and number, turgor pressure, and stomatal opening (Greenway & Munns, 1980; Brady & Weil, 2010; Maksimovic et al., 2010). Plants affected by water stress will often exhibit dull-surfaced dark-bluish green leaves, become wilted (lose turgor), and eventually necrotic beginning with the most mature (lower) leaves that have been transpiring the longest (Brady & Weil, 2010). Similarly, the most mature leaves will exhibit the symptoms associated with ion cytotoxicity, as they have been accumulating excessive ions for the longest period. Symptoms of cytotoxicity often begin with chlorosis and necrosis at the leaf margins and eventually lead to the senescence of the mature leaves, necrosis of the stem, and whole-plant death (Munns & Tester, 2008; Brady & Weil, 2010; Leonforte et al., 2013a). As transpiration rates and important metabolic processes decline as a result of water deficiency and ion cytotoxicity, the accumulation of excessive Reactive Oxygen Species (ROS) within leaf cells will result in cell death and further accelerate tissue necrosis and plant mortality (Ahmad et al., 2009).

Mechanisms

The mechanisms associated with salt stress are complicated, often overlapping and influencing the detrimental effects of the other. It is clear that high salinity negatively affects plant growth, but the precise cause of a plant response is extremely difficult to

determine (Greenway & Munns, 1980). Therefore, it is important to understand how each phase may affect the plant individually, so that the interactions between them can be speculated and the eventual manipulation of those interactions utilized to impose tolerance.

Osmotic Stress. As discussed previously, the stress imposed on plants by water imbalance is extremely detrimental to metabolic processes and overall plant vigor. High concentrations of soluble salts in the rhizosphere lower the water potential of the roots, inhibiting plant water uptake, and initiating metabolic and structural changes that mirror those imposed by drought stress (Munns et al, 2002). Total water potential is an important parameter in characterizing water status and is the sum of the osmotic potential, pressure potential, matric potential, and gravitational potential of a soil and/or plant (Giménez et al., 2005; Brady & Weil, 2010). Of the four components, osmotic potential and pressure potential are the most important in soil-plant interactions. Pressure potential is the measure of cell turgor resulting from the diffusion of water into cell protoplasts enclosed by mostly rigid cell walls and is directly related to osmotic potential (Giménez et al., 2005). Osmotic potential (also referred to as water potential) is the measure of the total energy potential of soil water or plant cell water in relation to the presence of solutes (Giménez et al., 2005, Brady & Weil, 2010). Water molecules tend to be attracted to and accumulate around solute ions, reducing the freedom of movement and potential energy of the water (Brady & Weil, 2010). Therefore, a solution containing a high concentration of solutes will have a low osmotic potential and vice versa.

Unlike soil in which solutes are relatively free to move and disperse to equalize concentrations, the semipermeable membrane isolating plant root cells from a soil solution requires the movement of water for osmotic adjustment (Brady & Weil, 2010). (Brady & Weil, 2010). When excessive soluble salt ions in the rhizosphere lower the water potential below that of the plant root cells, uptake of water into the plant is severely constrained (Brady & Weil, 2010). Under extreme conditions, the osmotic potential of the soil may be so low that water is pulled from the root cells, resulting in plasmolysis (cell collapse) (Brady & Weil, 2010).

Most plant species in semi-arid and arid regions have evolved mechanisms to tolerate the low external osmotic potential caused by drought and therefore have developed tolerance to the osmotic phase of salinity stress also common to those regions (Munns & Tester, 2008). When the soluble salt concentration of the soil is low or moderate, most plants can osmotically adjust by accumulating solutes in their roots, thus maintaining a gradient for water intake and preserving whole-plant cell turgor (Parihar et al., 2015). In addition to solute accumulation, plants will also initiate reduced shoot growth via long distance signals and close stomatal openings to minimize the requirement for water uptake (Shahid et al., 2012; Nadeem et al., 2019). Unfortunately, unlike drought stress responses, the osmotic phase of salinity stress in plants is so complicated, difficult to measure, and interconnected with ion toxicity that there has been little advancement in understanding made in this area of salinity research over the past few decades (Greenway & Munns, 1980; Roy et al, 2014).

Specific Ion Stress. As discussed previously, in response to low external osmotic potential resulting from excess soluble salts, plants will begin the excessive uptake of ions in an attempt to lower the osmotic potential in the roots, often leading to specific ion toxicities when exposed to high salinity environments. Specific ion toxicities associated with solute accumulation under low external osmotic potential conditions primarily involve Na^{+}, Cl^{-}, and SO_4^{-2} soluble salt ions (Flowers et al., 2010; Maksimovic et al., 2010; Isayenkov & Maathuis, 2019). A major consequence of salt ion accumulation is a decrease in the uptake of essential plant nutrients and the replacement of them in plant tissues. This often results in a nutritional imbalance and deficiency in N, phosphorus, and more importantly, K^{+} and Ca^{2+}, two key ions involved in various physiological mechanisms critical to normal plant growth (Maksimovic et al., 2010; Shahid et al., 2012; Parihar et al., 2015; Isayenkov & Maathuis, 2019). A very important but much less studied secondary consequence of excess salt ion accumulation in plant tissues is that many enzymes essential to vital metabolic processes will be inhibited (Munns et al., 2002). Excess Na^{+} ions are generally considered to be the most detrimental to ion homeostasis and plant processes, having been found to reach toxic concentrations before any other salt ion (Munns & Tester, 2008). Conflicting research conducted by Tarchoune et al. (2012) indicated that SO_4^{2-} anions reduced growth rates more substantially than Na^{+}; likely a result of unadapted root selectivity for the less common ion. In legumes, research conducted with faba bean (Tavakkoli et al., 2010a) and pea (Maksimovic et al., 2010) found that high concentrations of Na^{+}, rather than Cl^{-}, had the highest

accumulations in vegetative and reproductive plant tissues and were suspected of interfering with final K^+ and Ca^{2+} nutrient contents of mature grains.

The ability of a glycophyte (e.g. legume) to tolerate high salinity at the whole plant level is subject to the plant's capacity to control salt ion transport through selective root cell uptake, xylem loading, and ion removal from the xylem in important vegetative tissues (Munns et al., 2002). Salt-loving halophytes will utilize additional mechanisms such as excretion through salt glands or bladders and phloem loading to transport excess salt ions out of important plant tissues (Munns et al., 2002). The maintenance of appropriate ion ratios is extremely important to the normal physiological growth and development of a plant (Wang et al., 2003; Parihar et al., 2015). The initial mechanism for specific ion tolerance in most plant species is the exclusion of salt ions from root cells and the control of their transport within the root system to prevent subsequent toxic accumulations within vegetative tissues (Roy et al., 2014). As salt ions enter the root system, several signal cascades are activated that localize specific ion accumulation and restrict net Na^+ translocation (Isayenkov & Maathuis, 2019). It is inevitable that under excessive external soluble salt conditions, ions will accumulate to unmanageable concentrations in root cells, ultimately demanding translocation to vegetative tissues to prevent cytotoxicity. Most plants translocate excess salt ions to their leaves, where they will accumulate in the cytosol of leaf cells. There is no evidence that intracellular enzymes have adapted to the presence of excessive specific ion accumulation in plant cells, thus requiring the exclusion of these ions from the cytosol to prevent cytotoxicity (Munns et al., 2002). Furthermore, in vitro studies have found that concentrations of Na^+

exceeding 10 dS m^{-1} will completely inhibit enzyme activity, suggesting that specific ion accumulations in plant tissues must be maintained below this threshold to prevent deleterious effects (Blumwald, 2000; Munns et al., 2002).

Perhaps the most important mechanism associated with salt tolerant plants is the compartmentalization of excess cytosolic ions into the cell vacuole, where specific ion concentrations can exceed 20 dS m^{-1} and still allow normal cell function (Blumwald, 2000; Munns et al., 2002; Flowers et al., 2010; Roy et al., 2014). Sequestration of excess cytosolic specific ions in the cell vacuole is essential to averting deleterious effects on essential plant physiological mechanisms. Exchanges between specific salt ions and K^+ and organic solutes across the vacuolar membrane supports the maintenance of osmotic pressure balance between the cytosol and vacuole (Munns et al., 2002). The two strategies discussed here, ion exclusion and translocation for compartmentalization in leaf cell vacuoles, are the most widely studied specific ion tolerance mechanisms in plant salinity research. It is important to note that within the complexity of the whole plant, the precise damage caused by excess specific ion accumulation is still not completely understood by researchers and requires further exploration.

Reactive Oxygen Species. Alongside osmotic potential disruption and specific ion toxicity, a tertiary consequence of salinity stress is the accumulation of Reactive Oxygen Species (ROS) within plant cells. Hydrogen peroxide, singlet oxygen, superoxide, and hydroxyl radicals are reactive oxygen species that can be very harmful to vital plant cell mechanisms at high concentrations (Ahmad et al., 2009). The accumulation of ROS in plant cells can lead to the oxidation of proteins, inactivation of essential enzymes,

denaturation of DNA, and peroxidation of lipids (Ahmad et al., 2009). ROS are a natural byproduct of photorespiration, respiration, and photosynthesis and are rapidly detoxified by enzymatic and nonenzymatic mechanisms in cells under normal conditions (Ahmad et al., 2008). In times of severe water stress, most plants will close their stomata to conserve water, resulting in the inhibition of carbon fixation as carbon dioxide availability declines (Ahmad et al., 2008). Chloroplasts exposed to the subsequent excessive excitation energy will increase the production of detrimental ROS and under the ionic and osmotic imbalances associated with salt stress, will lead to catastrophic events. Plant tolerance to excessive ROS production has not been studied in detail by salinity researchers but is likely associated with mechanisms involved in osmotic stress tolerance and hormonal regulation. Further information on ROS and salinity stress can be found in the review and study conducted by Hernández et al. (2000), where antioxidant defenses in salt-stressed pea were observed on a molecular level.

Salinity Tolerance Breeding Efforts

Across the myriad of stresses that reduce yield potential in major crops, biotic pests and abiotic climatic stresses have been the primary focus, as abiotic soil stresses can typically be remedied through chemical amelioration or changes in cropping practices. Salt-affected soil is generally seeded to a different crop or native vegetation that can tolerate high salinity conditions or the land is simply taken out of production. However, as market demand for specific crops continues to grow and global salt-affected land

continues to increase annually, the improvement of salt tolerance in major crops has garnered intensified attention.

Two main approaches are generally taken for improving salt tolerance in a given crop; the first is to explore the natural diversity within the species or an inter-fertile species for salinity tolerance characteristics, and the second is to manipulate existing or introduce new genetic material into the crop via genetic engineering (Munns et al., 2002). However, the current lack of community acceptance and the market demand for genetically modified crops has impeded advancements (Munns et al., 2002). This is extremely relevant in pulse crops, as the primary pulse markets reject genetically modified products. To bypass genetic engineering limitations, breeders have embraced the use of molecular markers that assist in the identification of quantifiable trait differences between lines and allow them to track trait inheritance through backcrossing into cultivars (Munns et al., 2002).

Another limitation faced by breeders is the decision to target a particular phase of salinity stress in their tolerance improvement efforts, as some characteristics may be more favorable in various salinity environments. Tolerance to the osmotic phase may be more important for moderately saline environments and the ionic phase more important for high salinity ones; the interaction with other abiotic stresses also becomes a difficult component in breeding tolerance (Roy et al., 2014). As discussed previously, it is very difficult to differentiate between mechanisms for the various phases of salinity tolerance due to the difficulty in measuring them and their interconnectedness with one another and the various abiotic stresses also present. Therefore, one of the most difficult obstacles that

plant breeders must overcome is the screening for and measurement of plant salinity tolerance mechanisms.

Salinity Tolerance Screening

Munns et al. (2002) indicated that there is likely a great diversity in response to high salinity stress within crop species that has not yet been fully explored. This is due to the difficulty of distinguishing tolerance to salinity from tolerance to osmotic stress, accumulation of Na^+ or Cl^- in plant tissues, or to a deficiency in K^+ or Ca^{2+} (Greenway & Munns, 1980). The extreme difficulty associated with screening large numbers of genotypes in the field is another important concern, with the spatial heterogeneity of physical and chemical soil properties and the unpredictability of climatic fluctuations encouraging extreme confounding (Munns & James, 2003). Therefore, most salinity tolerance screening for all crops has been performed in semi-controlled environments where small differences in biomass production between individuals can be quantified and reproduced (Munns et al., 2002). To reduce the error associated with biological measurements, tolerance to high salinity is generally reported as the percent biomass production in control versus saline conditions over an extended period of time (Munns et al., 2002). Another important aspect of salinity tolerance screening is that special attention must be directed to the developmental stage at which the plant is being assayed. Studies have shown that salt tolerance characteristics will differ among growth stages within a species and often within a single cultivar, requiring screening at both the vegetative and germination stages of development to adequately understand overall salinity tolerance (Shahid et al., 2012).

Germination Screening for Salinity Tolerance. Many factors can contribute to germination rates, including climatic and storage conditions, the age of the seed, harvest time, and the nutritional composition of the seed (Shahid et al., 2012). Therefore, it is important to eliminate confounding factors when screening plants at the germination stage by using seeds of uniform quality, size, age, and storage conditions (Shahid et al., 2012). Notable germination screening in legumes include research on pea (Shahid et al., 2012; Ouerghi et al., 2016), wild legume (Sunita et al., 2013), and common bean (Cokkizgin, 2012). Tolerance to salinity at germination is relatively easy to measure, but little or no correlations between salt tolerance at the germination stage and seedling or mature plant stage has been found for any species (Munns et al., 2002). Therefore, it is insufficient to rely solely on salinity assay results at the germination stage of development when attempting to adequately establish crop salinity tolerance (Shahid et al., 2012). Some crops have been shown to exhibit tolerance to salinity concentrations exceeding 20 dS m^{-1} at the germination stage, but few studies have gone beyond this initial growth phase to explore later plant development stage response at salinity levels more indicative of actual field conditions (Munns & James, 2003).

Seedling Screening for Salinity Tolerance. Conducting a high salinity assay with crop seedlings in a controlled environment where results are replicable can be very difficult. Historically, hydroponic screening of seedlings for salinity tolerance was the standard, but the method has met resistance in recent years as it is extremely expensive and not representative of field soil conditions. This is supported in a study conducted by

Tavakkoli et al. (2010b), where barley seedling response to salinity stress was found to be significantly different under hydroponic and soil-based systems. Research conducted by Wang (2002), found that graded coarse river sand medium in pots provided the best environment for rapid solution exchange in the root zone and achieved desired high salinity growth conditions. The hydraulic and thermal properties within the sand media were found to be comparable to those observed in field soils, further supporting the use of sand media in pots to conduct controlled and efficient salinity screening (Wang, 2002). Likewise, Ledesma et al. (2016) found sand media salinity screening of soybean to produce good results and the group concluded that the practice would be essential to low-cost (unlike hydroponics), high-throughput greenhouse screening with short trials (as soon as genotypes start to differ). Many other recent salinity tolerance studies in legumes at the seedling stage (Shahid et al., 2012; Leonforte et al., 2013a; Javid et al., 2015) have utilized sand media for screening under controlled conditions, indicating that the practice is quickly replacing hydroponic screening as the standard.

A concern that must be addressed when screening in sand media is that at high concentrations of Na^{+}, plant growth will be inhibited as a result of a breakdown in the Ca^{2+} regime of the media, leading to the reduced availability of calcium (Ca^{2+}) as a plant nutrient (Lahaye & Epstein, 1971). Inadequate levels of calcium will lead to poorly developed root systems, weakened selective ion transport, and increased cell permeability (Lauchli & Epstein, 1970; Lahaye & Epstein, 1971; Greenway & Munns, 1980). Plants deficient in Ca^{2+} have been found to transport large quantities of Na^{+} to the leaves of the plant rather than exclude it. Therefore, researchers will often supplement sand media for

pot screening with nutrient solutions containing additional Ca^{2+} in the form of calcium nitrate ($Ca(NO_3)_4$) (Leonforte et al., 2013a; Javid et al., 2015). This ensures that field soil conditions naturally containing adequate levels of Ca^{2+} are represented as accurately as possible within the artificial pot environment and that high salinity symptoms are not influenced. The significance and effects associated with Na^+/Ca^{2+} ratios in salinity tolerance experiments are discussed at length by Greenway & Munns (1980).

Salinity Tolerance Studies

Despite the significant distribution of Na_2SO_4 affected soils worldwide and the fact that most soil salinity in nature is a mixture of soluble salts, the overwhelming majority of salt toxicity studies in crop species have focused on NaCl salt. Similarly, most salt-influenced ionic stress and tolerance studies have focused primarily on Na^+ cation exclusion and tolerance mechanisms within plant tissues rather than looking at other important ions (e.g. Ca^{2+}, SO_4^{2-}, etc.) also involved in saline soils (Munns & Tester, 2008). This is not completely surprising as NaCl salt and Na^+ cations in particular are the most common features of salt-affected soils worldwide. Upon review, it appears that the detrimental effects of NaCl in relation to Na_2SO_4 have been explored to a small extent in limited studies, with results indicating that salt-induced symptom severity will depend on the species, growth stage, salt concentration, and the metabolic processes being observed (Poljakoff-Mayber & Gale, 1975; Chavan & Karadge, 1980; Bhivare & Chavan, 1987; Reich et al., 2017; Tarchoune et al., 2012). Although inconclusive, research exploring the effects of high Na_2SO_4 on legumes in particular can be found for peanut (Chavan &

Karadge, 1980), French beans (Bhivare & Chavan, 1987), and pea (Abd El-Samad & Shaddad, 1996).

In legumes, the majority of genetic studies on tolerance to high NaCl concentrations have been conducted using the model legume soybean, for which industry capital has pushed advancements (Jha et al., 2019). Despite capital limitations, many studies involving field pea and NaCl salinity tolerance have been conducted. Notable studies include growth, ion composition, and stomatal conductance responses (Maksimovic et al., 2010), antioxidant defense response (Hernandez et al., 2000), green and yellow cotyledon variation response (Steppuhn et al., 2001), and response variability in height, growth rate, and biomass among 780 pea accessions (Leonforte et al. 2013a). It is important to note that the conclusions rendered from such studies are difficult to relate to actual field conditions as they were conducted in semi-controlled environments that did not have a significant interaction with other plant stresses. In a real-world field scenario, interactions with other abiotic and biotic stresses are inevitable and the concentration of natural soil salinity is rarely at a constant level, often fluctuating with periods of precipitation (or irrigation) and drought (Poljakoff-Mayber & Gale, 1975). Therefore, the screening of any crop to high salinity conditions must include years of field trials at diverse locations and released salt-tolerant cultivars will likely need to be supplemented with other salt-affected soil reclamation practices to maintain successful yields in high salinity environments.

Germplasm Collections

Increasing concerns regarding the impact of predicted climate change on global food security and biodiversity has prompted the conservation of wild, landrace, and cultivated accessions to become a high priority among plant researchers worldwide (Coyne et al., 2011; Maxted et al., 2010; Smýkal et al., 2012). Nikolai Vavilov was the first to highlight the importance of identifying centers of origin, collecting diverse cultivars, and conserving crop wild relatives (CWR) (Dzyubenko, 2018). The relevance of such activities at present day is expressed in detail by Maxted et al. (2010). National gene banks now hold germplasm collections for most crops and wild species found on Earth. *Pisum* germplasm collections are currently found in excess of 2,000 accessions in at least 15 national gene banks around the world, maintaining important genetic diversity for the genus (Coyne et al., 2011). The aim of a core collection is to capture the genetic diversity of the genus within 20% of the total number of accessions (Symkal et al., 2012; Coyne et al., 2005). By maintaining non-elite and wild germplasm pools that are generally selected against in breeding programs, novel favorable alleles can be protected within core collections and easily accessed by researchers (Holdsworth et al., 2017). Providing a pre-determined, genetically diverse set of accessions presents an efficient genetic resource for plant breeders who would otherwise be forced to sift through thousands of genetically redundant accessions (Kwon et al., 2012).

The USDA *Pisum* Collection

The USDA *Pisum* core collection (UPCC) maintained by the Plant Germplasm Introduction and Testing Research Station in Pullman, Washington consists of more than 5,400 accessions that have been genotyped, phenotyped, and sorted into a smaller core (Smýkal et al., 2012). Major efforts have been put forth in recent decades to abbreviate the large UPCC into a smaller core representing the genetic diversity present within the larger collection. The evaluation and classification of UPCC accessions has historically been based on agronomic, geographic, and morphological traits, but with advancements in technology, genomic studies have been frequently utilized in recent decades (Coyne et al., 2005, Holdsworth et al., 2017, Kwon et al., 2012). The results of these ongoing studies propel the constant evolution of the UPCC and the number of accessions included therein.

USDA Pea Single Plant (PSP) Collection

To aid researchers, the USDA developed a genetically diverse core collection subset of homozygous *Pisum* single plant (PSP) accessions derived from the UPCC. The number of accessions within the PSP core collection fluctuates with the availability of seed and the number of accessions constituting the ever-evolving UPCC. Accession availability ranged from 384, 312, and 287 accessions in 2015, 2018, and 2019, respectively (Cheng et al., 2015; GRIN-NPGS, 2019). To develop the homozygous accessions for the PSP core collection, single plant selections were made from the UPCC and seed quantity was increased through single-seed descent (Holdsworth et al., 2017). The PSP collection consists of inbred lines (IDs designated with a PI) derived from a

single plant of each accession in the UPCC and advanced breeding lines (IDs designated with a W6; Table 2, Appendix A; Cheng et al., 2015). Up to date agronomic, morphological, and genotype data for the PSP core accessions can be accessed through the USDA Agricultural Research Service's (ARS) Germplasm Resources Information Network (GRIN) National Plant Germplasm System (NPGS) online database (www.ars-grin.gov/npgs/).

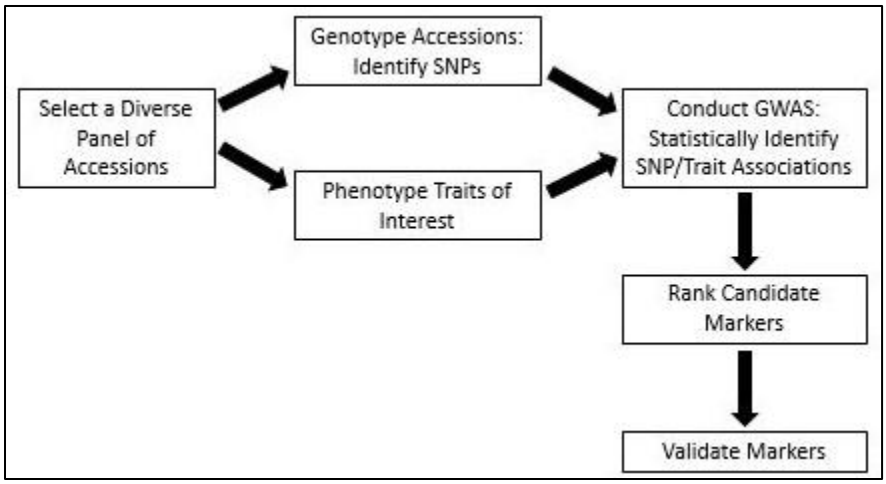
PSP Core Collection Studies. Research involving the PSP core has increased in tandem with the release of genotypic and phenotypic data for the collection. A vast range of studies involving the PSP core are explored by Smýkal et al. (2012) and Kwon et al. (2012). The use of association mapping in identifying marker-trait associations between phenotypes and SNP markers within the PSP collection of 384 accessions was highlighted by Cheng et al. (2015). An association mapping study conducted by Carpenter et al. (2017) was successful in utilizing a subset of 92 PSP accessions to find associations between starch chain length distribution and amylose content. In 2017, Holdsworth et al. assembled the USDA Pea Single Plant Plus Collection (PSPPC) containing 431 *P. sativum* accessions with taxonomic, morphological, and geographic diversity. The group used genotyping-by-sequencing (GBS) to generate 66,591 high-quality SNPs for the PSPPC with the aim of assisting trait mapping and genomics-assisted breeding efforts in pea (Holdsworth et al., 2017). Recently, researchers have utilized this invaluable set of 66,591 SNPs to conduct marker-trait association studies involving the PSPPC (Chang et al., 2018).

Genome Wide Association Studies

Association mapping, also referred to as linkage disequilibrium (LD) mapping, is a method for studying the statistical analysis of the associations between the genetic make-up and the observable traits of an individual within a larger collection (Rafalski, 2010). Genome Wide Association Studies (GWAS) are a recently developed, increasingly popular, and effective association mapping approach for understanding trait variation genetics (Norrgard, 2008; Carpenter et al., 2017). GWAS aim to identify the Single Nucleotide Polymorphisms (SNPs), either individual or haplotype, that are linked to a causal mutation influencing a phenotype of interest in plants and animals (Carpenter et al., 2017; Norrgard, 2008, Rafalski, 2010).

The process of association analysis (Figure 5) begins with the selection of a genetically diverse germplasm panel. More statistical power is associated with a larger number of individuals within the panel, but collections ranging from 100-500 individuals are considered adequate for plant breeding purposes (Rafalski, 2010). Each accession is genotyped at densely distributed loci covering all chromosomes and then divided into groups that share individual or haplotype SNPs at each locus (Rafalski, 2010; Burghardt et al., 2017). Precise phenotype data for each accession is collected and the haplotypes (alleles) of each accession are statistically compared to distributions of these phenotypic values (Rafalski, 2010; Burghardt et al., 2017). The strength of statistical associations, genomic annotations, and prior biological knowledge of the trait of interest are then used to rank candidate markers for further validation experiments (Rafalski, 2010; Burghardt et al., 2017).

Figure 5. The process of association mapping studies; adapted from Burghardt et al. (2017).



Historically, popular genetic mapping techniques such as QTL mapping, required the time-consuming creation of family-based mapping populations consisting of segregating progeny descended from parents differing for a particular trait of interest (Rafalski, 2010). Association mapping involves the use of germplasm panels that harness the genetic diversity naturally occurring among diverse genotypes, rather than the family-based populations that limit QTL mapping (Rafalski, 2010; Bohra, 2013). This results in a higher potential for capturing a wider range of alleles and eliminates the time required for generating experimental mapping populations (Carpenter et al., 2017).

All emerging genomic approaches to crop improvement, especially association mapping studies, greatly rely on the precision, cost, and high-throughput effectiveness of phenotyping and genotyping techniques (Bohra, 2013; Jha et al., 2019). For many crop species, large high-density genotypic datasets for diverse populations are now available to the public for download from government agencies and educational institutions. Many

opensource and private GWAS software programs requiring minimal computational power, time, and prior knowledge are also easily accessible. In plant improvement research, rapid advancements in low-cost and high-throughput genotyping and sequencing and the public availability of high-density genetic maps and haplotype maps (HapMaps) has led to the emergence of GWAS as an effective method for facilitating Marker Assisted Selection (MAS) within breeding programs and the subsequent rapid development of superior cultivars that can meet current crop production needs (Rafalski, 2010; Lipka et al., 2012; Bohra, 2013).

Association Study Software

There are many easily accessible software packages for conducting GWAS that can be used effectively on most datasets. However, packages may differ in their input formatting, computing power requirements, and the handling of missing values, epistatic interactions, and response variables (Burghardt et al., 2017). A current list of common software packages used for conducting GWAS, along with the capabilities and limitations of each, is discussed by Burghardt et al. (2017). A list of less popular GWAS and marker-trait association programs is presented by Bohra (2013). To date, the GWAS software package most widely used by plant geneticists is Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) (Burghardt et al., 2017). A detailed description of TASSEL's capabilities and requirements can be found in Bradbury et al. (2007). In recent years, with the emergence of R Studio® coding instruction and popularity, the Genome Association and Prediction Integrated Tool (GAPIT) has become the go-to package for conducting GWAS.

GAPIT is a R-based software package developed by Dr. Zhiwu Zhang and the Buckler Lab at Cornell University and was first released to the public in 2011 (Zhang, 2018). GAPIT implements the advanced statistical methods of Efficient Mixed Model Association (EMMA), Compressed Mixed Linear Model (CMLM), and Population Parameters Previously Determined (P3D) to make genomic predictions and conduct GWAS (Zhang, 2018). GAPIT is unique among other GWAS programs in that it is an easily accessible, user-friendly R package that can process datasets exceeding 10,000 individuals, 1,000,000 SNPs, and multiple traits with minimal computational time required (Lipka et al., 2012). The average computing time per SNP is 7-fold greater than the TASSEL program and 180-fold faster than the EMMA R package (Lipka et al., 2012). GAPIT can process large datasets with minimal computational time through importing small genotype files separately and selecting random samples of SNPs for calculations when memory limits are exceeded (Lipka et al., 2012; Zhang, 2018). Population structure and kinship are the primary sources of false positives in association mapping studies (Rafalski, 2010); by default, GAPIT will analyze and automatically assign the appropriate principle components for a particular population (Lipka et al., 2012; Zhang, 2018). The GWAS output from GAPIT is presented in the R Studio® interface for further analytical options and in external files as publication-ready Manhattan plots, Quantile-Quantile (Q-Q) plots, organized Excel® result datasheets, and many other graphics.

Field Pea Association Studies

Until its recent release in 2019, the previous lack of a publicly available reference genome for *P. sativum* severely limited the amount of association studies released in relation to other major crops with fully sequenced open-access genomes. Many SNPs have been identified in pea, but the previously incomplete position and chromosome information rendered SNP annotation difficult and hindered the efficiency of MAS (Chang et al., 2018). For pea, the largest publicly available SNP dataset is for the USDA PSPP Core Collection and contains 66,591 SNPs developed specifically for use in association studies of traits within the diverse panel (Holdsworth et al., 2017). In a study by Chang et al. (2018), the researchers were able to better understand SNP annotations by retrieving the Genotyping by Sequencing (GBS) raw data for this large set of SNPs and searching against RNA-seq data using BLASTN. This is a high cost and time-consuming method, but nonetheless they were able to identify 206 and 118 SNPs associated with lesion and nodal resistance in pea, respectively (Chang et al., 2018). Most association studies in pea use smaller SNP datasets developed from SNP arrays of relatively small and less diverse populations. Many studies have utilized the 10,000 SNPs developed by Tayeh et al. (2015) for the specific purpose of advancing pea genomics. Recent notable association studies in pea include research on root architecture and *Aphanomyces* resistance (Desgroux et al., 2017), seed mineral concentrations and content (Ma et al., 2017), and starch chain length distribution and amylose content (Carpenter et al., 2017). With the recent release of the pea genome in 2019, it is anticipated that many more association studies involving pea will begin to surface.

CHAPTER TWO

SCREENING FIELD PEA FOR TOLERANCE TO HIGH SALINITY CONDITIONS

Introduction

Field pea (*Pisum sativum* L.) is an important crop utilized in rotation with cereals in semi-arid cropping systems in the Northern Great Plains (NGP). In Montana, it is estimated that over 4.3 million ha are impacted by saline soil conditions (EC > 4 dS m^{-1}) and that over 121,405 ha have been removed from production due to increasing soil salinity (Scianna, 2002; Kharel, 2016). The Montana Salinity Control Association (MSCA) estimates that 80,937 ha of Montana cropland are currently negatively affected by soil salinity (MSCA, personal communication, May 22, 2018). Montana is the second largest producer of dry peas in the United States behind North Dakota and accounted for nearly one third of all dry pea production in the country at 255,129 metric tons in 2018 (USDA-NASS, 2019). Producers in Montana seeded and harvested 135,569 and 125,452 ha of dry peas in 2018, respectively (USDA-NASS, 2019). The value of dry pea production in the state exceeded 48.8 million dollars in 2018 (USDA-NASS, 2019). The relatively shallow root system, nitrogen-fixing potential, and high water use efficiency associated with field pea makes it an excellent rotation crop with small grains in the semi-arid climate of Montana, where soil N and moisture conservation are critical (Endres et al., 2016).

Despite its global importance, few studies have explored field pea response to high sodium chloride (NaCl) conditions outside of germination testing (Leonforte et al., 2013a, 2013b; Javid et al., 2015) and even fewer have looked at tolerance to sodium sulfate (Na_2SO_4). In field pea, no genes conferring salinity tolerance have been identified, and relatively few Quantitative Trait Loci (QTLs) and Single Nucleotide Polymorphism (SNP) markers have been reported (NPGS, 2018; Leonforte et al., 2013a).

The objective of this study was to screen the 312 accessions comprising the genetically diverse USDA *Pisum* single plant (PSP) core collection for tolerance to Na_2SO_4, the dominant salt affecting plant growth in the Northern Great Plains (Miller et al., 1981). Phenotypic response measurements from growth chamber germination and greenhouse seedling experiments were used as indicators for tolerance. A Genome Wide Association Study (GWAS) was conducted using the collected phenotypic data and a large dataset of 68,222 Single Nucleotide Polymorphisms (SNPs) developed from the USDA *Pisum* Single Plant Plus Core (PSPPC) collection. The significant marker-trait associations discovered were analyzed for potential marker-assisted selection breeding efforts to improve salinity tolerance in field pea. Candidate breeding germplasm conferring high salinity tolerance was also assessed.

Materials and Methods

Germplasm

A total of 312 *Pisum* single plant (PSP) core collection germplasm accessions were obtained from the USDA Western Regional Plant Introduction

Station at Pullman, WA. The PSP core collection represents the morphological, taxonomic, and geographic diversity within the *Pisum* genus. The 312 accessions obtained from the PSP core collection were composed of 270 *Pisum sativum*, 14 *P. sativum* variety *arvense*, 10 *P. sativum* subspecies *sativum*, 10 *P. sativum* subsp. *elatius*, 3 *P. sativum* subsp. *asiaticum*, 2 *P. sativum* var. *pumilio*, 1 *P. sativum* subsp. *transcaucasicum*, 1 *Pisum abyssinicum*, and 1 *P. sativum* var. *sativum*. The USDA PSP core collection is composed of collected, donated, and developed *Pisum* lines from 61 countries across the six arable continents of the world.

Germination Screening

Preliminary Experiments. Nineteen *Pisum sativum* lines were selected from North Dakota State University (NDSU) breeding germplasm for NaCl and Na_2SO_4 germination screening trials. NDSU lines were selected for preliminary screening due to the accessibility of abundant quantities of semi-adapted seed. Experiment strategies were adapted from high salinity germination screening studies of pea (Shahid et al., 2012; Ouerghi et al., 2016), wild legume (Sunita et al., 2013), and common bean (Cokkizgin, 2012). Each trial was designed as a randomized complete block with three salt-treated replicates and one non-saline water control replicate. The preliminary NaCl and Na_2SO_4 screening trials were repeated three times. The salt treatment solutions consisted of a 150 mM NaCl (~16 dS m^{-1}) (Fischer Scientific®) stock solution and a 95 mM Na_2SO_4 (~16 dS m^{-1}) (BDH® Chemical) stock solution. Germination response was screened at the

NRCS-designated "strongly saline" (≥ 16 dS m^{-1}) level to account for the extreme end of field salinity conditions. This saline level provided high selection pressure and was supported by previous studies that had observed high tolerance to salinity at the germination stage in legume development (Cokkizgin, 2012; Sunita et al., 2013; Ouerghi et al., 2016). All glassware, Whatman™ filter papers, and deionized (DI) water for experiment solutions were autoclaved prior to trial set-up. A total of one hundred seeds from each pea breeding line were sterilized in a 5% sodium hypochlorite (NaOCl) solution for approximately 20 minutes and rinsed with autoclaved DI water three times. All seeds were screened for cracks, disease symptoms, and below average size. Each 100 mm x 15 mm VWR™ petri dish received 20 seeds placed with equidistant spacing and the seed hilum facing down on a double layer of Whatman™ 85 mm diameter filter papers. Each dish received 10 ml of treatment solution (NaCl or Na_2SO_4) or non-saline DI water (control). The blocks were arranged in plastic lid-topped bins and placed in a Conviron® growth chamber with the lights turned off and the temperature held at 25°C +/- 2°C. Each experiment was conducted over a period of 8 days with the number of germinated seed recorded on days three, five, and eight. If a dish required additional moisture, 2-5 ml of solution was added on days three and/or five. A seed was considered germinated when the radicle had extended to at least 1 cm in length. Upon germination, seeds were removed from the plate and discarded. On the third day (72hrs), hard (dormant) seeds that did not imbibe were counted and discarded. The final percent germination for each entry was calculated using the following equation:

$$\%\ Germination = \frac{\#\ of\ Germinated\ Seeds}{20\ seeds - (\#\ of\ hard\ seeds)} * 100$$

The percent germination compared to the control response was then calculated for all three treatment replicates. All the values indicated in this investigation are the mean of three replicates. Experimental design was generated using Microsoft® Excel® software and data analysis was performed using R Studio® software in which P values were derived using Type III ANOVA and accession responses were compared using Tukey's Honestly Significant Difference (HSD) test.

PSP Core Collection. A total of 312 USDA *Pisum* single plant (PSP) core collection germplasm accessions were obtained from the USDA Western Regional Plant Introduction Station at Pullman, WA. The limited quantity of seeds acquired from the USDA required that all accessions be grown in the field at the Post Research Farm in Bozeman, MT to increase seed in the 2018 growing season. After harvest and processing of the seed increase plots in September of 2018, similar methods to the preliminary germination screenings were implemented for screening the PSP accessions with 95 mM Na_2SO_4 (~16 dS m^{-1}). The PSP accessions were divided into 14 sets (runs) due to the space constraints within the growth chamber. Each set contained 22 accessions and two control genotypes. The adapted cultivar 'CDC Amarillo' and the semi-adapted breeding line NDP121622 were selected as controls for their good and poor salinity tolerance performance in the preliminary trials, respectively. All accessions were scarified with a scalpel prior to sterilization to account for hard seed genotypes and to ensure uniform imbibition. Experimental design was generated using Microsoft® Excel® software and data analysis was performed using R Studio® software in which P values were derived using Type III ANOVA and accession responses were compared using Tukey's HSD test.

Greenhouse Screening

Concentration Series. Two semi-adapted breeding germplasm lines, NDP121622 and NDP121587 were selected for preliminary greenhouse seedling screening with Na_2SO_4 based on their low and high performance across NaCl and Na_2SO_4 germination experiments. Greenhouse seedling screening strategies were adapted from salinity tolerance pot screening studies for pea (Noreen et al., 2009; Leonforte et al., 2013a, 2013b; Javid et al., 2015), chickpea (Maliro et al., 2008), and lentil (Maher et al., 2003). To determine a salinity tolerance threshold level at which to screen the PSP core accessions, an experiment with a two-factor (germplasm lines and concentration levels) factorial design arranged in a randomized complete block was conducted. A total of seven concentration levels of Na_2SO_4 (0, 3, 6, 9, 12, 15, and 18 dS m^{-1}) were used to determine tolerance thresholds. Plastic pots (10 cm x 10 cm x 12 cm) with a coffee filter placed in the bottom were filled with autoclaved coarse grade sand media. Five seeds were sown 2 cm deep in each pot with equidistant spacing. Each pot received one bamboo stake with clothespin hoops to minimize crowding and shading effects. The blocks were arranged on a wire bench in a temperature-controlled lean-to greenhouse with GE Multi-Vapor® metal halide lighting in the Plant Growth Center at Montana State University in Bozeman, MT. All pots were thinned to three plants at three days post-emergence (DPE) and watered daily with tap water from sowing through ten DPE. Beginning at ten DPE, all pots were watered with a nutrient solution containing a supplemental calcium source and all treated plots received an additional EC concentration derived from the addition of Na_2SO_4. The nutrient solution was composed

of Jack's Professional® water-soluble fertilizer 20-20-20 General Purpose blend (diluted to the label recommendation for 10% N) and the supplemental calcium source was a Hoagland's solution half-strength concentration of $CaNO_3$, mixed using calcium nitrate tetrahydrate (BDH® Chemical). To prevent plant shock, the EC of the watering solution was raised by 3 dS m^{-1} every three days (3 dS m^{-1} at 10 DPE, 6 dS m^{-1} at 13 DPE, etc.) until a final EC for each level was reached and maintained. A Hanna® Instruments combo EC & pH meter was used to test the EC of each solution. The total volume of watering solution added to each pot was 100 ml per day to keep the media saturated and prevent drought stress. Salinity symptom scores were assessed using a visual growth response scale of 1 – 9 (Table 3), adapted from a scale (1 – 10) previously developed for visually assessing salt stressed pea (Leonforte et al., 2013a) and lentil (Maher et al., 2003). Salinity tolerance scoring began on day 21 post-sowing and was conducted every seventh day until day 42 post-sowing (Leonforte et al., 2013b). The salinity symptom scores were assessed for each pot, considering all three plants as one. At 42 days post-sowing, final individual plant heights were measured and averaged across the plot. Above-ground biomass and roots were separately bulked by plot, oven-dried, and the dry biomass weights were recorded. All the values indicated in this investigation are the mean of three replicates. Experimental design was generated using Microsoft® Excel® software and data analysis was performed using R Studio® software in which P values were derived using Type III ANOVA and accession responses were compared using Tukey's HSD test.

Table 2. Salinity Symptom Score Descriptions.

Salinity Symptom Score	Description of Plant
1	Healthy, Green
2	Some chlorosis/wilting beginning on leaf margins
3	Surface area of leaves becoming chlorotic/wilted, no necrosis
4	Chlorosis, wilting and/or necrosis on 50% of leaves
5	50-75% of leaves chlorotic, wilted, and/or necrotic, stem is green
6	> 75% of plant chlorotic, wilted, and/or necrotic, stem necrosis beginning
7	Youngest leaves green, stem necrosis > 50%, rest of plant dead or wilted/brittle
8	Only small part of stem is still green and not wilted/brittle, rest of plant dead
9	Plant is dead

PSP Core Collection. A total of 311 *Pisum* single plant (PSP) core collection germplasm accessions were screened in the greenhouse at the seedling stage with Na_2SO_4 following methods similar to those used in the concentration series experiment. The primary differences of this study included termination of the experiment at 38 days post-sowing, due to time constraints; as well as the screening of all accessions at the 9 dS m^{-1}

Na_2SO_4 level previously determined by the concentration series results. The PSP accessions were divided into nine sets (runs) due to the space constraints within the greenhouse. The first run consisted of 28 PSP accessions and two control genotypes. Each following run contained a set of 36 PSP accessions and two control genotypes. The adapted cultivar 'CDC Amarillo' and the semi-adapted breeding line NDP121622 were selected as controls for their good and poor salinity tolerance performance in the preliminary trials, respectively. Each trial was designed as a randomized complete block with three treated replicates and one non-saline control replicate. All accessions were scarified with a scalpel prior to sowing to account for hard seed genotypes and to ensure uniform germination and emergence. Upon the termination of the experimental run and the final scoring of visual growth response symptoms, the Area Under the Injury Curve (AUIC) was calculated for each accession using the following equation:

$$AUIC = \sum_{i=1}^{n} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

where "y" represents the visual growth response score given, "i" represents the scoring interval, and "t" represents the number of days post-sowing. The AUIC values were used to supplement final scores and to represent a more comprehensive understanding of the salinity tolerance for each accession, with smaller AUIC values indicating greater predicted tolerance to high salinity.

The multiple experimental runs (e.g. GC01, GH01, GH02) within the growth chamber and greenhouse studies were combined for analysis of specific traits only if the F-max value for that trait did not exceed the threshold value of ten. The F-max value is

derived from calculating the ratio between the largest mean square error and the smallest mean square error within a set of experimental runs.

GWAS

A genome-wide association study was performed on 311 lines in the USDA Pea Single Plant (PSP) core collection and their responses to salinity stress at different times of development. The phenotypic dataset was formatted in the comma-separated values (CSV) format and included five traits measured for plants screened under high salinity conditions in growth chamber germination and greenhouse seedling studies. The traits measured were percent germination compared to the control, percent biomass compared to the control, percent height compared to the control, final salinity symptom score, and the Area Under the Injury Curve (AUIC).

The genotypic dataset was formatted in the HapMap format and consisted of 68,222 SNPs derived from the USDA PSPPC collection. A semi-filtered set of 163,902 SNPs was obtained from the USDA in which all SNPs that contained missing values for more than 40% of the accessions were excluded. Using Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) 5.0 software, SNPs were filtered further, excluding those with a Minor Allele Frequency (MAF) of less than 0.03; resulting in the set of 68,222 SNPs. The SNP filtering parameters were selected to increase statistical power within the GWAS and to minimize the number of SNPs excluded. The raw SNP dataset is provided to the public on the NCBI database (Holdsworth et al., 2017).

The GAPIT software package in R was used to conduct the GWAS. Initial coding included four models and the default of three principal components (PCs) to assess the

most appropriate settings to account for the population structure and specific dataset. The four models selected were the Generalized Linear Model (GLM), Mixed Linear Model (MLM), Multiple Locus Mixed Linear Model (MLMM), and Fixed and random model Circulating Probability Unification (FarmCPU). Graphical output in the form principle component (PC) scree plots, 3-dimensional principle component analysis (3D PCA) graphs, and Q-Q plots were analyzed to determine the best model and principle component parameters for each trait. A Bonferroni correction was utilized by the GAPIT program to account for the multiple testing problem and determine the statistically significant threshold for SNP-trait associations (Holdsworth et al., 2017). Manhattan plots and GWAS result data output were analyzed to identify significant marker-trait associations and further determine model performance (fit).

Phenotypic data for percent germination compared to the control was only available for 300 accessions due to seed quantity constraints on growth chamber screening methods. SNP data were not available for 32 accessions and in combination with missing percent germination data, allowed for only 268 accessions to be included in the GWAS for the percent germination compared to the control trait. Phenotypic data for all greenhouse experiment traits were available for 311 accessions. The missing SNP data allowed for only 279 accessions to be included in the GWAS for the percent height and biomass compared to the control, final score, and AUIC traits. Two genome-wide association studies were conducted separately for the percent germination trait and for the four additional greenhouse screening traits to account for accession/SNP discrepancies

and to increase statistical power. The JBrowse genome browser was utilized to locate significant SNPs on the *Pisum* genome and explore their surrounding genomic regions.

Results

Germination Screening

The preliminary germination screening trials using 19 NDSU breeding germplasm lines indicated that the protocols developed were adequate for detecting differences in genotype response (data not shown). Limited seed quantities for 12 PSP accessions allowed for a total of 300 PSP accessions to be screened under 16 dS m^{-1} Na_2SO_4 conditions in 14 growth chamber germination studies. The combined analysis of all runs showed accession (name) and run to be significant while rep was not significant (Table 3). The F-max value for all 14 growth chamber runs (Appendix B Table B1) did not exceed the threshold of 10 and, therefore, allowed for analysis to be conducted treating all runs as one experiment. The percent germination compared to the control across the 14 runs ranged from 3.7 to 100 percent and the mean of the percent germination compared to the control for all accessions was 67.3 percent (Table 4). The distribution of percent germination compared to the control values favored high germination under saline conditions (Figure 6) with 62 accessions falling into the 90^{th} percentile for percent germination. A scatter plot displaying the mean percent germination compared to the control for each accession (Figure 7) showed no significant trend (*P value < 0.05*) for germination response to high salinity when progressing through the PSP collection by inventory order. A Pearson's product correlation test found no significant correlations (*P*

value < 0.05) between percent germination compared to the control and any of the four other responses measured (Table 5).

Table 3. Combined ANOVA for percent germination compared to the control for 300 *Pisum* plant introduction accessions and three controls.

Source	DF	Sum Square	F value	Pr>F
Name	302	537117	8.422	< 0.0001
Rep	2	1143	2.706	0.06755
Run	13	11597	4.224	< 0.0001

Figure 6. Distribution of percent germination compared to the control for 300 *Pisum* single plant core accessions challenged with 16 dS m^{-1} Na_2SO_4.

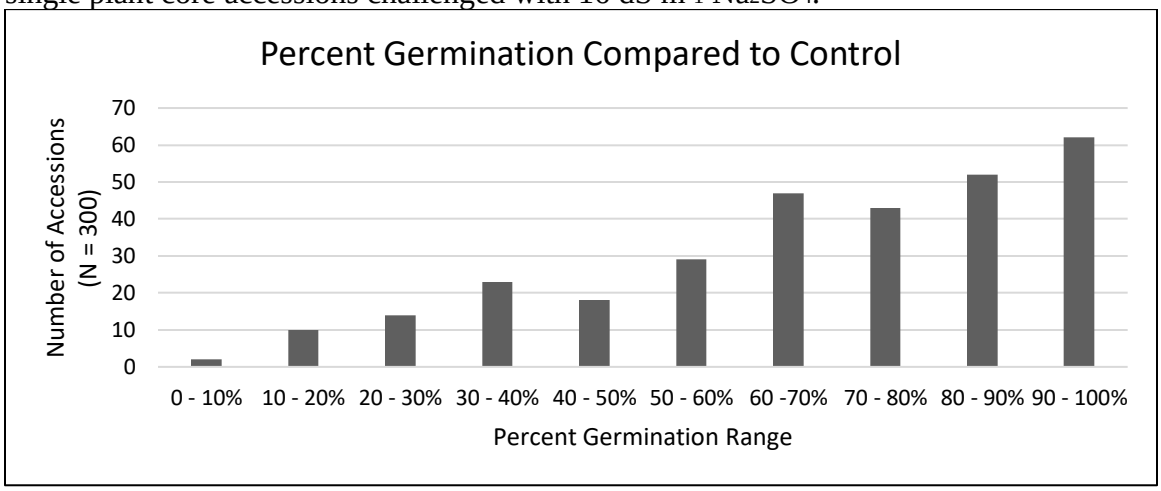


Figure 7. Data scatter plot for mean percent germination compared to the control for 300 *Pisum* single plant core accessions arranged by date of submission to the collection.

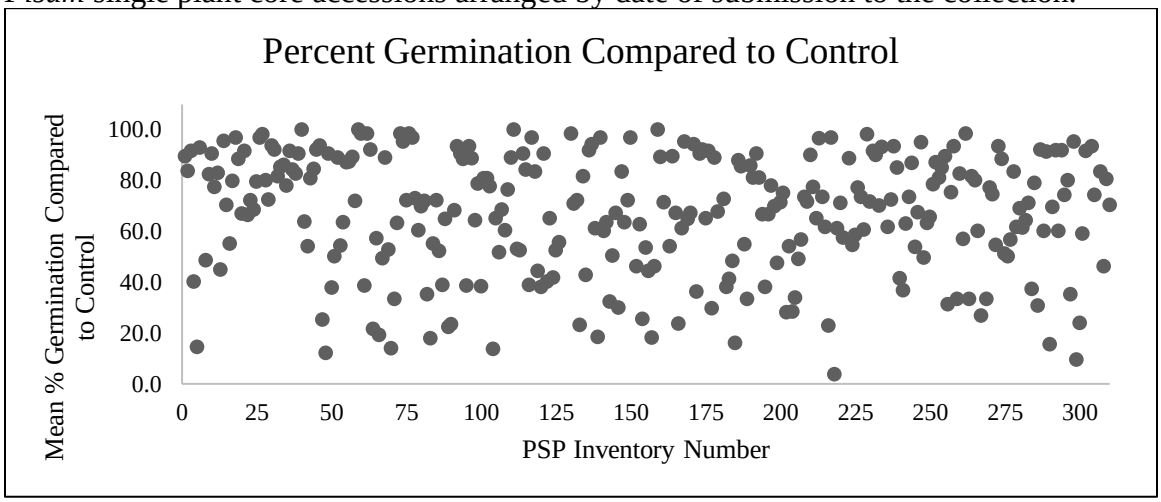


Table 4. Mean and range of all traits measured for 300 *Pisum* single plant core accessions screened for high salinity tolerance.

Trait	Mean	Range	N
% Germination Compared to Control	67.3	3.7 - 100.0	300
% Biomass Compared to Control	48.1	20.2 - 134.9	310
% Height Compared to Control	53.9	31.8 - 78.7	311
Score*	6.3	1.7 - 9.0	311
AUIC**	49.4	19.7 - 96.2	311

* Scale 1-9 (Healthy - Dead)
** Lower Values Indicate Tolerance
AUIC = Area Under the Injury Curve

Table 5. Pearson's correlation coefficients among all traits measured in the growth chamber and greenhouse high salinity screening studies.

	% Germination Compared to Control	% Biomass Compared to Control	% Height Compared to Control	Final Score	AUIC
% Germination Compared to Control	-	-	-	-	-
% Biomass Compared to Control	0.071	-	-	-	-
% Height Compared to Control	0.101	0.683***	-	-	-
Final Score	-0.022	-0.739***	-0.524***	-	-
AUIC	-0.046	-0.660***	-0.499***	0.877***	-

"***" = P-value < 0.0001
AUIC = Area Under the Injury Curve

Greenhouse Concentration Series

The two semi-adapted breeding lines NDP121622 and NDP121587 were selected for the concentration series salinity screening experiment to determine the appropriate screening concentration for the PSP core collection. An interaction plot displaying the mean final salinity symptom score for both genotypes at each salt concentration level (Figure 8) indicated that minimal final score increase was gained past the 9 dS m^{-1} Na_2SO_4 level, suggesting that it was a good candidate for a concentration threshold. Likewise, an interaction plot displaying the percent height compared to the control for both genotypes at each concentration (Figure 9) indicated that minimal reductions in percent height compared to the control were observed past the 9 dS m^{-1} level for the

NDP121622 line. A least square means for multiple comparisons test was conducted to determine if there was a significant difference between treatment levels and final salinity symptom score response (not shown). Parameters for this test involved using the mean response values between the two genotypes, Tukey-adjusted p-values, and a 95 percent confidence interval. A significant difference (*P value < 0.0001*) in mean final score for both genotypes combined was observed between the 6 dS m^{-1} and 9 dS m^{-1} Na_2SO_4 concentration levels. No significant differences (*P value < 0.05*) in mean final score were observed between the 9 dS m^{-1} level and the 12, 15, and 18 dS m^{-1} levels.

Figure 8. Interaction plot of mean final score for two pea genotypes across seven salinity concentration treatments. The mean final score is on a 1 – 9 (healthy – dead) scale.

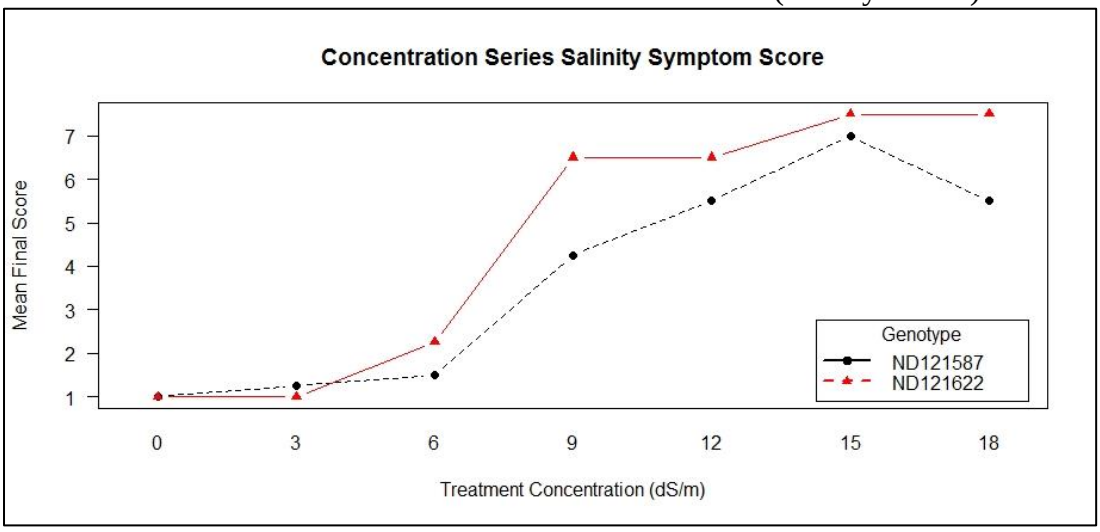
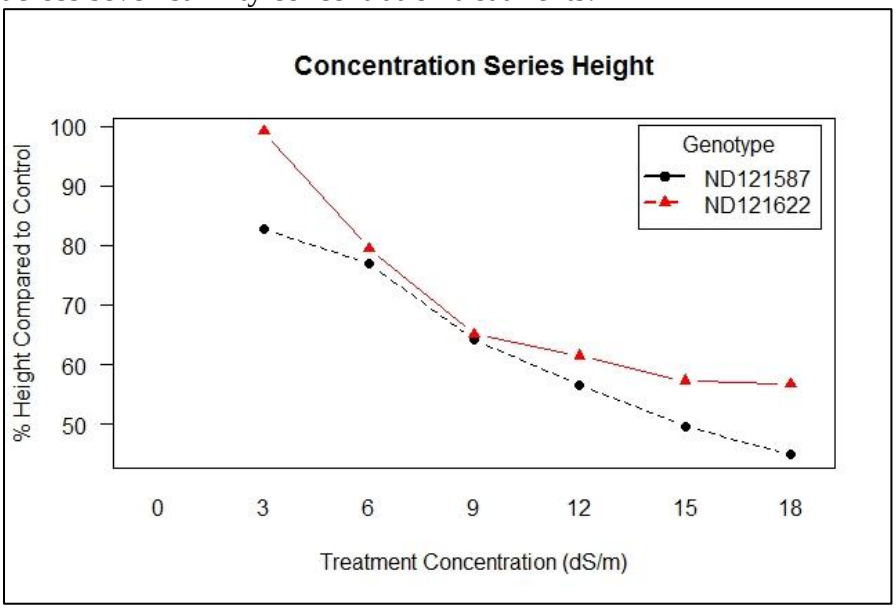


Figure 9. Interaction plot of percent height compared to the control for two genotypes across seven salinity concentration treatments.



Greenhouse Screening of PSP Core

A total of 311 PSP accessions were screened under 9 dS m^{-1} Na_2SO_4 conditions in 9 greenhouse studies. The F-max values for all traits across the nine greenhouse runs (Appendix B Table B1) did not exceed the threshold value of 10, therefore allowing combined analysis across runs. The percent biomass compared to the control ranged from 20.2 to 134.9 percent and the mean was 48.1 percent (Table 4). The distribution of percent biomass compared to the control was normally distributed and slightly skewed (Figure 10). Of the 310 accessions screened (one accession had missing values for percent biomass), 232 accessions (75%) exhibited 30 to 60 percent biomass compared to the control. A Pearson's correlation found a positive significant correlation of 0.55 (*P value* $< 2.2 \times 10^{-16}$) between percent biomass response and time of submission into the PSP core collection; the trendline is observed in the scatter plot showing the mean

percent biomass compared to the control for each accession (Figure 11). A Pearson's correlation found a significant positive correlation of 0.683 (*P value < 0.0001*) between percent biomass compared to the control and percent height compared to the control (Table 5). Significant negative correlations (*P value < 0.0001*) were observed between percent biomass compared to the control and the mean final score (r = -0.739) and AUIC (r = -0.660) traits (Table 5).

Figure 10. Distribution of percent total biomass compared to the control for 310 *Pisum* single plant core accessions challenged with 9 dS m^{-1} Na_2SO_4.

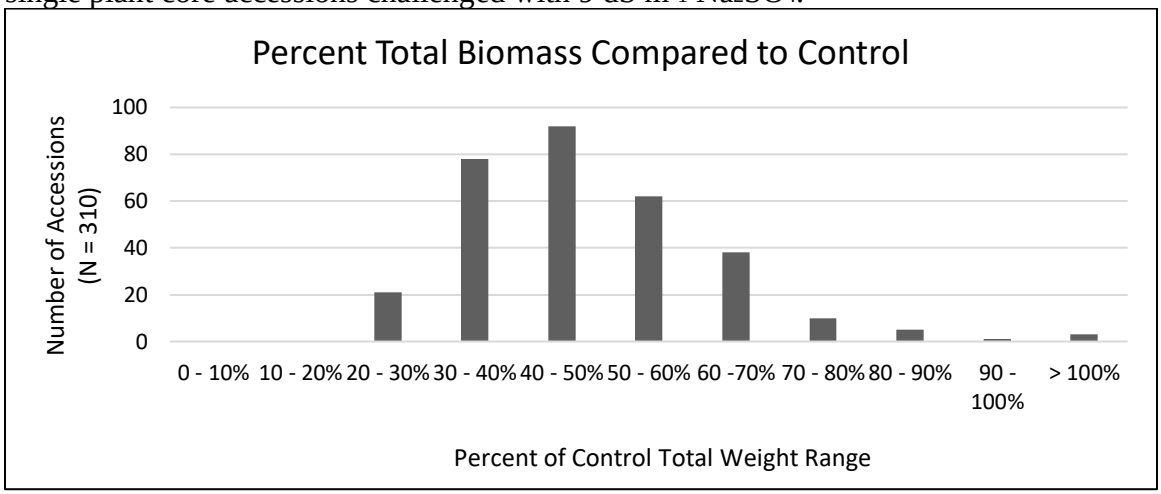
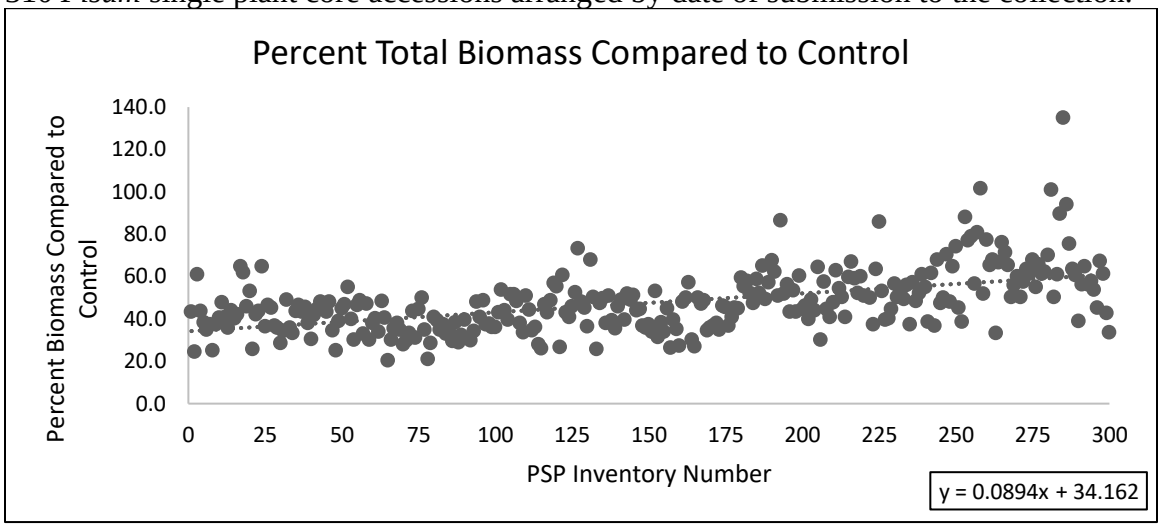


Figure 11. Data scatter plot for mean percent total biomass compared to the control for 310 *Pisum* single plant core accessions arranged by date of submission to the collection.



r = 0.55 (*P value < 2.2 x 10-16*)

Percent height compared to the control ranged from 31.8 to 78.7 percent and the mean of the percent height compared to the control was 53.9 percent (Table 4). The distribution of percent height compared to the control was normally distributed with 45.3 percent of the accessions exhibiting 50 to 60 percent height compared to the control (Figure 12). A scatter plot showing the mean percent height compared to the control for each accession (Figure 13) displayed a positive significant Pearson's correlation of 0.32 (*P value < 4.0 x 10-9*) between plant height response to high salinity and PSP accession based on its time of submission to the collection. Pearson's correlation identified a significant positive correlation of 0.683 (*P value < 0.0001*) between percent height compared to the control and percent biomass compared to the control (Table 5). Significant correlations (*P value < 0.0001*) were observed between percent height compared to the control and the mean final score (r = -0.524) and AUIC (r = -0.499) traits (Table 5).

Figure 12. Distribution of percent height compared to the control for 311 *Pisum* single plant core accessions challenged with 9 dS m^{-1} Na_2SO_4.

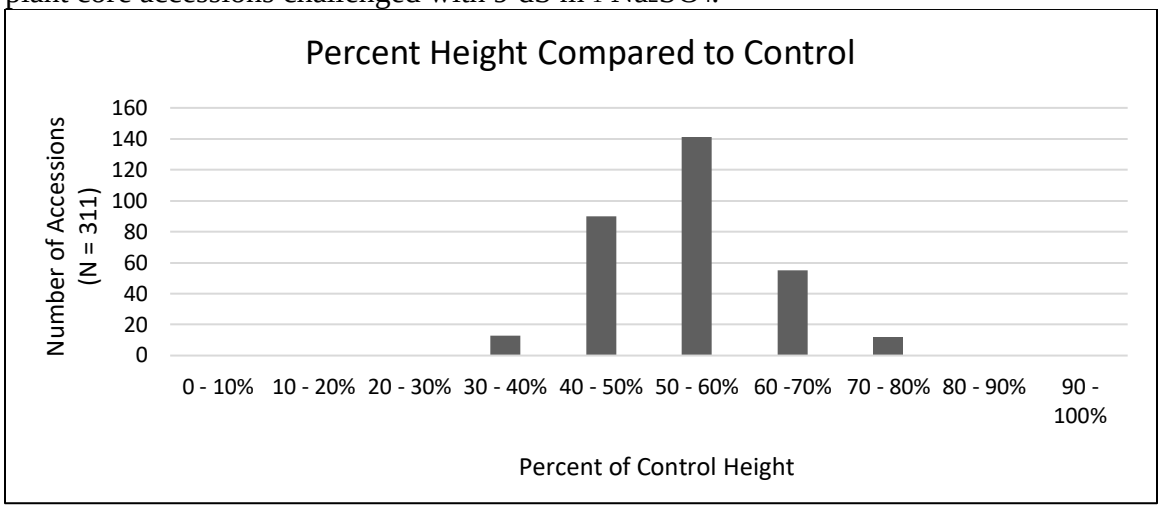
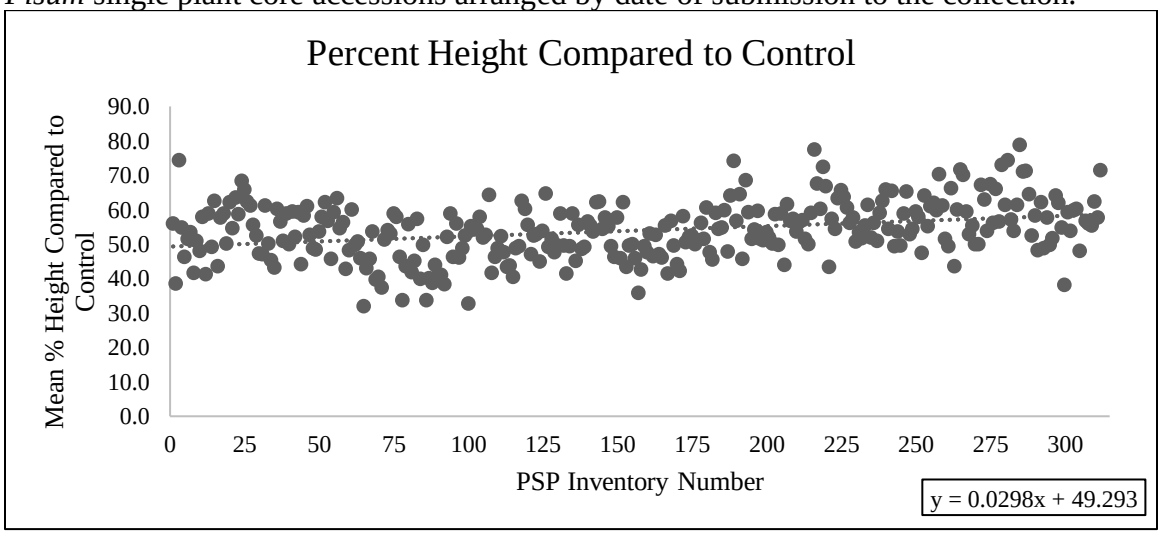


Figure 13. Data scatter plot for mean percent height compared to the control for 311 *Pisum* single plant core accessions arranged by date of submission to the collection.



r = 0.32 (*P value < 4.0 x* 10^{-9})

The mean final salinity symptom score ranged from 1.7 to 9.0 and the mean final score for all accessions was 6.3 (Table 4). The distribution of mean final salinity symptom score exhibited a non-normal distribution with scores nearly equally distributed across the 4 to 9 score ranges (Figure 14). A scatter plot showing the mean final score for

each accession (Figure 15) demonstrated a negative Pearson's correlation of -0.62 (*P value* < *2.2 x 10-16*) between final score response to high salinity and accession inventory number when organized by date of entry to the collection. A Pearson's correlation test found a significant positive correlation of 0.877 (*P value* < *0.0001*) between mean final salinity symptom score and AUIC (Table 5). Significant negative correlations (*P value* < *0.0001*) were observed between mean final score and the percent biomass (r = -0.739) and percent height (r = -0.524) compared to the control traits (Table 5).

Figure 14. Distribution of mean final score for 311 *Pisum* single plant core accessions challenged with 9 dS m^{-1} Na_2SO_4.

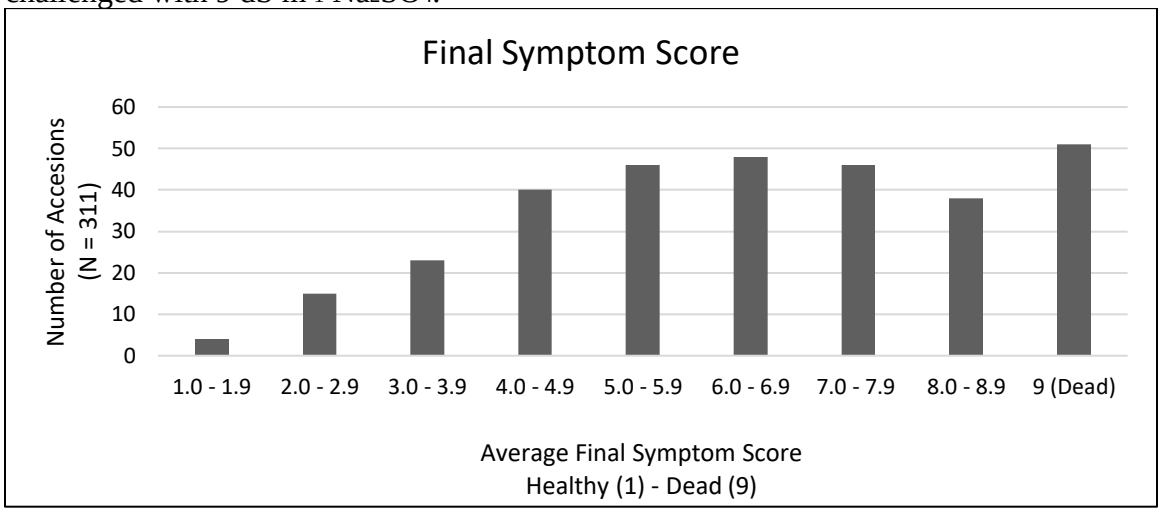
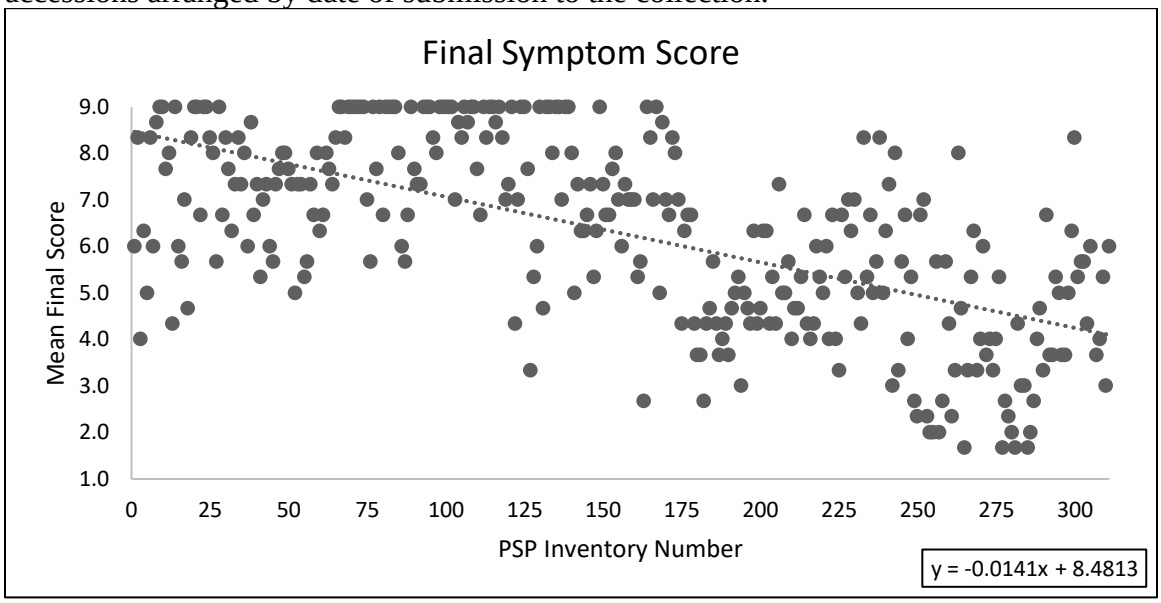


Figure 15. Data scatter plot for mean final score for 311 *Pisum* single plant core accessions arranged by date of submission to the collection.



r = -0.62 (*P value < 2.2 x 10-16*)

The AUIC value ranged from 19.7 to 96.2 and the mean was 49.4 (Table 4). The distribution of mean AUIC value exhibited a skewed normal distribution (Figure 16). A scatter plot showing the mean AUIC value for each accession (Figure 17) demonstrated a significant negative Pearson's correlation of -0.55 (*P value < 2.2 x 10-16*) when PSP accessions were organized by date of entry to the collection. A Pearson's correlation test identified a significant positive correlation of 0.877 (*P value < 0.0001*) between mean AUIC value and mean final salinity symptom score (Table 5). Significant negative correlations (*P value < 0.0001*) were observed between mean AUIC and the percent biomass (r = -0.660) and percent height (r = -0.499) compared to the control traits (Table 5).

Figure 16. Distribution of area under the injury curve (AUIC) values for 311 *Pisum* single plant core accessions challenged with 9 dS m^{-1} Na_2SO_4.

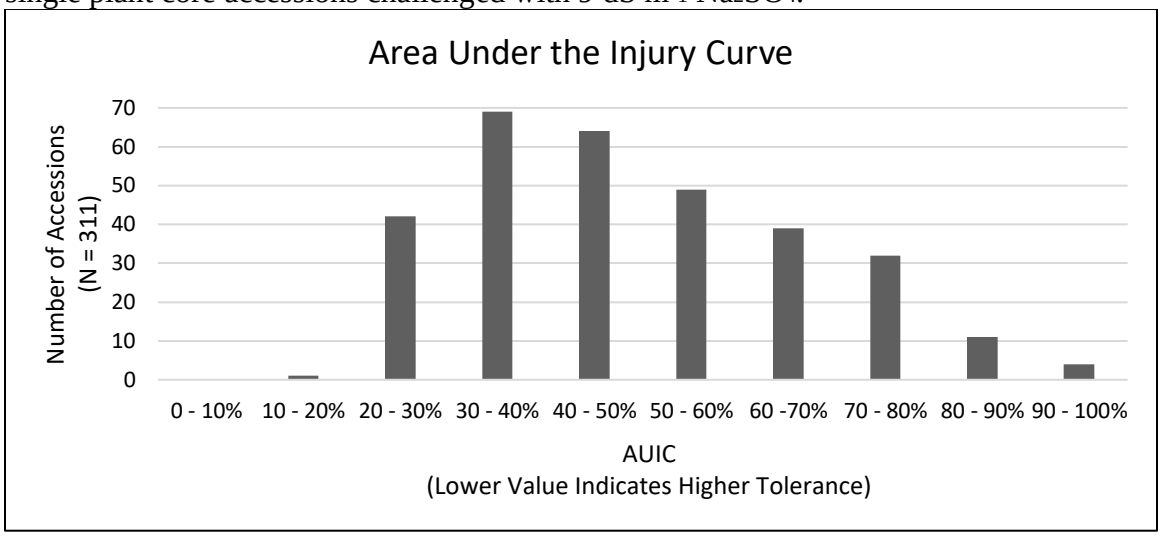
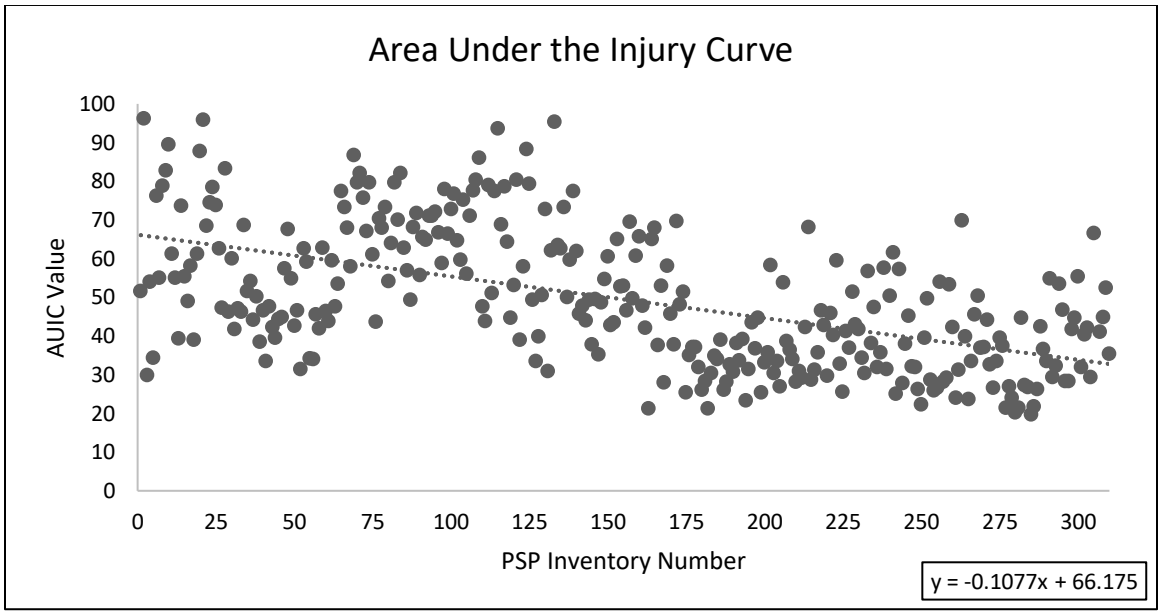


Figure 17. Data scatter plot for area under the injury curve (AUIC) values for 311 *Pisum* single plant core accessions arranged by date of submission to the collection.



r = -0.55 (*P value < 2.2 x* 10^{-16})

Top performing accessions were ranked based on their overall performance across the germination and greenhouse seedling response traits (Table 6). A complete table

displaying the top 10 accessions for percent germination, total biomass, and plant height, as well as the top 20 accessions for mean final score and AUIC can be found in Appendix B (Table B2). The USA-developed cultivar 'Stirling' (PI634571) was the top performing accession for all four seedling response traits and the Netherlands-developed cultivar 'Solara' (PI601516) was in the top 5 accessions for each trait (Table B2). The Afghanistan-collected line Musus (PI429839) was the only accession that was in the top 5 percent for percent germination and the top 10 percent for percent total biomass, plant height, and mean final score (Table B2). Cotyledon color and development status was split evenly across the top 10 performing accessions (Table 6).

Table 6. The overall top performing PSP core collection accessions across the germination and greenhouse screening experiments along with plant name, country of origin, and cotyledon color descriptors.

Overall Rank	Inventory Number	PSP Name	Plant Name	Origin	Cotyledon
1	285	PI634571	Stirling	USA$_D$	Green
2	281	PI601516	Solara	Netherlands$_D$	Green
3	286	PI638516	SW Carousel	USA$_D$	Yellow
4	279	PI505144	ILCA 5115	Spain $_C$	Green
5	265	PI429849	Uzbekskij	Uzbekistan $_C$	Yellow
6	287	PI639967	VIR K-7290	India $_C$	Yellow
7*	258	PI413685	NZ 51	Hungary$_D$	Green
8**	262	PI429839	Musus	Afghanistan $_C$	Yellow
9	253	PI411141	Pania	New Zealand$_D$	Green
10	277	PI505122	ILCA 5089	Albania $_C$	Yellow

* = Top 10% for Germination
** = Top 5% for Germination
D = Developed
C = Collected

To understand the geographic distribution of Na_2SO_4 salinity tolerance within the *Pisum* genus, all accessions were grouped into geographic regions based on their country

of origin and the mean response for every trait measured was calculated for each world region (Table 7). The Oceania region had the highest mean for percent total biomass, mean final score, and AUIC (Table 7). The North America and Mediterranean regions had the highest mean for percent germination and plant height, respectively (Table 7). The overall least salt tolerant geographic region based on the traits measured in this study was Central America (Table 7).

Table 7. The mean trait values for all accessions within a world region. The top performing value in each trait column is underlined. Three accessions were excluded due to missing data.

Region	# of Accessions	% Germ	% Biomass	% Height	Score	AUIC
Africa	20	68.1	45.0	56.3	6.5	48.8
Asia	70	73.6	46.1	54.5	6.5	50.6
Central America	6	62.7	32.9	49.6	7.7	65.4
Eastern Europe	38	60.2	52.7	54.6	5.5	43.1
Mediterranean	30	69.7	50.0	**57.1**	5.8	45.8
Middle East	8	58.8	38.2	50.6	7.0	48.4
North America	22	**75.1**	55.3	56.7	5.4	45.1
Oceania	8	68.4	**58.8**	53.7	**5.1**	**41.0**
South America	6	66.0	39.4	45.9	6.7	60.2
Western Europe	101	63.5	47.3	52.2	6.7	52.4
Total:	309					

GWAS

The discrepancy in the availability of accessions between percent germination and the seedling traits made it necessary to run two genome-wide association studies separately to optimize statistical power. The percent germination GWAS was conducted using the percent germination phenotype data and the large set of SNPs. GWAS conducted using four GAPIT R package models discovered significant (*P value* <

0.000001) associations between the percent germination trait and the 68,222 SNPs within the panel of 268 PSP accessions (Table 8). The PCA scree plot (Figure 18) indicated that the first three principle components explained approximately 15% of the genetic variance and that additional PCs explained less than 2%. The 3D PCA plot (Figure 18) indicated that variability for the trait existed within the population with accessions (represented by red dots) clustering in different regions of the plot. The Bonferroni-adjusted statistical significance for SNP-trait associations in this GWAS was set at P value < 0.000001. The FarmCPU model Q-Q plot for percent germination compared to the control (Figure 19) indicated normality of the data. Values deviating from the null hypothesis line in the top right of the plot were observed, indicating that significant associations exist between those SNPs and the percent germination trait. The Manhattan plot (Figure 20) and FarmCPU model output (Table 8) indicated that five significant (*P value < 1 x 10-6*) marker-trait associations were identified within the study.

Figure 18. Principle component analysis graphs of percent germination compared to the control with a scree plot (left) and a 3D principle component analysis plot (right) displaying the population structure.

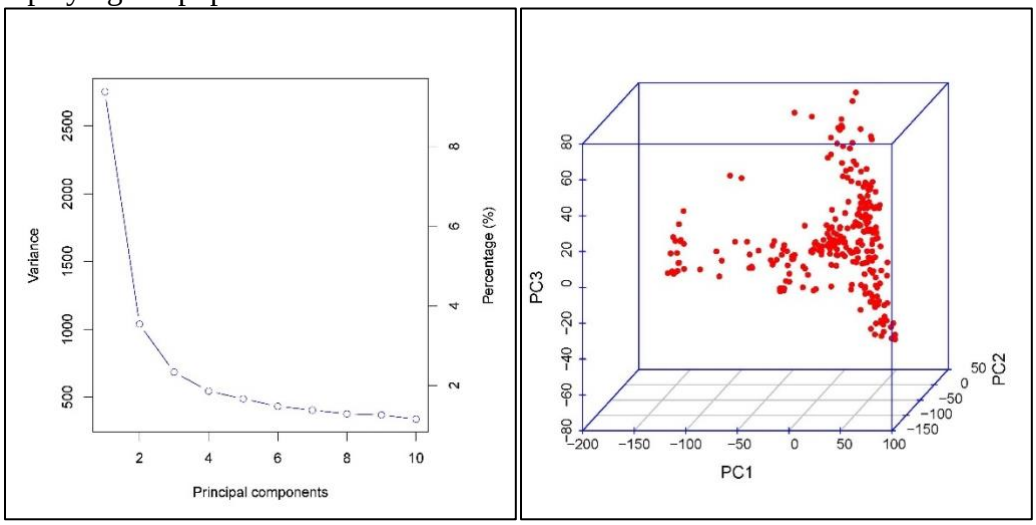


Figure 19. A FarmCPU analysis of percent germination GWAS Q-Q plot displaying the deviation of observed significance levels (black dots) from the expected significance values (red line) under the null hypothesis of no marker-trait association.

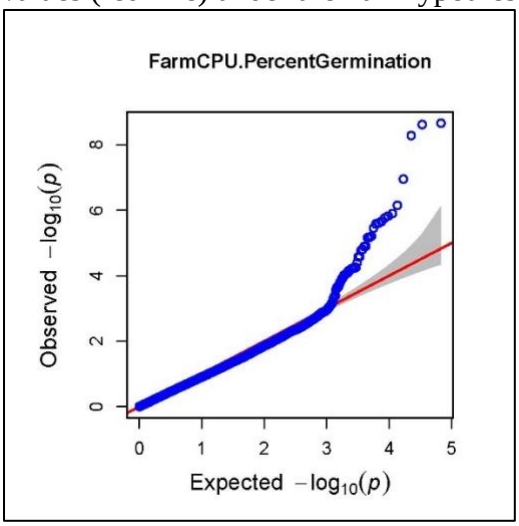
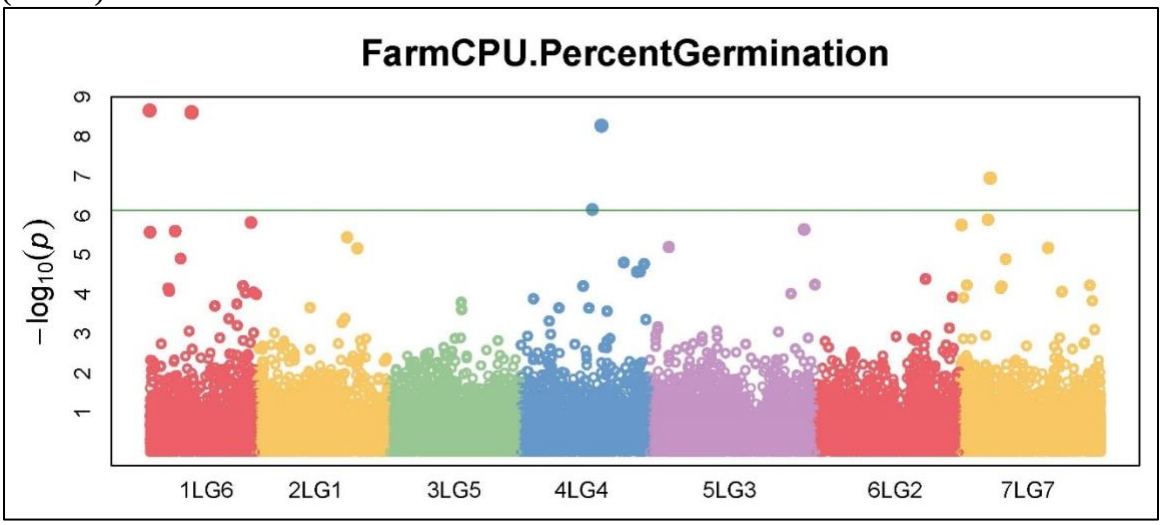


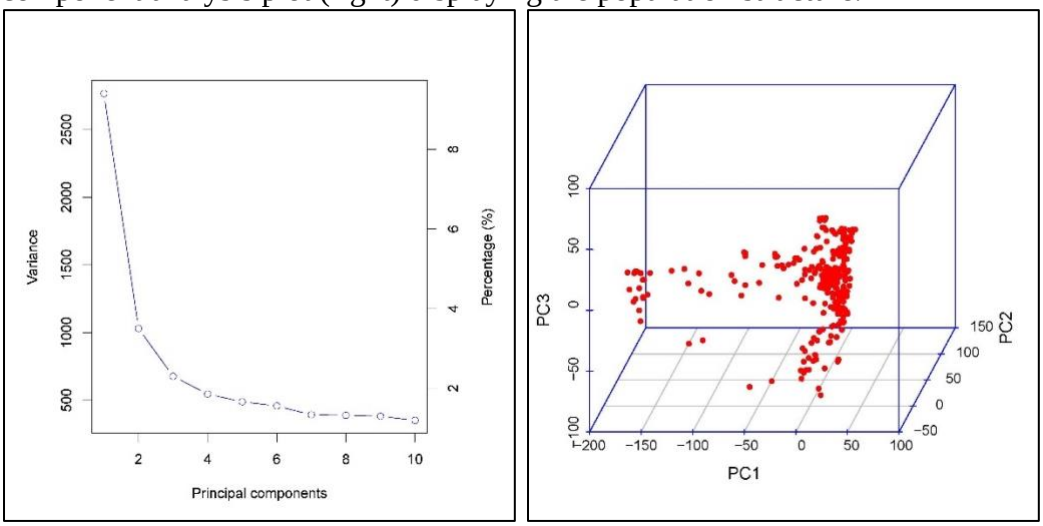
Figure 20. A FarmCPU model Manhattan plot for percent germination displaying SNPs plotted against their chromosome location or base position (X axis) and significance level (Y axis).



A GWAS was conducted using the percent biomass and height, final score, and AUIC phenotype data and the large set of SNPs. Significant (*P value < 1 x 10-6*) marker-trait associations were found for all four greenhouse screening traits among the 279 PSP

core accessions. The PCA scree plot (Figure 21) indicated that the first three principle components explained approximately 15% of the genetic variance and that additional PCs explained less than 2%. The 3D PCA plot displayed two distinct cluster groups and a few intermediaries, indicating variability for accession responses to the traits measured within the population (Figure 21). The Bonferroni-adjusted statistical significance for SNP-trait associations in this GWAS was set at P value < 0.000001. The FarmCPU model was selected for the percent biomass, percent height, and final score traits and the MLMM was selected for the AUIC trait based on Q-Q plot, Manhattan plot, and marker-trait association data output (Table 8, 9).

Figure 21. Principle component analysis graphs of percent height and biomass compared to the control, final score, and AUIC with a scree plot (left) and a 3D principle component analysis plot (right) displaying the population structure.



The FarmCPU model Q-Q plot for percent biomass compared to the control (Figure 22) indicated normality of the data. Values deviating from the null hypothesis line in the top right of the plot were observed, indicating that significant associations

exist between those SNPs and percent biomass. Significance values deviated from the null hypothesis line area in the top right of the plot, but only one value exceeded the threshold, indicating that associations were approaching statistical significance, but did not within the parameters of the model. The Manhattan plot (Figure 23) indicated that significant (*P value* < *1 x 10-6*) marker-trait associations were discovered for at least one SNP and the FarmCPU model output validated this, showing that one SNP was observed to have significant (*P value* < *1 x 10-6*) associations with percent biomass (Table 8).

Figure 22. A FarmCPU model analysis of percent biomass Q-Q plot displaying the deviation of observed significance levels (black dots) from the expected significance values (red line) under the null hypothesis of no marker-trait association.

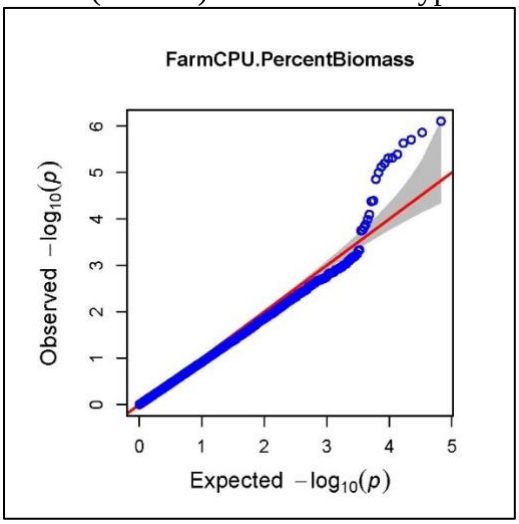
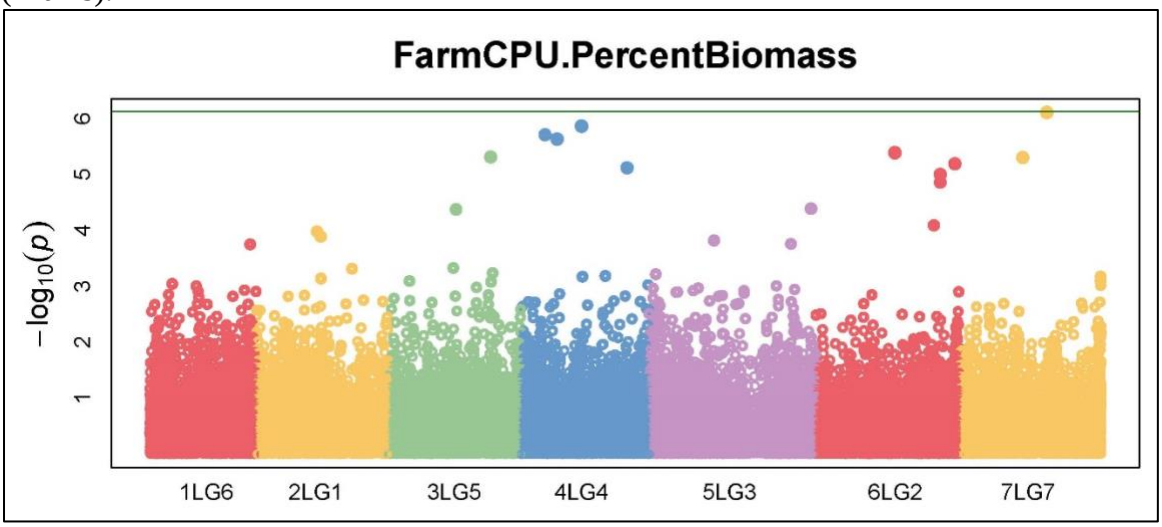


Figure 23. A FarmCPU model Manhattan plot for percent biomass displaying SNPs plotted against their chromosome location or base position (X axis) and significance level (Y axis).



The FarmCPU Q-Q plot for percent height (Figure 24) indicated normality of the data. Values deviating from the null hypothesis line in the top right of the plot were observed, indicating that significant associations exist between those SNPs and the percent height trait. The Manhattan plot (Figure 25) indicated that significant (*P value* < *1 x 10-6*) marker-trait associations were discovered for at least 8 SNPs. In the FarmCPU model output, eight SNPs were observed to have significant (*P value* < *1 x 10-6*) associations with percent height (Table 8).

Figure 24. A FarmCPU model analysis of percent height Q-Q plot displaying the deviation of observed significance values (blue dots) plotted against the expected significance values (red line) under the null hypothesis of no marker-trait association.

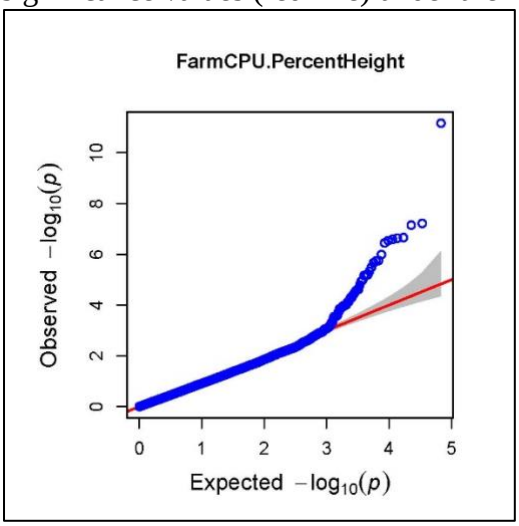
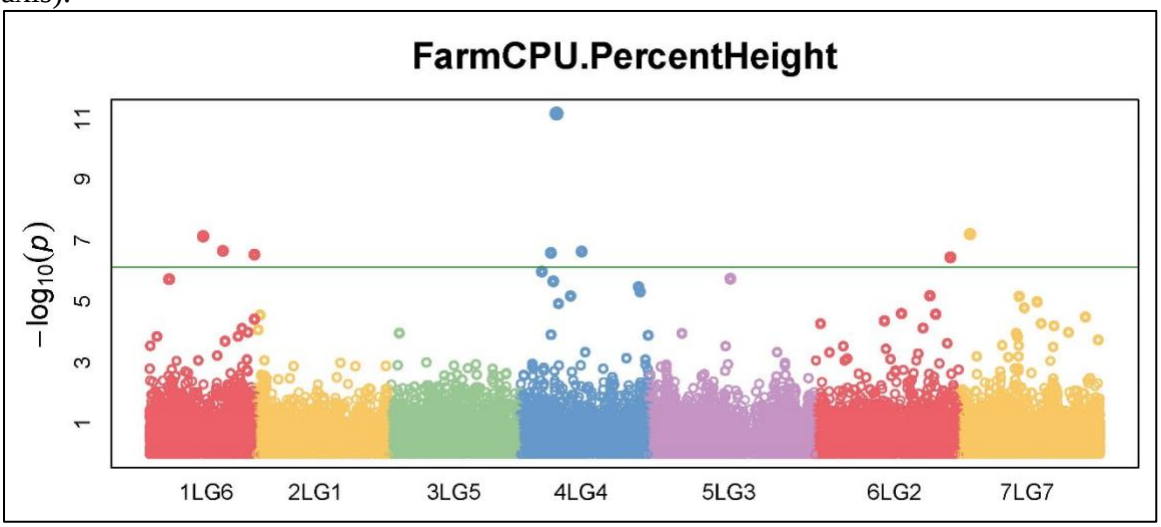


Figure 25. A FarmCPU model Manhattan plot for percent height displaying SNPs plotted against their chromosome location or base position (X axis) and significance level (Y axis).



The FarmCPU model Q-Q plot for mean final score (Figure 26) indicated normality of the data. Values deviating from the null hypothesis line in the top right of the plot were observed, indicating that significant associations exist

between those SNPs and the mean final score. The Manhattan plot (Figure 27) indicated that significant (*P value < 1 x 10-6*) marker-trait associations were discovered for at least five SNPs. The FarmCPU model output validated this, displaying five SNPs having significant (*P value < 1 x 10-6*) associations with the trait (Table 8).

Figure 26. A FarmCPU model mean final score Q-Q plot displaying the deviation of observed significance values (black dots) plotted against the expected significance values (red line) under the null hypothesis of no marker-trait association.

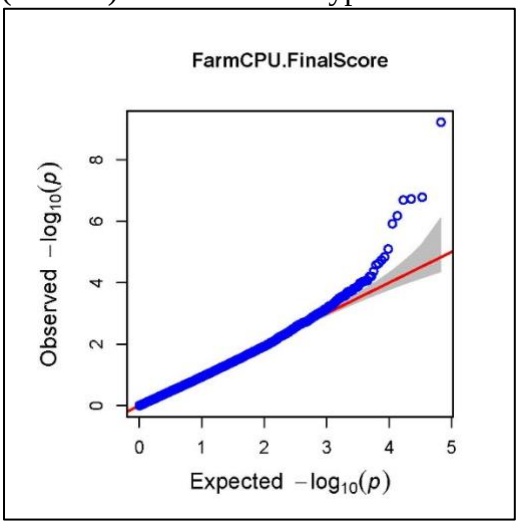
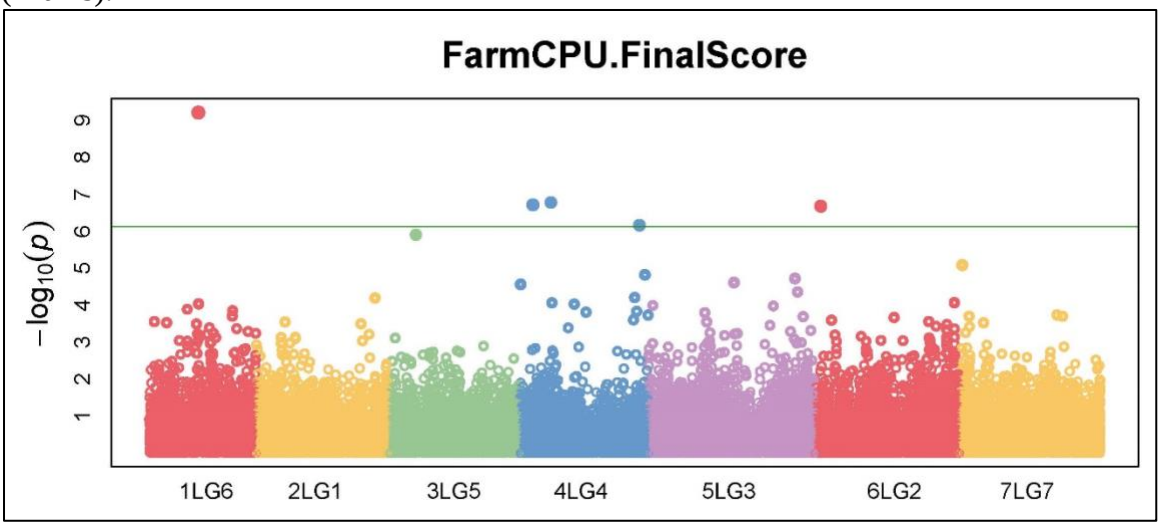


Figure 27. A FarmCPU model Manhattan plot for mean final score displaying SNPs plotted against their chromosome location or base position (X axis) and significance level (Y axis).



The MLMM Q-Q plot for mean AUIC (Figure 28) indicated normality of the data and exhibited values deviating from the null hypothesis line in the top right of the plot. The Manhattan plot (Figure 29) indicated that one significant (*P value* $< 1 \times 10^{-6}$) marker-trait association was discovered; this was supported by the MLMM GWAS output data (Table 9).

Figure 28. A MLMM mean AUIC Q-Q plot displaying the deviation of observed significance values (black dots) plotted against the expected significance values (red line) under the null hypothesis of no marker-trait association.

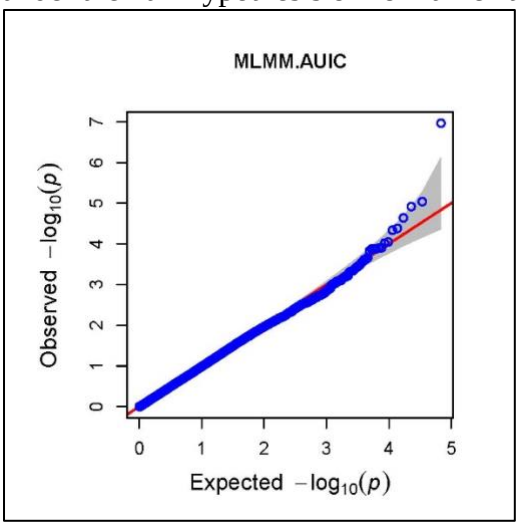
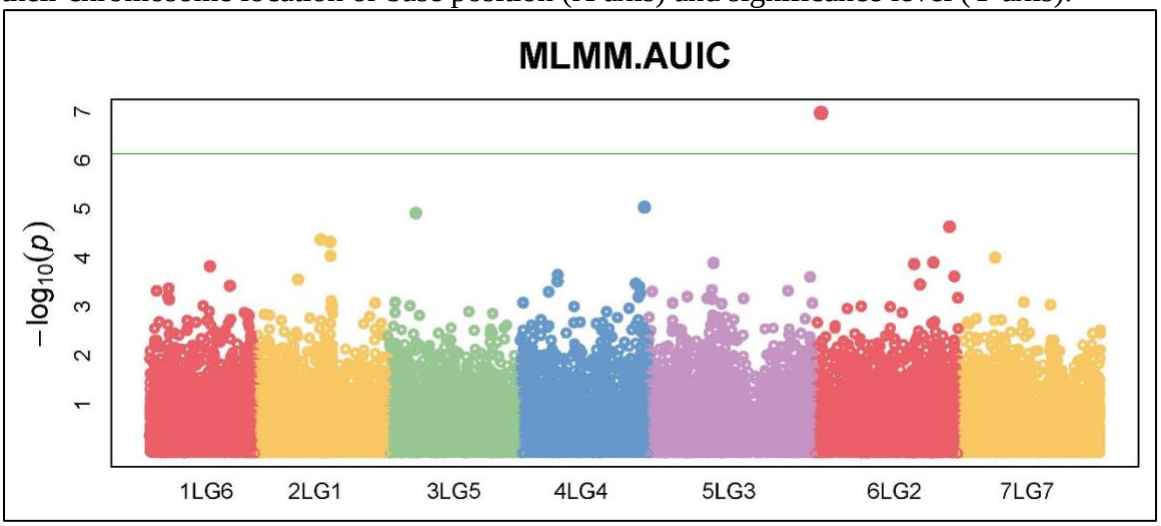


Figure 29. A MLMM Manhattan plot for mean AUIC displaying SNPs plotted against their chromosome location or base position (X axis) and significance level (Y axis).



The GWAS identified 19 different significant SNPs (*P value < 1 x 10-6*) across chromosomes 1, 4, 6, and 7 (Linkage groups 6, 4, 2, & 7) (Table 8, 9). The 'S6LG2_20798185' SNP on chromosome 6 (LG 2) was discovered to have significant (*P value < 1 x 10-6*) associations with both the mean final score and mean AUIC greenhouse

screening traits (Table 8, 9). The GWAS output indicated that other significant SNP/trait associations (*P value < 0.0001*) existed between multiple traits but did not meet the strict significance threshold (*P value < 1 x 10*$^{-6}$) established for this experiment (data not shown). The percent plant height compared to the control trait had the largest amount of significant associations (*P value < 1 x 10*$^{-6}$) within the 68,222 SNPs, with 8 different SNPs observed (Table 8). The 'S4LG4_126644638' SNP was the most significant SNP observed within the study and had an association significance of 6.92 x 10^{-12} with the percent height trait (Table 8). Chromosome 4 (LG 4) contained the largest number of significant SNPs identified within the study, with 9 SNPs associated with four of the five traits measured (Table 8, 9).

Table 8. GWAS output for FarmCPU model analysis of percent germination, percent biomass, percent height, and mean final score traits.

Trait	SNP	Chrm[1]	Position	P value	MAF	N	FDR Adjusted P value	Effect
Germ	S1LG6_245742	1	245742	2.2E-09	0.3	268	8.20E-05	7.3
	S1LG6_147015922	1	147015922	2.429E-09	0.0	268	8.20E-05	-10.5
	S4LG4_281280017	4	281280017	5.258E-09	0.1	268	1.10E-04	2.4
	S7LG7_105919792	7	105919792	1.126E-07	0.1	268	0.002	7.7
	S4LG4_248138686	4	248138686	7.134E-07	0.3	268	0.010	5.4
Biomass	S7LG7_301416933	7	301416933	7.851E-07	0.2	279	0.040	4.9
	S4LG4_210864939	4	210864939	1.38E-06	0.1	279	0.040	-4.8
Height	S4LG4_126644638	4	126644638	6.927E-12	0.5	279	0.000	0.9
	S7LG7_37363293	7	37363293	6.151E-08	0.1	279	0.002	-3.1
	S1LG6_186944518	1	186944518	7.203E-08	0.0	279	0.002	3.2
	S1LG6_256164035	1	256164035	2.225E-07	0.3	279	0.003	-0.6
	S4LG4_213083009	4	213083009	2.346E-07	0.2	279	0.003	-0.4
	S4LG4_106172629	4	106172629	2.594E-07	0.3	279	0.003	-1.8
	S1LG6_365631772	1	365631772	2.93E-07	0.1	279	0.003	-3.1
	S6LG2_471384294	6	471384294	3.608E-07	0.3	279	0.003	2.3
Score	S1LG6_171378678	1	171378678	6.044E-10	0.1	279	0.000	-0.9
	S4LG4_106172647	4	106172647	1.667E-07	0.2	279	0.004	0.1
	S4LG4_44294109	4	44294109	1.906E-07	0.4	279	0.004	-0.2
	S6LG2_20798185	6	20798185	2.068E-07	0.2	279	0.004	0.4
	S4LG4_415350284	4	415350284	6.748E-07	0.2	279	0.009	-0.7

[1]Chromosome number

Table 9. Marker association significance based on MLMM analysis of mean AUIC.

SNP Name	Chrm[1]	Position	P value	MAF	N	FDR Adjusted P value	Effect
S6LG2_20798185	6	20798185	1.08907E-07	-	279	0.0074	-
S4LG4_431363654	4	431363654	9.23631E-06	-	279	0.277726818	-

[1]Chromosome number

The JBrowse *Pisum sativum* genome browser revealed that 13 of the 19 different SNPs fall within known genes and that 12 of these genes have known functions (Table 10, 11). Six of the identified genes are purported to be involved with domains and

binding sites and the remaining genes have various functions ranging from photosynthetic proteins to sugar transport proteins (Table 10, 11). The ‘S4LG4_106172647’ and ‘S4LG4_106172629’ SNPs for the mean final score and percent height traits, respectively, fell within the same gene (‘Psat4g065320’) (Table 10, 11). The genes observed closest to the SNP-linked genes varied widely in their distances, functions, and abundance (Table 10, 11). The ‘S1LG6_245742’, ‘S4LG4_281280017’, and ‘S7LG7_37363293’ significant SNPs fell within or near gene-rich genomic regions (data not shown).

Table 10. Significant SNPs for percent germination, biomass, and height compared to the control and their proximities to known genes within the *Pisum sativum* genome. The closest neighboring gene to the SNP is reported as well.

Trait	Chrm[1]	SNP	P value	Gene Name	Description
Germ	1	S1LG6_245742	2.2E-09	Psat1g000640	B3 DNA binding domain
				Psat1g000600	B3 DNA binding domain
	1	S1LG6_147015922	2.429E-09	Psat1g088720	Myb-like DNA-binding domain
				Psat1g088640	Prokaryotic membrane lipoprotein lipid attachment site profile
	4	S4LG4_248138686	7.134E-07	Psat4g130880	Eukaryotic translation initiation factor eIF2A
				Psat4g130920	RNA recognition motif
	4	S4LG4_281280017	5.258E-09	None[2]	-
				Psat4g142920	Regulation of cellular nucleobase + nucleoside + nucleotide and nucleic acid metabolic process
	7	S7LG7_105919792	1.126E-07	Psat7g065680	Peptidase family S41
				Psat7g065720	Aldo/keto reductase family
Biomass	7	S7LG7_301416933	7.851E-07	None[2]	
				Psat7g157920	Unknown Function
Height	1	S1LG6_186944518	7.203E-08	Psat1g103520	AP2 domain
				Psat1g103560	AP2 domain
	1	S1LG6_256164035	2.225E-07	Psat1g128400	WD40/YVTN domain
				Psat1g128360	WD domain + G-beta repeat
	1	S1LG6_365631772	2.93E-07	None[2]	-
				Psat1g216720	Pumilio-family RNA binding repeat
	4	S4LG4_106172629	2.594E-07	Psat4g065320	Photosynthetic reaction centre protein
				Psat4g065280	Proton-conducting membrane transporter
	4	S4LG4_126644638	6.927E-12	None[2]	-
				Psat4g076800	Glycosyl hydrolase family 14
	4	S4LG4_213083009	2.346E-07	Psat4g115000	PPR repeat family
				Psat4g114960	Unknown Function
	6	S6LG2_471384294	3.608E-07	None[2]	-
				Psat6g236200	Homeobox domain
	7	S7LG7_37363293	6.151E-08	Psat7g024280	ATPase family associated with various cellular activities (AAA)
				Psat7g024240	Acyltransferase C-terminus

[1]Chromosome number

[2]SNP not located within known gene

Table 11. Significant SNPs for mean final score and mean AUIC traits and their proximities to known genes within the *Pisum sativum* genome. The closest neighboring gene to the SNP is reported as well.

Trait	Chrm	SNP	P value	Gene Name	Description
Score	1	S1LG6_171378678	6.044E-10	Psat1g098880	MFS/sugar transport protein
				Psat1g098840	Unknown Function
	4	S4LG4_44294109	1.906E-07	Psat4g030680	Protein kinase domain
				Psat4g030720	Ribosomal protein S8
	4	S4LG4_106172647	1.667E-07	Psat4g065320	Photosynthetic reaction center protein
				Psat4g065280	Proton-conducting membrane transporter
	4	S4LG4_415350284	6.748E-07	Psat4g203520	Unknown gene
				Psat4g203560	Gamma-thionin family
	6	S6LG2_20798185*	2.068E-07	None[2]	-
				Psat6g026960	Unknown Function
AUIC	6	S6LG2_20798185*	1.089E-07	None[2]	-
				Psat6g026960	Unknown Function

[1]Chromosome number

[2]SNP not located within known gene

* Found in multiple traits

Discussion

Germination Screening

The percent germination data observed in this study supported previous research in pea (Shahid et al., 2012; Ouerghi et al., 2016) and wild legumes (Sunita et al., 2013) in that significant reductions in percent germination were observed in salinity treatments exceeding 10 dS m^{-1}. Measurement of percent germination compared to the control was adequate in evaluating accession response to strongly saline Na_2SO_4 conditions, however, percent germination was not a good indicator for seedling response. Correlation analysis between percent germination and the four seedling salinity response traits showed no significant relationship (Table 5). This is unfortunate, due to the high-throughput

phenotyping possibilities associated with screening for whole-plant salt stress response at the germination stage of development.

Greenhouse Screening

The concentration series screening experiment results indicated that the 9 dS m^{-1} Na_2SO_4 level would provide adequate selection pressure for subsequent greenhouse seedling screening. This was supported by the minimal reductions in plant performance observed at higher concentration levels (Figures 8, 9). The inability to direct seed field pea and the poor soil structure associated with soils with EC values exceeding 8 dS m^{-1}, further supported the screening of the PSP core collection at the 9 dS m^{-1} level (MSCA, personal communication, May 22, 2018). High sensitivity of pea to salinity in relation to other important crops was supported by the threshold determined in this study, as research conducted by Munns & James (2003) discovered that a minimum threshold of 15 dS m^{-1} was necessary to observe any significant differences between control and salt-treated diverse germplasm accessions of tetraploid and hexaploid wheat. Likewise, research conducted by Ledesma et al. (2016) discovered that a screening threshold of 12 dS m^{-1} was necessary for detecting significant differences in response to salinity stress among 14 soybean cultivars.

The assumption that the PSP core greenhouse seedling screening experimental runs were equal within the experiment was possible due to the controlled setting of the climate controlled greenhouse conditions and the F-max calculations of the combined greenhouse runs supported this statistically (Table B1). Significant positive correlations were observed between both the percent biomass ($P\ value < 2.2 \times 10^{-16}$) and plant height

(*P value* < *4.0 x 10*$^{-9}$) responses and PSP accession based on the time of submission into the collection (Figure 11, 13). Likewise, significant (*P value* < *2.2 x 10*$^{-16}$) negative correlations were observed between both the mean final score (Figure 15) and AUIC (Figure 17) traits and PSP accession based on its time of submission into the collection. These findings suggest that the later submissions into the PSP core collection, dominated by developed accessions, contain characteristics that favor higher salinity stress tolerance in the *Pisum* genus. The poor salinity tolerance performance associated with the earlier submissions, dominated by collected (less developed) accessions, suggests that improving specific tolerance characteristics in adapted cultivars may be the most efficient method for improving salinity tolerance in current breeding programs; rather than further searching of the wild accessions for novel germplasm conferring salinity tolerance. The adapted cultivar performance is likely due to the stacking of traits that confer tolerance to other abiotic and biotic stresses that may also impose salinity tolerance. This observation is in contradiction of findings by Leonforte et al. (2013a) that observed landraces to be more tolerant to high salinity (NaCl) than the adapted cultivars found in the Australian Temperate Field Crops Collection (ATFCC). This may be a result of specific ion effects (NaCl vs. Na_2SO_4), but further screening of the PSP core collection under high NaCl and the ATFC collection under high Na_2SO_4 salinity conditions must be conducted to verify this hypothesis.

Significant correlations between percent biomass and percent plant height were observed (Table 5); this was anticipated due to the biological relatedness of the two traits. As plant height increases, there is the expectation that total plant biomass will increase

and vice versa. Likewise, as the mean final score increased, the area under the injury curve (AUIC) increased as well. The AUIC is a calculation of injury (score) progression throughout the scoring days within the study; it is derived from the mean score, and therefore, they are directly related.

The bimodal distribution of salinity symptom scores observed in a study conducted by Javid et al. (2015) was not observed in this research (Figure 14). In their study, the 193 field pea lines screened under 18 dS m^{-1} NaCl primarily were placed into categories of tolerant (score ≤ 2.0) or sensitive (score > 6.0) on a one to ten visual growth response scale (Javid et al., 2015). In this research, the distribution of mean final scores exhibited a more uniform distribution across the one to nine scale with a skew towards sensitivity (Figure 14). These findings are more in support of research conducted with diverse germplasm sets in field pea (Leonforte et al., 2013a) and chickpea (Maliro et al., 2008) that found salinity symptom scores to exhibit a skewed distribution toward sensitivity in high NaCl seedling screening. In the study conducted by Leonforte et al. (2013a), 747 lines comprised of ATFCC accessions, Chinese landrace accessions, and Australian cultivars were screened under 18 dS m^{-1} NaCl (Leonforte et al., 2013a). The differences observed between the Javid et al. (2015) study and this research and Leonforte et al. (2013a) study suggest that larger genetically diverse germplasm collections (e.g. PSP core collection) are able to capture the true variation within the *Pisum* genus more adequately than the less diverse breeding lines and cultivars used in the study conducted by Javid et al. (2015).

The EC level discrepancies (9 dS m^{-1} vs. 18 dS m^{-1}) between this research and the studies conducted by Leonforte et al. (2013a) and Javid et al. (2015) may suggest that field pea response to high salinity varies significantly in relation to the salt anion (e.g. Cl^- or SO_4^{2-}) present; as they conducted screening at the 18 dS m^{-1} NaCl level and still observed significant tolerance across accessions. However, this is speculative with research exploring the differences in plant response to specific ion toxicities remaining inconclusive and the scope of this research project not including the observation of that comparison. The aim of this study was not to observe any particular mechanism of tolerance, quantify specific ion tissue accumulation, or observe response differences across specific ions but rather to determine a more overall understanding of the variation for high Na_2SO_4 salinity response in field pea and to identify salt tolerant accessions and associated markers.

The two phases comprising general plant response to high salinity that is discussed in detail by Munns et al. (2008) were observed in this study. Dramatic height discrepancies between the control and treated blocks beginning at 21 to 28 days post-sowing (DPS) suggested the first osmotic shock phase of high salinity pressure. Furthermore, this initial osmotic potential imbalance was observed in the seedling experiments with some plants exhibiting wilting symptoms when the growing medium was still saturated. The second phase, specific ion toxicity effect, was observed in the presence of chlorosis beginning on the margins of lower leaves and spreading to the whole plant from 28 to 38 DPS.

The pea germplasm displaying the highest salinity tolerance was morphologically diverse, varying in leaf type, cotyledon color, and plant height (Table 6). In the top 10 performing accessions, the top three were semi-leafless and the remaining where normal leaf types (not shown). Mean plant height across the top accessions under non-saline conditions was 54.1 cm (data not shown), suggesting that shorter lines may exhibit greater tolerance to high Na_2SO_4 conditions. Cotyledon color was green or yellow for five of the top ten accessions, respectively (Table 6), suggesting that tolerance is not likely to be linked to cotyledon color. Taxonomically, the ten highest performing accessions were all *Pisum sativum* with one accession designated as *P. sativum* subsp. *asiaticum*; implying that salinity tolerance is more associated with the *sativum* species within the *Pisum* genus, as well as with domestication (Tables A1, B2). The geographic distribution of the top performing accessions was vast, but the highest performance based on the traits measured was associated primarily with Oceania, North America, Eastern Europe, and the Mediterranean (Tables 7, A1, B2). This observation is not unexpected, as some countries within these regions have minimal annual precipitation that regularly imparts drought stress upon crops; suggesting that the development of cultivars in these regions has likely unintentionally selected for tolerance to the osmotic stress phase of salinity stress.

The most tolerant accession was the developed cultivar ‘Stirling’ (PI634571), obtained from the USA. ‘Stirling’ is a short (28 cm) and semi-leafless field pea cultivar with a green cotyledon; these traits increase its value as a potential parental line in targeted breeding of high Na_2SO_4 tolerance, as they match current trait preferences in

breeding programs. The other tolerant to moderately tolerant accessions identified (e.g. ‘Solara’, ‘SW Carousel’) will further provide a basis for selecting recurrent parents to use in targeted breeding for salinity tolerance.

GWAS

The discrepancy in the availability of accessions between percent germination and the seedling response traits made it necessary to run two genome-wide association studies separately to optimize statistical power. Scree plots showed that for percent germination, biomass, and plant height, mean final score, and AUIC, the three PCs used in conducting GWAS explained approximately 15% of the genetic variance within the population and minimal gain (< 2%) was attained by adding additional principle components. This indicated that the use of three PCs in the percent germination and seedling response trait GWAS runs was optimal for explaining genetic variance within the PSP core population while still maintaining statistical power. A 3D PCA plot displays the distribution of a population’s genetic variance across the principle components selected in the model. The 3D PCA plots for both GWAS runs (Figures 18, 21) indicated that the genotypes within this panel of 279 accessions vary for response to the traits measured. Model selection for analysis of the percent germination, biomass, plant height, mean final score, and AUIC was based on Q-Q plots, Manhattan plots, and marker-trait association data output. Observed deviations from the null hypothesis at lower significance levels in the Q-Q plots prompted the dismissal of that model in this experiment, as non-normal distributions of data and potential over or under-conservativeness of model parameters

have the potential to negatively impact the discovery rate of significant marker-trait associations.

The FarmCPU Q-Q plot for percent germination (Figure 19) exhibited the most normality among the four GAPIT models analyzed, with expected and observed significance values remaining on the null hypothesis line until the top right corner of the plot. This indicated that the FarmCPU model was the best fit for the data; this was further validated by the presence of significant ($P\ value < 1 \times 10^{-6}$) marker-trait associations (Table 8). The MLMM Q-Q plot for mean AUIC (Figure 28) exhibited the most normality among the four GAPIT models analyzed and produced a significant SNP association, indicating that it was the best fit model for that trait. The FarmCPU Q-Q plots for the percent biomass, percent height, and mean final score (Figures 22, 24, 26) indicated that the FarmCPU model was the best fit for the three traits despite the presence of significant ($P\ value < 1 \times 10^{-6}$) marker-trait associations in the three alternative models.

The primary objective of a Manhattan plot is to identify significant marker-trait associations through the presence of ‘peaks’ rising above the significance threshold line and to identify the genomic regions that may be associated with the trait of interest; this objective was possible within the scope of this study due to the annotated SNP position data within the PSPPC SNP dataset. The GWAS output data format was subject to the capabilities of the model that was selected. The FarmCPU model output included the SNP name, position information, P-value, MAF, False Discovery Rate (FDR) adjusted P

value, and effect (Table 8); while the MLMM did not include the MAF or effect in its analysis output (Table 9).

A total of 19 different statistically significant (*P value < 1 x 10-6*) associations between all five traits and the set of 68,222 SNPs were discovered using the strict parameters in this study (Figure 20, Table 8, 9). The 'S6LG2_20798185' SNP on chromosome 6 was the only SNP within this dataset discovered to have significant (*P value < 1 x 10-6*) associations between multiple traits (Table 8, 9) and, therefore, represents one of the best candidates for marker-assisted selection moving forward. Likewise, the 'S4LG4_126644638' SNP was the most significant SNP observed within this study (*P value 1 x 10-12*) (Table 8, 9), suggesting that it may play an important role in plant response to salinity. Chromosome 4 contained the largest number of significant SNPs, with 9 SNPs associated with four of the five traits measured (Table 8, 9), indicating that further gene annotation within this region of the pea genome would benefit future salinity response research. The presence of two statistically significant SNPs within the gene 'Psat4g065320' (Table 10, 11), suggests that it is also a good candidate gene for MAS. The 'S4LG4_281280017' SNP found to have a significant association with percent germination did not fall within a known gene (Table 10) but was in close proximity to a gene-rich region on chromosome 4 (LG 4) that contained many genes with known functions that may be of interest to high salinity response research (not shown). The ion transport protein ('Psat4g142960') and intracellular membrane-bound organelle protein ('Psat4g142880') genes found within this region are of particular interest, as they describe important functions that are critical in plant responses to high

salinity. Similarly, the ‘S1LG6_245742’ and ‘S7LG7_37363293’ SNPs significantly associated with percent germination and percent height, respectively, fell within gene-rich regions that need to be explored further (data not shown).

The efficacy of utilizing the GAPIT R program for conducting GWAS in important agricultural crops has been demonstrated in recent studies on spring wheat (Garcia et al., 2019), maize (Zhu et al., 2018), rice (Ma et al., 2016), and pea (Holdsworth et al., 2017; Desgroux et al., 2018). In rice, Ma et al. (2016) identified 29 SNP-trait associations among 270 diverse accessions using a large set of 1,019,883 SNPs and phenotypic data for three traits associated with height and yield under contrasting moisture regimes. Zhu et al. (2018) utilized the FarmCPU model to identify 20 SNP-trait associations among 292 inbred maize lines with a set of 25,331 SNPs and measurements of five ear traits. Likewise, this study was successful in discovering a comparable amount of SNP-trait associations (20) using a similar number of traits (5) and an intermediate sized SNP dataset (68,222). Holdsworth et al. (2017) had previously demonstrated the breeding potential of the large SNP set utilized in this study by identifying 25 SNP-trait associations between flower color and the set of 66,591 SNPs among 431 accessions comprising the USDA *Pisum* single plant plus core (PSPPC) collection. Their research and the results of this study indicate that the GAPIT program is an effective and valuable tool for conducting GWAS and identifying genomic markers associated with traits of interest in pea using the SNP dataset and accessions of the USDA PSP core and PSPPC collections.

Future Direction

Additional testing of the PSP core accessions screened in this study is necessary to validate results and provide greater insight into the underlying genetic control for field pea response to high Na_2SO_4 conditions. Subsequent studies should include the use of other widely used measurement techniques to better quantify plant responses. Phenotypic scoring can be effective in quantifying plant responses to salinity when used in conjunction with other measurements and should be a component of further research. Empirical evidence supporting the efficacy of visual growth response or "scorch scale" scoring is observed in this study as well as in other studies of pea (Leonforte et al., 2013a; Javid et al., 2015), chickpea (Maliro et al, 2008), and soybean (Ledesma et al., 2016). Utilization of spectrophotometry to identify specific ions, proteins, and the plant tissue accumulation areas in important crops subjected to high salinity has been demonstrated in studies on soybean (Ledesma et al., 2016), wheat (Munns & James, 2003), rice (Rana et al., 2019), and pea (Abd El-Samad & Shaddad, 1996; Shahid et al., 2012). This method would be invaluable in discovering the specific ions responsible for plant responses to salinity and identifying those areas exhibiting the highest salt ion accumulations.

Other measurement techniques utilized in quantifying salt response in legumes include observations of chlorophyll content changes over time using a hand-held chlorophyll meter (Tavakkoli et al., 2010a), leaf epidermal cell reactions and stomatal density using light microscopy (Maksimovic et al., 2010), and stomatal conductance using a portable porometer (Hernandez et al., 2000; Maksimovic et al., 2010). The

measurement of traits such as plant height and biomass of roots and shoots is ubiquitous across salinity tolerance studies in important crops and was likewise a significant contribution to the quantification of pea response to salinity in this study. The protocols developed for this study using the available resources were adequate in fulfilling the objectives of the experiment but could certainly be improved upon if resources were available. Primary additions and alterations to the seedling response protocol would include the utilization of larger pots to reduce the competition for resources among the three plants in each and more space to conduct the experiment all in one place and at one time. These alterations would significantly reduce the confounding effects associated with greenhouse seedling screening over the period of one year.

Conclusion

The germination and seedling screening protocols developed using the available resources in this study were successful in detecting significant differences between *Pisum* accessions in response to high Na_2SO_4 conditions. Results supported previous research that found the germination stage in legumes to be the most tolerant to high salinity conditions and that there is no correlation between the germination and seedling stages of development in field pea for response to high Na_2SO_4. Furthermore, genetic variation within the PSP core collection for germination and seedling response to high Na_2SO_4 conditions was observed. Salt tolerant and moderately tolerant accessions were identified based on high performance across the five salinity response traits measured, providing potential parental germplasm for the targeted breeding of salinity tolerance in field pea.

The identification of multiple statistically significant associations between SNPs and salinity tolerance traits within the PSP core collection offer potential markers for marker assisted selection (MAS) in targeted field pea breeding efforts.

Going forward, validation of the trait response, top performing accessions, and SNP-trait associations is required. Development and implementation of studies exploring the response of pea to high Na_2SO_4 in real field conditions will be imperative to the identification of field pea lines that will perform well for growers. Further exploration of the genomic regions harboring the SNPs of interest identified in this study will be necessary for MAS breeding efforts and understanding of the quantitative genetics imparting salinity tolerance in field pea.

The information provided by this research will be invaluable to the future development of salt tolerant field pea varieties; this will become particularly relevant as an increase in global climate change events and the continued mismanagement of agricultural lands are anticipated to accelerate the salinization of arable soils. The high water-use efficiency, soil health benefits, nutritional qualities, and crop rotation suitability associated with legume crops like field pea, will continue to push their demand globally. The results presented in this study should provide field pea breeders and researchers with reliable preliminary data for pea response to Na_2SO_4 salinity. This data also provides breeders of other crops with simple protocols that can be followed to relatively quickly identify salt tolerant germplasm and markers using genetically diverse collections for their crop and simple phenotyping methods and genome-wide association

studies. These techniques will allow researchers to address the needs of our constantly growing and changing world.

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APPENDICES

APPENDIX A

USDA PSP CORE COLLECTION PASSPORT DESCRIPTORS

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
1	PI 102888 PSP	No. 8	Pisum sativum	China	Asia	Collected	Yellow
2	PI 109866 PSP	Arvejas Verdes	Pisum sativum	Venezuela	South America	Collected	Green
3	PI 116056 PSP	Matar	P. sativum subsp. sativum	India	Asia	Collected	Yellow
4	PI 116844 PSP	Mattar	Pisum sativum	Pakistan	Asia	Collected	Mixed
5	PI 116944 PSP	Moshong	Pisum sativum	Afghanistan	Asia	Collected	Yellow
6	PI 117264 PSP	No. 215	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
7	PI 117998 PSP	Ervilha Torta Flor Roxa	Pisum sativum	Brazil	South America	Collected	Yellow
8	PI 118501 PSP	Ervilha Branca	Pisum sativum	Brazil	South America	Collected	Yellow
9	PI 121352 PSP	No. 1891	Pisum sativum	India	Asia	Collected	Yellow
10	PI 124478 PSP	Matar	Pisum sativum	Pakistan	Asia	Collected	Mixed
11	PI 125839 PSP	Mizhik	Pisum sativum	Afghanistan	Asia	Collected	Yellow
12	PI 125840 PSP	Moshong	Pisum sativum	Afghanistan	Asia	Collected	Yellow
13	PI 134271 PSP	No. 10	Pisum sativum	Afghanistan	Asia	Collected	Yellow
14	PI 137119 PSP	Gray's	Pisum sativum	Canada	North America	Collected	Yellow
15	PI 140298 PSP	No. 6618	Pisum sativum	Canada	North America	Collected	Mixed
16	PI 142775 PSP	Alaberga	Pisum sativum	Mexico	North America	Collected	Mixed
17	PI 143485 PSP	-	Pisum sativum	Azerbaijan	Western Europe	Collected	Yellow
18	PI 155109 PSP	-	Pisum sativum	USA	North America	Donated	Yellow
19	PI 156647 PSP	-	Pisum sativum	Ethiopia	Africa	Collected	Mixed
20	PI 156720 PSP	Thirty-Days	Pisum sativum	Japan	Asia	Donated	Yellow
21	PI 162909 PSP	L.P. No. 4	Pisum sativum	Paraguay	Central America	Collected	Mixed
22	PI 163126 PSP	Matar	Pisum sativum	India	Asia	Collected	Yellow
23	PI 163129 PSP	Matar	Pisum sativum	India	Asia	Collected	Mixed
24	PI 164548 PSP	Matar	Pisum sativum	India	Asia	Collected	Mixed

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
25	PI 164612 PSP	Patani	Pisum sativum	India	Asia	Collected	Mixed
26	PI 164779 PSP	Matar	Pisum sativum	India	Asia	Collected	Yellow
27	PI 164971 PSP	No. 17	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
28	PI 164972 PSP	No. 48	Pisum sativum	Turkey	Mediterranean	Collected	Mixed
29	PI 165949 PSP	Matar	Pisum sativum	India	Asia	Collected	Yellow
30	PI 166084 PSP	Matar	Pisum sativum	India	Asia	Collected	Yellow
31	PI 166159 PSP	No. 9705	Pisum sativum	Nepal	Asia	Collected	Yellow
32	PI 169603 PSP	No. 2187	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
33	PI 169608 PSP	Bezelya Yesil	Pisum sativum	Turkey	Mediterranean	Collected	Green
34	PI 171810 PSP	No. 6754	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
35	PI 172339 PSP	Mansholt Pluk	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
36	PI 173840 PSP	-	Pisum sativum	India	Asia	Collected	Mixed
37	PI 174320 PSP	Sultani	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
38	PI 174921 PSP	Kalaon	Pisum sativum	Nepal	Asia	Collected	Yellow
39	PI 175231 PSP	Kolung	Pisum sativum	Nepal	Asia	Collected	Mixed
40	PI 179450 PSP	No. 9747	Pisum sativum	Syria	Middle East	Collected	Mixed
41	PI 179451 PSP	No. 9749	Pisum sativum	Syria	Middle East	Collected	Mixed
42	PI 179459 PSP	No. 10072	Pisum sativum	Turkey	Mediterranean	Collected	Mixed
43	PI 179722 PSP	No. 10898	Pisum sativum	India	Asia	Collected	Yellow
44	PI 179970 PSP	Matar	Pisum sativum	India	Asia	Collected	Mixed
45	PI 180329 PSP	Watana	Pisum sativum	India	Asia	Collected	Mixed
46	PI 180693 PSP	Hoenheimer Pink-Flowered	Pisum sativum	Germany	Western Europe	Donated	Yellow
47	PI 180696 PSP	Mahndorfer Viktoria	Pisum sativum	Germany	Western Europe	Donated	Yellow
48	PI 180699 PSP	Rimpaus Green Viktoria	Pisum sativum	Germany	Western Europe	Donated	Mixed

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Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
49	PI 180702 PSP	Strengs Weihenstephaner Felderbse	Pisum sativum	Germany	Western Europe	Donated	Mixed
50	PI 181801 PSP	No. 9922	Pisum sativum	Lebanon	Middle East	Collected	Mixed
51	PI 181958 PSP	Homs No. 334	Pisum sativum	Syria	Middle East	Collected	Mixed
52	PI 183467 PSP	Matar	Pisum sativum	India	Asia	Collected	Yellow
53	PI 184130 PSP	No. 309	Pisum sativum	Croatia	Mediterranean	Collected	Mixed
54	PI 184784 PSP	-	Pisum sativum	Guinea	Africa	Collected	Mixed
55	PI 193578 PSP	No. 8508	Pisum sativum	Ethiopia	Africa	Collected	Yellow
56	PI 193584 PSP	No. 8696	Pisum sativum	Ethiopia	Africa	Collected	Yellow
57	PI 193590 PSP	No. 8736	Pisum sativum	Ethiopia	Africa	Collected	Yellow
58	PI 195020 PSP	No. 9292	Pisum sativum	Ethiopia	Africa	Collected	Yellow
59	PI 195404 PSP	Quezal Tenango	Pisum sativum	Guatemala	Central America	Collected	Mixed
60	PI 195631 PSP	No. 9886	Pisum sativum	Ethiopia	Africa	Collected	Yellow
61	PI 197044 PSP	No. 3005	Pisum sativum	Honduras	Central America	Collected	Mixed
62	PI 197990 PSP	Vinco	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
63	PI 198072 PSP	Brioart	Pisum sativum	Sweden	Western Europe	Donated	Yellow
64	PI 198074 PSP	Gorsdagsart III	Pisum sativum	Sweden	Western Europe	Donated	Mixed
65	PI 198735 PSP	-	Pisum sativum	Afghanistan	Asia	Collected	Yellow
66	PI 200755 PSP	No. 3498	Pisum sativum	Guatemala	Central America	Collected	Mixed
67	PI 201390 PSP	No. 3153	Pisum sativum	Mexico	North America	Collected	Yellow
68	PI 203064 PSP	-	Pisum sativum	Finland	Western Europe	Collected	Yellow
69	PI 203066 PSP	-	Pisum sativum	Finland	Western Europe	Collected	Green
70	PI 203067 PSP	-	Pisum sativum	Finland	Western Europe	Collected	Mixed
71	PI 203068 PSP	-	Pisum sativum	Finland	Western Europe	Collected	Mixed
72	PI 203069 PSP	-	Pisum sativum	Finland	Western Europe	Collected	Mixed

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Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
73	PI 204306 PSP	Dunn	Pisum sativum	Australia	Oceana	Donated	Yellow
74	PI 206006 PSP	Debut 149	Pisum sativum	Sweden	Western Europe	Donated	Mixed
75	PI 206816 PSP	-	Pisum sativum	USA	North America	Developed	Yellow
76	PI 206838 PSP	Everbearing	Pisum sativum	USA	North America	Developed	Mixed
77	PI 206861 PSP	Carters Market Gardener	Pisum sativum	USA	North America	Developed	Yellow
78	PI 207508 PSP	No. 12616	Pisum sativum	Afghanistan	Asia	Collected	Yellow
79	PI 209507 PSP	No. 3843	Pisum sativum	Costa Rica	Central America	Collected	Mixed
80	PI 210558 PSP	No. S-653	Pisum sativum	China	Asia	Collected	Mixed
81	PI 210561 PSP	No. S-688	Pisum sativum	Russia	Eastern Europe	Collected	Mixed
82	PI 210568 PSP	Kelina	Pisum sativum	Finland	Western Europe	Collected	Mixed
83	PI 210569 PSP	Sivikka	Pisum sativum	Finland	Western Europe	Collected	Mixed
84	PI 210571 PSP	Flo	Pisum sativum	Finland	Western Europe	Collected	Yellow
85	PI 210583 PSP	Rogers Ace	Pisum sativum	USA	North America	Developed	Mixed
86	PI 212031 PSP	No. 4	Pisum sativum	Iran	Middle East	Donated	Yellow
87	PI 220174 PSP	No. 13	Pisum sativum	Afghanistan	Asia	Collected	Mixed
88	PI 220189 PSP	Moshong	Pisum sativum	Afghanistan	Asia	Collected	Mixed
89	PI 221697 PSP	No. 12	Pisum sativum	Indonesia	Oceana	Donated	Mixed
90	PI 222071 PSP	Moshong	Pisum sativum	Afghanistan	Asia	Collected	Mixed
91	PI 222117 PSP	Moshong	Pisum sativum	Afghanistan	Asia	Collected	Yellow
92	PI 223527 PSP	Lobia	Pisum sativum	Afghanistan	Asia	Collected	Mixed
93	PI 227258 PSP	No. 14918	Pisum sativum	Iran	Middle East	Collected	Yellow
94	PI 236492 PSP	Lamprecht #368	Pisum sativum	USA	North America	Donated	Yellow
95	PI 240516 PSP	Lucknow Boniya	Pisum sativum	India	Asia	Donated	Mixed
96	PI 241593 PSP	-	Pisum sativum	Taiwan	Asia	Donated	Yellow

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Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
97	PI 242027 PSP	-	P. sativum subsp. sativum	Denmark	Western Europe	Donated	Yellow
98	PI 242028 PSP	-	Pisum sativum	Denmark	Western Europe	Donated	Mixed
99	PI 244093 PSP	Apollo	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
100	PI 244121 PSP	Crescent	Pisum sativum	Netherlands	Western Europe	Donated	Yellow
101	PI 244150 PSP	Goldkongin	Pisum sativum	Netherlands	Western Europe	Donated	Yellow
102	PI 244175 PSP	Lage Smelpeul	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
103	PI 244191 PSP	Morgenster	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
104	PI 248181 PSP	Col. No. 23171	Pisum sativum	Rwanda	Africa	Collected	Yellow
105	PI 249645 PSP	B.R. 178	Pisum sativum	India	Asia	Developed	Yellow
106	PI 250438 PSP	Libochovicky Rany	Pisum sativum	Czech Republic	Western Europe	Donated	Yellow
107	PI 250439 PSP	Konservova Kralovna (CSR)	Pisum sativum	Czech Republic	Western Europe	Donated	Green
108	PI 250440 PSP	Karlovna Konservy	Pisum sativum	Czech Republic	Western Europe	Donated	Yellow
109	PI 250441 PSP	Prebohaty	Pisum sativum	Czech Republic	Western Europe	Donated	Green
110	PI 250446 PSP	Libochovicky Urodny	Pisum sativum	Czech Republic	Western Europe	Donated	Mixed
111	PI 250447 PSP	Lincoln	Pisum sativum	Czech Republic	Western Europe	Donated	Mixed
112	PI 250448 PSP	Libochovicky Rany	Pisum sativum	Czech Republic	Western Europe	Donated	Yellow
113	PI 253968 PSP	Col. No. K1722	Pisum sativum	Afghanistan	Asia	Collected	Yellow
114	PI 257244 PSP	Tung Haun	Pisum sativum	China	Asia	Collected	Yellow
115	PI 257592 PSP	-	Pisum sativum	Ethiopia	Africa	Donated	Yellow
116	PI 261622 PSP	Col. No. D-43	Pisum sativum	Spain	Mediterranean	Collected	Mixed
117	PI 261623 PSP	Guisante Pirabesque	Pisum sativum	Spain	Mediterranean	Collected	Yellow
118	PI 261624 PSP	Col. No. D-45	Pisum sativum	Spain	Mediterranean	Collected	Green
119	PI 261636 PSP	No. D-88	Pisum sativum	Spain	Mediterranean	Collected	Mixed
120	PI 261671 PSP	Degrace	Pisum sativum	Netherlands	Western Europe	Collected	Mixed

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Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
121	PI 261677 PSP	Col. No. D-236	Pisum sativum	Netherlands	Western Europe	Collected	Mixed
122	PI 263014 PSP	Slier of Krombek	Pisum sativum	Netherlands	Western Europe	Collected	Yellow
123	PI 263027 PSP	Mangetout Corne de Beleir	Pisum sativum	France	Western Europe	Collected	Mixed
124	PI 263030 PSP	Pois du Chemen Long	Pisum sativum	France	Western Europe	Collected	Yellow
125	PI 263031 PSP	Mangetout Carouby	Pisum sativum	France	Western Europe	Collected	Mixed
126	PI 263032 PSP	Mangetout Fonant de St Diserat	Pisum sativum	France	Western Europe	Collected	Mixed
127	PI 263871 PSP	-	Pisum sativum	Greece	Mediterranean	Collected	Mixed
128	PI 266070 PSP	Line NO. 930	Pisum sativum	Sweden	Western Europe	Donated	Yellow
129	PI 269543 PSP	Col. No. 431	Pisum sativum	Pakistan	Asia	Collected	Yellow
130	PI 269761 PSP	Aa135	P. sativum var. arvense	Czech Republic	Western Europe	Collected	Mixed
131	PI 269762 PSP	Aa38	P. sativum subsp. sativum	UK	Western Europe	Donated	Yellow
132	PI 269770 PSP	CN 31212	Pisum sativum	UK	Western Europe	Donated	Mixed
133	PI 269777 PSP	Aa87	Pisum sativum	UK	Western Europe	Donated	Mixed
134	PI 269778 PSP	Aa88	Pisum sativum	UK	Western Europe	Donated	Yellow
135	PI 269782 PSP	Aa92	Pisum sativum	UK	Western Europe	Donated	Mixed
136	PI 269791 PSP	Brochette	Pisum sativum	UK	Western Europe	Donated	Mixed
137	PI 269802 PSP	Aa112	Pisum sativum	UK	Western Europe	Donated	Yellow
138	PI 269804 PSP	Aa117	Pisum sativum	UK	Western Europe	Donated	Mixed
139	PI 269812 PSP	Aa128	Pisum sativum	UK	Western Europe	Donated	Mixed
140	PI 269818 PSP	Aa134	Pisum sativum	UK	Western Europe	Donated	Yellow
141	PI 269822 PSP	Perfection	Pisum sativum	UK	Western Europe	Donated	Mixed
142	PI 269825 PSP	Aa175	Pisum sativum	UK	Western Europe	Donated	Mixed
143	PI 270536 PSP	-	Pisum sativum	Denmark	Western Europe	Donated	Mixed
144	PI 271035 PSP	Hindukusch	Pisum sativum	Sweden	Western Europe	Donated	Mixed

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
145	PI 271038 PSP	Granart	Pisum sativum	Nepal	Asia	Collected	Mixed
146	PI 271116 PSP	Granerbse	Pisum sativum	China	Asia	Collected	Yellow
147	PI 271511 PSP	1295	Pisum sativum	India	Asia	Collected	Yellow
148	PI 272148 PSP	Navale Artturi	Pisum sativum	Finland	Western Europe	Collected	Mixed
149	PI 272171 PSP	Zeylanicum, Selectas Peluschke	Pisum sativum	Germany	Western Europe	Donated	Yellow
150	PI 272175 PSP	Zeylanicum	Pisum sativum	Germany	Western Europe	Donated	Mixed
151	PI 272184 PSP	Arvense	Pisum sativum	Greece	Mediterranean	Collected	Mixed
152	PI 272194 PSP	Concolon	Pisum sativum	Germany	Western Europe	Donated	Mixed
153	PI 272204 PSP	Hodinger Futtererbse	Pisum sativum	Germany	Western Europe	Donated	Mixed
154	PI 272215 PSP	Pop. Lucienhofer Wintererbse	Pisum sativum	Germany	Western Europe	Donated	Mixed
155	PI 272216 PSP	Winter-Futtererbse	Pisum sativum	Bulgaria	Western Europe	Collected	Yellow
156	PI 272218 PSP	Population	Pisum sativum	Poland	Western Europe	Collected	Yellow
157	PI 273209 PSP	9009/60	P. sativum subsp. elatius	Russia	Eastern Europe	Collected	Mixed
158	PI 273605 PSP	-	Pisum sativum	Ecuador	South America	Collected	Mixed
159	PI 274307 PSP	809	Pisum sativum	Pakistan	Asia	Collected	Mixed
160	PI 274308 PSP	815	Pisum sativum	Pakistan	Asia	Collected	Yellow
161	PI 274584 PSP	-	Pisum sativum	Norway	Western Europe	Collected	Mixed
162	PI 275821 PSP	Line 3	Pisum sativum	Sweden	Western Europe	Donated	Yellow
163	PI 275822 PSP	Witham Wonder	Pisum sativum	Sweden	Western Europe	Donated	Mixed
164	PI 275825 PSP	Line 8	Pisum sativum	Sweden	Western Europe	Donated	Mixed
165	PI 277852 PSP	No. 8090	Pisum sativum	Ethiopia	Africa	Collected	Yellow
166	PI 279823 PSP	Edelperle	Pisum sativum	Germany	Western Europe	Developed	Yellow
167	PI 279825 PSP	Foli NO. 2	Pisum sativum	Germany	Western Europe	Donated	Mixed
168	PI 279827 PSP	Mansholt's Pluk	Pisum sativum	Germany	Western Europe	Donated	Green

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
169	PI 280252 PSP	No. 8120	Pisum sativum	Ethiopia	Africa	Collected	Yellow
170	PI 280603 PSP	Palestinicum	Pisum sativum	Israel	Middle East	Collected	Mixed
171	PI 280609 PSP	Amplissimo Local	Pisum sativum	Russia	Eastern Europe	Donated	Mixed
172	PI 280611 PSP	Amplissimo Viktoria Ukrainskaya	Pisum sativum	Ukraine	Eastern Europe	Collected	Mixed
173	PI 280613 PSP	Amplissimo Borec	Pisum sativum	Russia	Eastern Europe	Collected	Mixed
174	PI 280614 PSP	Amplissimo Neistoschimyj	Pisum sativum	Russia	Eastern Europe	Collected	Mixed
175	PI 280616 PSP	Amplissimo Zazerskij	Pisum sativum	Belarus	Eastern Europe	Collected	Mixed
176	PI 280617 PSP	Amplissimo Hamisepp	Pisum sativum	Estonia	Eastern Europe	Collected	Mixed
177	PI 280619 PSP	Amplissimo Tygevskij Kirju	Pisum sativum	Estonia	Eastern Europe	Collected	Yellow
178	PI 280626 PSP	Amplissimo Yjulskil	Pisum sativum	Russia	Eastern Europe	Collected	Mixed
179	PI 285710 PSP	Kujawski Pozny	Pisum sativum	Poland	Western Europe	Donated	Mixed
180	PI 285718 PSP	Pomorska	Pisum sativum	Poland	Western Europe	Donated	Mixed
181	PI 285722 PSP	Konserwowy I Har	Pisum sativum	Poland	Western Europe	Donated	Mixed
182	PI 285724 PSP	Lincoln-Freege	Pisum sativum	Poland	Western Europe	Donated	Yellow
183	PI 285727 PSP	Majowy	Pisum sativum	Poland	Western Europe	Donated	Mixed
184	PI 285730 PSP	Cud Ameryki	Pisum sativum	Poland	Western Europe	Donated	Green
185	PI 285737 PSP	Foli	Pisum sativum	Poland	Western Europe	Donated	Mixed
186	PI 285739 PSP	June Wonder	Pisum sativum	Poland	Western Europe	Donated	Yellow
187	PI 285740 PSP	Juvel	Pisum sativum	Poland	Western Europe	Donated	Mixed
188	PI 285747 PSP	Konservenkonigin	Pisum sativum	Poland	Western Europe	Donated	Green
189	PI 286430 PSP	-	Pisum sativum	Nepal	Asia	Collected	Mixed
190	PI 286431 PSP	Matar	Pisum sativum	Nepal	Asia	Collected	Yellow
191	PI 286607 PSP	-	Pisum sativum	Thailand	Asia	Collected	Mixed
192	PI 288025 PSP	Quiki	Pisum sativum	France	Western Europe	Donated	Mixed

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
193	PI 288263 PSP	Mark-Erbsen	Pisum sativum	Germany	Western Europe	Donated	Mixed
194	PI 293426 PSP	No. 30	Pisum sativum	Bulgaria	Western Europe	Donated	Mixed
195	PI 306591 PSP	Pjeljuska Passzkaja	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
196	PI 307666 PSP	Verja	Pisum sativum	Costa Rica	Central America	Collected	Mixed
197	PI 308796 PSP	Sylvia	Pisum sativum	India	Asia	Donated	Mixed
198	PI 314794 PSP	CPI 12559	Pisum sativum	Australia	Oceana	Donated	Green
199	PI 314795 PSP	CPI 12561	Pisum sativum	Australia	Oceana	Donated	Mixed
200	PI 319374 PSP	Col. No. 22074	Pisum sativum	Mexico	North America	Collected	Mixed
201	PI 320972 PSP	-	Pisum sativum	Hungary	Eastern Europe	Donated	Yellow
202	PI 324695 PSP	Arakass	Pisum sativum	Hungary	Eastern Europe	Donated	Yellow
203	PI 324697 PSP	Gris D'Hiver	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
204	PI 324702 PSP	UNRRA/972/	Pisum sativum	Hungary	Eastern Europe	Donated	Yellow
205	PI 324703 PSP	Wunterbergische-Wintererbse Pop.	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
206	PI 324706 PSP	No. 833	Pisum sativum	Romania	Western Europe	Collected	Mixed
207	PI 331413 PSP	Col. R-44	Pisum sativum	Ethiopia	Africa	Collected	Mixed
208	PI 331414 PSP	Col. 795-B	Pisum sativum	Ethiopia	Africa	Collected	Mixed
209	PI 340128 PSP	1-301	Pisum sativum	Turkey	Mediterranean	Donated	Yellow
210	PI 340130 PSP	1-305	Pisum sativum	Turkey	Mediterranean	Donated	Yellow
211	PI 341889 PSP	Elwy	Pisum sativum	Netherlands	Western Europe	Donated	Mixed
212	PI 343292 PSP	-	Pisum sativum	USA	North America	Donated	Mixed
213	PI 343321 PSP	-	Pisum sativum	USA	North America	Donated	Yellow
214	PI 343331 PSP	-	Pisum sativum	USA	North America	Donated	Mixed
215	PI 343338 PSP	-	Pisum sativum	USA	North America	Donated	Mixed
216	PI 343824 PSP	Col. 6946	Pisum sativum	Uganda	Africa	Collected	Yellow

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
217	PI 343958 PSP	Biselia	Pisum sativum	Turkey	Mediterranean	Collected	Mixed
218	PI 343987 PSP	22718	P. sativum subsp. sativum	Turkey	Mediterranean	Collected	Mixed
219	PI 344003 PSP	22703	Pisum sativum	Turkey	Mediterranean	Collected	Yellow
220	PI 344010 PSP	22732	P. sativum subsp. elatius	Greece	Mediterranean	Collected	Yellow
221	PI 344013 PSP	22735	P. sativum subsp. elatius	Greece	Mediterranean	Collected	Mixed
222	PI 344538 PSP	2	P. sativum subsp. elatius	Italy	Mediterranean	Collected	Mixed
223	PI 347281 PSP	PLP 10	Pisum sativum	India	Asia	Collected	Yellow
224	PI 347295 PSP	PLP 26	Pisum sativum	India	Asia	Collected	Mixed
225	PI 347329 PSP	-	Pisum sativum	India	Asia	Collected	Mixed
226	PI 347457 PSP	PLP 264	Pisum sativum	India	Asia	Collected	Yellow
227	PI 347477 PSP	PLP 503	Pisum sativum	India	Asia	Collected	Mixed
228	PI 347490 PSP	PLP 82	Pisum sativum	India	Asia	Collected	Mixed
229	PI 347496 PSP	PLP 105A	Pisum sativum	India	Asia	Collected	Mixed
230	PI 355906 PSP	Komidori	Pisum sativum	Japan	Asia	Donated	Mixed
231	PI 356973 PSP	PLP 35	Pisum sativum	India	Asia	Collected	Mixed
232	PI 356974 PSP	PLP 120	Pisum sativum	India	Asia	Collected	Green
233	PI 356980 PSP	PLP 16A	Pisum sativum	India	Asia	Collected	Yellow
234	PI 356984 PSP	PLP 70	Pisum sativum	India	Asia	Collected	Yellow
235	PI 356986 PSP	PLP 174	Pisum sativum	India	Asia	Collected	Yellow
236	PI 356991 PSP	PLP 196	Pisum sativum	India	Asia	Collected	Mixed
237	PI 356992 PSP	PLP 197A	Pisum sativum	India	Asia	Collected	Yellow
238	PI 357290 PSP	Brzak	Pisum sativum	Macedonia	Eastern Europe	Collected	Mixed
239	PI 358300 PSP	27b	Pisum sativum	Ethiopia	Africa	Collected	Yellow
240	PI 358613 PSP	22867	Pisum abyssinicum	Ethiopia	Africa	Collected	Yellow

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
241	PI 358620 PSP	22758	Pisum sativum	Ethiopia	Africa	Collected	Mixed
242	PI 358633 PSP	22778	Pisum sativum	Ethiopia	Africa	Collected	Yellow
243	PI 358640 PSP	22791	Pisum sativum	Ethiopia	Africa	Collected	Mixed
244	PI 365419 PSP	BR 1-49-9	Pisum sativum	Canada	North America	Donated	Mixed
245	PI 371796 PSP	-	Pisum sativum	New Zealand	Oceana	Developed	Yellow
246	PI 378157 PSP	Red Flower No. 1	Pisum sativum	Malaysia	Asia	Collected	Yellow
247	PI 381334 PSP	Imposant Brown	Pisum sativum	Netherlands	Western Europe	Donated	Yellow
248	PI 393488 PSP	Klatovska Ozima	Pisum sativum	Czech Republic	Western Europe	Donated	Yellow
249	PI 393489 PSP	Munchen Weisbluhende	Pisum sativum	Czech Republic	Western Europe	Donated	Mixed
250	PI 393490 PSP	-	Pisum sativum	Czech Republic	Western Europe	Donated	Yellow
251	PI 404225 PSP	Pinskij Mestnyj	Pisum sativum	Belarus	Eastern Europe	Collected	Yellow
252	PI 409031 PSP	Hohenheimer Rosabluehende	Pisum sativum	Germany	Western Europe	Donated	Yellow
253	PI 411141 PSP	Pania	Pisum sativum	New Zealand	Oceana	Developed	Mixed
254	PI 411142 PSP	Patea	Pisum sativum	New Zealand	Oceana	Developed	Mixed
255	PI 411143 PSP	Piri	Pisum sativum	New Zealand	Oceana	Donated	Green
256	PI 413678 PSP	Zloty Olbrzymi	Pisum sativum	Hungary	Eastern Europe	Donated	Yellow
257	PI 413683 PSP	Lancet	Pisum sativum	Hungary	Eastern Europe	Donated	Green
258	PI 413685 PSP	NZ 51	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
259	PI 413688 PSP	Ujmajori Korai Viktoria	Pisum sativum	Hungary	Eastern Europe	Donated	Yellow
260	PI 413698 PSP	Grune Perle	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
261	PI 413703 PSP	I.P. 2	Pisum sativum	Hungary	Eastern Europe	Donated	Mixed
262	PI 429839 PSP	Musus	Pisum sativum	Afghanistan	Asia	Collected	Yellow
263	PI 429843 PSP	Torsdag	Pisum sativum	Latvia	Eastern Europe	Collected	Yellow
264	PI 429845 PSP	Kombajnovyj 5	Pisum sativum	Russia	Eastern Europe	Collected	Yellow

Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
265	PI 429849 PSP	Uzbekskij	Pisum sativum	Uzbekistan	Eastern Europe	Collected	Yellow
266	PI 430702 PSP	Bardi	Pisum sativum	Hungary	Eastern Europe	Donated	Green
267	PI 476409 PSP	Rota	Pisum sativum	Latvia	Eastern Europe	Collected	Yellow
268	PI 476413 PSP	Ul'Ianovskij-72	Pisum sativum	Russia	Eastern Europe	Collected	Yellow
269	PI 477371 PSP	Rosakrone	Pisum sativum	Denmark	Western Europe	Developed	Yellow
270	PI 486131 PSP	E8454-A-F	Pisum sativum	Ecuador	South America	Collected	Yellow
271	PI 494077 PSP	-	Pisum sativum	Chile	South America	Collected	Mixed
272	PI 499982 PSP	-	Pisum sativum	China	Asia	Donated	Yellow
273	PI 505059 PSP	ILCA 5076	P. sativum subsp. elatius	Sudan	Africa	Collected	Yellow
274	PI 505062 PSP	ILCA 5005	P. sativum subsp. sativum	Greece	Mediterranean	Collected	Yellow
275	PI 505080 PSP	ILCA 5039	P. sativum subsp. sativum	Cyprus	Mediterranean	Collected	Yellow
276	PI 505108 PSP	ILCA 5072	P. sativum subsp. sativum	Greece	Mediterranean	Collected	Yellow
277	PI 505122 PSP	ILCA 5089	P. sativum subsp. sativum	Albania	Mediterranean	Collected	Yellow
278	PI 505127 PSP	ILCA 5094	P. sativum subsp. sativum	Albania	Mediterranean	Collected	Yellow
279	PI 505144 PSP	ILCA 5115	P. sativum subsp. sativum	Spain	Mediterranean	Collected	Green
280	PI 594358 PSP	Delta	Pisum sativum	USA	North America	Developed	Yellow
281	PI 601516 PSP	Solara	Pisum sativum	Netherlands	Western Europe	Developed	Green
282	PI 619079 PSP	Shawnee	Pisum sativum	USA	North America	Developed	Yellow
283	PI 628276 PSP	Lifter	Pisum sativum	USA	North America	Developed	Green
284	PI 633698 PSP	Ds Admiral	Pisum sativum	Denmark	Western Europe	Developed	Yellow
285	PI 634571 PSP	Stirling	Pisum sativum	USA	North America	Developed	Green
286	PI 638516 PSP	SW Carousel	Pisum sativum	USA	North America	Developed	Yellow
287	PI 639967 PSP	VIR K-7290	P. sativum subsp. asiaticum	India	Asia	Collected	Yellow
288	PI 639968 PSP	-	P. sativum subsp. asiaticum	Nepal	Asia	Collected	Yellow

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Experiment Inventory #	PSP Plant ID	Plant Name	Taxonomy	Origin	Region	Status	Cotyledon Color
289	PI 639969 PSP	-	P. sativum subsp. asiaticum	Nepal	Asia	Collected	Yellow
290	PI 639974 PSP	VIR 2743	P. sativum subsp. elatius	Georgia	Eastern Europe	Collected	Yellow
291	PI 639976 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
292	PI 639977 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
293	PI 639978 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
294	PI 639979 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
295	PI 639980 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
296	PI 639981 PSP	-	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
297	W6 12723 PSP	Pleven 2	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
298	W6 12739 PSP	Markova 1	P. sativum var. arvense	Bulgaria	Western Europe	Donated	Yellow
299	W6 15008 PSP	VIR 2524	P. sativum subsp. elatius	Israel	Middle East	Collected	Yellow
300	W6 15010 PSP	VIR 2998	P. sativum subsp. elatius	Latvia	Eastern Europe	Collected	Yellow
301	W6 15019 PSP	VIR 7328	Pisum sativum var. pumilio	Turkey	Mediterranean	Collected	Yellow
302	W6 15028 PSP	VIR K-2381	P. sativum subsp. transcaucasicum	-	-	Collected	Yellow
303	W6 15044 PSP	54-760	P. sativum subsp. elatius	-	-	Collected	Yellow
304	W6 15048 PSP	54-764	Pisum sativum var. pumilio	-	-	Collected	Yellow
305	W6 17293 PSP	PAK10198	P. sativum var. arvense	Pakistan	Asia	Collected	Yellow
306	W6 26109 PSP	G0801	P. sativum subsp. elatius	Georgia	Eastern Europe	Collected	-
307	W6 26154 PSP	G2501	P. sativum var. arvense	Georgia	Eastern Europe	Collected	Yellow
308	W6 26157 PSP	-	P. sativum var. arvense	Georgia	Eastern Europe	Collected	Yellow
309	W6 26160 PSP	Levakhane	P. sativum var. arvense	Georgia	Eastern Europe	Collected	Yellow
310	W6 26161 PSP	-	P. sativum var. arvense	Georgia	Eastern Europe	Collected	Yellow
311	W6 31707 PSP	-	P. sativum var. sativum	Russia	Eastern Europe	Collected	Green
312	W6 39726 PSP	Columbian L1	Pisum sativum	USA	North America	Developed	Green

APPENDIX B

DATA TABLES

Table B1. Mean squares error, degrees of freedom, mean, coefficient of variation, MSD, and F_{max} calculation for all runs of the PSP core collection for tolerance to salt conditions.

Trait	Run	MSError	Df	Mean	CV	MSD	F-max
% Germination Compared to Control	GC01	210.27	44	72.27	20.06	45.57	
	GC02	105.59	45	78.70	13.06	-	
	GC03	159.02	46	63.71	19.79	39.78	
	GC04	295.91	45	61.04	28.18	-	
	GC05	196.81	46	61.98	22.63	44.26	
	GC06	248.65	45	64.12	24.59	-	
	GC07	153.74	45	56.61	21.90	-	
	GC08	163.01	43	68.45	18.65	-	
	GC09	203.58	46	58.73	24.30	45.02	
	GC10	173.83	45	60.44	21.81	-	
	GC11	225.69	43	69.32	21.67	-	
	GC12	350.60	44	64.31	29.12	58.85	
	GC13	171.73	46	61.67	21.25	41.34	
	GC14	130.41	37	66.82	17.09	-	
							3.32
% Weight Compared to Control	GH01	132.03	55	42.72	26.90	-	
	GH02	32.78	74	40.74	14.05	18.83	
	GH03	41.95	74	35.63	18.18	21.30	
	GH04	58.98	74	43.53	17.64	25.25	
	GH05	55.88	74	41.38	18.06	24.58	
	GH06	78.88	72	53.59	16.57	-	
	GH07	59.42	71	54.74	14.08	-	
	GH08	92.50	74	70.09	13.72	31.63	
	GH09	81.82	72	55.75	16.22	29.67	
							4.03
% Height Compared to Control	GH01	127.01	55	55.44	20.33	-	
	GH02	32.00	74	52.93	10.69	18.60	
	GH03	33.91	74	45.94	12.67	19.15	
	GH04	58.42	74	51.46	14.85	25.13	
	GH05	36.80	74	51.29	11.83	19.95	
	GH06	34.20	73	56.05	10.43	-	
	GH07	46.75	74	59.05	11.58	22.48	
	GH08	39.57	74	61.80	10.18	20.69	
	GH09	34.35	72	54.58	10.74	19.23	
							3.97
Score	GH01	2.57	55	7.49	21.41	-	
	GH02	1.75	74	7.00	18.91	4.35	
	GH03	1.33	74	8.30	13.91	3.80	
	GH04	1.44	74	8.34	14.39	3.95	
	GH05	2.39	74	6.75	22.94	5.09	
	GH06	2.23	74	4.73	31.60	4.91	
	GH07	1.31	74	5.46	20.97	3.77	
	GH08	1.03	74	3.43	29.64	3.34	
	GH09	2.16	72	4.75	30.93	4.82	
							2.49
AUIC	GH01	289.08	55	64.74	26.26	-	
	GH02	146.15	74	47.69	25.35	39.75	
	GH03	173.34	74	68.34	19.26	43.29	
	GH04	204.02	74	66.62	21.44	46.97	
	GH05	155.34	74	47.40	26.30	40.98	
	GH06	92.98	74	34.57	27.90	31.71	
	GH07	74.33	74	39.02	22.09	28.35	
	GH08	54.86	74	33.28	22.26	24.36	
	GH09	101.93	72	40.14	25.16	33.12	
							5.27

Table B2. The overall top performing PSP core collection accessions across the germination and greenhouse screening experiments along with country of origin and cotyledon color descriptors.

Trait	Inventory Number	Name	Origin	Cotyledon	% Germination	% Biomass	% Height	Score	AUIC
Germination	59	PI195404	Guatemala	Mixed	100.0	30.0	42.8	8.0	62.8
	111	PI250447	Czech Republic	Mixed	100.0	44.1	52.3	6.7	43.8
	159	PI274307	Pakistan	Mixed	100.0	35.1	49.3	7.0	60.7
	40	PI179450	Syria	Mixed	99.8	30.3	49.8	7.3	46.5
	60	PI195631	Ethiopia	Yellow	98.4	38.0	48.1	6.3	46.3
	62	PI197990	Netherlands	Mixed	98.4	33.9	49.7	8.0	59.5
	73	PI204306	Australia	Yellow	98.4	43.5	53.9	9.0	67.0
	76	PI206838	USA	Mixed	98.4	49.8	57.9	5.7	43.7
	130	PI269761	Czech Republic	Mixed	98.4	36.5	49.5	9.0	72.7
	262	PI429839	Afghanistan	Yellow	98.3	67.8	66.2	3.3	31.2
Total Biomass	285	PI634571	USA	Green	78.9	134.9	78.7	1.7	19.7
	258	PI413685	Hungary	Mixed	93.3	101.4	70.1	2.7	29.2
	281	PI601516	Netherlands	Green	61.4	100.8	74.2	1.7	21.3
	286	PI638516	USA	Yellow	30.7	93.9	71.0	2.0	21.8
	284	PI633698	Denmark	Yellow	37.3	89.6	61.4	3.0	26.7
	253	PI411141	New Zealand	Mixed	80.9	87.9	64.1	2.3	28.7
	193	PI288263	Germany	Mixed	81.0	86.3	68.5	5.3	39.2
	225	PI347329	India	Mixed	58.5	85.8	65.5	3.3	25.5
	257	PI413683	Hungary	Green	75.4	80.8	59.7	2.0	28.2
	255	PI411143	New Zealand	Green	89.5	78.7	61.0	2.0	26.5

Table B2. The overall top performing PSP core collection accessions across the germination and greenhouse screening experiments along with country of origin and cotyledon color descriptors (Continued).

Trait	Inventory Number	Name	Origin	Cotyledon	% Germination	% Biomass	% Height	Score	AUIC
Plant Height	285	PI634571	USA	Green	78.9	134.9	78.7	1.7	19.7
	216	PI343824	Uganda	Yellow	22.9	67.0	77.4	4.0	31.2
	281	PI601516	Netherlands	Green	61.4	100.8	74.2	1.7	21.3
	3	PI116056	India	Yellow	91.6	60.9	74.2	4.0	29.8
	189	PI286430	Nepal	Mixed	33.3	57.0	74.1	4.3	32.7
	279	PI505144	Spain	Green	61.7	62.2	72.9	2.3	24.0
	219	PI344003	Turkey	Yellow	61.1	60.0	72.3	5.3	42.8
	265	PI429849	Uzbekistan	Yellow	80.0	76.0	71.5	1.7	23.7
	312	W639726	USA	Green	89.6	56.1	71.4	4.7	34.8
	287	PI639967	India	Yellow	92.1	75.3	71.2	2.7	26.2

Table B2. The overall top performing PSP core collection accessions across the germination and greenhouse screening experiments along with country of origin and cotyledon color descriptors (Continued).

Trait	Inventory Number	Name	Origin	Cotyledon	% Germination	% Biomass	% Height	Score	AUIC
Final Score	285	PI634571	USA	Green	78.9	134.9	78.7	1.7	19.7
	281	PI601516	Netherlands	Green	61.4	100.8	74.2	1.7	21.3
	265	PI429849	Uzbekistan	Yellow	80.0	76.0	71.5	1.7	23.7
	277	PI505122	Albania	Yellow	56.7	65.6	65.9	1.7	21.3
	286	PI638516	USA	Yellow	30.7	93.9	71.0	2.0	21.8
	280	PI594358	USA	Yellow	69.0	69.9	61.2	2.0	20.2
	255	PI411143	New Zealand	Green	89.5	78.7	61.0	2.0	26.5
	257	PI413683	Hungary	Green	75.4	80.8	59.7	2.0	28.2
	254	PI411142	New Zealand	Mixed	85.0	77.0	55.1	2.0	25.8
	279	PI505144	Spain	Green	61.7	62.2	72.9	2.3	24.0
	253	PI411141	New Zealand	Mixed	80.9	87.9	64.1	2.3	28.7
	250	PI393490	Czech Rep.	Yellow	65.6	74.0	59.4	2.3	22.3
	261	PI413703	Hungary	Mixed	56.9	65.3	49.2	2.3	24.0
	287	PI639967	India	Yellow	92.1	75.3	71.2	2.7	26.2
	258	PI413685	Hungary	Mixed	93.3	101.4	70.1	2.7	29.2
	278	PI505127	Albania	Yellow	83.3	61.3	56.4	2.7	26.8
	249	PI393489	Czech Rep.	Mixed	63.3	64.7	54.3	2.7	26.2
	163	PI275822	Sweden	Mixed	54.0	57.0	52.6	2.7	21.2
	182	PI285724	Poland	Yellow	38.1	57.9	45.4	2.7	21.2
	242	PI358633	Ethiopia	Yellow	63.0	61.5	65.3	3.0	25.0

Table B2. The overall top performing PSP core collection accessions across the germination and greenhouse screening experiments along with country of origin and cotyledon color descriptors (Continued).

Trait	Inventory Number	Name	Origin	Cotyledon	% Germination	% Biomass	% Height	Score	AUIC
AUIC	285	PI634571	USA	Green	78.9	134.9	78.7	1.7	19.7
	280	PI594358	USA	Yellow	69.0	69.9	61.2	2.0	20.2
	163	PI275822	Sweden	Mixed	54.0	57.0	52.6	2.7	21.2
	182	PI285724	Poland	Yellow	38.1	57.9	45.4	2.7	21.2
	281	PI601516	Netherlands	Green	61.4	100.8	74.2	1.7	21.3
	277	PI505122	Albania	Yellow	56.7	65.6	65.9	1.7	21.3
	286	PI638516	USA	Yellow	30.7	93.9	71.0	2.0	21.8
	250	PI393490	Czech Rep.	Yellow	65.6	74.0	59.4	2.3	22.3
	194	PI293426	Bulgaria	Mixed	66.7	51.8	59.1	3.0	23.3
	265	PI429849	Uzbekistan	Yellow	80.0	76.0	71.5	1.7	23.7
	279	PI505144	Spain	Green	61.7	62.2	72.9	2.3	24.0
	261	PI413703	Hungary	Mixed	56.9	65.3	49.2	2.3	24.0
	242	PI358633	Ethiopia	Yellow	63.0	61.5	65.3	3.0	25.0
	175	PI280616	Belarus	Mixed	65.0	45.6	52.4	4.3	25.3
	199	PI314795	Australia	Mixed	47.3	60.2	51.1	4.3	25.3
	225	PI347329	India	Mixed	58.5	85.8	65.5	3.3	25.5
	254	PI411142	New Zealand	Mixed	85.0	77.0	55.1	2.0	25.8
	180	PI285718	Poland	Mixed	-	59.2	60.4	3.7	26.0
	187	PI285740	Poland	Mixed	85.4	65.1	47.8	3.7	26.0
	287	PI639967	India	Yellow	92.1	75.3	71.2	2.7	26.2

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection.

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
1	PI 102888 PSP	89.6	43.2	55.8	6.0	51.5
2	PI 109866 PSP	83.7	24.3	38.4	8.3	96.2
3	PI 116056 PSP	91.6	60.9	74.2	4.0	29.8
4	PI 116844 PSP	40.2	43.4	54.6	6.3	54.0
5	PI 116944 PSP	14.4	38.2	46.2	5.0	34.3
6	PI 117264 PSP	92.9	34.9	51.5	8.3	76.2
7	PI 117998 PSP	-	37.2	53.3	6.0	55.0
8	PI 118501 PSP	48.5	25.1	41.5	8.7	78.8
9	PI 121352 PSP	82.5	37.2	50.8	9.0	82.7
10	PI 124478 PSP	90.4	40.3	48.0	9.0	89.5
11	PI 125839 PSP	77.3	47.7	57.8	7.7	61.2
12	PI 125840 PSP	82.8	40.7	41.2	8.0	55.0
13	PI 134271 PSP	44.8	35.7	58.8	4.3	39.3
14	PI 137119 PSP	95.4	43.8	49.0	9.0	73.7
15	PI 140298 PSP	70.2	40.1	62.4	6.0	55.3
16	PI 142775 PSP	55.2	41.9	43.5	5.7	49.0
17	PI 143485 PSP	79.8	64.6	57.7	7.0	58.2
18	PI 155109 PSP	96.8	61.7	58.8	4.7	39.0
19	PI 156647 PSP	88.3	45.6	50.0	8.3	61.2
20	PI 156720 PSP	67.0	53.1	62.1	9.0	87.7
21	PI 162909 PSP	91.6	25.7	54.5	9.0	95.8
22	PI 163126 PSP	66.4	42.0	63.5	6.7	68.5
23	PI 163129 PSP	72.1	43.5	58.6	9.0	74.5
24	PI 164548 PSP	68.3	64.6	68.3	9.0	78.5
25	PI 164612 PSP	79.4	36.4	65.8	8.3	73.8
26	PI 164779 PSP	96.8	46.4	62.3	8.0	62.7
27	PI 164971 PSP	98.1	45.2	61.2	5.7	47.2
28	PI 164972 PSP	80.0	36.7	55.4	9.0	83.3
29	PI 165949 PSP	72.4	35.6	52.3	6.7	46.2
30	PI 166084 PSP	93.7	28.3	47.2	8.3	60.0
31	PI 166159 PSP	92.0	34.2	47.0	7.7	41.7
32	PI 169603 PSP	81.7	48.8	61.2	6.3	47.0
33	PI 169608 PSP	85.2	35.6	50.1	7.3	46.2
34	PI 171810 PSP	86.1	33.2	45.2	8.3	68.7
35	PI 172339 PSP	77.8	43.6	43.2	7.3	51.5

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
36	PI 173840 PSP	91.7	46.3	60.1	8.0	54.2
37	PI 174320 PSP	84.1	43.3	56.4	6.0	44.2
38	PI 174921 PSP	82.5	45.5	50.9	8.7	50.2
39	PI 175231 PSP	90.6	38.0	58.8	6.7	38.5
40	PI 179450 PSP	99.8	30.3	49.8	7.3	46.5
41	PI 179451 PSP	63.8	41.6	59.3	5.3	33.5
42	PI 179459 PSP	54.1	44.5	51.9	7.0	47.7
43	PI 179722 PSP	80.9	48.0	59.1	7.3	42.2
44	PI 179970 PSP	84.3	44.4	44.0	6.0	39.5
45	PI 180329 PSP	92.0	43.2	58.2	5.7	44.3
46	PI 180693 PSP	93.6	47.9	60.8	7.3	44.8
47	PI 180696 PSP	25.0	34.5	52.6	7.7	57.3
48	PI 180699 PSP	11.9	24.9	48.6	8.0	67.5
49	PI 180702 PSP	90.5	38.9	48.3	8.0	54.8
50	PI 181801 PSP	37.8	44.8	53.6	7.7	42.7
51	PI 181958 PSP	50.0	46.6	57.9	7.3	46.5
52	PI 183467 PSP	88.9	55.0	62.1	5.0	31.3
53	PI 184130 PSP	54.4	39.9	56.6	7.3	62.5
54	PI 184784 PSP	63.5	29.9	45.6	7.3	59.2
55	PI 193578 PSP	87.0	46.5	59.2	5.3	34.2
56	PI 193584 PSP	87.3	48.6	63.2	5.7	34.0
57	PI 193590 PSP	89.3	32.7	54.5	7.3	45.5
58	PI 195020 PSP	72.0	47.0	56.2	6.7	41.8
59	PI 195404 PSP	100.0	30.0	42.8	8.0	62.8
60	PI 195631 PSP	98.4	38.0	48.1	6.3	46.3
61	PI 197044 PSP	38.6	40.1	60.0	6.7	43.8
62	PI 197990 PSP	98.4	33.9	49.7	8.0	59.5
63	PI 198072 PSP	92.1	48.3	50.6	7.7	47.7
64	PI 198074 PSP	21.6	40.5	45.8	7.3	53.5
65	PI 198735 PSP	57.1	20.2	31.8	8.3	77.3
66	PI 200755 PSP	19.1	30.0	42.9	9.0	73.3
67	PI 201390 PSP	49.2	35.5	45.7	9.0	68.0
68	PI 203064 PSP	88.9	37.8	53.5	8.3	58.0
69	PI 203066 PSP	52.6	34.7	39.6	9.0	86.7
70	PI 203067 PSP	13.9	27.9	40.4	9.0	79.7

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
71	PI 203068 PSP	33.3	29.7	37.3	9.0	82.0
72	PI 203069 PSP	63.3	33.3	51.3	9.0	75.7
73	PI 204306 PSP	98.4	43.5	53.9	9.0	67.0
74	PI 206006 PSP	95.2	31.1	52.9	9.0	79.7
75	PI 206816 PSP	72.2	44.4	58.8	7.0	61.0
76	PI 206838 PSP	98.4	49.8	57.9	5.7	43.7
77	PI 206861 PSP	96.7	34.9	46.1	9.0	70.3
78	PI 207508 PSP	73.0	21.0	33.7	7.7	68.0
79	PI 209507 PSP	60.3	28.5	43.4	9.0	73.3
80	PI 210558 PSP	69.6	40.7	55.7	6.7	54.2
81	PI 210561 PSP	71.9	39.2	41.7	9.0	64.0
82	PI 210568 PSP	35.2	34.7	45.1	9.0	79.7
83	PI 210569 PSP	17.8	37.3	57.2	9.0	70.0
84	PI 210571 PSP	55.0	32.8	39.8	9.0	82.0
85	PI 210583 PSP	72.2	33.8	49.6	8.0	62.8
86	PI 212031 PSP	52.1	29.5	33.5	6.0	56.8
87	PI 220174 PSP	38.9	38.1	39.9	5.7	49.3
88	PI 220189 PSP	64.7	28.8	38.6	6.7	68.2
89	PI 221697 PSP	22.2	32.3	43.9	9.0	71.7
90	PI 222071 PSP	23.3	39.3	41.0	7.7	55.7
91	PI 222117 PSP	68.3	30.7	40.9	7.3	65.5
92	PI 223527 PSP	93.3	29.9	38.2	7.3	64.8
93	PI 227258 PSP	90.5	34.2	52.0	9.0	71.0
94	PI 236492 PSP	88.3	48.0	58.7	9.0	71.0
95	PI 240516 PSP	38.5	40.7	46.1	9.0	72.0
96	PI 241593 PSP	93.3	48.7	55.9	8.3	66.7
97	PI 242027 PSP	88.7	37.3	46.1	8.0	58.8
98	PI 242028 PSP	64.3	37.6	48.7	9.0	78.0
99	PI 244093 PSP	78.6	36.1	52.5	9.0	66.3
100	PI 244121 PSP	38.3	36.1	32.7	9.0	72.7
101	PI 244150 PSP	80.7	42.8	55.0	9.0	76.7
102	PI 244175 PSP	80.8	53.8	54.0	9.0	64.7
103	PI 244191 PSP	77.8	43.9	54.4	7.0	59.7
104	PI 248181 PSP	13.7	39.5	57.8	8.7	75.2
105	PI 249645 PSP	65.1	51.6	51.9	8.3	56.0

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
106	PI 250438 PSP	51.6	51.6	52.6	9.0	71.0
107	PI 250439 PSP	68.3	48.4	64.2	8.7	77.5
108	PI 250440 PSP	60.3	37.9	41.5	9.0	80.3
109	PI 250441 PSP	76.2	33.4	46.1	9.0	86.0
110	PI 250446 PSP	88.9	50.8	48.8	7.7	47.7
111	PI 250447 PSP	100.0	44.1	52.3	6.7	43.8
112	PI 250448 PSP	52.9	34.3	47.5	9.0	79.0
113	PI 253968 PSP	52.4	36.0	43.2	8.3	51.0
114	PI 257244 PSP	90.5	27.8	43.7	9.0	77.3
115	PI 257592 PSP	84.1	25.8	40.4	9.0	93.7
116	PI 261622 PSP	38.8	46.8	48.7	8.7	68.8
117	PI 261623 PSP	96.7	42.9	49.3	9.0	78.7
118	PI 261624 PSP	83.4	48.8	62.5	8.3	64.3
119	PI 261636 PSP	44.4	56.7	60.1	7.0	44.7
120	PI 261671 PSP	38.1	55.1	55.6	7.3	53.2
121	PI 261677 PSP	90.5	26.6	47.0	9.0	80.3
122	PI 263014 PSP	40.0	60.4	52.4	4.3	39.0
123	PI 263027 PSP	65.1	42.8	52.8	7.0	58.0
124	PI 263030 PSP	41.7	40.8	44.8	9.0	88.3
125	PI 263031 PSP	52.4	45.7	53.8	9.0	79.3
126	PI 263032 PSP	55.6	52.3	64.6	7.7	49.3
127	PI 263871 PSP	-	73.1	49.1	3.3	33.5
128	PI 266070 PSP	-	47.9	51.4	5.3	39.8
129	PI 269543 PSP	-	45.3	47.5	6.0	50.5
130	PI 269761 PSP	98.4	36.5	49.5	9.0	72.7
131	PI 269762 PSP	70.8	67.7	58.8	4.7	30.8
132	PI 269770 PSP	72.2	50.2	49.4	9.0	62.0
133	PI 269777 PSP	22.9	25.7	41.3	9.0	95.3
134	PI 269778 PSP	81.7	47.3	49.3	8.0	63.5
135	PI 269782 PSP	42.8	49.5	58.7	9.0	62.7
136	PI 269791 PSP	91.7	37.8	45.1	9.0	73.3
137	PI 269802 PSP	94.1	50.9	55.6	7.0	50.0
138	PI 269804 PSP	61.2	39.0	48.5	9.0	59.7
139	PI 269812 PSP	18.3	35.2	49.1	9.0	77.3
140	PI 269818 PSP	96.8	45.8	56.3	8.0	61.8

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
141	PI 269822 PSP	60.0	49.0	55.2	5.0	45.7
142	PI 269825 PSP	63.5	39.5	53.4	7.3	47.8
143	PI 270536 PSP	32.3	51.8	62.0	6.3	44.0
144	PI 271035 PSP	50.3	48.9	62.2	6.3	49.3
145	PI 271038 PSP	67.3	51.2	54.4	6.7	37.8
146	PI 271116 PSP	29.8	44.0	57.6	7.3	49.5
147	PI 271511 PSP	83.4	44.4	55.1	5.3	35.2
148	PI 272148 PSP	63.5	36.7	49.2	6.3	48.7
149	PI 272171 PSP	72.2	35.8	46.2	9.0	54.7
150	PI 272175 PSP	96.8	37.4	57.7	7.3	60.5
151	PI 272184 PSP	-	33.9	45.8	6.7	42.8
152	PI 272194 PSP	46.0	53.1	62.1	6.7	43.5
153	PI 272204 PSP	62.7	31.2	43.2	7.7	65.0
154	PI 272215 PSP	25.4	38.3	49.5	8.0	52.8
155	PI 272216 PSP	53.4	33.7	49.8	7.0	53.0
156	PI 272218 PSP	44.4	44.9	45.8	6.0	46.5
157	PI 273209 PSP	17.9	26.2	35.7	7.3	69.5
158	PI 273605 PSP	46.2	39.6	42.5	7.0	49.7
159	PI 274307 PSP	100.0	35.1	49.3	7.0	60.7
160	PI 274308 PSP	89.1	27.3	47.5	7.0	65.7
161	PI 274584 PSP	71.4	48.1	52.9	5.3	47.8
162	PI 275821 PSP	-	49.8	46.3	5.7	42.0
163	PI 275822 PSP	54.0	57.0	52.6	2.7	21.2
164	PI 275825 PSP	89.5	30.1	47.0	9.0	65.0
165	PI 277852 PSP	67.2	26.8	45.9	8.3	68.0
166	PI 279823 PSP	23.5	49.9	55.3	7.0	37.7
167	PI 279825 PSP	61.1	47.0	41.4	9.0	53.0
168	PI 279827 PSP	95.2	48.7	56.5	5.0	28.0
169	PI 280252 PSP	64.8	34.4	49.4	8.7	58.2
170	PI 280603 PSP	67.2	35.8	44.0	7.0	45.7
171	PI 280609 PSP	94.3	36.6	42.1	6.7	37.8
172	PI 280611 PSP	36.3	37.9	57.9	8.3	69.7
173	PI 280613 PSP	90.4	34.7	50.3	8.0	48.2
174	PI 280614 PSP	92.2	46.2	51.9	7.0	51.3
175	PI 280616 PSP	65.0	45.6	52.4	4.3	25.3

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
176	PI 280617 PSP	91.6	36.8	49.9	6.3	35.0
177	PI 280619 PSP	29.7	40.6	51.0	6.7	37.2
178	PI 280626 PSP	88.9	45.0	56.1	6.7	37.2
179	PI 285710 PSP	67.8	44.9	51.4	4.3	32.0
180	PI 285718 PSP	-	59.2	60.4	3.7	26.0
181	PI 285722 PSP	72.5	55.1	47.5	3.7	28.3
182	PI 285724 PSP	38.1	57.9	45.4	2.7	21.2
183	PI 285727 PSP	41.2	52.1	58.9	4.3	30.3
184	PI 285730 PSP	48.3	47.4	54.3	4.7	34.8
185	PI 285737 PSP	15.9	58.8	54.7	5.7	34.0
186	PI 285739 PSP	87.9	52.0	59.7	4.3	39.0
187	PI 285740 PSP	85.4	65.1	47.8	3.7	26.0
188	PI 285747 PSP	54.9	49.4	63.9	4.0	28.2
189	PI 286430 PSP	33.3	57.0	74.1	4.3	32.7
190	PI 286431 PSP	85.7	67.6	56.7	3.7	30.7
191	PI 286607 PSP	81.0	62.0	64.4	4.7	38.2
192	PI 288025 PSP	90.6	50.9	45.6	5.0	33.7
193	PI 288263 PSP	81.0	86.3	68.5	5.3	39.2
194	PI 293426 PSP	66.7	51.8	59.1	3.0	23.3
195	PI 306591 PSP	38.1	56.2	51.4	5.0	31.3
196	PI 307666 PSP	66.7	43.4	54.1	4.7	43.5
197	PI 308796 PSP	77.8	53.2	59.5	4.3	36.7
198	PI 314794 PSP	69.8	43.4	51.3	6.3	44.7
199	PI 314795 PSP	47.3	60.2	51.1	4.3	25.3
200	PI 319374 PSP	71.4	44.5	53.4	4.7	33.2
201	PI 320972 PSP	75.0	46.1	51.7	6.3	35.7
202	PI 324695 PSP	28.0	39.9	50.1	6.3	58.3
203	PI 324697 PSP	54.0	48.8	58.5	4.3	30.3
204	PI 324702 PSP	28.3	43.9	49.7	5.3	33.5
205	PI 324703 PSP	33.8	64.4	58.7	4.3	27.0
206	PI 324706 PSP	49.0	30.1	43.8	7.3	53.8
207	PI 331413 PSP	56.7	57.4	61.4	5.0	38.7
208	PI 331414 PSP	73.3	44.6	56.6	5.0	36.3
209	PI 340128 PSP	71.7	40.6	57.3	5.7	34.0
210	PI 340130 PSP	90.0	47.7	53.6	4.0	28.2

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
211	PI 341889 PSP	77.3	62.7	56.3	4.7	30.8
212	PI 343292 PSP	65.0	54.3	56.9	4.7	29.2
213	PI 343321 PSP	96.5	50.1	51.4	5.3	42.2
214	PI 343331 PSP	73.3	40.7	49.9	6.7	68.2
215	PI 343338 PSP	61.7	59.5	58.9	4.3	28.7
216	PI 343824 PSP	22.9	67.0	77.4	4.0	31.2
217	PI 343958 PSP	96.7	58.9	67.4	4.3	35.7
218	PI 343987 PSP	3.7	52.0	60.1	6.0	46.5
219	PI 344003 PSP	61.1	60.0	72.3	5.3	42.8
220	PI 344010 PSP	71.1	50.8	66.7	5.0	29.7
221	PI 344013 PSP	57.5	-	43.2	6.0	45.8
222	PI 344538 PSP	-	49.8	57.2	4.0	40.2
223	PI 347281 PSP	88.7	37.4	54.4	6.7	59.5
224	PI 347295 PSP	54.5	63.4	63.3	4.0	32.8
225	PI 347329 PSP	58.5	85.8	65.5	3.3	25.5
226	PI 347457 PSP	77.2	52.9	63.7	6.7	41.2
227	PI 347477 PSP	73.3	39.4	60.5	5.3	36.8
228	PI 347490 PSP	60.6	40.1	56.0	7.0	51.3
229	PI 347496 PSP	98.2	44.5	57.6	6.3	43.0
230	PI 355906 PSP	71.7	56.6	50.6	7.0	41.7
231	PI 356973 PSP	91.7	50.2	53.3	5.0	34.3
232	PI 356974 PSP	90.0	53.1	51.6	4.3	30.3
233	PI 356980 PSP	70.0	49.3	55.2	8.3	56.7
234	PI 356984 PSP	93.2	55.8	61.3	5.3	38.2
235	PI 356986 PSP	-	37.2	51.7	6.7	47.5
236	PI 356991 PSP	61.7	57.1	56.1	5.0	32.0
237	PI 356992 PSP	72.4	48.3	50.9	5.7	35.7
238	PI 357290 PSP	93.3	52.1	59.0	8.3	57.7
239	PI 358300 PSP	85.0	60.8	62.4	5.0	31.3
240	PI 358613 PSP	41.5	55.0	65.7	6.3	50.3
241	PI 358620 PSP	36.7	38.4	54.3	7.3	61.5
242	PI 358633 PSP	63.0	61.5	65.3	3.0	25.0
243	PI 358640 PSP	73.3	36.5	49.3	8.0	57.2
244	PI 365419 PSP	86.7	67.7	53.6	3.3	27.8
245	PI 371796 PSP	53.7	47.3	49.5	5.7	38.0

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
246	PI 378157 PSP	67.4	49.8	58.7	6.7	45.2
247	PI 381334 PSP	95.0	70.3	65.1	4.0	32.2
248	PI 393488 PSP	49.5	48.1	52.7	5.3	31.8
249	PI 393489 PSP	63.3	64.7	54.3	2.7	26.2
250	PI 393490 PSP	65.6	74.0	59.4	2.3	22.3
251	PI 404225 PSP	78.3	45.2	57.7	6.7	39.5
252	PI 409031 PSP	87.0	38.5	47.4	7.0	49.7
253	PI 411141 PSP	80.9	87.9	64.1	2.3	28.7
254	PI 411142 PSP	85.0	77.0	55.1	2.0	25.8
255	PI 411143 PSP	89.5	78.7	61.0	2.0	26.5
256	PI 413678 PSP	31.3	56.4	61.9	5.7	54.0
257	PI 413683 PSP	75.4	80.8	59.7	2.0	28.2
258	PI 413685 PSP	93.3	101.4	70.1	2.7	29.2
259	PI 413688 PSP	33.3	51.7	61.0	5.7	53.3
260	PI 413698 PSP	82.6	77.2	51.4	4.3	42.3
261	PI 413703 PSP	56.9	65.3	49.2	2.3	24.0
262	PI 429839 PSP	98.3	67.8	66.2	3.3	31.2
263	PI 429843 PSP	33.3	33.1	43.5	8.0	69.8
264	PI 429845 PSP	81.7	66.5	59.8	4.7	39.8
265	PI 429849 PSP	80.0	76.0	71.5	1.7	23.7
266	PI 430702 PSP	60.2	71.3	69.9	3.3	33.5
267	PI 476409 PSP	26.7	65.4	59.3	5.3	45.5
268	PI 476413 PSP	-	50.0	52.8	6.3	50.3
269	PI 477371 PSP	33.4	55.7	55.2	3.3	36.8
270	PI 486131 PSP	77.2	60.0	49.8	4.0	37.2
271	PI 494077 PSP	74.5	50.3	49.9	6.0	44.2
272	PI 499982 PSP	54.4	57.5	67.0	3.7	32.7
273	PI 505059 PSP	93.3	63.5	62.7	4.0	26.5
274	PI 505062 PSP	88.3	61.2	53.8	3.3	33.5
275	PI 505080 PSP	51.1	67.7	67.3	4.0	39.5
276	PI 505108 PSP	50.0	54.8	56.1	5.3	37.5
277	PI 505122 PSP	56.7	65.6	65.9	1.7	21.3
278	PI 505127 PSP	83.3	61.3	56.4	2.7	26.8
279	PI 505144 PSP	61.7	62.2	72.9	2.3	24.0
280	PI 594358 PSP	69.0	69.9	61.2	2.0	20.2

Table B3. The mean germination and seedling salinity response trait values for all 312 accessions within the *Pisum* single plant core collection (Continued).

Experiment Inventory #	PSP Plant ID	% Germination	% Biomass	% Plant Height	Score	AUIC
281	PI 601516 PSP	61.4	100.8	74.2	1.7	21.3
282	PI 619079 PSP	64.1	50.3	57.0	4.3	44.7
283	PI 628276 PSP	71.1	60.9	53.7	3.0	27.3
284	PI 633698 PSP	37.3	89.6	61.4	3.0	26.7
285	PI 634571 PSP	78.9	134.9	78.7	1.7	19.7
286	PI 638516 PSP	30.7	93.9	71.0	2.0	21.8
287	PI 639967 PSP	92.1	75.3	71.2	2.7	26.2
288	PI 639968 PSP	60.0	63.3	64.3	4.0	42.5
289	PI 639969 PSP	91.4	60.7	52.4	4.7	36.5
290	PI 639974 PSP	15.5	38.8	58.2	3.3	33.5
291	PI 639976 PSP	69.5	56.2	48.1	6.7	54.8
292	PI 639977 PSP	91.8	64.5	62.0	3.7	29.3
293	PI 639978 PSP	60.0	56.3	48.6	3.7	32.3
294	PI 639979 PSP	91.7	57.6	57.5	5.3	53.5
295	PI 639980 PSP	74.2	53.7	49.7	5.0	46.7
296	PI 639981 PSP	80.0	45.0	51.6	3.7	28.3
297	W6 12723 PSP	35.0	67.3	63.9	3.7	28.3
298	W6 12739 PSP	95.3	61.1	61.8	5.0	41.7
299	W6 15008 PSP	9.4	42.7	54.8	6.3	44.7
300	W6 15010 PSP	23.8	33.4	38.0	8.3	55.3
301	W6 15019 PSP	59.0	53.2	59.1	5.3	31.8
302	W6 15028 PSP	91.7	54.9	53.7	5.7	40.3
303	W6 15044 PSP	-	51.5	59.7	5.7	42.0
304	W6 15048 PSP	93.3	65.0	60.1	4.3	29.3
305	W6 17293 PSP	74.1	51.5	47.9	6.0	66.5
306	W6 26109 PSP	-	-	-	-	-
307	W6 26154 PSP	83.3	55.7	56.6	3.7	41.0
308	W6 26157 PSP	46.2	57.2	55.8	4.0	44.8
309	W6 26160 PSP	80.4	55.5	55.2	5.3	52.5
310	W6 26161 PSP	70.3	73.9	62.3	3.0	35.3
311	W6 31707 PSP	87.1	56.1	57.7	6.0	51.2
312	W6 39726 PSP	89.6	56.1	71.4	4.7	34.8