DETERMINING SALT TOLERANCE AMONG SUNFLOWER GENOTYPES

A Thesis

by

LAURA LEE MASOR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Plant Breeding

Determining Salt Tolerance Among Sunflower Genotypes

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Approved by:

Chair of Committee,	Steve Hague
Committee Members,	Kevin Crosby
	C. Wayne Smith
Head of Department,	David D. Baltensperger

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ABSTRACT

Determining Salt Tolerance Among Sunflower Genotypes. (December 2011)

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Chair of Advisory Committee: Dr. Steven Hague

Crop lands around the world are becoming more salt-affected due to natural processes and agricultural practices. Due to this increase of salinization, acquisition of saline tolerant germplasm for breeding purposes is becoming a priority. Although cultivated sunflower is classified as a moderately salt tolerant crop, highly tolerant germplasm may be of value. The goal of this study was to screen *Helianthus* spp. in order to determine the salt tolerance of different genotypes. To accomplish this goal, a novel method of rapid screening was developed. Screening for tolerance at initial growth stages was accomplished by germinating seeds in varying concentrations of NaCl solution in petri dishes. Radicle lengths were measured as an indicator of tolerance. This method identified genotypes that are more tolerant than others during germination. Greenhouse trials were also conducted to ascertain morphological measurements during vegetative stages. Two field locations were chosen to screen germplasm for tolerance through physiological maturity; College Station, TX with low salt concentrations and Pecos, TX with high concentrations of salt in the soil and water. Vegetative growth measurements showed a significant genotype by environment interaction. Due to insect

infestation in both locations, yields could not be accurately measured and thus compared between sites in 2010. Yields between locations in 2011 showed significant differences and identified germplasm more suited for cropping in salt affected soil. Seed oil content was determined with Fourier Transform Near-Infrared Spectroscopy. Seed oil content was not significantly different between locations, but was highly significant between genotypes. These screenings identified genotypes that are more salt tolerant than others.

DEDICATION

For Julie, Jane and June

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I am also very grateful for the support of the National Sunflower Association and the Texas Department of Agriculture. These entities provided the funds that made this study possible.

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1. INTRODUCTION

Salt-affected soils comprise over six percent of the world's arable land. These lands have become salty due to natural processes and human practices. Because these processes are ongoing, lands are continuously becoming affected and abandoned. Salts in the soil solution have adverse effects on plant growth. Plants commonly experience stunting, reduced biomass and leaf burn which ultimately result in reduced yields. Efforts to remediate soils are often expensive and ineffective. In addition, remediation efforts can require vast amounts of fresh water which may be in short supply. Low yields along with the expense of high crop inputs leave growers with lower profits. An alternative to soil remediation is growing crops with salt tolerance. Therefore, farmers around the world in saline and sodic zones could benefit from more suitable crop options.

The genus *Helianthus*, to which sunflower belong, is comprised of 51 species. Cultivated sunflower *(Helianthus annuus L.)* is classified as moderately tolerant to salt, though some wild species are more tolerant (Ashraf and Tufail, 1995). Phenotypic and genotypic studies have shown that sunflower has potential as a crop suitable for farming in places where others cannot be grown. Because of the vast genetic variation found in sunflowers along with the ease of hybridization, breeding for high salt tolerance within this crop is possible.

This thesis follows the style of *Crop Science.*

Sunflower is mainly used as a source of edible seed oil and is gaining in popularity due to newly developed healthful oil (or fatty acid) profiles. In addition to its use as an oil source, sunflowers are grown for ornamental purposes and direct consumption (confectionary or kernel) markets. Sunflower is grown around the world and is a popular crop in countries that have salt affected soils such as India and China. Because of these reasons, breeding for salt tolerant lines of sunflower can be warranted.

One of the first, and perhaps the most important steps, in a breeding program is to identify germplasm with a trait of value. Screening wild species for use in future breeding programs and existing commercial hybrids for immediate use by growers is a worthy goal.

Many methods of screening for salt tolerance in plants have been used in past studies. Some of these experiments take years to complete and only screen a small number of accessions. Because of the necessity for profitable salt tolerant crops, more rapid screening methods need to be developed. Development of rapid, high throughput, effective screening methods is another goal of this project.

Lastly, calibration curves were created for Fourier Transform Near-Infrared Spectroscopy. These calibrations will allow rapid determination of seed oil and dry matter content, which can be affected by soil nutrient and mineral concentrations. The FT-NIRS model can therefore be a selection tool for sunflower breeders.

Feeding the growing world population is an ever increasing concern. Utilization of marginal and salt affected lands may prove to be an option for diverting widespread

famine. These lands will only be successful though if crops grown on them are suited to the conditions.

2. SALT-AFFECTED SOILS

2.1 Salts in Soils

A salt is defined as a combination of two ions, one possessing a positive charge and the other a negative charge. Some salts found in soil are sodium chloride, also known as table salt, magnesium chloride, calcium chloride, and calcium sulfate (McFarland et al., 2000). Many of these salts are deposited with the addition of irrigation water.

The amount of salt in a soil is reported as its electrical conductivity (EC) or the amount of total dissolved solids (TDS). EC and TDS are loosely related and the equation $TDS(mgl^{-}1)=EC(\mu S/cm^{-}1)*0.67$ can be used to calculate one if the other is known. The amount of sodium in a soil relative to calcium and magnesium is the sodium adsorption ratio (SAR).

Exchangeable sodium percentage (ESP) is another measure used to asses a soil's salinity hazard. In addition to these direct measurements of salt, soil pH can be indicative of soil salt hazard (Fipps). These measurements work in conjunction with one another to classify and describe the physical condition of a soil (Table 1.).

Table 1. Relation of soil classification, EC, SAR and pH to each other and the soil physical condition (Davis et al., 2010).

Soil Classification	EC (mmhos/cm)	SAR	pH	Soil physical condition
Saline	>4.0	<13	<8.5	normal
Sodic	<4.0	>13	>8.5	poor
Saline-sodic	>4.0	<13	<8.5	normal

The electrical conductivity of a soil can be determined by measuring a prepared soil solution extract with a conductivity meter. Electrical conductivity can be reported as either mmhos/cm, µmhos/cm or dS/m. Atomic adsorption spectrometry, and ion chromatography can be used to quantify the amounts of cations, Ca^{2+}, Mg^{+}, K^{+} and Na^{+} in a soil extract (Rhoades and Miyamoto, 1990) and thus determine the SAR. Theses cations are generally reported in parts per million (ppm).

Salt affected soils are classified as saline, sodic or saline-sodic. A saline soil has accumulated water soluble salts. Sodic soils contain an abundance of exchangeable sodium. Saline-sodic soils contain both water soluble salts and exchangeable sodium (Thomas and Morini, 2005). A soil's EC and SAR will determine how a soil is classified.

2.2 Creation of Salt-Affected Lands

Salt affected lands are created by what is classified as primary and secondary processes. Primary processes are those which are natural and not induced by humans. Secondary processes are the processes that increase the salt content of a soil due to human actions.

One of the primary processes that contribute to salt accumulation in soils is weathering rock or parent material. This weathering releases magnesium chloride, calcium chloride, and most commonly sodium chloride. Sulphates and carbonates are also released in the chemical reactions that reduce parent material.

Another important primary process that results in the deposition of salts in soil is that of wind and rain deposition. The salts deposited with wind and rains are referred to as cyclic salts. This process is of more importance in coastal farming areas.

Human induced salinity can be found in irrigated or non-irrigated areas. In dryland agriculture, salt hazards are found when the water table is high and contains considerable amounts of salts. In some cases this hazard was created by humans when native vegetation such as trees and perennial species that utilized the large amounts of ground water were replaced with annual crops (Pannell and Ewing, 2006).

Salt affected soils are also created by humans through the application of saline irrigation water. The need to irrigate with wastewater, saline groundwater and drainage water has increased. This is because urban water demand has increased, leaving farmers in shorter supply of good quality water (Beltrán, 1999). Shannon (1997) estimates that

irrigation can add up to 60 tons of salt to a hectare of land in one year. In poorly drained areas, shallow saline water tables can be created by crop irrigation (Maas and Grattan, 1999).

2.3 Impact of Salts on Soil

Salts in the soil matrix greatly affect soil structure. Salts cause swelling and dispersion of clays and ultimately cause aggregate breakdown. Swelling of clay particles and dispersion of aggregates reduce water permeation into the soil. Another negative impact of salts on soil is that crusts may form on the surface of the soil which impede water infiltration, gas exchange and seed emergence (Rolston et al., 1984).

2.4 Reclamation of Salt-Affected Soil

Reclamation of salt affected soils utilize chemical and physical means (Frenkel et al., 1989). Saline, sodic and saline-sodic soils require different methods of reclamation. All methods utilize water to move salts from plant root zone downward. This water can be applied in split applications or all at once. The most effective method is determined by the amount of salt in the soil and soil type (McCauley and Jones, 2005).

Saline soils can be remediated with the application of good quality irrigation water. The water is applied by flooding or sprinkler irrigation. The type and rate of application is also dependent on EC, SAR and soil type (McCauley and Jones, 2005).

The saline water is then carried away from the area by a series of drains (Hoffman and Durnford, 1999).

Reclamation of sodic and saline-sodic soil also involves the use of large amounts of water, but application of a calcium containing compound is also needed. Ca^{2+} is needed during this process to replace the Na^{+} cation from the soil colloid exchange site. Gypsum ($CaSO_4$) and lime ($CaCO_3$) are two calcium containing compounds often incorporated into the soil for the purpose of reclamation. Calcium chlorite can also be used with good results, but is more expensive (Davis et al., 2010). If lime is present in the soil, it can be dissolved by the addition of sulfuric acid to the soil (Casiday and Frey, 1998). Large amounts of water are then needed to push the released sodium downward through the soil profile (Ilyas et al., 1997).

Reclamation of salt affected soils can be costly, both monetarily and environmentally. The monetary cost of reclamation increases with the amount of amendment needed, the amount of electricity required to pump water, and the cost of equipment for incorporation. Where drainage is needed, the cost of reclamation is significantly higher due to labor and materials.

The dissolution of sodium and other salt forming ions into water can be of consequence to the environment. Irrigation leachate may have a salt concentration 20-100 times higher than the initial irrigation water. This water then has the potential to pollute a stream or the groundwater (Bouwer, 2000). If the effluent flows into a closed system, such as a lake, the salt accumulation may be enough to pose a threat to inhabiting organisms (Hoffman and Durnford, 1999).

2.5 Plant Salt Tolerance

Plants show a range in salt tolerance and can be classified as sensitive, moderately sensitive, moderately tolerant and tolerant (Maas and Hoffman, 1976). Halophytic plants grow well in medium that contains high salt concentrations while glycophytes are not naturally adapted. Some dicotyledonous halophytic plants will only grow at high salt concentrations (Munns and Tester, 2008). Different physiological mechanisms for salt tolerance are found among plant families, and halophytes are believed to utilize at least three (Glenn et al., 1999) .

The tolerance of a plant can be measured by yield decline in the presence of salt (Fig.1). Other physical measurements such as shoot, root and fruit weights, germination rates, and leaf damage ratings may be made to determine tolerance (Shannon, 1997).

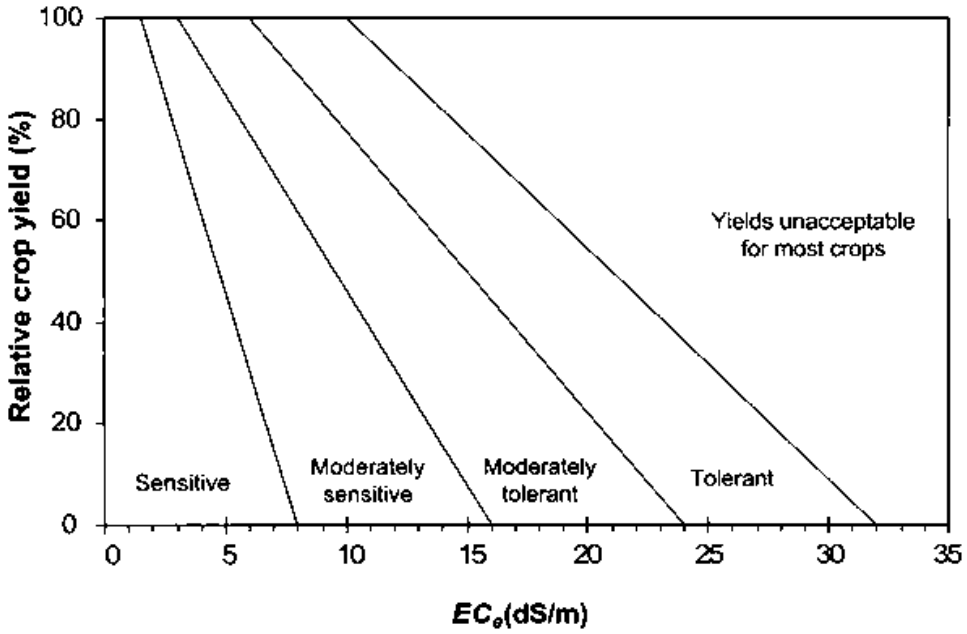

Fig.1. Crop salt tolerance can be classified according to percent yield loss when grown in soils that have particular EC (MAAS, 1993)

Fruit and nut trees are sensitive to salt even at low salt levels. Moderately sensitive crops include clover, oats and rice, while more tolerant crops include cotton, alfalfa, tomatoes, barley, and beets (FAO, 1988; Greenway and Munns, 1980).

Three types of adaptations to salt affected environments are found in plants: [1] osmotic stress tolerance, [2] Na^+ or Cl^- exclusion, and [3] Na^+ or Cl^- tolerance in tissues. There are many mechanisms and processes for salt tolerance found in plants that fall into these categories. For example, sodium can be sequestered in root vacuoles so that it is excluded from the leaf tissues thus preventing toxic accumulation (Munns and Tester, 2008).

Many mechanisms for salt tolerance under regulation of many genes may be found in an organism. Because of the array of phenotypic selection criteria and multigenetic nature of salt tolerance, breeding for high salt tolerance may be difficult (Ashraf, 2004).

3. SUNFLOWER

3.1 Introduction to Sunflower

Sunflower is one of the few crops native to North America. It is believed that thousands of years ago these plants were cultivated by Native American Indians (Rogers et al., 1982). During this cultivation, seed size was increased and plants with the non-shattering trait were selected (Burke et al., 2002). Sunflowers were introduced to Europe as an ornamental plant in the 1600s. From the 1920s to 1970s, Russians bred for high oil content in the achene by utilizing recurrent selection in open pollinated populations (Rogers et al., 1982). Cytoplasmic male sterility in sunflower was found in 1969 and restorer genes were discovered in 1970 (Fernandez-Martinez et al., 2009).

There are 51 species of sunflower that can be found in varying niches across the United States. The genetics of the plant also vary considerably among species. Sunflower can be divided into perennial and annual groups. Annuals are diploids while perennials can be diploids, tetraploids or hexaploids. Crossing between species can occur, as can hybridization of ploidy groups. Crossing of ploidy groups may result in triploid progeny (Chittarajan, 2010).

Sunflower can be bred for specific types of oil with differing fatty acid composition, such as midoleic (NuSun®), linoleic (traditional) or high oleic oils. These fatty acids differ in carbon atoms and double bonds. The fatty acid composition of the seed can determine its healthfulness and oxidative stability (Flagella et al., 2004). Hybrid

confection (Fig 2.) and ornamental sunflower are also created by commercial breeders (Fernandez-Martinez et al., 2009).

Fig. 2. Large seeded confection hybrid (left) and wild species seed (right).

In 2005, sunflower was the second largest hybrid crop grown, and the origin of 10% of the edible seed oil in the world (2010). In 2009, nearly 24 million hectares of sunflower were harvested from around the world (FAOSTAT, 2011). While the amount of land dedicated to sunflower cultivation has dropped in the United States since the 1970s, other countries still grow considerable acreage (Fig.3.)

Fig. 3. Hybrid sunflower farm in South Dakota.

3.2 Salt Tolerance in Sunflower

Sunflower are classified as being a moderately salt tolerant crop (Maas and Hoffman, 1976); however, there are significant differences in tolerance among cultivars (Ashraf and Tufail, 1995). Sunflower can show signs of salt induced stress despite being classified as a moderately tolerant crop. More salt sensitive lines may show reduction in leaf area, dry matter and ultimately yield (Katerji et al., 1994). Seed oil content can be affected by soil salt concentrations (Ashraf and Tufail, 1995; Flagella et al., 2004).

Helianthus paradoxus is a wild species of sunflower known for its ability to thrive in saline and alkaline environments. Also known as the Pecos or puzzle sunflower this plant can be found in far west Texas and New Mexico, but permits are required for

collection because this plant is listed as threatened under the Endangered Species Act of 1973. Its threatened status is due in part to the damages suffered to its environment caused by highway construction, invasive species encroachment and agriculture disturbance. *H. paradoxus* is believed to be a hybrid that originated between 75,000 and 280,000 years ago (Welch and Rieseberg, 2002b) from a cross of two salt sensitive species, *H. annuus* and *H. petiolaris* (Lexer et al., 2003a). Because of this hybridization event and its ability to thrive in soils with high salt concentrations, the Pecos sunflower has been the subject of much sunflower research.

The Pecos sunflower's greater leaf succulence, or increased leaf thickness, is believed to contribute to its salt tolerance (Welch and Rieseberg, 2002a). An increase of root exposure to NaCl can induce leaf succulence in salt tolerant genotypes (Longstreth and Nobel, 1979). Leaf succulence is believed to be an adaptation for salt tolerance because the condition can ultimately increase photosynthesis.

Miller et al. (1995) believed that salt tolerance in sunflower can be attributed to one major gene along with possible recessive modifier genes. Other studies suggest that genetic regulation is not as simple as Miller proposed. Lai et al. (2005), identified six genes that may be responsible for the regulation of uptake of mineral ions in sunflower. The identified genes are designated HT089, HT175, HT185, HT 215, HT 216 and HT227.

Calcium is believed to play an important role in stress tolerance and may be responsible for the observation of salt-tolerance QTL's (Lexer et al., 2003b) . Experiments suggest that sodium, boron, manganese and magnesium exclusion coupled

with greater calcium uptake can contribute to salt tolerance (Lexer et al., 2004). It has also been shown that the genes showing response to salinity tolerance also express when the plant undergoes drought stress (Liu and Baird, 2003).

Breeding for abiotic stress, though laborious and time-consuming, can prove worthwhile. The USDA recognized the need for the creation of salt resistant germplasm with a cultivated background and released two lines from an interspecific cross of *H. paradoxus* and *H. annuus.* These lines are registered as HA 429 and HA 430 (Miller and Seiler, 2003).

4. MATERIALS AND METHODS

4.1 Introduction to Material and Methods

Three experiments were designed and conducted to evaluate the salt-tolerance of USDA breeding material, wild populations and commercial hybrids. The experiments were designed to evaluate the salinity tolerance of 24 sunflower accessions at different stages of growth and in differing environments. The experiments consisted of petri dish, greenhouse and field comparisons. In addition to these experiments, a FT-NIRS was calibrated to determine total seed oil content.

4.2 Germplasm

Germplasm lines and populations were acquired from the United States Department of Agriculture- Agricultural Research Service (USDA-ARS) National Plant Germplasm System (USPGS) Working Collection for Sunflower located at Ames, IA in the spring on 2010 and once again in the spring of 2011 when stocks of certain seed were not adequate. The initial shipment contained 80 accessions recommended for screening by the NCRPIS oilseeds curator, Laura Marek, and USDA-ARS sunflower breeders Gerald Seiler and Brent Hulke. None of the accessions received from the USPGS were treated with an insecticide or fungicide.

Twelve of the 80 accessions received from the USDA were originally chosen for the research reported herein. Of these twelve, four were *Helianthus paradoxus* accessions (PAR-1671, 'PAR-1084-1', 'PAR-1673-1', and 'PAR-1673-2') and two ('HA 429' and 'HA 430') were bred from crosses containing the salt tolerant *H. paradoxus* in the pedigree. DEB-CUC-1810 was chosen because the species *Helianthus debilis* Nutt. ssp. *cucumerifolius* [Torrey & Gray] Heiser is native to dry environments and therefore may have salt tolerance. The accessions 'ARG-420' and' ARG-1575' were chosen because the species *Helianthus argophyllus* [Torrey and Gray] is native to sandy beaches of Texas and Florida and therefore may have tolerance to salt.

Helianthus negelectus Heiser, the parental species of 'NEG-1255', is native to west Texas and southeastern New Mexico in areas with an average of 25 to 50 cm of precipitation per year. 'GIG-1616-1' and 'GIG-1616-2' are accessions developed from the species *Helianthus giganteus* L., also known as the giant sunflower. This species is a perennial native to the northeastern United States and Canada. The 'TUB-1709-1' and 'TUB 1709-3' germplasm was bred from populations of *Helianthus Tuberosus* L. This species has as haploid chromosome number of n=51 where the normal haploid number for sunflower is n=17, but crosses easily with annual sunflower (Rogers et al., 1982) TUB-365 was a late entry into the project and has not been deveoped.

Four commercial seed companies also contributed seed to the project. These companies were Advanta, Seeds 2000, Syngenta and Triumph. All of these companies sent 1 kg or more of seed in the spring of 2010 and again in 2011. All of the seed sent from the seed companies had a seed treatment pre-applied.

Advanta is a company based in Fargo, North Dakota. In addition to sunflower breeding, the company also works with grain and forage sorghum. Advanta contributed the varieties 'HySun 454', 'Aguara 4', and 'F 30294'.

Seeds 2000 donated seed of the varieties 'Panther II' and 'Firebird'. Ross Hakes, vice President of sales and marketing, states, "Firebird is a single cross, medium-full maturity, NuSun® (mid-oleic) sunflower hybrid having a fatty acid composition ranging between 55 to 75% and an oil content ranging between 40 to 44%. It ranges in height between 60 to 66 inches and has tolerance to verticillium wilt, rust, and sclerotinia stalk rot. Firebird is tolerant to Express herbicide (tribenuron-methyl) produced by Dupont."

Hakes describes Panther II as, "… a 3-way, medium-early maturity, large stripe seeded confectionery hybrid having linoleic acid oil composition ranging between 60 to 68%, and low oil content ranging between 25 to 30%. It ranges in height between 60 to 72 inches."

Triumph is a seed company located in the West Texas town of Ralls, TX.. The sunflower breeder at Triumph, Joseph Legako, sent four lines to be screened for tolerance. These cultivars were Triumph s668, s678, 768c and 664. The "s" in s668 and s678 designates that the sunflower is short stature. Short stature is a trait obtained by crossing dwarf sunflower with sunflower of normal height. The line s678 has heads that turn down during maturity to protect from bird loss. The line s668 has excellent rust tolerance and overall plant health. The "c" in 768c denotes that the cultivar is confectionary. This cultivar is noted for its seed length and color. Triumph 664 is a NuSun® variety noted for its rust tolerance (Triumph Seeds, 2009)

Syngenta is a large global producer of sunflower hybrids. Syngenta contributed the hybrids '4651 NS', '3732 NS' and '3845 HO' for salt tolerance screening in the spring of 2010. Syngenta 3732 NS is a NuSun® cultivar listed as "Premium Class Hybrid" and has downy mildew resistance. The hybrid 4651 is a NuSun® variety and is listed as being on average 11 inches shorter than 3732 NS at 58-64 inches tall. The hybrid line 3845 HO is a high oleic oil brand that is not as early maturing as 3732 NS and 4651 NS.

In the spring 2011, Syngenta sent another high oleic for screening, '4596 HO'. Syngenta 4596 HO is a hybrid reported to have a high degree of drought tolerance and resistance to downy mildew (Inc., 2010). All seeds sent from Syngenta were treated with a combination insecticide/fungicide called CruiserMaxx®, which is a patented product of Syngenta.

During the course of the study, some accessions were dropped due to low germination or low seed supply. When this occurred, a different accession was added so that at all times there were 24 entries in the experiment, except for in the case of the third repetition of petri dish screenings. The accessions excluded due to low germination were ARG-420, ARG-1575, DEB-CUC-1810.

4.3 Petri Dish Salt Tolerance Screening

A laboratory experiment was designed and conducted in order to determine salinity tolerance among sunflower accessions at germination. More specifically, the goal of the experiment was to note differences in percent germination and radicle lengths of sunflower seeds germinated in varying concentrations of NaCl solution.

Twenty-five seeds from each accession were chosen based on seed uniformity and absence of abnormality or insect damage. These seeds were surfaced sterilized by soaking them in a commercial seed sterilant, Physan20™, for five minutes. The Physan20™ was prepared according to labeled instructions; by mixing 1.95 mL Physan20™ into 1L of water. After sterilization, the seeds were rinsed with distilled water.

The NaCl solution was made by mixing a calculated weight of sodium chloride in distilled water. The molar weight of NaCL is 58.44 grams per mole. To achieve a 1M solution of NaCl, 58.44 grams of NaCl would be mixed into distilled water then more water would be added to bring to volume. This study was performed using three concentrations.

- Control- 0M=distilled water only
- 5.84g NaCl in ~ 1L dH_20=100 mM solution NaCl or 100 moles per cubic meter (moles/$m^{3)}$
- 17.53g Nacl in ~ 1L dH_20=300 mM solution NaCl

Three sterile petri dishes per accession were designated and each one labeled with the accession number and assigned NaCl molar concentration. A fourth concentration, 500 mM, was used in preliminary experiments, but no seeds germinated in this concentration.

Five sterilized seeds of each accession were placed into a petri dish labeled with the accession designation (Fig. 4). The assigned solution was then slowly poured into the petri dish so that half the seed was immersed. Petri dishes were stacked on trays then placed in a dark seed germinator maintained at 22.2˚C (Fig. 5).

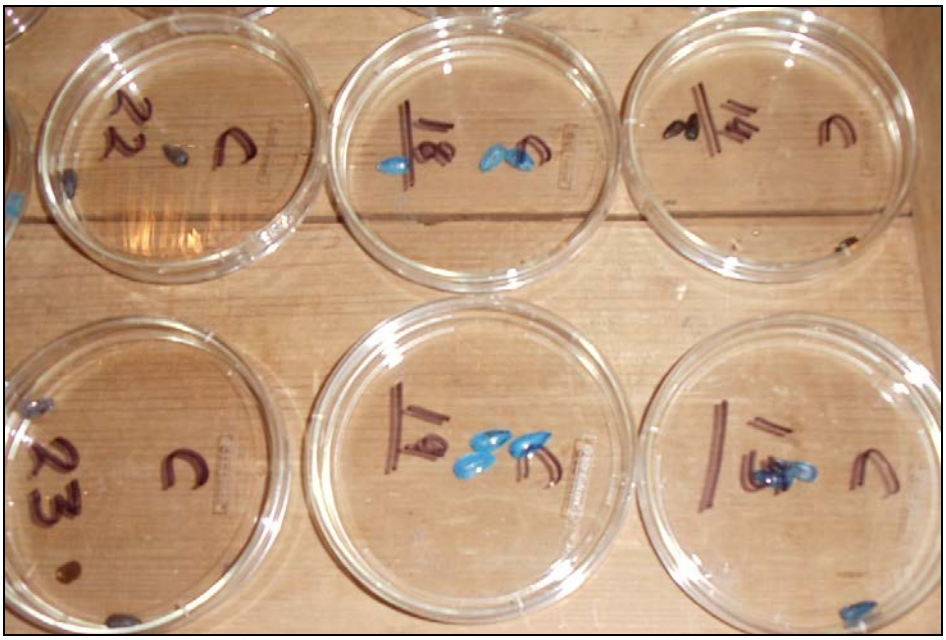

Fig. 4. Seeds placed in petri dishes for salt tolerance screening. Each petri dish is marked with the entry number and solution concentration.

Fig. 5. Petri dishes for salt tolerance screening are stacked in trays then placed in a germination chamber.

Petri dishes were checked on the third day for adequate moisture. If there was not adequate moisture for germination, then solution would be added. If fungi began to grow in the petri dish, then the seed would be rinsed with the solution and returned to the germination chamber.

After ten days in the germination chamber, petri dishes were removed from the germination chamber. The seeds were removed from the petri dishes and radicle lengths individually measured. Measurements were made with the use of a small flexible retracting tape measure in mm increments. When the radicles were not straight, the tape measure was bent to conform to the shape of the radicle. When the long radicles curled like corkscrews they were broken and measured in pieces and total length calculated.

4.4 Greenhouse Salt Tolerance Screening

In late winter of 2011, greenhouse screening trials were initiated. The greenhouse trials took place in the plant growth facilities at the Borlaug Center for Southern Crop Improvement on the Texas A&M campus. The rationale for the greenhouse screening was to determine salt tolerance under controlled conditions through vegetative stages. Data taken from greenhouse experiments consisted of plant height, leaf length and width, chlorophyll concentration, and fresh and dry biomass weight.

Twenty-four accessions of sunflower were screened during each replication. Two pots were designated for each accession; one control and one treatment. In total, 48 black plastic one gallon pots were filled with vermiculite wetted to field capacity. Horticultural vermiculite was chosen as a potting medium due to its absence of salts and high water holding capacity. Eight seeds of each accession were planted, four in the control pot and four in the treatment pot. At the two to four leaf stage, misshapen, short or tall plants were removed leaving the one most average plant per pot.

Approximately two weeks after planting, the salt treatment was initiated. During the first solution treatment, all pots were fertilized with half strength fertilizer. This concentration was made by adding 37 mL of water soluble Miracle-Gro® all-purpose plant food to 18.9 L of reverse osmosis water. The salt treated plants received supplemental salt in the solution that was created by mixing in an amount of laboratory grade NaCl. The target EC for the first watering was 13 Ds/cm, and the EC was measured using a Hanna HI991301 ph/EC/TDS temperature meter.

Plants were watered twice after the initial fertilizer application. Salt treated plants were watered with a salt solution with a target EC of 16, and control plants were watered with the greenhouse supplied RO water. There were three reps that took place over time, which equaled about one replication per every 5 weeks.

Plant heights were measured in week five and number of leaves per plant counted. Leaf lengths and widths were taken for each set of leaves. Leaf areas were obtained by squaring the mean width of the lower two leaves of each plant (Rouphael et al., 2007). SPAD™ meter readings were taken for the bottom and top sets of leaves on each plant. SPAD™ meter readings could not be taken on the last replication due to leaf desiccation. This leaf desiccation was most likely due to the high temperatures in the greenhouse.

Plants were harvested after all of the morphological data was obtained. To harvest the biomass, the plant was cut with hand-pruners where the first root protruded from the main stem. The leaf and stem were placed in a paper bag while still intact. The plant accession number and treatment were identified on the outside of the bag.

Upon arrival to the CIL, the plant tissue was weighed with a digital scale. After recording the fresh weights of the plants, the bags were left sitting upright with the tops open to allow the leaf tissue to air dry. When the plant tissue was thoroughly desiccated the dry weights were taken.

4.5 Field Trials

The goal of the field trials was to observe and compare differences in morphology and yield of the sunflower accessions between College Station and Pecos, TX. Pecos, TX, is located in far west Texas in Reeves County, and known for growing conditions with high concentrations of salts in the soil and irrigation water. The amount of salts in the soil and water can be partly explained from an excerpt written by R. Boghici and N. G. VanBroekhoven in Hydrogeology of the Rustler Aquifer, Trans-Pecos Texas (2001), which describes the geologic makeup of the area. "The Rustler Formation consists of up to 500 ft of carbonate and evaporite strata of Permian age deposited in the Delaware Basin of West Texas. The formation yields moderate to large quantities of fresh to brackish groundwater, primarily from solution openings in its upper section. Recharge takes place by cross-formational flow from deeper aquifers and percolation of surface water through the formation outcrop. Discharge is predominantly to pumping wells and by flow into overlying aquifers. Geochemical data indicate the main processes impacting the groundwater chemical composition are the dissolution of calcite, dolomite, gypsum, and halite and cation exchange," (Boghici and VanBroekhoven, 2001).

College Station is located in Burleson County Texas and in comparison to Pecos, has relatively low salt levels in the soil (Table 2) and irrigation water (Table 3). The field trial was located at the Texas AgriLife Research Farm. Trials were irrigated with water from the Brazos River.

Table 2. Soil analysis results from samples taken from sunflower plots in College Station and Pecos, TX

Soil Analysis				
	College Station, TX		Pecos, TX	
Routine Analysis	2010	2011	2010	2011
pH	7.7	7.8	8.0	8.2
Conductivity, µmhos/cm	389	511	2360	9240
Nitrate, ppm	4.00	14.25	71.00	167.25
Phosphorus, ppm	38.00	42.25	37.00	39.50
Potassium, ppm	513	494	490	586.25
Calcium, ppm	10124	10037	14265	15442
Magnesium, ppm	446	447	535	732
Sulfur, ppm	19	26	595	2771
Sodium, ppm	133	159	1120	5759
Detailed Salinity Analysis				
pH	7.1	7.2	7.1	7.5
Conductivity, mmhos/cm	0.06	0.90	5.40	877.85
Sodium, ppm	55	88	875	15372
Potassium, ppm	15	17	37	220
Calcium, ppm	93	119	426	1748
Magnesium, ppm	9	12	67	502
SAR	1.46	2.05	10.41	86.89

Table 3. Well water analysis report from the Pecos, TX field location.

Pecos, TX Irrigation Water Report	
pH	8
Sodium Adsorption Ratio	11.4
Adjusted SAR	12.6
Electrical Conductivity, mmho/cm	2364
Cations/Anions, me/L	3.94

The field design used for this study was a randomized complete block design with four repetitions. Twenty-four genotypes were planted at both locations with a cone planter. In 2010, 3845 HO was used for border planting, and in 2011, the hybrid 4651 NS was used.

One month after planting, plant populations were counted at each location. From each plot's two inner rows, ten plants were randomly chosen and plant height measured and vegetative or reproductive stages recorded. When the plants reached physiological maturity, the middle two rows of each plot were harvested.

The trial at Pecos was planted 19 April 2010. Before planting, the preemergent herbicide Prowl® was incorporated at a rate of 0.57 L per ha. The beds were than shaped. The plots were four row plots with 76.2 cm rows. The plot length in Pecos in 2010 was 6.7 m with 2.4 m alleys. One hundred and twenty seeds were planted per plot.

During the blooming stages, the sunflower trial could not be sprayed with insecticide due to field flooding even though sunflower head moth, *Homoeosoma electellum*, populations were present (Fig. 6). Because of severe insect infestation and subsequent seed loss (Fig. 7), the field was not harvested.

Fig. 6. Adult sunflower head moth, *Homoeosoma electellum,* on cultivated sunflower.

Fig. 7. Damage caused by *H. electellum* to sunflower during seed production.

College Station was planted on 3 May 2010. Prior to planting, the field was pre-irrigated. The field was then plowed and beds shaped. The plot length in College Station was 6.1 m with 1.8 m alleys. Two hundred seeds were planted per plot in College Station. In the plots where the seed quantity was insufficient, the inner two rows of the plot were planted with the seed to be screened while the outer rows were planted with an alternate, "fill" seed.

A synthetic pyrethroid insecticide was sprayed in College Station when sunflower head moth and sunflower midge, *Contarinia schulzi*, (Fig. 8) damage occurred.

Fig. 8 Sunflower head with deformation due to *C. schulzi* infestation.

When plants reached physiological maturity, the middle two rows of each plot were harvested. The heads were harvested by cutting them off with hand-pruners then placed in plastic mesh bags.

In 2010, some of the heads were harvested in College Station, approximately one physiological stage before physiological maturity and therefore the heads had not begun desiccation. The heads were left inside the Texas A&M Cotton Improvement Lab for two weeks. Because these heads still contained a large amount of moisture, they became infested with mold and small insects. The sunflowers that were left in the field the extra two weeks were thoroughly dry upon harvest.

The heads were threshed with an Almaco thresher (Fig. 9). The seed was then further cleaned with a seed blower (Fig. 10). Weed seeds, dust and sunflower shells with immature kernels, "pops" are blown up and out of the system while good seed, because of its weight, is not.

Fig. 9. Almaco thresher used to separate sunflower seed from the heads.

Fig. 10. Erie Magnetics seed blower used to separate seed from debris.

The trial at Pecos was planted 15 April 2011. The same bed preparation procedure and seed planting population was used in 2011 that was used in 2010. The

plots length was 7.62 m long with 1.5 m alleys. The field was flood irrigated on 18April 2011 to encourage plant emergence.

Phosphorus (P_2O_5) was knifed in after emergence. Plots were irrigated every fourteen days. Sunflower head moth populations did not reach a threshold that warranted pesticide application in 2011 at Pecos. Bird feeding caused seed loss (Fig. 11) so netting was draped over the middle two rows of each plot to prevent further bird damage.

Fig. 11. Sunflower with seed loss due to bird feeding.

The trial at College Station was planted 28 April 2011. The plots were 7.62 m long with 1.8 m alleys. This year the sunflower was thinned to one plant per 0.5 m, and off- types rogued.

In 2011, three pyrethroid insecticide applications were made in July when head moth damage occurred. There was no evidence of sunflower midge in 2011 at College Station. Reflective tape and scare eyes were placed in and around the trial to deter birds.

In 2011, the College Station location was harvested with use of a Massey Ferguson 8 XP combine (Fig. 12). When the sunflower plots were harvested, the seed was put into canvas bags. The combine harvested some weed seed and high-moisture biomass along with sunflower seeds. The contents of the bag were dried if there was excessive moisture in the plot sample. After drying, samples were cleaned with the seed blower.

Fig.12. Massey Ferguson 8 XP combine used to harvest the College Station field in 2011.

After being cleaned, samples from each location were placed into paper bags. These bags were identified with the location and plot number. The plot yield was then weighed and estimated as kg^{-1} ha. One hundred seeds from each plot were counted and weighed and from this 100 seed weight per accession was calculated. Seed samples from each plot were also taken and cleaned by hand to be scanned with the FT-NIRS.

4.6 Fourier Transform Near-Infrared Spectroscopy

Fourier Transform Near-Infrared (FT-NIRS) was conducted with the goal of determining seed oil content of the sunflower seeds grown during this project. In order to determine the oil content of all of the accessions, a calibration model must be generated.

In order to create a calibration model, seed samples were drawn from the yield of each plot harvested. Samples were hand cleaned to remove debris or degraded seed. Seed was scanned with the Thermo Fischer Anteris II NIRS using a glass spin cup and 64 points to make the seed profile. Samples from all harvested plots were scanned in this manner. One hundred and three samples were sent to Ward laboratories in Kearney, NE for fat analysis with use of chemical methods.

The reflectance of most importance is the wavelength from 4,000 to 10,000 wavenumbers. The reflectance was converted to absorbance so that calibration standards could be developed. The software, “TQ Analyst”, was utilized to evaluate spectral data. This software utilizes scanned absorbance data and the oil content of each sample obtained through wet chemistry to create the calibration.

5. RESULTS AND DISCUSSION

5.1 Petri Dish Salt Tolerance Screening

In the evaluation of seeds of multiple genotypes at different salt levels in petri dishes, the analysis of variance indicated significant differences among genotypes and salt concentrations (Table 4). Interactions among salt and genotype were highly significant.

Table 4. ANOVA of radicle lengths of seeds of sunflower genotypes germinated in a petri dish at different concentrations of salt (NaCl).

Source	df	MS	F-value	Pr >F
Salt	2	14,568.0	88.91	<0.00
Salt(rep)	6	2,502.3	20.87	<0.00
Genotype	21	449.4	1.49	0.14
Salt*Genotype	42	302.5	2.95	<0.00
Error	659	102.4		

Table 5. ANOVA of the effect of differing salt treatments on *Helianthus* radicle elongation germinated in petri dishes.

	0 mM NaCl			100mM NaCl			300mM NaCl		
Source	df	MS	Pr >F	df	MS	Pr >F	df	MS	Pr >F
Rep	2	6153.6		2	251.69		2	4.87	
Genotype	21	956.3	<0.00	21	84.53	<0.00	21	1.3	<0.00
Error	221	273.3		217	32.01		221	0.54	

Within concentrations of salt in solution, the ANOVA states differences among genotype were highly significant (Table 5). The high coefficients of variation for these tests indicate that the tests are not precise (Table 6).

Table 6. Radicle lengths (mm) of sunflower genotypes in differing concentrations of NaCl solution germinated in petri dishes.

	Salt (NaCl) Concentration		
Genotype	0 mM	100 mM	300 mM
Advanta 454	10c-f	3b-e	*
Advanta Aguara	6c-f	1e	*
Advanta F3029	30ab	6a-e	*
ARG-1575-1	1e-f	0e	1a
ARG-420-1	1d-f	1e	*
GIG-1616-2	16b-e	5a-f	1a
HA 429	30a	4a-e	1ab
HA 430	13c-f	5a-e	0a-c
NEG-1255	10c-f	4b-e	0bc
PAR-1084-1	17b-d	7a-d	0a-c
PAR-1673-1	14c-f	4a-e	1a-c
PAR-1673-2	19bc	7a-c	1a-c
Seeds 2000 Firebird	20bc	5a-e	0bc
Seeds 2000 Panther	0f	2c-e	*
Syngenta 3732 NS	14c-f	8ab	0a-c
Syngenta 3845 HO	19bc	4a-e	1a-c
Syngenta 4651 NS	4c-f	1de	*
Triumph 664	38a	5a-e	*
Triumph 786c	6c-f	0e	*
Triumph s668	4c-f	2c-e	*
Triumph 678	14c-f	9ab	*
TUB-1709-3	18bc	10a	0a-c
Mean	15	4	0
C.V., %	108.6	129.9	239.4

*None of the seed germinated.

Waller Duncan k-ratio T-test for means separation was performed on the radicle length data. Of the 22 genotypes analyzed in the petri dish experiment, Triumph 664,

Advanta F3029 and HA 429 had the longest radicles in the control solution. In the 300 mM solution, many seeds did not germinate. TUB-1709-3 had among the largest radical length in the 100 mM concentration. ARG-1575 radicle length did not change from the control solution to the 300 mM solution.

The petri dish experiments showed significance for both genotypes and salt concentration sources of variation. There were significant genotype x salt interactions, which suggests that salt tolerance, even at the seedling stage, involves a complex physiological mechanism.

This screening experiment showed significant reductions of radicle lengths in all genotypes screened with increasing salt concentration, except for ARG-1575-1. This genotype is bred from the drought tolerant species, *H. argophyllus*, and therefore may also contain enhanced genetic mechanisms for salt tolerance. In general, genotypes bred for salt tolerance had significantly longer radicles following germination in the highest salt concentration than most commercial hybrids.

The coefficients of variation of these tests were high, and may be due to seeds not germinating in any of the solutions. Germination was affected by the category of sunflower in which each genotype belonged. Confection genotypes (large seeded) required the most moisture to germinate and therefore germinated slower than hybrid oilseed genotypes. Wild sunflower genotypes have lower germination percentages than the other groups of germplasm. The germination percentage was higher in hybrid sunflower because that set of germplasm has been selectively bred to overcome seed dormancy.

In order to improve the efficiency of this test, more seeds of a genotype may need to be used per repetition. Genotypes may also need to be limited in number or grouped with similar genotypes. For example, confections should not be compared to oilseed cultivars, and wild species or races should not be evaluated against commercial hybrids.

This experiment did not group similar genotypes and only screened a small amount of seed. There were also many significant sources of variation beyond genotype. Therefore, evaluation techniques need further refinement before results can be used to differentiate salt tolerant lines with a high degree of accuracy.

5.2 Greenhouse Salt Tolerance Screening

Genotype and treatment were significant for height, but the interaction between genotype and treatment was not. Solution applied, genotype and the interaction between treatment and genotype were all found to be significant for leaf area (Table 7). The C.V. of the test of significance of height was 15.4% which is acceptable, and the C.V. for the leaf area test was 34.3%, which indicates the test was not effective.

Table 7. ANOVA of plant height and leaf area of sunflower genotypes grown with salt (NaCl) and without salt in a greenhouse.

	Plant Height			Leaf Area		
Source	df	MS	Pr>F	df	MS	Pr>F
Salt	1	162.14	<0.00	1	189.87	<0.00
Salt(rep)	4	16.17	0.38	4	23.13	0.32
Genotype	22	86.74	<0.00	22	46.34	<0.00
Salt*Genotype	22	12.96	0.66	22	9.35	0.95
Error	86	15.31		82	17.18	

Table 8. ANOVA of plant height of genotypes by salt (NaCl) treatments in a greenhouse.

	No salt			Salt		
Source	df	MS	Pr>F	df	MS	Pr>F
Genotype	22	63.49	<0.00	22	36.22	<0.00
Rep	2	8.42	0.65	2	29.47	0.09
Error	43	19.17		43	11.47	

Table 9. ANOVA of leaf area of genotypes by salt (NaCl) treatments in a greenhouse.

	No salt			Salt		
Source	df	MS	Pr>F	df	MS	Pr>F
Genotype	22	27.02	0.16	22	28.29	0.05
Rep	2	3.97	0.81	2	36.81	0.11
Error	43	18.87		43	15.66	

Within salt treatments, height was significantly different among genotypes (Table 8). Plants not treated with salt showed no significant difference for leaf area. Salt treated plants showed differences in leaf area among genotypes (Table 9).

Table 10. Plant height and leaf area of sunflower genotypes grown in the greenhouse with salt (NaCl) and no salt.

	Height (cm)		Leaf Area (cm^2)	
Genotype	No salt	Salt	No salt	Salt
Advanta 454	25a-e	22a-e	14.02ab	9.47a-c
Advanta Aguara	26a-d	23a-c	18.52a	17.05a
Advanta F3029	27a-c	22a-e	14.42ab	9.63a-c
GIG-1616-1	13gh	18c-h	9.44ab	5.02bc
GIG-1616-2	17f-h	17c-g	8.90ab	6.60bc
HA 429	23a-f	21a-e	12.34ab	8.12a-c
HA 430	23a-f	20a-f	11.83ab	7.12bc
HIR-828-1	20c-h	20a-g	9.65ab	7.20bc
NEG-1255	18d-h	15f-h	7.85ab	7.49bc
PAR-1084-1	18e-h	13gh	9.48ab	4.90bc
PAR-1673-1	18d-h	16f-h	9.12ab	12.50ab
PAR-1673-2	21b-g	19b-g	9.23ab	7.43bc
Seeds 2000 Firebird	23a-f	21a-e	16.24ab	11.01a-c
Seeds 2000 Panther	31a	19ab	9.22ab	11.24a-c
Syngenta 3732 NS	24a-f	21a-e	11.71ab	7.63bc
Syngenta 3845 HO	28ab	25ab	13.08ab	8.97a-c
Syngenta 4651 NS	21b-g	19a-g	11.02ab	9.88a-c
Triumph 664	23a-f	25a	11.97ab	12.11a-c
Triumph 786c	26a-e	21ab	9.83ab	11.83a-c
Triumph s668	18e-h	16f-h	15.01ab	9.45a-c
TUB-1709-1	12h	12gh	7.57ab	3.1248c
TUB-1709-3	20c-h	17d-h	6.54b	4.83bc
TUB-365	17f-h	18c-h	7.38ab	6.25bc
Mean	21	19	11.19	8.79
C.V., %	20.6	17.7	38.8	45.0

Waller-Duncan k-ratio T-test means separation was performed based on mean genotype height and leaf area (Table 10). The genotypes that showed no reduction were TUB-1709-3, GIG-1616-2 and HIR- 828-1. TUB-365, Triumph 664 and PAR-1084-1 showed an increase in mean height when watered with salt solution.

The means separation shows that salt reduced the mean leaf area of many genotypes. The genotypes that did not have a decreased mean leaf area were PAR-1673-1, and the confection cultivars Seeds 2000 Panther and Triumph 786c.

Table 11. ANOVA of height and mean area ratios between sunflower genotypes grown with and without salt (NaCl) in a greenhouse.

	Height ratio			Leaf area ratio		
Source	df	MS	Pr > F	df	MS	Pr > F
Genotype	22	0.05	0.19	22	0.21	0.91
Rep	2	0.14	0.02	2	0.42	0.32
Error	43	0.03		39	0.36	

In an effort to compare the differential response of a genotype grown in salt and without salt in a greenhouse environment, the ratio of height as well as the ratio of leaf area of salt affected and non-salt affected plants was calculated by dividing the mean of the salt treated plans with the mean of the control plants. Analysis of variance of the ratio indicated no significant difference among genotypes for height or leaf area ratio (Table 11).

Table 12. ANOVA of fresh weight and dry weight for plants grown for greenhouse salt tolerance screening.

	Fresh weight			Dry weight		
Source	df	MS	Pr>F	df	MS	Pr>F
Salt	1	114.51	<0.00	1	2.29	<0.00
Salt(rep)	2	5.53	0.60	4	0.20	0.41
Genotype	22	19.21	0.05	22	0.72	<0.00
Salt*Genotype	22	4.70	0.98	22	0.05	0.99
Error	41	10.53		82	0.20	

Table 13. ANOVA of moisture of plants grown for greenhouse salt tolerance screening.

Source	df	MS	F-value	Pr >F
Salt	1	74.96	3.32	0.08
Salt(rep)	2	192.59	8.53	<0.00
Genotype	22	16.05	0.70	0.80
Salt*Genotype	22	22.90	1.01	0.47
Error	40	22.61		

From the dry and fresh weights of plants harvested from the greenhouse screenings, percent moisture was obtained. Analysis of variance was performed on these three data points. For fresh weight, salt was highly significant. For dry weight genotype and salt were significant, but the interaction was not (Table 12).

There were no significant differences between genotype, salt, or the interaction for percent moisture (Table 13). No sources of variance for the SPAD™ were significant (data not shown).

The salt tolerance screening that took place in the greenhouse demonstrated that plant height and leaf area are reduced, with the exception of a few genotypes, when plants are grown with salts in the soil solution. The coefficient of variation for the height analysis was low, which suggests precision of the trial. Therefore, using average heights as an indicator of salt tolerance may be an effective methodology. Because there were no significant differences among genotypes for height, one genotype was not clearly more tolerant than another.

The interaction between genotype and salt concentration in the treatment solution was not significant for mean leaf area. Three genotypes in the salt applied group showed

an increase of leaf area. These genotypes were PAR-1673-1, Seeds 2000 Panther and Triumph 786c.

Height ratio analysis was performed in order to differentiate the performances between the two salt levels and within a genotype. These ratios were not significant across genotypes.

Fresh weight, dry weight and percent moisture of the plants grown in the greenhouse, also showed no significant difference between genotypes. The coefficient of variation of this test, was high enough to suggest that the either the testing environment or application of treatments needed to be improved. Another consideration as to why the C.V. was high may be due to inherent differences in the amount of biomass each genotype produces regardless of salt. It is possible that if commercial hybrids and wild genotypes were analyzed separately, the C.V. would be lower.

5.3 Field Trials

Height measurements were analyzed using the GLM procedure. At College Station and in Pecos, the three height measurements (Table 14), yield and 100-weight of the seeds were significantly affected by year (Table 15).

Table 14. ANOVA of three height measurements of sunflower genotypes in 2010 and 2011 at College Station and Pecos, TX.

	Height-1		Height-2		Height-3	
Source	df	MS	df	MS	df	MS
Year	1	399**	1	112.07**	1	45.48**
Rep	3	11	3	4.26	3	5.48
Error	283	11	379	1.32	378	1.79

** Highly significant (p-value < 0.01)

Table 15. ANOVA of seed yield and hundred-weight of seed of sunflower genotypes in 2010 and 2011 at College Station and Pecos, TX.

	Yield		100-Weight	
Source	df	MS	df	MS
Year	1	3,142,754**	1	76.87**
Rep	3	348,122	3	0.38
Error	277	350,856	379	5.91

** Highly significant (p-value < 0.01)

Table 16. ANOVA of three height measurements of sunflower by genotype and location in 2010.

	Height-1		Height-2		Height-3	
Source	df	MS	df	MS	df	MS
Location	-	-	1	5.44	1	111.28**
Location (rep)	-	-	6	31.18**	6	2.82**
Genotype	-	-	23	13.58**	23	39.80**
Genotype*Location	-	-	23	9.39**	23	24.46**
Error	-	-	138	1.45	138	3.16

** Highly significant (p-value < 0.01)

In 2010, among locations, for the data point height-2, the interaction between genotype and location was highly significant (Table 16).

Table 17. ANOVA of three height measurements of sunflower by genotype and location in 2011.

	Height-1		Height-2		Height-3	
Source	df	MS	df	MS	df	MS
Location	1	1387.88**	1	325939**	1	225082**
Location (rep)	6	21.28*	6	609	6	629
Genotype	23	8.26	23	721	23	1170*
Genotype*Location	23	6.31	23	485	23	1013
Error	138	7.69	138	469	137	682

*Significant (p-value<0.05)
** Highly significant (p-value < 0.01)

In 2011, plant heights were not significantly different (Table 17). In 2011 genotype and the interaction of genotype and location was a significant source of variance for seed yield and 100-weight (Table 18).

At College Station in 2010 genotype was highly significant for height-2, height-3, seed yield and 100-seed weight (Table 19). At Pecos, TX in 2010, all genotypes had significantly different heights at each measurement (Table 20). Plants were not harvested in Pecos, 2010.

Table 18. ANOVA of seed yield and hundred-weight of seed of sunflower genotypes in 2011 at College Station and Pecos, TX.

	Seed yield		100-seed weight	
Source	df	MS	df	MS
Location	1	20.99**	1	8.48*
Location (rep)	6	1.60	6	2.80
Genotype	23	12.88**	23	25.18**
Genotype*Location	23	3.04*	23	2.60**
Error	132	1.75	131	1.27

* Significant (p-value < 0.05)
** Highly significant (p-value < 0.01)

Table 19. ANOVA of plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2010 at College Station, TX.

		Mean Squares				
Source	df	Height-1	Height-2	Height-3	Seed Yield	100-seed weight
Genotype	23	-	1133**	4964**	635041**	26.31**
Rep	3	-	4631	4752	1096515	3.74
Error	69	-	207	503	246385	2.73

** Highly significant (p-value < 0.01)

Table 20. ANOVA of plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2010 at Pecos, TX.

		Mean Squares				
Source	df	Height-1	Height-2	Height-3	Seed Yield	100-seed weight
Genotype	23	8.93	1164**	1462**	-	-
Rep	3	3.25	1605	896	-	-
Error	69	0.25	82.4	130	-	-

** Highly significant (p-value < 0.01)

Table 21. ANOVA of plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2011 at College Station, TX.

		Mean Squares				
Source	df	Height-1	Height-2	Height-3	Seed Yield	100-seed weight
Genotype	23	9.67	612	1861	986632**	18.88**
Rep	3	40.69	718	603	179792	0.98
Error	69	14.58	846	1340	197819	1.23

** Highly significant (p-value < 0.01)

Table 22. ANOVA of plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2011 at Pecos, TX.

		Mean Squares				
Source	df	Height-1	Height-2	Height-3	Seed Yield	100-seed weight
Genotype	23	4.91**	594.1**	320.7**	607907**	8.42**
Rep	3	1.86	500.3	688.8	140803	4.64
Error	69	0.80	92.2	32.4	154387	1.32

** Highly significant (p-value < 0.01)

In 2011, at College Station, seed yield and 100-seed weight were highly significant (Table 21). At Pecos in 2011, for the three height measurements, yield and 100-weight, genotype was a highly significant source of variation (Table 22).

Among the tallest genotypes in 2010 at College Station at the final height measurement was Advanta Aguara. Among the shortest was ARG-1575-1 and ARG-420-1. The confection cultivars Seeds 2000 Panther and Triumph 786c and the commercial oilseed hybrid Advanta 454 had among the highest yields (Table 23).

Table 23. Plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2010 at College Station, TX.

Genotype	Height-1 cm	Height-2 cm	Height-3 cm	Seed Yield kg ha^{-1}	100-seed wt. g
Advanta 454	-	69a-e	145a-c	929a-d	6.36b
Advanta Aguara	-	77a	169a	1188a	7.17b
Advanta F3029	-	71a-d	153ab	690a-e	5.37b-f
ARG-1575-1	-	38h	82j	31e	2.19gh
ARG-420-1	-	47f-h	83ij	153c-e	2.11gh
DEB-CUC-1810	-	44gh	104g-j	231d-e	5.58b-e
GIG-1616-1	-	55d-g	101g-i	261b-e	4.97b-f
GIG-1616-2	-	53d-g	112e-i	140de	3.44e-g
HA 429	-	45gh	122d-g	93e	3.96d-g
HA 430	-	55c-h	100g-j	30e	3.44e-g
NEG-1255	-	36h	90h-j	33e	4.12c-g
PAR-1084-1	-	8i	-	-	-
PAR-1671	-	-	-	-	-
PAR-1673-1	-	45f-g	101g-j	161c-e	2.37g
PAR-1673-2	-	38h	107f-j	158c-e	3.64e-g
Seeds 2000 Firebird	-	52g-g	113d-h	197d-e	2.72g
Seeds 2000 Panther	-	63a-g	136b-f	940a-d	10.34a
Syngenta 3732 NS	-	76ab	152ab	916a-d	6.23bc
Syngenta 3845 HO	-	80a	139b-e	809a-e	5.99b-d
Syngenta 4596 HO	-	-	-	-	-
Syngenta 4651 NS	-	74a-c	151a-c	955a-c	6.18bc
Triumph 664	-	77a	142a-d	756a-e	6.36b
Triumph 786c	-	65a-f	150a-c	1055ab	11.82a
Triumph s668	-	51e-h	108f-j	656a-e	5.5b-e
Triumph s678		56b-h	136b-f	611a-e	5.06b-f
TUB-1709-1	-	-	-	-	-
TUB-1709-3	-	50f-h	97g-j	133e-f	3.29fg
TUB-365	-	-	-	-	-
Mean		55	116	446	4.89
C.V. %	-	26.9	19.3	107.5	33.7

Table 24. Plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2010 at Pecos, TX.

Genotype	Height-1 cm	Height-2 cm	Height-3 cm	Seed Yield kg ha^{-1}	100-seed wt. g
Advanta 454	5hi	63d-h	66e-i	-	-
Advanta Aguara	6d-f	54g-j	49j	-	-
Advanta F3029	5i	54f-i	68d-h	-	-
ARG-1575-1	6c-e	71a-d	74b-f	-	-
ARG-420-1	-	-	-	-	-
DEB-CUC-1810	5f-i	60d-i	77b-e	-	-
GIG-1616-1				-	-
GIG-1616-2	6c-e	74a-c	73c-g	-	-
HA 429	5hi	53g-j	53ij	-	-
HA 430	5hi	64d-g	77b-d	-	-
NEG-1255	8a	70a-d	84a-c	-	-
PAR-1084-1	.	33k	56h-j	-	-
PAR-1671	5f-h	56e-i	60f-i	-	-
PAR-1673-1	7bc	78ab	78b-e	-	-
PAR-1673-2	5hi	50ij	49j	-	-
Seeds 2000Firebird	6e-g	80a	77b-e	-	-
Seeds 2000 Panther	6c-e	80a	97a	-	-
Syngenta 3732 NS	6b-d	66d-f	81b-d	-	-
Syngenta 3845 HO	7ab	72a-d	74a-f	-	-
Syngenta 4596 HO	-	-	-	-	-
Syngenta 4651 NS	7b-d	51h-j	86a-c	-	-
Triumph 664	6c-e	64d-g	68e-i	-	-
Triumph 786c	6d-f	54g-j	88ab	-	-
Triumph s668	5hi	43jk	59g-i	-	-
Triumph s678	6c-e	67b-e	74c-f	-	-
TUB-1709-1	-	-	-	-	-
TUB-1709-3	6c-e	60d-i	75c-f	-	-
TUB-365	-	-	-	-	-
Mean	5.6	59	68	-	-
C.V. %	9.0	15.4	16.7	--	-

Table 25. Plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2011 at College Station, TX.

Genotype	Height-1 cm	Height-2 cm	Height-3 cm	Seed Yield kg ha^{-1}	100-seed wt. g
Advanta 454	9a	121a	134b-d	1199b-e	5.36c-g
Advanta Aguara	10a	143a	150a-d	1442a-c	8.20b
Advanta F3029	10a	115a	154a-d	1158b-f	5.25c-g
ARG-1575-1	-	-	-	-	-
ARG-420-1	-	-	-	-	-
DEB-CUC-1810	-	-	-	-	-
GIG-1616-1	8a	118a	128cd	255hi	3.04i
GIG-1616-2	12a	137a	140a-d	590e-i	5.29c-g
HA 429	12a	121a	159ac	599e-i	5.33c-g
HA 430	12a	138a	165ab	116i	5.07c-h
NEG-1255	12a	140a	149a-d	282hi	4.89c-h
PAR-1084-1	-	-	-	-	-
PAR-1671	12a	128a	128cd	287hi	4.34f-i
PAR-1673-1	12a	151a	162ab	200hi	4.32f-i
PAR-1673-2	12a	130a	162ab	166hi	4.28f-i
Seeds 2000Firebird	11a	130a	140a-d	1257a-d	4.78d-h
Seeds 2000 Panther	12a	134a	141a-d	804c-h	12.28a
Syngenta 3732 NS	12a	139a	154a-d	1820a	5.97c-e
Syngenta 3845 HO	12a	129a	136b-d	511f-i	6.16cd
Syngenta 4596 HO	12a	132a	141a-d	1015b-g	4.38c-g
Syngenta 4651 NS	12a	161a	171a	1346a-c	6.32c
Triumph 664	12a	122a	134b-d	1377a-c	5.93c-e
Triumph 786c	12a	154a	127cd	674d-i	12.82a
Triumph s668	12a	139a	147a-d	1475ab	5.45c-g
Triumph s678	12a	138a	154a-d	1026b-f	5.73c-f
TUB-1709-1	12a	115a	123d	197hi	3.62hi
TUB-1709-3	12a	133a	154a-d	559e-i	4.18g-i
TUB-365	12a	114a	141a-d	373g-i	4.23e-h
Mean	10	132	145	804	5.76
C.V. %	35.0	21.9	12.6	55.3	19.2

In 2010 at Pecos, among the tallest at the last height measurement were Syngenta 4651 NS, NEG-1255and Seeds 2000 Panther (Table 24). Advanta Aguara and the short stature cultivar Triumph s668 were among the shortest.

In 2011 at College Station, HA 430 and PAR-1673-1 and PAR 1673-2 were among the tallest genotypes while TUB-1709-1 was among the shortest. Syngenta 4596 HO and 4651 NS were among the highest yielding lines, and HA 430 was among the lowest (Table 25).

In 2011 at Pecos, (Table 26) HA 430 and Advanta F3029 were among the tallest genotypes. PAR-1084-1 was among the shortest genotypes in the test. Triumph s668 and s678 performed well for yield. Syngenta 3732 NS and 4596 HO had among the highest 100-seed weights, and GIG-1616-1 had among the lowest.

Table 26. Plant heights, seed yield and hundred-weight of seed of sunflower genotypes in 2011 at Pecos, TX.

Genotype	Height-1 cm	Height-2 cm	Height-3 cm	Seed Yield kg ha^{-1}	100-seed wt. g
Advanta 454	6c-g	62a-d	82d-g	1013bc	6.26b-e
Advanta Aguara	7a-d	51e-i	99a	509c-f	6.24b-e
Advanta F3029	7a-d	49e-j	91bc	460c-f	6.3b-e
ARG-1575-1	-	-	-	-	-
ARG-420-1	-	-	-	-	-
DEB-CUC-1810	-	-	-	-	-
GIG-1616-1	5g-j	37j-l	82d-f	186f	3.56f
GIG-1616-2	6a-f	57c-f	79e-g	418e-f	5.53de
HA 429	6a-e	35kl	75g-i	158f	5.54de
HA 430	5g-j	37j-l	89b-d	227ef	6.1c-e
NEG-1255	5g-k	56c-g	78e-g	240ef	5.47de
PAR-1084-1	3k	32kl	60k	250ef	5.11d-f
PAR-1671	-	-	-	-	-
PAR-1673-1	4i-k	39i-l	75f-h	258ef	4.71ef
PAR-1673-2	4jk	30l	79e-g	455c-f	5.41de
Seeds 2000Firebird	5g-k	52d-i	84c-e	483c-f	5.87c-e
Seeds 2000 Panther	7a-c	52d-i	78e-g	405ef	10.22a
Syngenta 3732 NS	5e-h	54c-h	66jk	633b-f	7.18bc
Syngenta 3845 HO	6a-f	67a-c	84c-e	765b-e	7.79b
Syngenta 4596 HO	7ab	73a	96ab	995b-d	6.41c-d
Syngenta 4651 NS	7a	59b-e	83d-f	727b-f	6.01c-e
Triumph 664	6a-g	71ab	84c-e	1681a	6.4b-d
Triumph 786c	7a-c	63a-d	80e-g	715b-f	10.09a
Triumph s668	5g-j	45f-k	68ij	1140ab	5.92c-e
Triumph s678	5f-i	55c-g	82d-g	1057ab	6c-e
TUB-1709-1	4h-k	44g-k	80e-g	287ef	5.22de
TUB-1709-3	5d-g	44g-k	79e-g	328ef	6.1c-e
TUB-365	4i-k	42h-l	68h-j	176f	5.86c-e
Mean	5	50	80	570	6.23
C.V. %	16.1	19.3	7.11	60.8	18.4

Year and location has an effect on the expression the plants. Height, total yield and 100- seed weight were all affected. Years may have been significant to all of these traits because of the differing climatic conditions between 2010 and 2011. Amount of salts in the soil may be one of the reasons for the differential trait expression of the same genotypes between the two locations. Climatic differences between the two locations may have also had an effect on the differential expression of the genotypes between locations.

There was only one height stage (height-2) that showed genotype by location interaction. Because there were significant interactions between genotype and location for total yield and 100 seed weight, the most efficient method for selecting for salt tolerance would be to evaluate only these traits.

Based on the stability of yield from one location to the next, genotypes can also be chosen. GIG-1616-2, PAR-1673-1, PAR-1673-2, and Syngenta 3845 HO could be chosen for future tests. All of the Triumph cultivars could be recommended for cultivation at Pecos and possibly considered for other high-salt environments. It would be preferred if the C.V. of the test had been less than measured, but based on the data and the diversity of germplasm in this trial, this test was fairly accurate. Syngenta 3732 NS could be recommended for cropping in College Station because of high yields over the two years of the test.

The weight of 100 seed was often higher from plants grown in Pecos. One possible explanation for this occurrence may have been the high frequency of empty

kernels or “pops” due to lack of pollination or insect predation at College Station. If empty kernels were erroneously weighed, it would reduce the 100 seed weight.

If genotype were to be chosen based on the 100 seed weight test then the oilseed cultivar, Advanta Aguara would be recommended for the College Station area. For Pecos, Syngenta 3732 NS and 3845 HO would be recommended for cropping. This test has a moderately low C.V., therefore if weight difference is due to actual seed weight and not faulty weighing, then the test should be reliable.

5.4 Fourier Transform Near-Infrared Spectroscopy

The Thermo Scientific FT-NIRS was calibrated with 103 oilseed sunflower genotypes. The correlation coefficient of the model is 0.96, which shows good prediction capability (Fig. 13). This model was used to predict oil content of the seed that was harvested in 2010 and 2011.

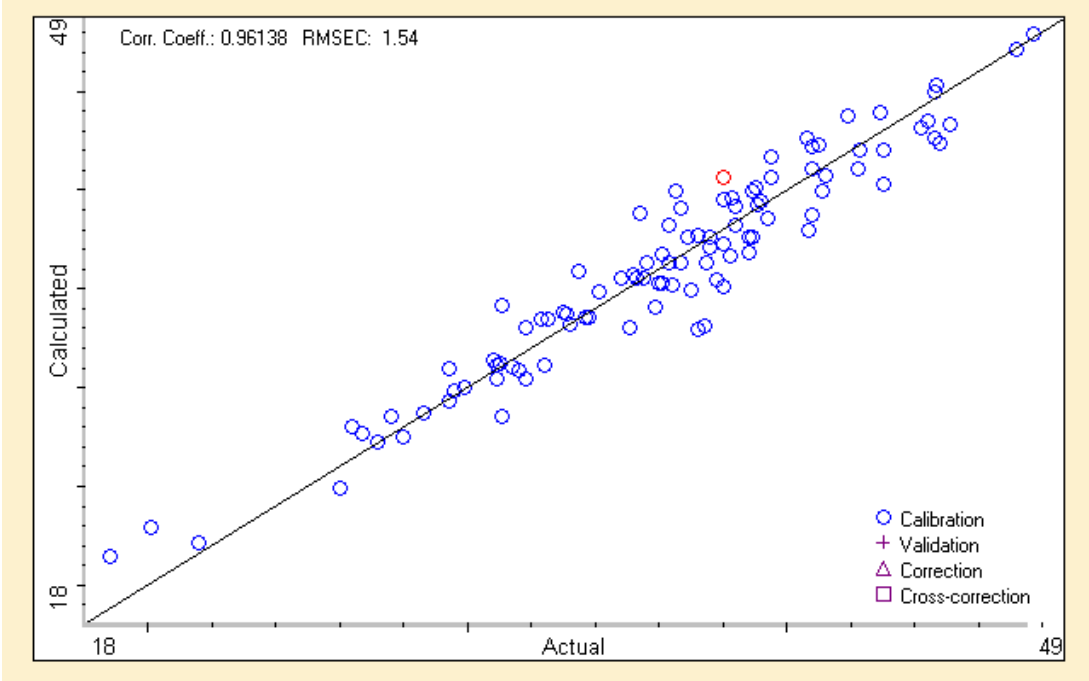

Fig. 13. Correlation of prediction of the FT-NIR software with actual oil content determined by Ward Laboratories.

Table 27. ANOVA of dry matter and seed oil content of sunflower genotypes in 2010 and 2011 at College Station and Pecos, TX.

	Dry Matter		Seed Oil %	
Source	df	MS	df	MS
Year	1	1.36**	1	285.69**
Rep	3	0.09	3	43.17
Error	227	0.04	227	17.41

** Highly significant (p-value < 0.01)

Table 28. ANOVA of dry matter and seed oil content of sunflower genotypes and location in 2011.

	Dry Matter		Seed Oil %	
Source	df	MS	df	MS
Location	1	0.06	1	8.93
Location (rep)	6	0.04	6	13.71
Genotype	21	0.15**	21	88.43**
Genotype*Location	21	0.01	21	87.72
Error	119	0.01	119	5.96

Table 29. Seed oil content of sunflower genotypes grown at College Station and Pecos, TX, in 2011.

	Seed oil
Genotype	%
Advanta 454	34.8c-e
Advanta Aguara	31.8e-h
Advanta F3029	30.7gh
GIG-1616-1	25.82i
GIG-1616-2	30.2h
HA 429	35.6bc
HA 430	30.7gh
NEG-1255	31.9e-h
PAR-1084-1	32.1e-h
PAR-1673-1	34.7b-d
PAR-1673-2	27.9i
Seeds 2000 Firebird	32.8e-h
Syngenta 3732 NS	36.7ab
Syngenta 3845 HO	36.11bc
Syngenta 4596 HO	27.1i
Syngenta 4651 NS	36.2bc
Triumph 664	35.5bc
Triumph s668	36.7ab
Triumph s678	36.0bc
TUB-1709-1	34.1c-e
TUB-1709-3	38.7a
TUB-365	33.1d-f
Mean	33.3
C.V. %	7.3

Oil content and dry matter were analyzed using the GLM procedure and means were generated. Year was a source of significant difference in the seed oil content (Table 27). The ANOVA shows that for dry matter and oil content, genotype is highly significant (Table28). The mean oil content of all samples predicted is 33.3%. This test has a C.V. of 7.3%.

Genotypic means were separated by the Waller-Duncan k-ratio T-test of 100 (Table 29). The genotype that had among the highest average oil content of both locations was TUB-1709-3 at 38.7% oil. GIG-1616-1 had the lowest oil content, 25.82%. The coefficient of variation of this test was 8.3% which indicates reliability. The TUB-1709-3, even if not proven to be salt tolerant has high oil content. Because of this trait, it may prove useful in future breeding programs.

The model that was created for the Thermo Fischer Anteris II NIRS instrument had a degree of accuracy high enough for the needs of this study. Accuracy of the model will improve as more samples validate the prediction curve. The FT-NIRS is a promising breeding tool to determine and select for oil content.

6. SUMMARY

Each experiment in this study contributed to the task of identifying salt tolerant germplasm. The different screenings used different methodology through different stages of sunflower growth to help identify a salt-tolerant genotype of sunflower. These methods, when statistically analyzed, also helped to identify the more effective ways to screen germplasm.

The cultivars that can be recommended for cultivation in College Station are Advanta Aguara, Syngenta 4651 NS, Syngenta 3732 NS, Triumph 664 and Triumph s668. Advanta Aguara was among the highest for yield and seed weight in this location. The other commercial hybrids also yielded among the highest.

There are many commercial hybrids that could be recommended for salt-affected soils. Triumph 664 is a promising cultivar because of its ability to maintain a consistent stature in the greenhouse and in the field. This hybrid also had high seed oil content at Pecos coupled with high yields. The other genotypes that could be recommended for cultivation in Pecos are Triumph s668 and Triumph s678. Another year of yield data in this location would be desired to make more confident recommendations.

The other hybrid that could be recommended for saline growing conditions is Syngenta 3845 HO. This hybrid exhibited an ability to germinate in an environment with a high concentration of salts. This hybrid also performed well in the field by

providing high yields and seeds with high oil content. The 100-seed weight was among the highest of the oilseed varieties at Pecos.

There was one hybrid of interest, Syngenta 4596 HO, but due to its late entry into the study could not be definitively defined as salt tolerant. Another genotype, ARG-1575-1, had low germination rates in both field locations in the petri dish and greenhouse. So though salt tolerance was suspected it could not be confidently confirmed.

TUB-1709-3, PAR-1673-1, and PAR-1673-2 are other candidates for future breeding. TUB-1709-3 had among the highest oil seed content and longest radicles in the 100 mM NaCl solution. PAR-1673-1 and PAR-1673-2 had increased yields and 100-seed weights in Pecos compared to College Station.

Overall this study examined various forms of salt tolerance screening for sunflower. The petri dish system was a novel, high-throughput screening method that can identify salt tolerant germplasm. Although this method needs refinement for greater accuracy, it is a rapid, cost-effective and simple method of analysis.

The greenhouse trials identified salt tolerant lines. The greenhouse data should be used in conjunction with other data because of the frequent lack of significance among genotypes. It is possible that if the plants were grown in a greenhouse with precise temperature and light controls, results may have been more telling.

The field trials generated seed yields for commercial hybrids grown in College Station and in West Texas. This information is valuable for growers and breeders. From

the field trials, mean genotype yields and 100-seed weights could be ascertained. Oil content of genotypes could be determined from the seed harvested from the field trials.

Lastly, this project was productive because oil content of sunflower seeds can now be quickly estimated with confidence through use of the FT-NIRS. Oil content is a crucial breeding objective in sunflower breeding. The use of this instrument saves money and time for the breeding program.

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