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CHARACTERIZATION OF SALT AND DROUGHT TOLERANCE IN SUNFLOWER (Helianthus annuus L.)

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CHARACTERIZATION OF SALT AND DROUGHT TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)

by

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Consequent to the increasing world population, food sources are needed to be increased to meet the nutritional needs. However, due to natural processes and agricultural activities, the most destructive environmental factors that limit crop production, soil salinity, and drought-exposed areas are growing. As one of the major oilseed crops, sunflower (*Helianthus annuus* L.), is considered to be moderately tolerant to salt and drought. Although it can grow in arid to semi-arid regions, increasing salinity and drought might adversely affect sunflower production. This study aimed to investigate several sunflower germplasms' morphological responses to salt and drought stresses. For this purpose, greenhouse and field trials were conducted at University of Nebraska-Lincoln facilities during 2020-2021. For the greenhouse salinity experiment, germplasms PI 539899, PI 539900, PI 539901, PI 539902, PI 539903, and PI 599984 were used and exposed to three different salt concentrations (0, 150, and 250 mM). In addition, PI 632338/HA 429 and PI 632339/HA 430 were tested for drought response under three different irrigation levels for drought experiments in both greenhouse and field. For the greenhouse, treatments consisted of full irrigation (2L/pot), limited irrigation of 50% (1L/pot), 25% irrigation (.5L/pot), and while for the field, full irrigation treatment

(FIT), limited irrigation treatment (LIT), and rain-fed (RF) treatments were applied. In the greenhouse experiments, while the plant height was observed as the highest under the 150 mM salinity treatment, it was seen in the 50% irrigation treatment for the drought experiment. The salt treatment effect was significant with the Soil Plant Analysis Development (SPAD), and the Normalized Difference Vegetation Index (NDVI) with a downward trend over time, and canopy temperature showed an upward trend for salinity and drought trials. In the field experiment, irrigation treatments were not found significant for over time data, however, the effect of time was significant in all data sets, while the germplasm effect and its interaction on canopy temperature, and NDVI was significant. In the salinity experiment, the treatment effect was found to be significant for dry root and shoot weight, while only the germplasm effect was found statistically significant for dry head weight. Different irrigation treatments for the greenhouse drought experiment were only significant for dry shoot weight and head weight. In the field trial, the highest values for head diameter, head weight, whole seed weight g/head, and hundred seed weight were observed in full-irrigated plants. Post-harvest data for the field experiment, different irrigation applications significantly affected the oil amount, and not the crude protein and fatty acids composition. This study indicated that there are differences in genotypes' response to both drought and salinity that could be used for sunflower improvement.

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1. INTRODUCTION

1.1. Sunflower

A member of the family Asteraceae (Compositae), Sunflower (*Helianthus annuus* L.) is one of the widely cultivated annual oilseed crops across the globe. The genus Helianthus includes 51 species and 19 subspecies, which involves 14 annuals, and 37 perennials. While the Helianthus genius` chromosome number is n=17, there are diploid (2n=34, *H. annuus* L.), tetraploid (4n=68), and hexaploid (6n=102) species (Kaya et al.,2012).

Even though the time of domestication and first place of cultivation is uncertain, based on molecular, archaeological, and linguistic finds, the domestication center has existed in eastern North America. Therefore, Sunflower (*H. annuus* L.) is accepted to be native to North America. Additionally, with a long and varied history, unearthed findings of pre-Columbian sunflower ruins in the historical sites in Mexico revealed a second possible domestication place in Southern Mexico (Blackman et al., 2011).

Before the discovery of the New World, North American Indians used Sunflower as a source of diet, medicine, and also, they utilized pollens and petals for painting their body in their ceremonies. Early Spanish, English, and French discoverers grew sunflowers, which were carried from North America to Europe by the 16th century, in their gardens as common flowers. After spreading to Italy, Egypt, Afghanistan, India, China, and Russia, Sunflower became the main oilseed crop in Russia, which led to accepted all around Europe. Regarding usage in the U.S., even though before 1966, non-oilseed varieties were commonly cultivated, after 1966, oilseed types became economically valuable. While approximately 60% of the seeds are processed for oil

production, the remainder is used as bird feed, snacks, baking stuff, and food products (Seiler & Gulya, 2015).

According to the current report revealed by the U.S Department of Agriculture; USDA Foreign Agricultural Service in 2020/21, among annual crops in the world cultivated for edible oil, Sunflower (*H. annuus* L.) placed in 3rd rank after soybean [*Glycine max* (L.) Merr.], and rapeseed (*Brassica rapa* L., and *B. napus* L.), respectively. Based on recent data revealed by Food and Agriculture Organization (2020), Ukraine is the top producer with a production of more than 13 million metric tons of Sunflower seed, followed by Russia, Argentina, China, and Romania, with approximately 11 million, 3 million, 2,5 million and 2 million metric tons of seed production, respectively. In addition to these top five countries, Turkey is ranked as the 7th largest producer (~1.6 million metric tons), while the U.S is the 9th largest producer (~1.2 million metric tons) in the world.

Sunflower considerably contributes to human health owing to the presence of healthy oil and dietary fiber (Adeleke & Babalola, 2020). It is mainly grown for its oilseeds, which contain a considerable quantity of polyunsaturated and monounsaturated fats, phytosterols, tocopherols, protein, copper, folates, iron, zinc, vitamin E, and vitamin B. Being compromised by numerous essential fatty acids like palmitic, stearic, oleic, and linoleic acid makes sunflower a demandable oilseed crop (Tasneem et al., 2015). The protein amount that sunflower seed includes is relatively 30-50% as a percentage, which might be replaced with soybean when the cultivation is challenging. A study conducted in 2013 by Ivanova et al. showed that amino acids in sunflower seeds were glutamic acid (26.91), aspartic acid (10.50), arginine (9.75), phenylalanine, tyrosine, leucine (8.57),

methionine (6.18), and cysteine (3.47) as a percentage. Sunflower has been utilized for its different forms as flour, roasted, baked, or boiled as composite foods (Adeleke & Babalola, 2020).

Aside from being a significant oilseed crop as a food source, birdseed, edible oil, is also presented as a highly adaptive crop to various agroecological conditions. Its moderate tolerance in stress conditions makes sunflower one of the desirable oilseed crops. In general, sunflower is known as a suitable crop to grow in most semi-arid areas, from North America to South, and from Central Africa to Asia, which means it shows tolerance to changing temperatures. Whereas the temperature demand of sunflower for seed germination is at least 46 to 50°F, it shows germination in lower temperatures as 39°F, but temperatures like 28 °F may result in the death of developing seedlings. Regarding its growth stage, 70 to 78 °F is accepted as optimum temperatures, although higher temperatures around 91°F may affect productivity (Putnam et al., 1990).

In addition to its ability to grow in various temperatures, the sunflower is also adaptive to grow in different types of soils such as sands, loams, silts, and clays. In proper plant growth, micronutrients play a significant role. Sunflower macronutrients requirement is relatively less than some commonly cultivated crops including corn, wheat, or potato. The reason for its inefficient usage is that sunflower stover is composed of a large part of these elements, which eventually return the vast majority of macronutrients to the soil as stover (Putnam et al., 1990).

1.2. Sunflower Oil

"*The food that is good for the heart is likely to be good for the brain" – Hippocrates.*

Due to the rapid surge in human population, the 21st century has become more challenging for agriculture regarding increasing demands of food source concern for humans and animals. Because of these challenges, having alternative approaches for agricultural sectors to increase food production is essential. Not only increasing alternative sources is crucial, but also having nutritional sources is the key.

Sunflower is considered one of the most nutritious oilseed crops with containing health-wise beneficial fatty acids components, namely oleic acid (C18:1), palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2), which are liquid at room temperature and includes one or more double bonds and carbon chains less in hydrogen atoms. In 2016, Avni et al. demonstrated that sunflower oil content is mostly formed by linoleic acid (polyunsaturated oil) with 59%, followed by monounsaturated oils oleic acid, stearic acid, and palmitic acid with 30%, 6%, and 5%, respectively. With advanced breeding programs, Sunflowers containing high-linoleic with 69% linoleic acid, high-oleic with 82% oleic acid, mid-oleic with 65% oleic acid, and high-stearic with high-oleic 18% stearic acid, and 72% oleic acid, were also obtained (Gupta, 2014). In comparison to some other important oilseed crops safflower seed (28.2%), sesame (25.5%), flax (22.4%), cottonseed (18.1%), peanut (13.1%), and soybean (3.5%), sunflower is higher in polyunsaturated fatty acids with 31.0% (Saunders et al., 2013).

There are a variety of vegetable oils and grades regarding the quality of oils commonly used in daily life cooking and commercial purpose (López-Beceiro et al., 2011). Considering dominantly used major oilseed crops such as soybean, rapeseed, peanut, and sunflower, sunflower has been accepted as a premium-quality source of edible oil for kitchen use (Pal et al., 2015). Oxidation of oils at a higher temperature

during frying is one of the main health and safety concerns for human consumption. Therefore, stability at relatively high temperatures is one of the crucial criteria in oxidation prevention (Vorria et al., 2004). According to Food and Agricultural Organization (FAO), consuming oils and fats consisting of high oleic acid, which is more stable at higher temperatures and high tolerance to oxidation (Inturrisi, 2015), lowers the level of cholesterol and atherosclerosis risk. Thus, sunflower oil with high oleic acid-rich in vitamins and nutrients meets the demand for healthy diet standards compared to other oils (Romanic, 2020). A study showed that sunflower oil is rich in tocopherols (alpha, beta, gamma, and delta), which are chemical forms of vitamin E that provide a strong antioxidant, compared to soybean and canola (Grilo et al., 2014).

A recent study revealed by Vijayakumar et al. (2016), in India and South Africa, the cultivation of sunflower can be competitive with some of the major food sources such as maize, soybean, and sorghum. Based on the research conducted by Taher et al. (2017), it is emphasized that Sunflower production needs to be developed to alleviate the need for rising oilseed demand.

1.3. Salinity

1.3.1. Salts

Salts, as a term, are the chemical combination of an acid and metal, which are positively (cations) and negatively (anions) charged ions. Some widely known cations and anions namely sodium (Na^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^{-}), sulfate (SO_4^{2-}), and bicarbonate (HCO_3^{-}) contribute in formation of sodium chloride-NaCl (table salt) ,calcium chloride-$CaCl_2$ (common deicing agent), magnesium chloride-$MgCl_2$ (common deicing agent), sodium sulfate-Na_2SO_4 (thenardite), calcium sulfate-

$CaSO_4$ (gypsum), magnesium sulfate - $MgSO_4$ (Epsom salt), sodium bicarbonate- $NaHCO_3$ (baking soda), calcium carbonate- $CaCO_3$ (limestone), calcium-magnesium carbonate Ca Mg $(CO_3)_2$ (dolomite) (Bauder et al., 2008).

1.3.2. Salinity in Soil

Salinity is one of the common problems in irrigated and non-irrigated lands used for agricultural purposes. The amount of resolved salt in water is defined as salinity, detrimental abiotic stress restricting soil fertility and crop productivity (Parihar et al., 2015). The Food and Agriculture Organization (FAO, 2021) states that approximately 833 million hectares of soils are affected by salinity around the world, which equals around 8.7% of the globe. Salt-affected areas are primarily centered in arid or semi-arid environments in Africa, Asia, and Latin America. Since 20-50 % of irrigated lands on earth are considered excessively salty, food production becomes challenging for more than 1.5 billion humans (FAO, 2021). Concerning non irrigated areas, drylands, Australia is under a severe salinity threat compared to other countries. The National Land and Water Resources Audit (2001) predicted that by 2050, dryland salinity is to rise from 5.7 million to 17 million ha (Pannell & Ewing, 2006).

Soils become salty very rapidly for several reasons. Regarding the reasons, there are two groups classified as Natural or primary salinity and Secondary or human-induced salinity (Parihar et al., 2015).

1.3.2.1. Natural or Primary Salinity

Primary salinity is caused by the deposition of salts in the long term via the biological activities in the soil or underground water, resulting from two processes. One of two is the degradation of bedrocks comprised of resolvable salts. Breaking down the

bedrocks and minerals helps to loosen the salts such as chlorides of sodium (most soluble), calcium, magnesium, sulfates, and carbonates. The second one is the weathering of oceanic salt via rain and wind as cyclic salts, which mainly contain sodium chloride (Parihar et al., 2015).

1.3.2.2. Secondary or Human-Induced Salinity

Human mismanagements are the reason for secondary salinization, ending up unbalanced soil hydrology. Over usage of fertilizers, deforestation of green lands, aggressive irrigation strategies in agriculture, poor drainage of fields, irrigation of the lands with relatively high salt waters are examples of these mismanagements. As a result, fertility and productivity are lesser in human-induced salt-affected soils (Garg & Manchanda, 2008).

Augmentation of salts in the soil leads to the swell of the disperse of clays in soil. This occurrence eventually ends up with the breakdown of aggregation, which results in reduced water transmission (Masor, 2011). Additionally, the presence of salt causes to crust layer that blocks the absorption of water, gas diffusion, damages seedlings, and the activity of microorganisms associated with the cycle of nutrients (Rolston et al., 1984). Based on these reasons mentioned, salt-affected soils create unfavorable inhabitancy for plants.

1.3.3. Salinity Impact on Plants

Saline-exposed environments inhibit the uptake of water and minerals in plants, resulting in reduced plant development, directly affecting yield. Plants absorb water with the process known as osmoregulation, an internal balance of water and soluble materials, in which water is moving from root to all plant body. While the low or moderate salinity

(high soil water potential) provides a favorable atmosphere for regulating osmotic and sustaining a proper inflow of water to plants, the amount of salt in the soil is higher than in-plant, water cannot travel from soil to roots properly, which impacts transpiration and, most importantly, yield (Parihar et al., 2015). A study conducted by Khan et al. (2013) demonstrated a drastic reduction in water potential in *Cucumis sativa* when the salinity level increased.

During salt stress, vital biological activities such as photosynthesis, protein synthesis, and energy and lipid metabolism are primarily affected. The initial salinity response in plants is seen as reducing the rate of leaf area enlargement, while leaf expansion may be ceased as conditions worsen (Parida & Das, 2005).

Salinity adversely affects the fertility of plants, even when slight symptoms may be observed at all growth stages from germination to maturity. Plants showing chlorosis and necrosis, especially on the leaves, may end up with plant death in case of a higher level of salinity. Due to the accumulation of ions such as boron, sodium, and chloride in soil, causing water uptake restriction, salinity unearths the elemental toxicity for plants (Bouder, 2008).

1.3.3.1. Salinity Effect on Germination

Seed germination is the first stage of the plant life cycle closely associated with the crop yield. It has been revealed that the germination of seeds in various plants, including *Posidonia*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, and *Brassica* spp., is negatively influenced by salinity in many aspects (Parihar et al., 2015). The salinity decreases the absorption of water by seeds possessing a potentially low level of osmotic in germination media, creates a toxic environment causing changes in enzymatic

activities and protein metabolism, disrupts the balance of hormones, and restricts the usage of seed reserves (Othman et al., 2006). Kaveh et al. (2011) showed a notable negative association between salinity and seed germination in *Solanum lycopersicum* with the delayed and decreased germination rate. Bordi, (2010) revealed that there is a dramatic reduction in the germination percentage of *Brassica napus* seeds at 150 and 200 mM sodium chloride (NaCl). Similarly, over 90 mM of sodium chloride (NaCl) could be detrimental to cowpea cultivation (Ravelombola, 2021). Additionally, Khodarahmpour et al. (2012) claimed that in *Zea mays* seeds applied 240 mM NaCl shows a significant decrease in seed germination with 32%, length of radicle and plumule with 80% and 78%, respectively, and vigorous of seeds with 95%.

1.3.3.2. Salinity Effect on Photosynthetic Pigments And Photosynthesis

Photosynthesis is one of the essential biochemical processes that plants transform solar energy into chemical energy. The photosynthesis pathway is inhibited by salt stress predominantly because salinity reduces water absorption. Furthermore, the aggregation of Na+ and Cl- in the chloroplasts and chlorophyll, which correspond to the greenness and health, leads to the photosynthesis rate reduction (Zhang et al., 2005).

The chlorophyll content is a critical indicator of examining cell metabolism (Chutipaijit et al., 2011). To illustrate, a study done by Amirjani in 2011 on *O. sativa* depicted that after applying NaCl (200 mM) for 14 days, the leaves' chlorophyll a and b contents reduced. While the reduction amount was 41% for chlorophyll-b content, chlorophyll-a decreased 33 % as a percentage. Likewise, the application of 100 mM NaCl concentration on *O. sativa* caused a reduction in chlorophyll a, chlorophyll b, and carotenoids compared to the control groups, 30, 45, and 36 %, respectively (Chutipaijit et

al., 2011). In another study, under salt concentration treatments, the content of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, and xanthophylls in the mung bean (*Vigna radiata*) showed a linear drop (Saha et al., 2010).

1.3.3.3. Salinity Effect on Yield

Salinity over the tolerable level eventually results in crop loss which is the most calculable impact in agriculture as the grower's approach. A study conducted by Nahar & Hasanuzzaman (2009) showed that the yield and yield components, including pods number per plant, seeds per pod, and seed weight, of *V. radiata*, adversely influenced by salinity above the inhabitable limits, causing a significant decrease in all these yield components. The decline of yield due to salinity is closely associated with the low biological and physiological activities in the green part of the plants supported by the less photosynthetic rate (Wahid et al., 1997). For example, pollen viability is dramatically reduced under salt stress conditions, causing seed-forming failure (Abdullah et al., 2001). Greenway & Munns (1980) notified that 200 mM NaCl application might result in around 20% dry weight loss even in salt-tolerant species sugar beet; however, reach up to 60% crop loss in moderately tolerant species cotton dead in soybean as an intolerant to salinity. Additionally, the growth and yield components of *Foeniculum vulgare* Mill, such as plant height, fresh weight yield, and biomass are also negatively affected by irrigation with higher salinity (Semiz et al., 2012).

1.3.4. Salinity Tolerance in Plants

Salinity stress disturbs homeostasis in water content and ion dispersion actualizing at the cellular and the whole plant stages. The extreme alterations in ion and water homeostasis cause damage to plant cells and tissues, growth cease, and eventually

death. Therefore, there are three associate strategies to accomplish tolerance to high salinity, which are 1) inhibition of the detrimental effect of salinity, 2) reestablishment of the homeostatic conditions under salt stress, 3) continuing plant growth at a lowered rate (Zhu, 2001).

The cell integrity, nutrient obtaining and transport from roots to other organs, and the function of proteins and the photosynthetic tools are sensitive to the high salt level. The reason for damage might be the generation of the reactive oxygen species (ROS) due to salinity stress. Plants respond to salt stress by producing stress proteins such as glutathione peroxidase, superoxide dismutase, ascorbate peroxidases, glutathione reductases, and relevant osmolytes such as mannitol, fructans, trehalose, ononitol, proline, glycine betaine, and ectoine to detract from ROS for the detoxification (Zhu, 1997).

Plants are also required to develop water and osmotic homeostasis and ionic homeostasis at the cellular level. The plants deposit numerous osmolytes in the cytosol to maintain water uptake from the soil with high salinity (Zhu, 1997). The accumulation of organic osmolytes might play an important role in protecting cellular structures. Proteins in the water channel might also regulate the velocity of water transfer through cellular membranes in salt stress conditions (Chrispeels et al., 1999).

Lower growth is an adaptive trait responding to salinity stress for plant survival, which provides the ability to stand towards stress (Zhu, 2001). However, plants can continue to grow when the stress is relieved. Productivity could be enhanced through this fine-control responsiveness in the environment with salinity stress (Hanin et al., 2016).

1.4. Drought

Recently, the impact of climate change is being felt more and more. Climate change has begun to impact the magnitude and the distribution of rainfall, ultimately impacting the dynamics and availability of water resources. Increasing temperatures, changing precipitation regimes, and meteorological disasters are examples of some impacts that affect all vital human activities.

Evaluating the predicted impacts of climate change on agriculture and agricultural water management, the availability of water fed by rainfall, rivers, and aquifers is essential (Turral et al., 2011). By 2050, approximately 60 % more food needed to be assured of preserving global food security while protecting and developing natural sources (FAO, 2016). One of the main entries of food production is water, which requires from field to all the steps in the agro-value chain. Not only for food purposes, but water is also crucial for sustaining water-related ecosystems. However, while water demand is rising, at the same time, sources of water are decreasing, which is mainly caused by climate change. Alteration of climate not only limits the water sources but also leads to other reasons for droughts, namely high temperatures, high light density, and dry wind, all of which result in evaporation from the soil (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). Thus, the world is under threat of water scarcity that requires national and global action to lessen food needs in many areas, mainly in regions already facing water shortages (Khan et al., 2013). Drought is a hypothetical climatic hazard caused by severely insufficient precipitation, considered the most destructive natural disaster affecting the global population than any other catastrophe (Kallis, 2008).

1.4.1. Drought Types

According to the National Integrated Drought Information System (NIDIS), drought is commonly explained as "a deficiency of precipitation over an extended period (usually a season or more), resulting in a water shortage." Drought can be categorized as meteorological, hydrological, socioeconomic, and agricultural drought.

As reported by the National Drought Mitigation Center (NDMC) in the University of Nebraska-Lincoln, while meteorological drought is interpreted as dryness status during the dry conditions, compared to regular status, which is specific to regions, hydrological drought is related to the insufficient precipitation amount on the ground and underground water reservoir. Socioeconomic drought differs from other drought types with its existence since it is focused mainly on stock and demand of economic possessions. Lastly, agricultural drought is connected with all other drought types since it is about inadequate precipitation amount, potential evapotranspiration, lack of water in the soil, and low level of subterranean water (Wilhite & Glantz, 1985). Therefore, it can be seen that agricultural drought is commonly affected by meteorological drought and hydrological drought. In these scenarios, plants are the elements that are mostly affected.

1.4.2. Drought Effect on Plants

Environmental factors like stresses primarily control plant growth and development, which limits production (Dennis, 2000). One of the widespread abiotic stresses that essentially inhibit productivity is drought. Drought stress is accepted as a significant threat to crop production (Fathi & Tari, 2016) and therefore it is one of the most investigated environmental stresses by researchers on the globe.

1.4.2.1. Drought Effect on Morphological and Physiological Factors

Plant growth and development result from complex processes controlled by numerous morphological and physiological factors. Drought stress causes changes in the cytosol with the declining water potential and turgor of plant cells, causing the increase in the dissolved matters in the cytosol including free amıno acids, sugars, and proline (Benlloch-González et al., 2015), which scale down the cell elongation, limiting the plant growth (Lisar et al., 2012). Thus, lower carbon absorption, erratic mineral nutrients transfer from cell to cell, and abscisic acid (ABA) deposition present after growth reduction, ending with plant wilt (Farooq et al., 2012). Moreover, the leaf area reduction is a typical response of drought stress on the mineral matter and metabolic activities such as carbon assimilation disruption, followed by the chlorosis on the leaf and drop of leaves eventually lessen plant canopy (Hussain et al., 2018). Additionally, drought stress causes substantial alterations in crops' morphological and physiological characteristics, including root development, plant height, stem diameter, and chlorophyll contents. For example, Turhan & Baser (2004) reported a significant reduction in the plant height, stem diameter, number of nodes, and leaf area of sunflower growing under the drought stress condition. However, there is an increase in root length and weight compared to the untreated (control) group.

1.4.2.2. Drought Effect on Germination

The potential impact of agricultural drought is related to the period of deficit precipitation, plant species, and growth stages of crops (Gupta et al., 2020). One of the most deleterious effects of drought stress on plant growth is reducing germination and seedlings (Kaya et al., 2006), which is the initial step of the plant establishment in the

field. Seed germination is one of the most complicated phases in the plant life cycle, directly associated with soil moisture (Luan et al., 2014). An adequate amount of water moisture is necessary to induce seed germination through the absorption of water by seeds from the soil (Hussain et al., 2018). Therefore, the early impact of drought may begin with reduced and nonuniform germination leading to unsatisfactory plant development (Hussain et al., 2018). There is a positive correlation between soil water potential, seed germination, and seedling elongation (Wen, 2015). A study regarding water stress on seed germination of mung beans demonstrated that germination and seedling elongation rate declines with increasing dry conditions. Even though water stress seems to be comparatively less damaging to the seedlings in the short term (24 hours), a reduction in fresh seedling weight was still seen (De & Kar, 1995).

1.4.2.3. Drought Effect on Photosynthesis and Chlorophyll Content

Drought stress directly influences several vital metabolic pathways such as photosynthesis, contributing to plant growth and development by using sunlight, water, and CO_2 to obtain oxygen and energy. Photosynthesis is inhibited under water-deficient conditions due to decreased CO_2 conductivity between stomata and mesophyll cells (Havrlentová et al., 2021). Also, the lower stomatal activity due to drought stress may be a reason for reducing photosynthesis (Abid et al., 2018). In addition, the reduction in CO_2 assimilation negatively impacts the Rubisco function. It decreases the activity of nitrate reductase and sucrose phosphate synthase, and ribulose bisphosphate (RuBP) production (Singh & Thakur, 2018).

Drought stress also affects the chlorophyll content, another photosynthetic indication, deeply touched by water scarcity (Alghbari & Iksan 2018). Due to drought

stress, there may be an alteration in the chlorophyll synthesis and the ratio of chlorophyll a/b on the leaves. The reduction in the quantity of chlorophyll under the water deficit is the result of the enhancement of O_2 and H_2O_2 production, causing lipid peroxidation and degradation of chlorophyll (Havrlentová et al., 2021). In addition, the presence of Calvin cycle proteins like Rubisco goes down due to drought stress (Anjum et al., 2011). A reduced plant output may be encountered because of less photosynthesis and abundance of chlorophyll, changes in stomatal activity, and unbalanced internal water content of plants.

1.4.2.4. Drought Effect on Yield

The drought-induced loss is classified as the most extensive loss in the agronomical aspect (Daryanto et al., 2016). The drought impact on plants ranges from morphological to cellular levels and in all growth stages of the plants', scarcity of water has detrimental effects on all yield-associated physiological practices (Farooq et al., 2009).

In 2012, the United States experienced the dry seasons, similar to the drought periods in the 1980s, and the national crop yields reduction was approximately 21% when the last five years' yield average was considered (Boyer et al., 2013). While it is known that drought adversely affects productivity, some dynamic dimensions such as the duration of dryness, timing, and the adaptation of the plants are also needed to be taken into account (Irmak et al., 2019). To illustrate, a yield-related study conducted by Daryanto et al. (2016) evidenced that at a 40 percent water decrease, yield reduction in wheat was 20.6%, while in maize, this amount was 39.3 %. Additionally, researchers

claimed that wheat was more tolerant to dryness than maize, particularly during the flowering phase.

1.4.3. Drought Tolerance in Plants

Water is a vital source for plant viability, and water deficiency is a major limiting factor for plant growth. Drought resistance is described as sensing and responding to water shortage signals and inducing response strategies. Plants possess several mechanisms to inhibit water loss, provide the optimum amount of water to vital parts of plants to sustain the water content of cells in the duration of drought, which are a) drought escape through the completion of the reproductive stage before the severe water deficiency, b) drought avoidance by increasing internal water capacity with closing stomata and constraining leaf area, c) tolerance with the osmotic regulation and enhanced cell wall elasticity, and alteration in metabolic reactions like an uplifted rate of antioxidant metabolism (Abid et al., 2018).

Water presence signals are transferred through the plant vascular components, the xylem, and phloem, from below-ground parts to above-ground parts, and reverse transfer direction exists for the photosynthesis products (Scharwies & Dinneny, 2019). The formation of the vasculature tissues plays a significant role in response to drought resistance/tolerance, especially in the reproductive stage of the plant (Gupta et al., 2020). Concerning the drought escape, the transfer of the photoperiod-dependent protein FLOWERING LOCUS T (FT) from leaves to the shoot apical meristem via the phloem promotes early flowering in Arabidopsis thaliana(Andrés & Coupland, 2012).

Water stress activates phytohormone signaling as a drought response, causing a rapid increase in the abscisic acid (ABA) production, promoting the stomatal closure to

reduce inner water loss (Tardieu et al., 2018). In addition, the antioxidant production system is also triggered to sustain redox homeostasis and deliver peroxidase enzymes to maintain cellular functions and integrity (Bailey-Serres et al., 2019). Thus, metabolites, including proline and trehalose, stress protectants, are synthesized (Gupta et al., 2020).

The plants' below-ground parts (roots) are in the first line sensing the soil water deficiency. Roots exhibit morphological changes from the cellular level to the entire architecture in the roots as a response to drought to improve water and nutrient uptake from the soil, which is coordinately existing in all root parts (Dinneny, 2019). Longer and deeper roots with fewer branches can sufficiently uptake the moisture and nutrients from the soil that is dry at the surface but preserve moisture at the deeper levels. However, shallow roots can maximize water absorption from the ground surface in areas with low rainfall (Dinneny, 2019). Roots, developing in the habitat with inhomogeneous water supply, show hydropatterning by forming lateral roots in the direction of soil with an increased water capacity (Rellán-Álvarez et al., 2016). Hydrotropism is another strategy to adapt to the environment that water distribution is non-homogeneous through the soil to enhance water acquisition (Dietrich et al., 2017).

1.5. Drought and Salt in Sunflower

1.5.1. Drought Effect and Tolerance in Sunflower

Drought is multifaceted stress affecting most plants at different life cycle stages (Yordanov et al., 2000). One of the most used preventative practices among industrial and agricultural users is irrigation of planted areas to alleviate the impact of drought. However, it is expected that irrigated lands will be decreased in the near future, which will lead the watering lands to rainfed lands, resulting in most plants undergoing drought

stress (Alexandratos & Bruinsma, 2012). Therefore, in this scenario, almost all crops will be adversely affected by water stress, even moderately drought plants such as sunflower (Hussain et al., 2018).

The amount of yield diminution in sunflower is dependent on the growth stage, genotype, and intensity of the drought (Rauf, 2008). In several studies, it is stated that drought stress substantially lowers the yield of achene and oil in sunflowers (Soleimanzadeh et al., 2010), (Stone et al., 2011). Even though sunflower is moderately tolerant to drought (drought escape behavior), it may be susceptible to drought from early flowering to achene filling because due to water inefficiency, leaf enlargement and transpiration rates will be inadequate for proper development (García-López et al., 2014). Despite drought influencing sunflowers in every growth stage, the most detrimental effect in yield is seen during the reproductive stage (Rauf, 2008). An experiment carried out in Turkey by Karaata (1991) to observe the most sensitive stage of plant growth in a drought environment revealed that the highest yield decrease is seen during flowering time. Furthermore, according to a study done by Vijay (2004), the maximum yield is achieved when sunflower plants are irrigated properly during the reproductive stage. Likewise, Prabhudeva et al. (1998) tested sunflower genotypes in water stress during bud initiation and achene filling. As a result, a biological yield reduction in both stages, but stress in bud initiation was more damaging than the achene filling stage.

Declining the water amount in the soil triggers leaves to wilt, leading to yield reduction in low rainfall receiving lands (Aboudrare et al., 2006). Tahir et al. (2002) examined 25 inbred lines of sunflower under drought conditions and revealed that due to drought stress, while root growth was increasing, there was a reduction in plant height,

leaf area, head diameter, 100 seed weight, and plant biomass. Even if water scarcity adversely affects the achene yield and oil in sunflowers, it has been reported that the quality of oil content was not considerably affected (Petcu et al., 2001).

Water use efficiency (WUE) is a term presented over a hundred years ago to describe the relationship between plant development and water usage with an explanation of the measurement of biomass produced per unit of water uptake by the plant (Hatfield & Dold, 2019). A rise in water use efficiency is important for water stress tolerance, an indicator of adjustment to various environments. Regarding water use efficiency, since Sunflower creates deep roots to draw water in water shortage, it is accepted as good in water use efficiency. However, it depends on the duration development stage of water insufficiency since the yield and yield components (seed and oil rate) are affected adversely in the case of long-time dry conditions (García-Vila et al., 2012; Ahmad et al. 2014).

Regarding the planting of sunflower in semi-arid regions, Aboudrare et al. (2006) indicated that there are some suggested approaches to reduce the water stress in a sunflower; planting in the fall season (tropic regions), irrigation as needed, using drought-tolerant genotypes, reducing the density of plant, and applying fertilizer (N). In this regard, Talebi (2009) also emphasized that the usage of drought-resistant plant varieties is vital in regions with drought problems.

With the competence to grow in various environments and be moderately tolerant to drought, Sunflower may become the preferred oilseed oil in the future, most notably considering global climate changes (Miladinović et al., 2019). In the same point of view, Seiler (2018) agreed that the capability to survive in different agroecological

environments makes Sunflower a promising future industrial crop. Garcia-Vila et al. (2012) also pointed out that the preference for Sunflower in the future is associated with its adaptation to climate changes.

1.5.2. Salt Effect and Tolerance in Sunflower

In addition to drought, salt stress is one of the increasing detrimental abiotic stress for plants. In sunflower production, salinity is a significantly restricting component for growing, even though it is classified as moderately tolerant to salt stress (Miladinović et al., 2019).

For evaluation of salt tolerance in sunflower, there are some main agronomical parameters to use, such as yield, plant height, leaf area, leaf injury, leaf greenness, relative growth rate, relative growth reduction, and root parameters (Ashraf & Harris, 2004).

Various studies have shown that high salinity levels result in a decline of yield and yield components such as leaf area, dry matter in sunflower, especially in relatively nonresistant lines (Katerji et al., 1994). Miladinović et al. (2019) also argued that higher salinity levels induce the decline of seed number and weight per head, significantly affecting yield (Miladinović et al., 2019). Reduced osmotic potential of soil, less nutrition, and all related factors are correlated with the harmful effects of salinity in sunflowers that affect plant growth and development at physiological, biochemical, molecular, and whole plant levels (Rasool et al., 2013). Noreen & Ashraf (2008) stated that salinity induced a decrease in CO_2 absorption, rate of transpiration, and stomatal conductivity of sunflowers. Similarly, Ibrahim et al. (2006) alleged that salt stress lowers the photosynthetic rate due to stomatal limitations. Ashraf & Tufail (1995) affirmed that

sunflower planting in salinity conditions causes toxic ion aggregation, primarily in the old leaves. Likewise, Mutlu & Bozcuk (2005) indicated that some of the osmolites' concentration in the leaf, namely proline, betaine, and free and bound polyamines, has increased due to salinity.

According to several studies' results carried out by Ashraf & Tufail, 1995; El-Kheir et al., 2004; Flagella et al., 2004; Di Caterina et al., 2007, sunflower seed oil yield is also affected by salt stress. In this respect, Flagella et al. (2004) stated that while the oleic acid rate increased, there was a reduction in linoleic acid content.

Regarding salt tolerance in sunflower, Miller & Gulya (1995) believed that one major gene and possible recessive modifier genes are responsible for tolerance, while Lai et al. (2005) stated that six genes (*HT089*, *HT175*, *HT185*, *HT215*, *HT 216*, and *HT227)* could be attributed to regulation of ions, which responsible for tolerance. *H. annuus* and *H. petiolaris* have genes that enable potassium and calcium transportation, providing adaptation for salt to these species (Edelist et al., 2009). Many wild species are adapted to grow naturally in saline soil conditions, which plant breeders can use to examine genes for soil resistance (Miladinović et al., 2019). For example, *H.paradoxus* is believed to be three times more stable than cultivated sunflower in salt conditions with higher leaf succulence (Karrenberg et al., 2006; Edelist et al., 2009). The United States Department of Agriculture (USDA) released two lines from an interspecific cross of *H.paradoxus* and *H.annuus* as HA 429 and HA 430, which are meant to be tolerant to salt and drought (Miller & Seiler, 2003).

Some methods have been suggested as NaCl priming and K+, ascorbic, proline, calcium application to the leaf that increased growth and yield of sunflower under salinity

conditions to mitigate the negative effects of salinity on sunflowers (Bajehbaj, 2010; Akram et al., 2009; Khan et al., 2013; Khan et al., 2014; Lexer et al., 2003).

Even though sunflower is considered moderately tolerant to drought and salinity, high tolerance plants will be significant for breeders. In the light of this knowledge, this study aims to a) determine the effect of drought and salinity on yield and yield components of sunflower in greenhouse conditions, b) investigate the impacts of the different irrigation levels on Sunflower yield and yield components in semi-arid conditions c) examine the effect of different irrigation levels on sunflower seed oil yield and fatty acid contents.

2. MATERIALS AND METHODS

2.1. Greenhouse Experiments

Greenhouse experiments were conducted in the University of Nebraska-Lincoln East Campus Agronomy greenhouse complex in 2020 and 2021. For each experimental year, the room temperature was set at 25 ℃ days and 23 ℃ nights, with 15 hours of day length.

2.1.1. Germplasm Selection

For this experiment, germplasms were obtained from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) U.S National Plant Germplasm System, Ames, Iowa. All plant materials were authorized by curator Laura Marek.

The selected plant materials (*H.annuus*) with PI 539899, PI 539900, PI 539901, PI 539902, PI 539903, PI 599984, PI 632338, and PI 632339 were chosen based on the accession recommendation on the system. As described by USDA-ARS, PI 539899, PI

539900, PI 539901, PI 539902, PI 539903 were potentially salt-tolerant, while the PI 599984 was indicated as salinity susceptible. Therefore, these six lines were used for salinity stress experiments in the greenhouse condition.

For the drought stress experiments, PI 632338 (HA 429) and PI 632339 (HA 430) were used for both greenhouse and field conditions. These two lines were described as salt and drought tolerant, but they are used as drought-tolerant lines in the drought-associated experiments. All germplasms were developed in North Dakota, United States, and maintained in North Central Regional PI Station placed in Ames, Iowa, United States. Seeds were not treated with any fungicides and insecticides.

2.1.2. Soil Preparation

For the experiments, in each year of 2020 and 2021, a standard greenhouse mix (5 gallons peat, 3 gallons soil, 2.5 gallons sand, 2.5 gallons vermiculite) was used. Pots with 5 Gallon-11.3"D x 11.3"W x 12"H measurements were filled with standard greenhouse mix soil. In total, 120 pots were used. While 90 pots were used for the salinity test, 30 pots were used for the drought test as ;

- *Salinity experiment: 6 lines * 5 replications for each line * 3 treatments = 90 pots.*
- *Drought experiment: 2 lines * 5 replications for each line * 3 treatments= 30 pots.*

2.1.3. Experimental Design for Greenhouse Experiments

For 2020 and 2021, greenhouse experiments were designed as completely randomized designs with two factors. Randomizations for each experiment (salinity and drought) were arranged according to AGROBASE results.

- *Salinity experiments: plant material x different salt concentration levels.*
- *Drought Experiments: plant material x different irrigation levels.*

2.1.4. Preparation of Salt Concentrations for Salinity Experiment

Three levels of salt concentrations no salt, 150 mM, and 250 mM were used for the salinity experiments. No salt treatment was just greenhouse tab water, used as control. For salt treatment, 99% pure sodium chloride (Fisher Scientific, Waltham, Massachusetts) was used.

- **For 150 mM NaCl solution ;**

The molecular weight (g/mol) of NaCl equals 58.44 g

Since 150 mM=0.15 M ;

*0.15 M*58.44 g =8.76 g NaCl is used for 1 liter of 150 mM NaCl solution.*

In every application, 2L solution per pot was used. Therefore, to obtain 150 mM NaCl solution for each application time;

*6 lines*5 rep*2L=60L of the solution was prepared, in which 60L*8.76 g NaCl =525.6 g NaCl was used in every application.*

- **Similarly, for 250 mM NaCl solution ;**

The molecular weight (g/mol) of NaCl equals 58.44 g.

Since 250 mM=0.25 M ;

*0.25 M*58.44 g = 14.61 g NaCl is used for 1 liter of 250 mM NaCl solution.*

*With using 2L of solution per pot, 6 lines*5 rep*2L=60L solution was required each time. Therefore;*

*60L*14.61 g NaCl=876.6 g NaCl was used in each application.*

2.1.5. Irrigation Materials for Drought Experiment

Fisher Scientific low-form polypropene beakers were used to irrigate plants. For full irrigation treatment, 2L beaker, for limited irrigation level (50%) 1L beaker, and for 25% irrigation level 0.5L beaker was used. During experiment greenhouse tab water was used.

2.1.6. Data Collection for Greenhouse Salinity and Drought Experiments

A meter stick is used to calculate the plant height regularly. The readings were taken from the soil surface to the top leaf of the plants individually for each line. This measurement lasted to the reproductive stage since there was no significant change in the height after flowering was observed.

The SPAD-502 (Soil Plant Analysis Development) by Minolta Camera Co. Ltd., Japan, is a hand-held device that measures light transmittance (red 650 nm and infrared 940) through the leaf, which helps determine the chlorophyll concentration (Afonso et al., 2018). It is also called the chlorophyll meter, corresponding to N content. In this experiment, readings were taken from the three top leaves of each plant, which were relatively fresh and did not include any damage on the leaf surface.

The FieldScout CM 1000 meter (NDVI – Normalized Differences Vegetation Index) by Spectrum technologies Inc, USA, assesses the chlorophyll content by remote sensing based on the reflectance of chlorophyll in the leaves. It is a point-and-shoot technology that senses light from 660 nm to 840 nm to measure leaf greenness. The values were calculated based on the formula [(%Near Infrared − %Red) / (%Near Infrared + % Red)] and changes between -1 to 1. Data were taken from the same leaves

used for Spad-502 reading during the experiment to provide consistency since both readings are related to greenness.

Leaf surface temperature (IR temperature meter) by Spectrum technologies Inc, USA, is a hand-held device to measure the leaf temperature. In this experiment, readings were taken from each top three leaves of each plant, and the average was used for statistical analysis.

- *All data mentioned above were collected relatively at the same time, around noon, based on sunlight position.*

2.1.7. Post-Harvest Data Collection for Greenhouse Salinity and Drought Experiments

After plants completed their life cycles, the soil was removed from the root by washing. Then, each plant was individually separated by root, shoot, and head to be measured. For this process, firstly, plants' parts were completely dried in a drier at 70-75°C (over 160°F) for 10 hours. Afterward, dry root, dry shoot, and dry head were measured for each plant.

2.2. Field Experiment

The field experiment carried out at Eastern Nebraska Research and Extension Center of the University of Nebraska, Mead, NE, U.S. (41°08′44.4″ N, 96°26′20.6″ W, 369 m above sea level) with intending to observe and compare the morphology and yield of the sunflower accessions at different irrigation levels for the two accessions.

2.2.1. Field Design

The field design used for this study was a split-plot design in a randomized complete block design (RCBD). For this experiment, three different irrigation levels were randomly assigned as treatment, and two different germplasm were used. Irrigation levels were applied as full irrigation treatment (FIT/1.0 inch), limited irrigation treatment (LIT/0.60 inch) as 60% irrigation, and rain-fed (RF).

2.2.2. Germplasm Selection for Field Experiment

Regarding the germplasm, the seeds that were used for the field trial were PI 632338 (HA 429) and PI 632339 (HA 430), which were also used in the greenhouse drought experiment. According to the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) U.S National Plant Germplasm System, these accessions are mentioned to be relatively drought tolerant.

2.2.3. Cultural Practices

The planting site was disked in the fall, and just before planting, it was tilled with a field finisher. In addition, one month before planting, Nitrogen (N) was applied to the area in the form of urea (46-0-0) using a push-type rotary fertilizer applicator.

Planting was performed on May 15th using John Deere 7100 planters equipped with Almaco belt cones. After two weeks of planting, post-emergence herbicide Dual II Magnum (S-metolachlor - 83.7%) by Syngenta was applied.

2.2.4. Planting Site

The planting site included four blocks, and each block was divided into three plots; in total, twelve plots were placed in the experimental area. Each plot had six rows, including three rows for HA 429 and three rows for HA 430. Dimensions for each plot is

15' (east/west) by 21' 8" (north/south), 1'8" alley is cut out, leaving a plot length of 20'. The seeds were planted at two seeds/ft or about 40 to 45 seeds per row.

Irrigation drip lines are spaced on 30" at a depth of approximately 12". Rows are planted directly between drip tapes (15" from a drip tape in both directions).

The soil type of the experimental area is indicated as filbert silt loam with 0 to 1 percent slopes (USDA-NRCS, 2004).

Table 1. Field experiment site weather data for 2020 was obtained from the weather station adjacent to the field phenotyping site (Memphis 4N, Mead, NE, USA).

Months	Mean Maximum Temp. (ºC)	Mean Minimum Temp. (ºC)	Daily Mean Temp. (ºC)	Normal Daily Mean Temp. (ºC)	Temp. Deviation from Normal (ºC)	Precipitation (cm)	Normal Precipitation (cm)	Precipitation Deviation from Normal (cm)
January	1.04	-8.32	-3.64	-4.12	0.48	2.05	0.64	1.41
February	5.47	-8.08	-1.30	-1.49	0.19	0.00	0.80	-0.80
March	12.38	-0.78	5.80	4.53	1.27	3.29	1.93	1.36
April	17.68	0.47	9.08	10.89	-1.81	1.12	2.71	-1.59
May	20.05	9.28	14.67	16.86	-2.20	6.73	4.29	2.44
June	31.32	17.93	24.63	22.56	2.07	6.25	4.35	1.90
July	31.00	18.67	24.84	25.38	-0.54	4.06	3.40	0.66
August	30.57	15.85	23.21	24.09	-0.88	3.76	3.49	0.27
September	24.84	9.69	17.26	18.94	-1.68	3.88	3.02	0.86
October	15.90	1.68	8.79	11.81	-3.02	1.47	1.97	-0.50
November	13.61	-2.57	5.52	3.89	1.63	0.00	1.43	-1.43
December	4.56	-8.97	-2.21	-2.87	0.66	0.00	0.95	-0.95

2.2.5. Data Collection for Field Drought Experiment

Data were collected from six plots as two replication of different irrigation levels (randomly assigned two FIT, two LIT, and two RF) during the vegetative stage of plants. As stated above, considering each germplasm had three rows in each plot, the middle row of three was used for data collection. Five plants for each germplasm were randomly chosen from the selected plots (randomly assigned), where plant height, NDVI, SPAD, leaf surface thermometer, and **Leaf Area Index (LAI)** measurement were recorded. The same materials that were used in the greenhouse were applied to the field experiment. In addition to equipment used in the greenhouse experiment, LAI (LAI-2200C by LI-COR,

USA) was used in the field experiment. LAI is the quantity of leaf-covered area in a canopy, which means the ratio of one-sided leaf area per unit ground area. It is dimensionless.

2.3. Post-Harvest Measurements for Field Experiment

When the sunflower plants reached the maturity stage and almost dried they were hand-harvested. To acquire the post-harvest data set as yield parameters, ten heads were randomly chosen from each plot for each germplasm. Selected heads were from the middle rows of each germplasm for each plot.

Considering the twelve plots and two germplasm with ten plant heads, in total, there were two hundred and forty plant heads that were measured. Each ten headset measurements were recorded individually. After measuring the diameter of the heads and their weights, they were threshed with Almaco stationary plot thresher separately and sieved manually. The whole seed weight is measured per head, and a hundred-seed weight is recorded.

2.3.1. Preparation for Oil Extraction and Fatty Acid Content

Following the measurements done after harvest, seeds were processed for oil extraction to determine the effect of different irrigation levels on oil amounts. For this procedure, all equipment was provided by the Biosystem Engineering at the University of Nebraska-Lincoln.

For the oil extraction procedure, seeds were dehulled by hand from each plot and germplasm, dehulled seeds were arranged as 15 g. For oil extraction, approximately 5 g with two replications, and for calculation of the moisture content, 5 g was needed. For oil extraction, prepared samples were ground with an electronic grinder and were filled in

the vessels to be run in Dionex Accelerated Solvent Extractor 350 (ASE) by Thermo Scientific. To obtain the extract, hexane (by Fisher scientific) was used as a solvent. Ground samples were heated up to 150 ^{o}C for three static cycles of 20 minutes each, then purged with nitrogen for 100 seconds. At the end of the ASE process, oil and hexane mixed solution was ready to be placed to Genevac Rocket Synergy Evaporator by SP Scientific to evaporate the hexane and obtain pure oil. For oil amount calculation, the formula of ***Oil % = (oil weight/ (mass weight of the sample - moisture as a decimal))*100*** was used. Calculation results were recorded, and oil in the bottles was transferred to a small clear glass vial and capped tightly.

After oil was collected in small glass vials, they were kept at 4 ^{o}C and prepared for the micro-method to prepare the fatty acid methyl esters (FAMEs) protocol, which was proposed by Metcalfe et al. in 1966. Initially, 30-35 mg extracted oils for each seed set were weighted in 16x100 mm screw cap tubes, and 1 mL 0.5 M sodium hydroxide (NaOH) in methanol (CH_3OH) solution was added to oil samples and flashed with Nitrogen gas (N_2) then capped. After that, samples were heated for at least 10 minutes at 100 ^{o}C in a digital dry bath incubator and let to cool to room temperature. Following this, 1 mL, fresh and stored at 4^{o}C boron trifluoride (BF_3), added and flashed with Nitrogen gas (N_2). Then they were heated for 5 minutes at 100 ^{o}C and let to be cooled to room temperature. 2 mL saturated NaCl was added to cooled tubes and vortexed. After adding 2 mL hexane, tubes were put in the centrifuge for 5 minutes at 1000 x g. For the last step, the upper hexane layer was transferred to Gas Chromatography (GC) vials to be analyzed. For analyses, Sigma Aldrich Gas Chromatography was used.

2.3.2. Protein Analysis

Protein analysis was performed according to a crude protein-combustion method approved by AACC Approved methods of analysis (AACC International, 1999). Seed samples were dehulled and grounded. For combustion, equipment by LECO Corporation was used.

2.4. Statistical Analysis

Data analyses were performed with SAS (Statistical Analysis Software) for greenhouse and field experiments. Data collected over time in the greenhouse was evaluated as repeated measures structure between dates that were collected since all measurements were taken from the same plant at each time. Therefore, for greenhouse experiments (two years data mean , over time data was also included AR (1) structure on the residuals. LSD means graphs that used in the results section were set up with 95% confidence limits.

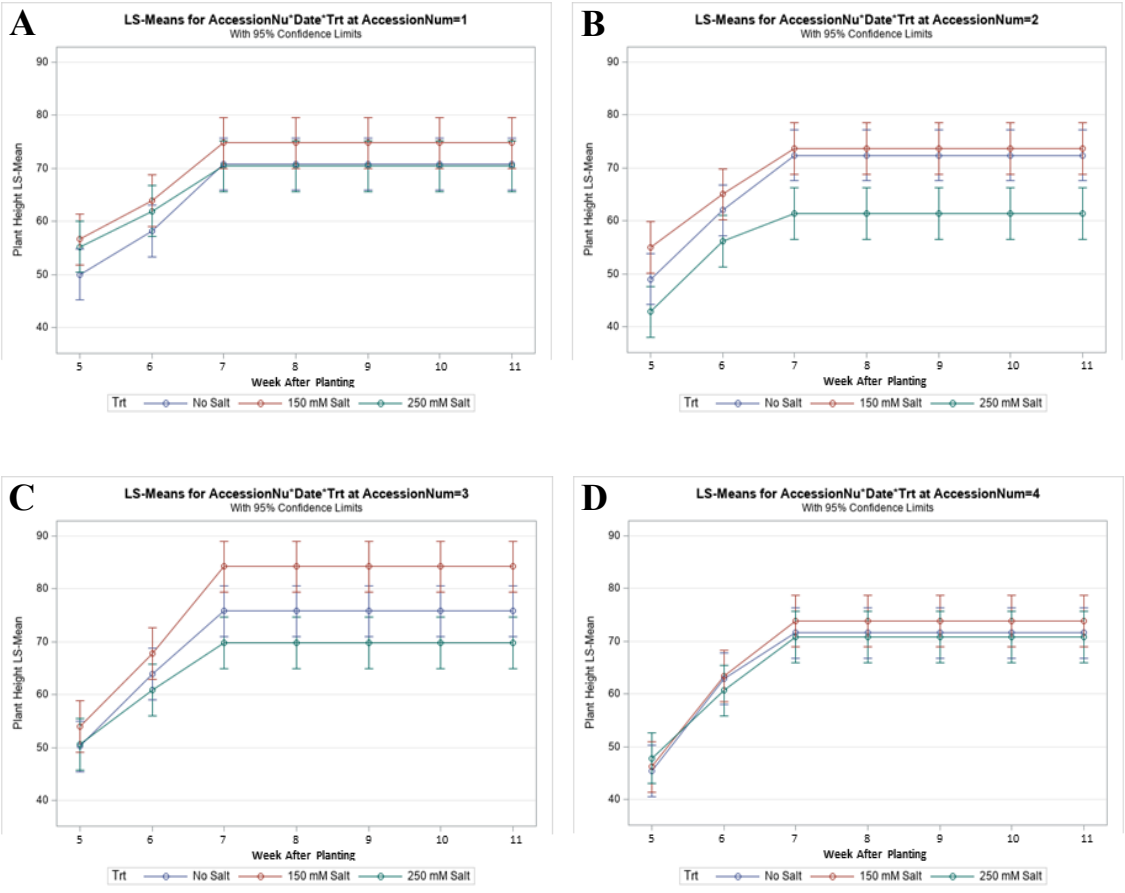
3. RESULTS

3.1. Over time Data for Greenhouse Salinity Experiment

3.1.1. Plant Height

Data was taken during eight weeks, starting from the fifth week after planting. When plants reached their maximum height, plant height data were recorded as the same as the final measurement. For the salt experiment, six germplasms PI 539899, PI 539900, PI 539901, PI 539902, PI 539903, and PI 599984 numbered as accession numbers 1 to 6, respectively. For example, PI 539899 is accession number 1.

According to the statistical analysis, the germplasm effect (P<.0001), treatment effect (P<.0001), the impact of time (P<.0001), germplasm x time interaction (P<.0001), and time x treatment interaction (P=.0108, P<.05) were found to be significant. Plants treated with 150 mM salt solution showed higher values than other treatments for all germplasms. While accessesion number 1, 2, 4, and 5 had reached their highest height as 74.8 cm, 73.7 cm, 73.8 cm, 74.2 cm, respectively, PI 539901 (accession number 3) and PI 599984 (accession number 6) had higher plant height as 84.2 cm, and 82.6 cm respectively. The lower plant heights for all germplasms were observed at the 250 mM salt treatment, compared to other treatments. The lowest plant height was observed in accession number 2 (PI 539900) with 61.4 cm, while the heights for this treatment were 71.4 cm in accession number 6 (PI 599984).



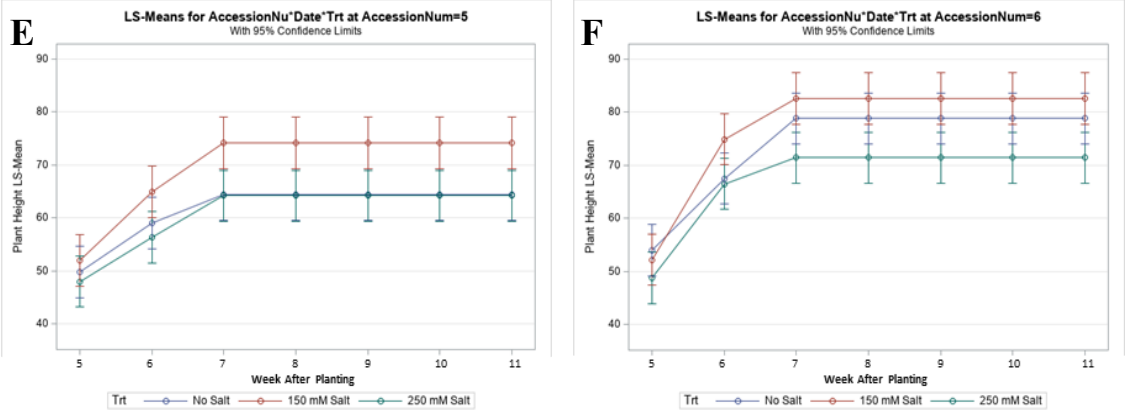


Figure 1. Over time plant height of germplasms under different salt concentrations. A) Accession number 1 (PI 539899). B) Accession number 2 (PI 539900). C) Accession number 3 (PI 539901). D) Accession number 4 (PI 539902). E) Accession number 5(PI 539903). F) Accession number 6 (PI 599984).

3.1.2. Over time SPAD Analysis

SPAD analysis showed that the effect of germplasm, treatments, germplasm x treatment interaction, time, germplasm x time interaction, time x treatment, germplasm x time x treatment interaction was significant. Over time, there was a decreasing trend for all germplasms, and the lowest values were seen at maturity. The lowest estimate values were observed at 250 mM salinity exposed plants in all germplasm used, except PI 539899 (accession number 1), with having lower SPAD values at 150 mM salinity (11.93 SPAD unit). Among all six germplasms used for the salinity experiment, the lowest value was 1.14 in PI 539903 (Accession number 5).

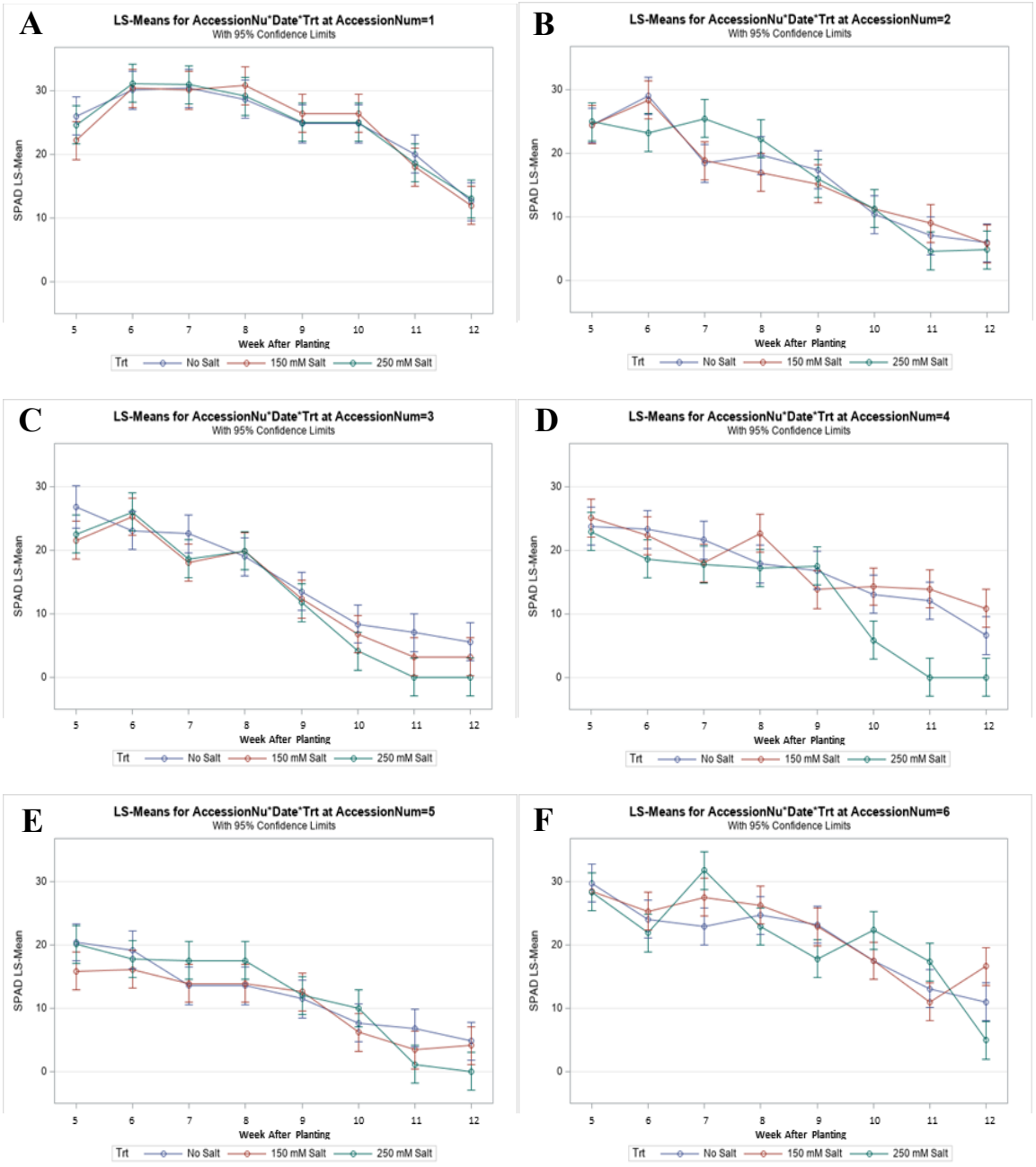
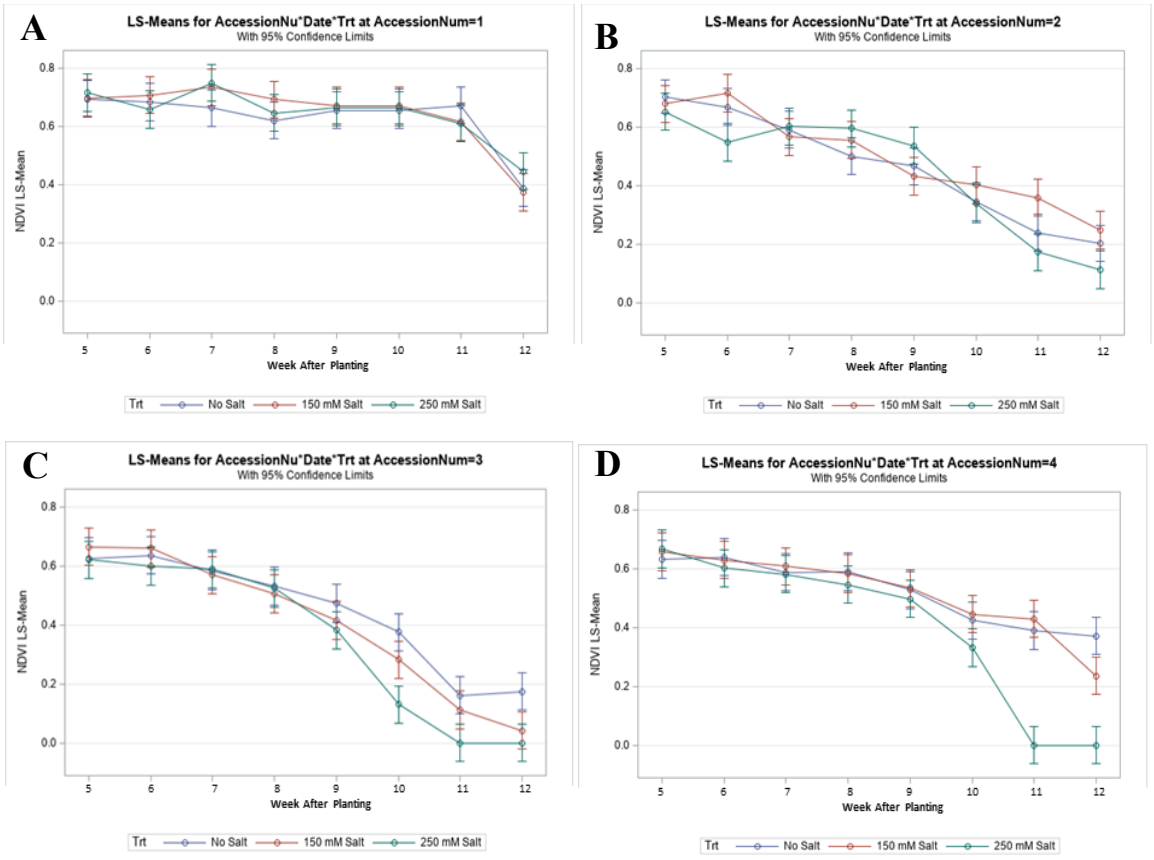


Figure 2. Over time SPAD analysis of germplasms under different salt concentrations. A) Accession number 1 (PI 539899). B) Accession number 2 (PI 539900). C) Accession number 3 (PI 539901). D) Accession number 4 (PI 539902). E) Accession number 5 (PI 539903). F) Accession number 6 (PI 599984).

3.1.3. Over time NDVI Analysis

The statistical analysis regarding the salinity effect on NDVI showed that the germplasm (P<.0001), treatment (P<.0001), germplasm x treatment interaction (P=0.0082), the impact of time (P<.0001), germplasm x time interaction (P<.0001), and time x treatment interaction (P<.0001), and germplasm x time x treatment interactions (P<.0001) were all significant.

Overall, NDVI values in all germplasms decreased over time. While the plants that were exposed to 250 mM salinity condition showed the lower NDVI values for accession number 2 (0.11), 3 (0.11), 4 (0.23), 5 (0.12), 6 (0.07), accession number 1 (0.37) had the lowest values at 150 mM salinity condition.



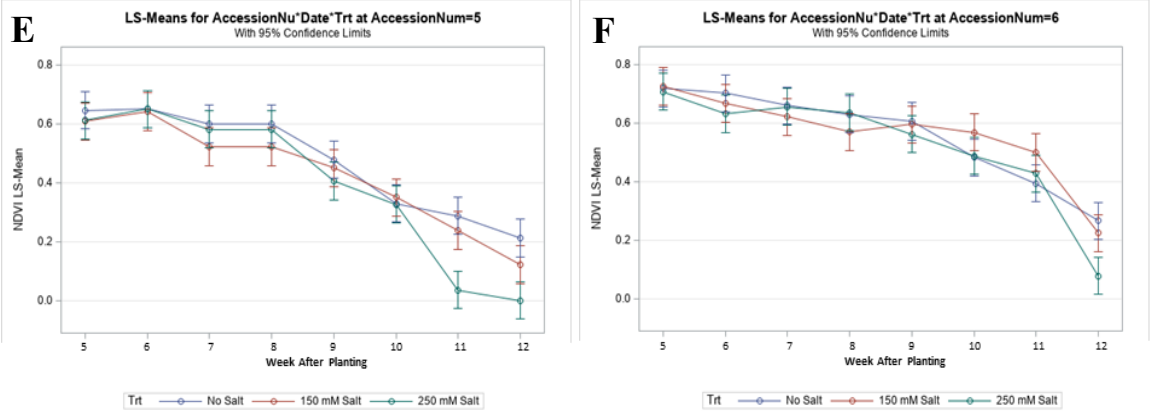


Figure 3. Over time NDVI analysis of germplasms under different salt concentrations. A) Accession number 1 (PI 539899). B) Accession number 2 (PI 539900). C) Accession number 3 (PI 539901). D) Accession number 4 (PI 539902). E) Accession number 5 (PI 539903). F) Accession number 6 (PI 599984).

3.1.4. Canopy Temperature

Regarding the canopy temperature analysis, germplasm, treatment, time effects, all two-way and three-way interactions were significant (P<.0001). As the plants got closer to maturity, the canopy temperature was higher than the vegetative stage. Based on the estimate graphs, the most elevated canopy temperatures were observed at 250 mM salinity treatments, except for accession number 3 (PI 539901), which reached the highest temperature at 150 mM salinity treatment with 89.16 ^{o}F. The highest temperature among all germplasms was recorded in accession number 2 (PI 539900) as 89.50 ^{o}F, while the lowest values were recorded for accession number 6 (PI 599984). Generally, plants under no-salt conditions have lower canopy temperatures.

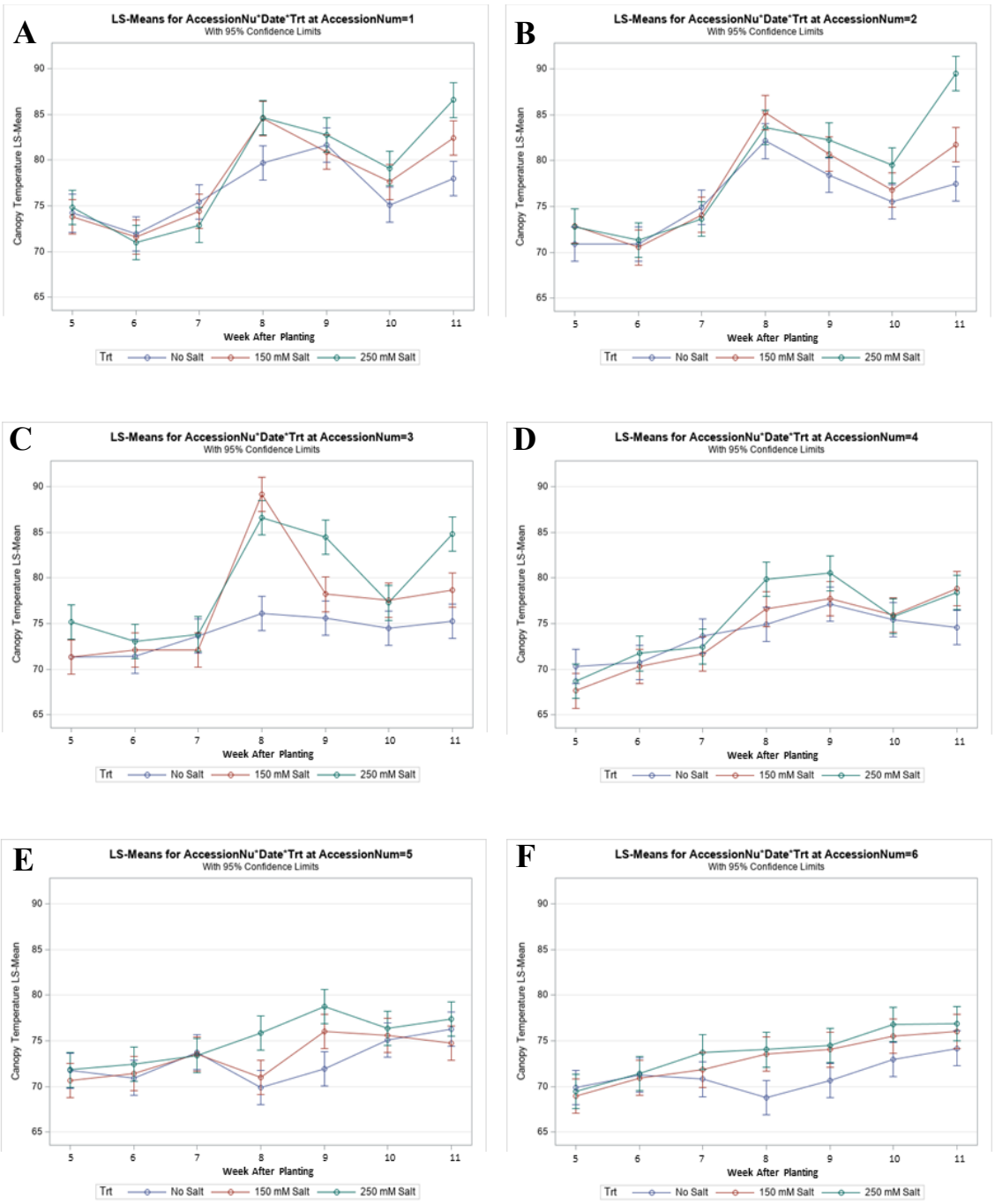
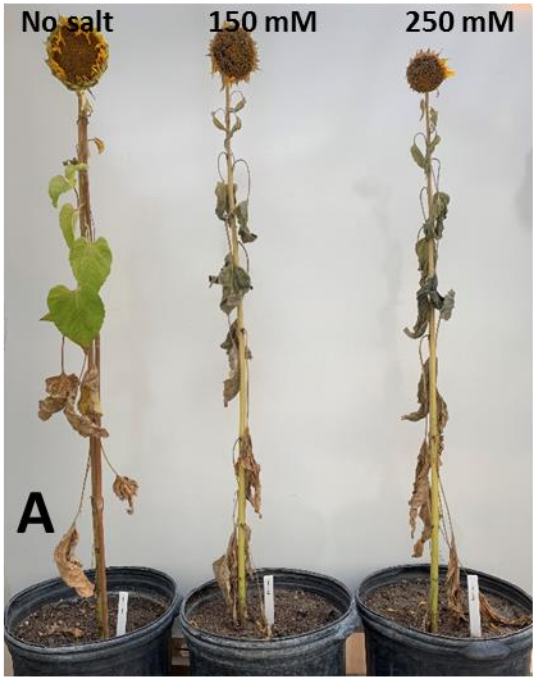


Figure 4. Over time canopy temperature of germplasms under different salt concentrations. A) Accession number 1 (PI 539899). B) Accession number 2 (PI 539900). C) Accession number 3 (PI 539901). D) Accession number 4 (PI 539902). E) Accession number 5 (PI 539903). F) Accession number 6 (PI 599984).


No salt
150 mM
250 mM
A


No salt
150 mM
250 mM
B


No salt
150 mM
250 mM
C


No salt
150 mM
250 mM
D

Figure 5. Sunflower plants treated with different levels of salt concentration A) Accession number 1 (PI 539899). B) Accession number 2 (PI 539900). C) Accession number 3 (PI 539901). D) Accession number 4 (PI 539902). E) Accession number 5 (PI 539903). F) Accession number 6 (PI 599984). Plants were randomly chosen from each treatment for each germplasm.

3.2. Over time Data for Greenhouse Drought Experiment

3.2.1. Plant Height

For the drought stress experiment, PI 632338 (HA 429) and PI 632339 (HA 430) were used as germplasms, and for statistical analyses, they were recorded as accession numbers 7 and 8, respectively.

Based on the statistical analysis, the effect of germplasm, treatments, time, and time x treatment interaction were significant on plant height. Regarding the irrigation levels, plants treated with 50% irrigation (1L/pot) had higher plant heights compared to other plants that were exposed to full irrigation (2L/pot) and 25% irrigation levels. PI

632339 (HA 430) had a higher plant height than PI 632338 (HA 429) in all irrigation levels. It reached the highest plant height at 50% irrigation level with 89.0 cm. On the other hand, the lowest plant height was observed at 25% (.5L/pot) irrigation levels in PI 632338 (HA 429) with 65.8 cm.

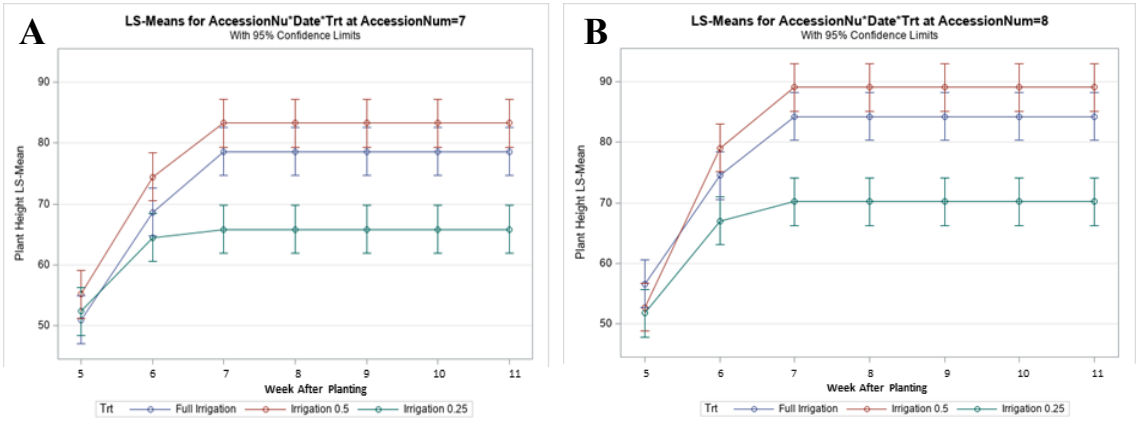


Figure 6. Over time plant height of germplasms under different irrigation levels in the greenhouse. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.2.2. Over time SPAD Analysis

According to the analysis, there is a significant difference between treatments ($P=0.0328$, $P<0.05$), germplasm x treatment interaction ($P=0.0265$, $P<0.05$), and time ($P<.0001$) effect. Germplasms used for the drought experiment showed a similar trend with the salinity experiment. Over time, SPAD values decreased when plants reached their full maturity. The lowest measurements were seen at the 50% irrigation (1L/pot) levels for both accession number 7 (PI 632338/HA 429) with 6.66 and accession number 8 (PI 632339 /HA 430) with 4.16 SPAD values.

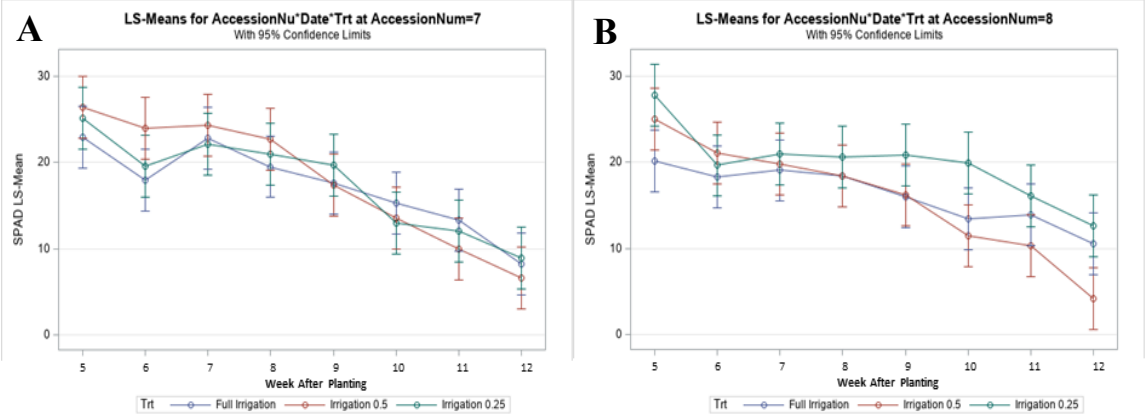


Figure 7. Over time SPAD analysis of germplasms under different irrigation levels in the greenhouse. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.2.3. Over time NDVI Analysis

Based on the outcome of the analysis, the effect of irrigation levels on NDVI was significant with the P-value of <.0001 for the germplasm effect, treatment effect, germplasm x treatment interaction, the impact of time, germplasm x time interaction, and time x treatment interaction, and germplasm x time x treatment interactions. As seen in figure 8, there was a dramatic decrease in the tenth week after planting for both germplasms. However, accession number 8 showed higher NDVI values at that period in each treatment, when number 7 accession was almost dried and had no significant NDVI values at 50% (1L/pot) and 25% (.5L/pot) irrigation levels.

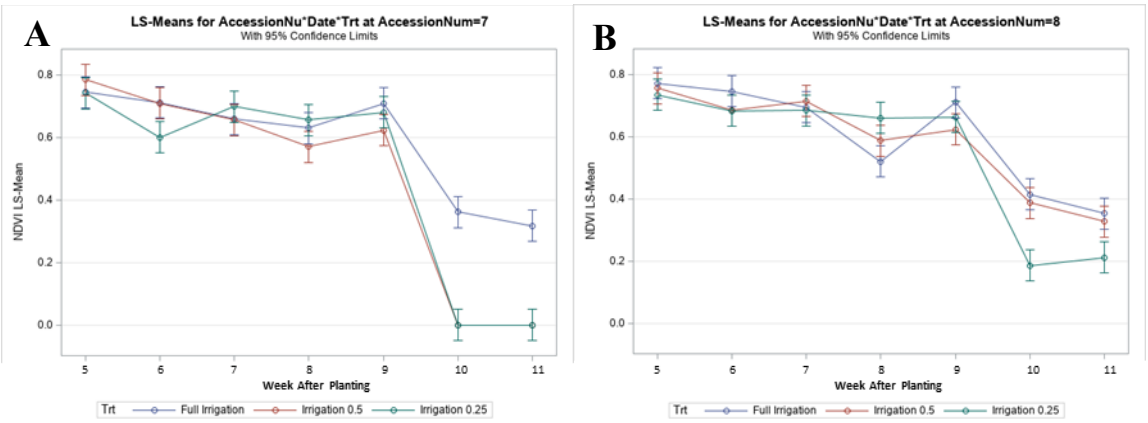


Figure 8. Over time NDVI analysis of germplasms under different irrigation levels in the greenhouse. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.2.4. Canopy Temperature

Canopy temperature analysis for the drought experiment showed that treatment effect, time effect (P<.0001), germplasm x time interaction (P value=0.0102<0.5), and time x treatment interactions (P<.0001) were significant. While the highest canopy temperature was measured in accessions number 8 (PI 632339/HA 430) with the value of 81.48 ^{o}F under 25% (.5L/pot) irrigation conditions, for accession number 7 (PI 632338/HA 429), the highest value was observed under 50% (1L per/pot) irrigation treatment with the value of 81.12 ^{o}F. The lowest values were measured at full irrigation (2L/pot) treatment for both germplasms.

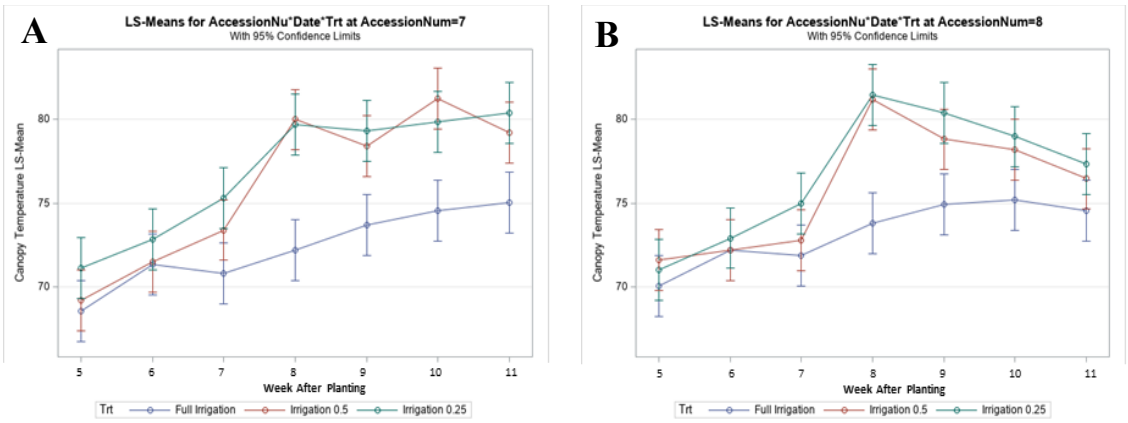


Figure 9. Over time canopy temperature analysis of germplasms under different irrigation levels in the greenhouse. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

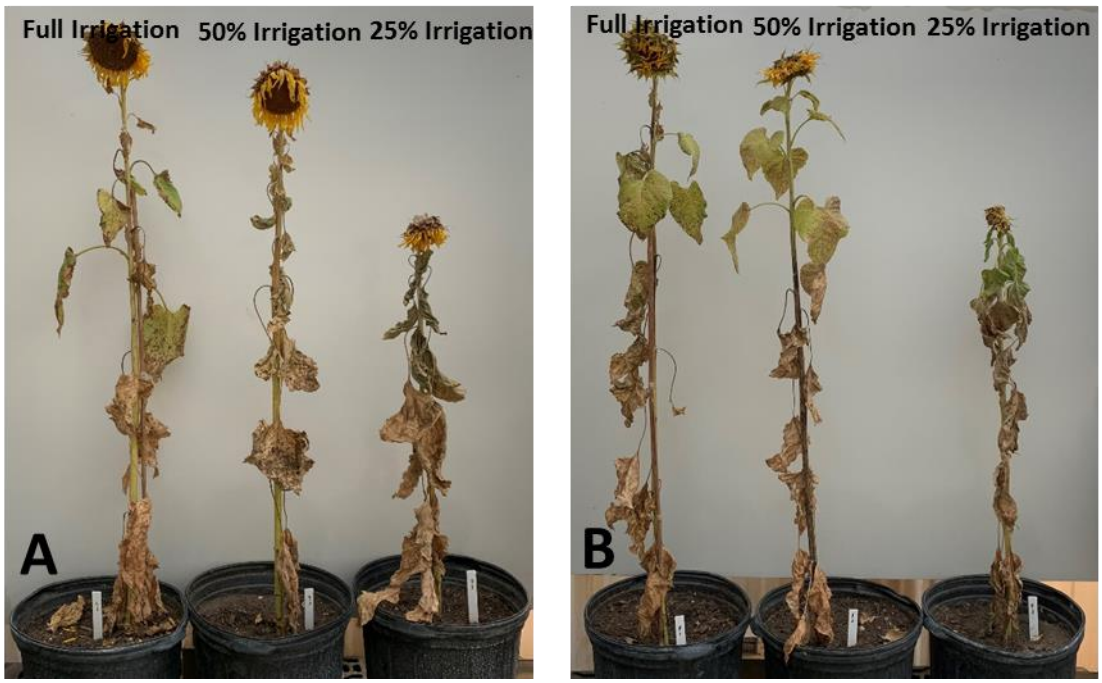


Figure 10. Sunflower plants treated with different irrigation levels A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)). Plants were randomly chosen from each treatment for each germplasm.

3.3. Over time Data for Field Drought Experiment

3.3.1. Plant Height

According to the statistical analysis, the time effect (P<.0001) and time x treatment interaction (P=0.0251), and germplasm x time x treatment interaction (P=0.0109) were significant. The highest plant height values were recorded in the eighth week after planting. After that time, there was no significant change in plant height since they were at the generative stage. While HA 429 had the highest values at full irrigation (164.87 cm), HA 430 had the highest values when plants were exposed to limited irrigation treatment (166.37 cm). Both germplasms had the lowest values at rain-fed treatment, with 158.50 cm in HA 429 and 161.37 cm in HA 430.

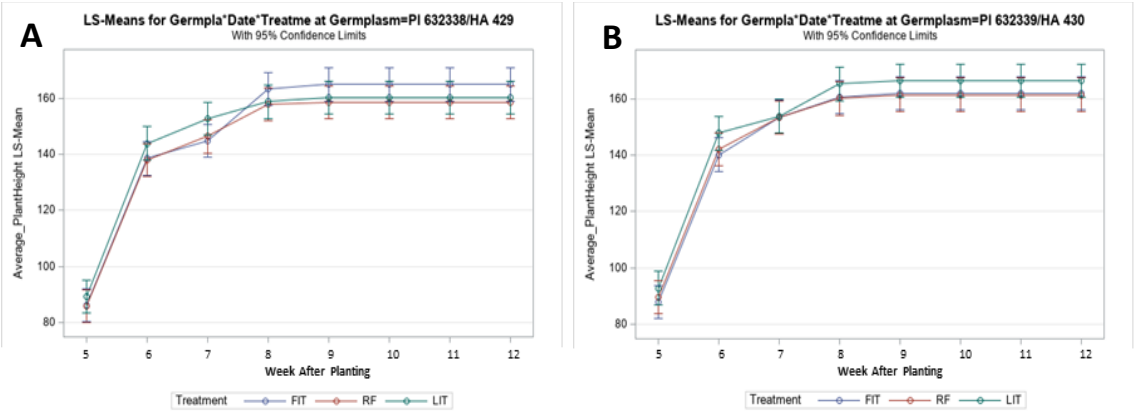


Figure 11. Over time plant height of germplasms under different irrigation levels in the field. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.3.2. Over time NDVI Analysis

Regarding the analysis, germplasm effect (P=0.0431), time effect (P<.0001), and germplasm x time interaction (P=0.0077) were significant. Overall, for both germplasms, the values decreased as time passed. Both germplasm HA 429 and HA 430 had the

highest value at limited irrigation treatment with 0.8812 and 0.8837 NDVI values, respectively. However, when plants were close to full maturity, these values were decreased to 0.5663 and 0.5650, respectively, in rain-fed treatment.

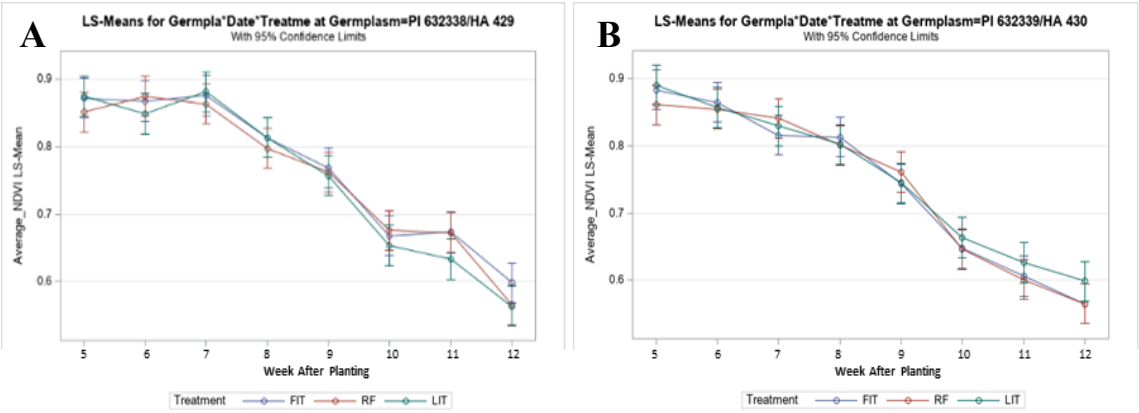


Figure 12. Over time NDVI analysis of germplasms under different irrigation levels in the field. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.3.3. Over time SPAD Analysis

Data analysis showed that only the time effect (P<.0001) was significant on SPAD values. While plants' SPAD average for HA 429 and HA 430 reached the highest value at the eighth week after planting with 38.97 (LIT) and 35.3 (RF), respectively, after the eighth week, the values showed a downward trend. Overall, even though there was no statistically significant difference between the germplasms, considering the mean Tables, the averages were higher in HA 429.

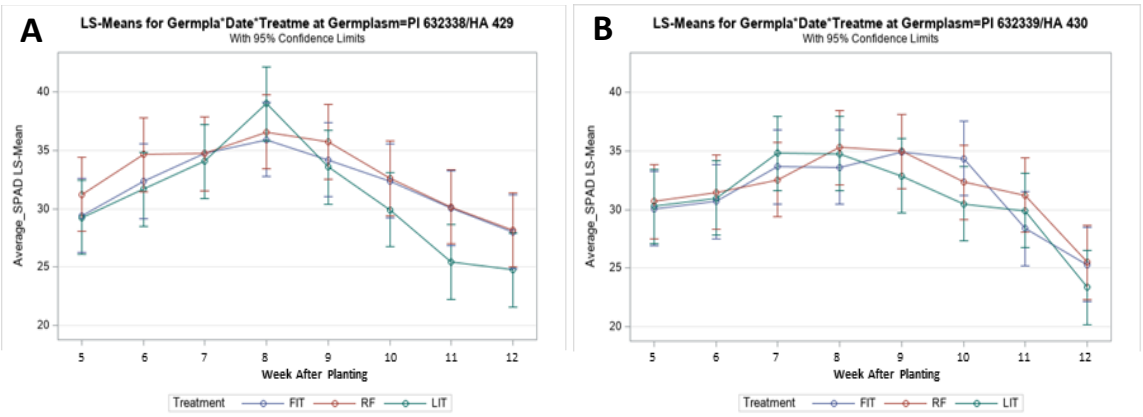


Figure 13. Over time SPAD analysis of germplasms under different irrigation levels in the field. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.3.4. Canopy Temperature

Based on over time average canopy temperature analysis, germplasm effect, time effect, and germplasm x time interaction were significant. For both germplasms, the highest temperature value was recorded at the eleventh week. HA 429 had the highest values at the rain-fed and limited irrigation treatments; the highest value for HA 430 was observed at full irrigation treatment; however, at the maturity stage for all irrigation levels, the values were close (FIT= 89.7 ºF, LIT= 87.8 ºF, and RF= 88.1 ºF).

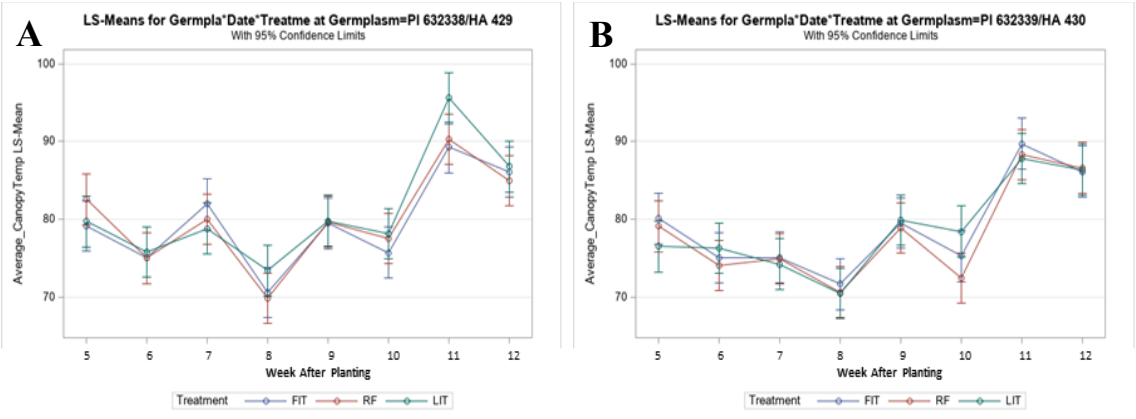


Figure 14. Over time canopy temperature of germplasms under different irrigation levels in the field. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.3.5. Over time LAI Analysis

Corresponding to the statistical analysis, only the time effect was found significant (P<.001). The highest rates for HA 429 and HA 430 were seen at the eighth week after planting; the highest LAI value was seen in the limited irrigation treatment for HA 429 with 3.43, while for HA 430, this value was seen in the full irrigation levels (3.03). In the final week of data collection, which was taken when plants started to dry, the lowest value for HA 429 was 1.2, recorded at rain-fed treatment; for HA 430 lowest values were noted in the rain-fed and full irrigation treatments with 1.17 and 1.12, respectively.

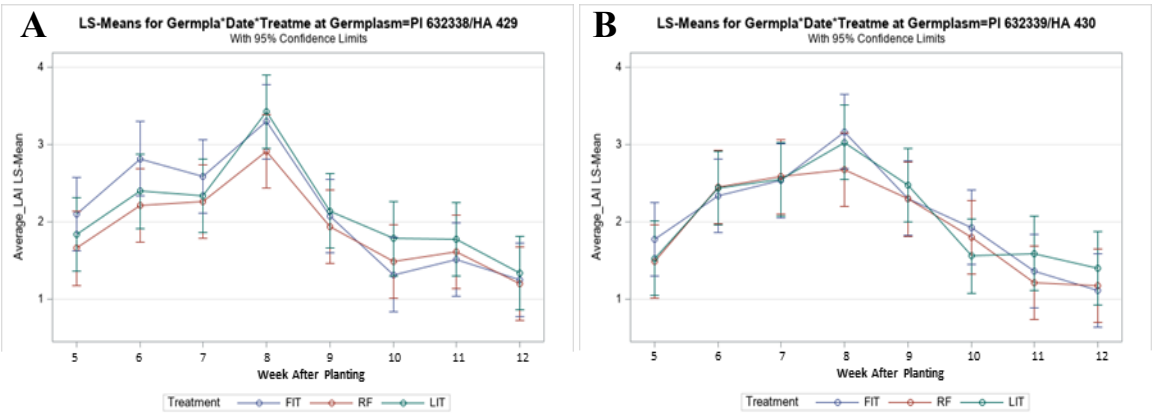


Figure 15. Over time LAI analysis of germplasms under different irrigation levels in the field. A) Accession number 7 (PI 632338 (HA 429)). B) Accession number 8 (PI 632339 (HA 430)).

3.4. Post-Harvest Data for Greenhouse Salinity Experiment

3.4.1. Dry Root Weight (g/plant)

The dry root weights of sunflower germplasms used for the greenhouse salinity experiment were shown in Table 2. Based on these results, the effect of germplasms and the level of salt (NaCl) application were found statistically significant. The means of dry

root weight of germplasms was ranged from 2.52 g/plant to 4.45 g/plant, while PI539899 and PI599984 had the lowest means. The highest dry root weight means was seen at 250 mM salt (NaCl) concentration application (4.45 g/plant). A statistical difference was not seen at no-salt treatment (3.37 g/plant) and 150 mM salt (NaCl) concentration (3.43 g/plant).

Table 2. Dry root weight of germplasms under different salt concentrations.

Germplasms	NaCl Concentrations			Mean
	No Salt	150 mM	250 mM	
PI539899	2.42	2.86	3.37	2.88[b]
PI539900	3.63	4.40	5.26	4.43[a]
PI539901	3.02	3.29	5.56	3.96[a]
PI539902	4.19	3.75	5.41	**4.45[a]**
PI539903	4.22	3.91	4.63	4.25[a]
PI599984	2.75	2.35	2.45	2.52[b]
Mean	3.37[b]	3.43[b]	**4.45[a]**	

3.4.2. Dry Shoot Weight (g/plant)

As seen in Table 3, the effects of genotype, different levels of NaCl concentrations, and genotype x NaCl concentrations interaction on shoot weights of sunflower germplasms were significant. Considering the estimates of dry shoot weights of the varieties, while the highest value was obtained from the PI 539901 germplasm, the lowest value was obtained from the PI 539903 germplasm. As the salt amount increased, a decrease in dry shoot weight was observed. The highest dry shoot weight of 15.42 g/plant was obtained from applying 150 mM salt solution to the PI 539901 genotype. In contrast, the lowest value was obtained from applying 150 mM salt solution to PI 539899. Considered as sensitive to salinity, PI 599984, has the highest values at no salt treatment.

Table 3. Dry shoot weight of germplasms under different salt concentrations.

Germplasms	NaCl Concentrations			Mean
	No Salt	150 mM	250 mM	
PI539899	11.57^{c-f}	8.73^{h}	9.9^{e-h}	10.07^{c}
PI539900	11.99^{c-f}	13.69^{a-d}	11.22^{d-g}	12.30^{b}
PI539901	13.74^{abc}	**15.42^{a}**	13.27^{a-d}	**14.14^{a}**
PI539902	14.7^{ab}	12.20^{cde}	11.89^{c-f}	12.93^{ab}
PI539903	12.35^{b-e}	8.29^{h}	8.83^{gh}	9.82^{c}
PI599984	10.59^{e-h}	9.60^{fgh}	10.13^{e-h}	10.11^{c}
Mean	**12.49^{a}**	11.32^{b}	10.87^{b}	

3.4.3. Dry Head Weight (g/plant)

Regarding Table 4, while the effect of the salt (NaCl) solution application and genotype x NaCl concentrations interaction was not significant, the genotype effect was statistically significant. The dry head measurements ranked as the lowest 5.31 g/plant (PI 539903) and the highest 9.19 g/plant (PI 539901).

Table 4. Dry head weight of germplasms under different salt concentrations.

Germplasms	NaCl Concentrations			Mean
	No Salt	150 mM	250 mM	
PI539899	7.07	6.40	5.4	6.29^{cd}
PI539900	6.66	7.19	7.38	7.08^{bc}
PI539901	9.08	9.97	8.52	9.19^{a}
PI539902	5.6	5.87	6.26	5.91^{cd}
PI539903	5.9	4.77	5.25	5.31^{d}
PI599984	9.26	7.33	7.24	7.94^{ab}
Mean	7.26	6.92	6.68	

3.5. Post-Harvest Data for Greenhouse Drought Experiment

3.5.1. Dry Root Weight (g/plant)

Table 5 shows the values for dry root weights of HA 429 and HA 430 germplasms grown under different irrigation levels. The effect of germplasms used for the greenhouse drought experiment, irrigation levels, and variety x irrigation levels interaction on dry

root weight was statistically insignificant. The estimate of dry root weight of the HA 429 was 1.96 g/plant, and for HA 430 genotype, it was 2.42 g/plant. Dry root weights were determined as 2.03 g/plant, 2.30 g/plant, and 2.25 g/plant at full irrigation, 50%, and 25% irrigation levels, respectively.

Table 5. Dry root weight of germplasms under different irrigation levels in the greenhouse.

	Irrigation Levels			
Germplasms	Full irrigation	50%	25%	Mean
HA 429	2.18	2.10	1.61	1.96
HA 430	1.88	2.50	2.89	2.42
Mean	2.03	2.30	2.25	

3.5.2. Dry Shoot Weight (g/plant)

Dry shoot weight values of sunflower germplasms are given in Table 6. The effect of genotype and irrigation levels on dry shoot weight was significant. HA 430 had the higher dry shoot weight value with 9.46 g/plant, while the dry shoot weight value of HA 429 was 7.59 g/plant. Regarding to the irrigation levels, the highest value was obtained from full irrigation with a mean of 10.17 g/plant shoot weight. The 50% irrigation (7.77 g/plant) and 25% irrigation (7.63 g/plant) levels were statistically in the same group.

Table 6. Dry shoot weight of germplasms under different irrigation levels in the greenhouse.

	Irrigation Levels			
Germplasms	Full irrigation	50%	25%	Mean
HA 429	9.08	7.40	6.28	7.59^{b}
HA 430	11.25	8.14	8.98	**9.46^{a}**
Mean	**10.17^{a}**	7.77^{b}	7.63^{b}	

3.5.3. Dry Head Weight (g/plant)

As shown in Table 7, only the effect of irrigation was significant on the weight of the dry head. Dry head weight value at full irrigation was 7.90 g/plant; for the 50% irrigation, it was 6.19 g/plant and 5.33 g/plant at the 25% irrigation level. Although there was no significant difference between germplasms used, the dry head weight value of the HA 429 was 5.94 g/plant, and the value of the HA 430 was 7.00 g/plant.

Table 7. Dry head weight of germplasms under different irrigation levels in the greenhouse.

	Irrigation Levels			
Germplasms	Full irrigation	50%	25%	Mean
HA 429	7.24	6.06	4.53	5.94
HA 430	8.56	6.32	6.13	7.00
Mean	**7.90**[a]	6.19[b]	5.33[b]	

3.6. Post-Harvest Data for Field Drought Experiment

3.6.1. Head Diameter (cm)

The effect of germplasm and irrigation on the head diameter of sunflowers was statistically significant, as shown in Table 8. The head diameter of HA 430 was 17.16 cm, while for HA 429, this measurement was 15.87 cm. Decreasing irrigation amount resulted in a decrease in head diameter. With full irrigation, the head diameter was reached 17.38 cm, 16.63 cm with limited irrigation (LIT), and 15.54 cm with rain-fed (RF) irrigations.

Table 8. Head diameter of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	16.65	15.63	15.33	15.87[b]
HA 430	18.10	17.63	15.75	**17.16[a]**
Mean	**17.38[a]**	16.63[ab]	15.54[b]	

3.6.2. Head Weight (g)

Head weight values of sunflower germplasms are given in Table 9. Only the effect of the germplasms on the head diameter was significant. The head weight value of HA 430 was 63.48 g/plant, while it was 52.65 g/plant for HA 429. Although it was not statistically significant, there was a decrease in head weight mean with the decrease of irrigation level. For instance, at full irrigation level, 64.21 g/plant head weight mean was seen, but this value was 52.14 g/plant in rain-fed treatment.

Table 9. Head weight of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	55.47	51.55	50.93	52.65[b]
HA 430	72.95	64.16	53.35	**63.48[a]**
Mean	64.21	57.85	52.14	

3.6.3. Whole Seed Weight (g/head)

The averages of whole seed weights of sunflowers grown at different irrigation levels are presented in Table 10. While the effect of germplasm on the whole seed weight g/head was significant, the effect of irrigation and germplasm x irrigation interaction was insignificant. HA 430 had 36.90 g/head whole seed weight; HA 429 had 28.70 g/head

whole seed weight. Statistically, the effect of irrigation was insignificant, but the decrease in irrigation caused a decrease in the whole seed weight values. Whole seed weight per plant head values of full irrigation, Limited irrigation, and rain-fed was 34.45, 33.72, and 30.23 g/per plant head, respectively.

Table 10. Whole seed weight of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	28.39	29.73	27.98	28.70[b]
HA 430	40.51	37.70	32.48	**36.90[a]**
Mean	34.45	33.72	30.23	

3.6.4. Hundred-Seed Weight (g)

Average hundred-seed weight values of sunflower germplasms are given in Table 11. Based on the table, only the germplasm affected a hundred-seed weight. For example, HA 429 weighted 4.48 g, while HA 430 had 4.09 g of hundred-seed weight.

Table 11. Hundred-seed weight of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	4.53	4.26	4.65	4.48[a]
HA 430	4.19	4.06	4.03	4.09[b]
Mean	4.36	4.16	4.34	

3.6.5. Crude Protein (%)

The effect of germplasm and germplasm x irrigation interaction on the crude protein content was statistically significant. The crude protein content of the HA 429 was 25.90%, this value for the HA 430 was 25.08%. In comparison, the highest crude protein

amount was obtained from the limited irrigation treatment for HA 429 with 27.00%, and the lowest value was obtained from the rain-fed treatment for HA 430 with 25.18% (Table 12).

Table 12. Crude protein of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	25.29[b]	27.00[a]	25.41[b]	25.90[a]
HA 430	25.69[b]	24.36[b]	25.18[b]	25.08[b]
Mean	25.49	25.68	25.29	

3.6.6. Crude Oil (%)

The effect of germplasm and irrigation on the crude oil content was significant, while the interaction was insignificant. The average crude oil content of the HA 429 was 56.25%, and for HA 430, this value was 54.75%. The highest crude oil was obtained from rain-fed treated plants (57.56%), whereas full irrigated plants yielded 54.32% and limited irrigated plants yielded 54.61% of oil.

Table 13. Crude oil of germplasms under different irrigation levels in the field.

	Irrigation Levels			
Germplasms	Full irrigation	LIT	RF	Mean
HA 429	55.93	54.49	58.33	**56.25[a]**
HA 430	52.70	54.74	56.80	54.75[b]
Mean	54.32[b]	54.61[b]	**57.56[a]**	

3.6.7. Fatty Acid Composition

The fatty acid composition of the germplasms under different irrigation levels is represented in Table 14. According to the results of the analysis, eight fatty acids were determined, where linoleic and oleic fatty acids were the primary fatty acids. The effect

of germplasms on the amount of palmitic, eicosanoic, stearic, and oleic fatty acids was significant, the effect of irrigation levels x germplasm interaction on the amount of linoleic acids was also significant. Palmitic acid rates of HA 429 and HA 430 were 6.09% and 6.38%, eicosanoic acid 0.32% and 0.25%, stearic acid 5.48% and 4.14, oleic acid 29.84% and 30.80%, respectively. Linoleic acid content ranged from 58.10% to 56.21%. The lowest linoleic acid content was obtained from rain-fed treatment of the HA 429 genotype, and the highest amount was obtained from limited irrigated HA 429.

Table 14. Fatty acids composition of sunflowers genotypes under different irrigation levels in the field.

Palmitic Acid					**Eicosanoic Acid**				
Genotypes	Irrigation Levels			Mean	Genotypes	Irrigation Levels			Mean
	100%	50%	25%			100%	50%	25%	
HA 429	5.96	6.08	6.22	6.09[b]	HA 429	0.33	0.30	0.34	0.32[a]
HA 430	6.53	6.26	6.33	**6.38[a]**	HA 430	0.24	0.23	0.27	**0.25[b]**
Mean	6.25	6.17	6.28		Mean	0.29	0.26	0.31	

Stearic Acid					**11-Eicosenoic Acid**				
Genotypes	Irrigation Levels			Mean	Genotypes	Irrigation Levels			Mean
	100%	50%	25%			100%	50%	25%	
HA 429	5.65	5.12	5.67	**5.48[a]**	HA 429	0.10	0.08	0.11	0.10
HA 430	3.95	4.41	4.06	4.14[b]	HA 430	0.09	0.10	0.09	0.10
Mean	4.80	4.77	4.86		Mean	0.10	0.09	0.10	

Oleic Acid					**Homogama Linolenic w6 Acid**				
Genotypes	Irrigation Levels			Mean	Genotypes	Irrigation Levels			Mean
	100%	50%	25%			100%	50%	25%	
HA 429	30.48	28.99	30.06	29.84[b]	HA 429	0.86	0.79	0.88	0.84
HA 430	30.46	31.20	30.73	**30.80[a]**	HA 430	0.76	0.86	0.81	0.81
Mean	30.47	30.09	30.39		Mean	0.81	0.82	0.85	

Linoleic Acid					**Lignoceric Acid**				
Genotypes	Irrigation Levels			Mean	Genotypes	Irrigation Levels			Mean

	100%	50%	25%	
HA 429	56.24[b]	**58.10[a]**	56.21[b]	56.85
HA 430	57.52[ab]	56.54[b]	57.24[ab]	57.10
Mean	56.88	57.32	56.72	

	100%	50%	25%	
HA 429	0.38	0.54	0.51	0.48
HA 430	0.44	0.39	0.47	0.43
Mean	0.41	0.47	0.49	

4. DISCUSSION

In the present experiment, the effect of salinity on six germplasms of sunflower mentioned previously was studied in greenhouse conditions. This study aims to investigate the effect of salinity stress on plant growth parameters. Interestingly, plant height showed an increase at 150 mM level of the salinity compared to the control group; however, the lowest plant heights were observed in plants exposed to 250 mM salt concentration. Similarly, Kumar et al. (2014) and Anwar-ul-Haq et al. (2013) proposed that moderately salt-treated sunflower plants demonstrated an increase in plant height compared to no salt treatments; however, higher salinity decreased the plant height compared to other treatments. On the other hand, in the research related to salinity effects on sunflowers, Hafeez et al. (2017) and Abd El-Kader (2006) stated that with increasing salinity levels, plant height was decreased in different sunflower genotypes.

Canopy temperature and salinity levels displayed a similar manner during the experiment. The canopy temperature of plants was increased over time with the increment of salinity levels, which is caused by the decrement of transpiration level during the salinity stress. Several studies showed that canopy temperature could be a convenient indicator of osmotic impacts of salinity stress in many plants. A study displayed that the canopy temperature of alfa alfa plants was increased with the increasing level of soil salinity (Tian et al., 2020). Gerard et al. (1992) showed that the

canopy temperature was increased in the sorghum plants with a high level of salinity in the soil.

According to the SPAD and NDVI readings, values were decreased over time, which is an indicator of reducing the plants' chlorophyll content and greenness. Correspondingly, Anwar-ul-Haq et al. (2013) stated that chlorophyll content value (SPAD) was significantly declined with rising salt exposure and the lowest SPAD value was recorded at the highest salinity treatment, while these values also changed by the genotypes. Likewise, Turhan et al. (2008) pointed out that NDVI and chlorophyll content value showed a similar trend with a noticeable reduction throughout the growth period. Besides the sunflower, similar evidence was found in a study focused on the effect of salt stress in sorghum, which depicted that the 250 mM of NaCl caused up to 68% declining chlorophyll content, directly affecting the NDVI and SPAD readings (Netondo et al., 2004).

Post-harvest data showed that salinity had an unfavorable impact on yield components. Surprisingly, dry root weight increased in higher salinity level (250 mM), which was not expected. In contrast to the present experiment, there are studies performed under salinity conditions showed that even though moderate salt concentrations increased the dry root weight, in higher salinity conditions, the dry root weight values were decreased (Kumar et al., 2014; Shila et al., 2016, Emerman & Kinsinger, 2003).

In addition to these parameters, dry shoot and dry head weight also declined as salinity increased. Farghaly et al. (2016) conducted an experiment related to the effect of different salinity levels (0, 80 mM, and 160 mM) on sunflower yield parameters,

resulting in dry shoot weight and head weight decreasing in tested salinity levels compared to control groups. In the same manner, a previous study on rice carried out by Puvanitha & Mahendran (2017) showed that salinity inhibited the dry shoot weight, which also resulted in reduced leaf production and a lower number of leaves causing a reduction in photosynthesis and dry matter accumulation.

For the drought experiments (greenhouse and field), two lines were used to investigate the response of plants in different irrigation levels. Over time data results and post-harvest data showed a similar trend in both greenhouse and field experiments.

In this study, in both experiments conducted in the greenhouse and field, plant height is adversely affected by the water deficiency. A comparable study led by Hussain et al. (2018) on drought stress in sunflowers showed that drought inhibits plant height. Regarding the effect of water limitation on plant height, Sari-Gorla et al. (1999) indicated that drought conditions cause a delay in plants' development, which causes plant height reduction. Likewise, for the reason of declining of the plant height, Nonami (1998); Kaya et al. (2006); Hussain et al. (2008) stated that water deficiency inhibits the water flow from the xylem to cells and affects cell division, which is consequently damaging mitosis, and cell elongation and therefore plant height is reducing.

Over time SPAD values for both experiments showed decreasing trend when plants reached their maturity. While SPAD values in limited irrigation treatments (greenhouse (50% irrigation) and field (LIT/0.60-inch irrigation)) were the lowest, interestingly, in the greenhouse experiment, the highest values for PI 632339 /HA 430 were observed at 25% level irrigated plants. A recent related study on sunflowers showed that the highest SPAD values were obtained from the plants under well-watered

conditions, while the minimum values were seen in severe drought conditions (Wasaya et al., 2021). Moreover, Wasaya et al. (2021) indicated that the reduction in values of SPAD might be due to loss of greenness and damaged chlorophyll in water deficit conditions. Similarly, Oraki & Aghaalikhana (2012); Ghobadi et al. (2013) observed that limited irrigated plants had a 15% to 25% reduction in chlorophyll content compared to watered plants. Other chlorophyll-related trait NDVI values also showed a decrease over time. Full irrigation treatments had the highest values in both greenhouse and field experiments. On the contrary, a decrease in water resulted in a decrease in NDVI values. Similar to these experiments, Thapa et al. (2019) revealed that the values from the plants growing in drylands were lower than well-watered exposed plants in winter wheat.

Canopy temperature is a commonly used indicator of the plant's response to water stress. The canopy temperature increases at the low level of available water for the plants due to lessening transpiration rates resulting from water shortage. Therefore, the canopy temperature of plants was higher at the limited irrigation level (LIT) over time, comparing the other irrigation levels of rain-fed (RF) and full irrigation treatment (FIT). Several studies illustrate a similar trend associated with the effect of water stress on canopy temperature. For instance, Taghvaeian et al. (2014) exhibited that canopy temperature raised with the increasing water deficiency.

One of the most critical responses of the plants in drought stress is a reduction in leaf area followed by photosynthesis decrease (Hussain et al., 2018), which might result from a decrement in cell size as a response to water deficiency (Cutler et al., 1977). In the present study, LAI over time data was recorded only in the field experiment. For both germplasms used in the field experiment, LAI values for plants increased from seedling

to reproductive stage, while the values decreased after the reproductive stage. Correspondingly, a study to observe drought stress in wheat illustrated that LAI was slowly increased after sowing; however, the values started to decrease after a period of time (DALIRIE et al., 2010).

Drought is one of the most challenging environmental stresses impacting the yield and yield components since water deficiency inhibits crop development and growth from cellular to plant organs. Even though sunflower is considered moderately tolerant to drought, as the duration of drought prolongs, it adversely affects sunflowers from seedling to maturity, ultimately lowering the yield and yield parameters (Andrianasolo et al., 2014). In this study conducted in a greenhouse and field, several yields and yield component parameters including dry root, shoot, head weight, head diameter, whole seed weight, hundred seed weight, crude protein, crude oil, and fatty acid composition were observed.

Sunflower has a long and deep root system that allows taking water from deeper soil levels, which is considered one of the reasons for being moderately tolerant to drought. Gunes et al. (2008) indicated that drought conditions reduced the root dry weight in sunflower. Conversely, there were no statistically significant differences in applying different irrigations for dry root weight in the present study. However, there is a slight increase in the dry root weight at 50% irrigation level compared to the other levels.

Unlike dry root weight, there were significant differences among the different irrigation levels in dry shoot weight, dry head weight, head diameter, and whole seed weight. Plants under full irrigation treatment produced the highest dry shoot weight fallowed by the 50% and 25% irrigation levels, respectively. In addition, head diameter,

whole seed weight, hundred seed weight were lessened with the decrease of irrigation levels in both germplasms used for the drought experiment. Many studies are demonstrating similar tendencies. For example, Pekcan et al. (2016), Turhan & Baser (2004), Onemli & Gucer (2010) pointed out that water stress caused a significant decrease in dry shoot weight in different sunflower lines. Additionally, Alahdadi & Oraki (2011), Buriro et al. (2015), and Pejić et al. (2009) stated that there is a dramatic decrement in the dry head weight, head diameter, whole seed weight, and hundred seed weight with the increment of water stress in sunflower hybrids.

Referring to crude protein, in the present study, differences in irrigation levels did not generate any statistically significant change in crude protein rate. Nel et al. (2001) illustrated that the water-deficient treatments did not cause any significant alteration in the crude protein content in sunflower seeds. However, Alahdadi et al. (2011) displayed that water stress caused a slight increase in the sunflower seed protein content compared to fully irrigated samples.

In the present study, the crude oil ratio was significantly changed based on different irrigation applications. While full irrigated sunflowers had the lowest level of crude oil, rain-fed irrigated sunflowers showed the highest amount of crude oil. Regarding oil amount, Dehkhoda et al. 2013, Alahdadi et al. (2011), Ali et al. (2009), and Daneshian et al. (2005) claimed that the oil content of sunflower seeds was decreased with increasing water stress different sunflower cultivars. Similarly, Jasso de Rodriguez et al. (2002) revealed that a light decrement was seen in the oil content of sunflower seeds when the water stress was present. On the contrary of these studies, although due to the reduction in oleate desaturase, sunflower oil yield and quality are impeded in drought

stress; there are some other studies showed that drought stress did not affect the oil quality of sunflower, which might be associated with genotypes used (Hussain et al., 2018).

In terms of fatty acid composition, eight different acids were observed in the sunflower seeds harvested from the field, which were predominated by oleic and linoleic acids. Different irrigation levels did not affect the seven fatty acids content, palmitic, eicosanoic, stearic, 11-eicosenoic, oleic, homogama linolenic w6, and lignoceric acids, except the linoleic acid content that was increased under limited irrigated condition in the genotype HA 429. Baldini et al. (2000) reported that the oleic acid content of high oleic hybrids sunflower increased under water stress conditions while going down in ordinary hybrids. On the contrary, Petcu et al. (2001) pointed out that there was an increase in the linoleic and palmitic acid content and a decrease in oleic acid and stearic acid contents in sunflower seeds grown under water stress. Jasso de Rodriguez et al. (2002) also reported the decrease of oleic acid content when drought conditions occurred. Furthermore, in the studies by Baldini et al. (2002) and Flagella et al. (2000), a reduction was observed in linoleic acid content under water stress conditions and an increment in the oleic acid content in sunflower hybrids.

5. CONCLUSION

In conclusion, the salt and drought stresses are growing environmental challenges for plant cultivation worldwide. To mitigate the effect of salinity and drought, it is essential to use tolerant plants. In this study, the response of different sunflower germplasms under different saline and irrigation levels was observed. For this aspect, sunflower is considered as one of the best sources of oil crop that could be grown in regions under the threat of water stress and high salinity since there was no significant decrease in yield and yield components under high salinity and water stress conditions. For breeding purposes, finding a high number of sources for salt and drought-tolerant and sensitive germplasms might be beneficial to alleviate the errors caused by limited germplasm variation. Moreover, some wild types of sunflowers can survive even in the desert area might provide a good source as a breeding material. Looking forward, further research from cellular to whole-plant level processes could be conducted to deeply understand the response of sunflower to salt and water stress.

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