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Morphological and Physio-Biochemical Responses and Gene Expression Analyses of Landscape Plants Under Salinity Stress

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MORPHOLOGICAL AND PHYSIO-BIOCHEMICAL RESPONSES AND GENE EXPRESSION ANALYSES OF LANDSCAPE PLANTS UNDER SALINITY STRESS

by

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ABSTRACT

Morphological and Physio-Biochemical Responses and Gene Expression Analyses of Landscape Plants under Salinity Stress

by

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Utah State University, 2024

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Soil salinity affects the growth and development of landscape plants worldwide, and salinity tolerance varies among species with unique mechanisms to cope with the detrimental effects of salt stress. Therefore, it is necessary to investigate the salinity tolerance of diverse landscape plants. This research aimed to investigate the salinity tolerance of nine landscape plants [*Albizia julibrissin* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), *Penstemon barbatus* 'Novapenblu' (rock candy blue® penstemon), *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue), *Punica granatum* 'Wonderful' (pomegranate), *Shepherdia ×utahensis* 'Torrey' (hybrid buffaloberry), and *Sophora japonica* (Japanese pagoda tree)] and determine their responses to salinity stress. These landscape plants were investigated for salinity tolerance in four separate greenhouse experiments with salinity levels ranging from electrical conductivity (EC) of 1.0 to 10.0 $dS \cdot m^{-1}$. Throughout the 8-week experiments, minimal to no foliar salt, such as leaf tip burn, leaf burn, or necrosis was

observed on *A. julibrissin, P. granatum* ‘Wonderful’, *S. japonica,* and *S. ×utahensis* ‘Torrey’. Whereas *A. uva-ursi* and *C. montanus* ‘Coy’ were dead when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Two penstemon species had severe foliar salt damage (leaf burn and necrosis) or were dead when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Elevated salinity reduced the shoot dry weight and net photosynthetic rates in all plants. Furthermore, sodium (Na^+) and chloride (Cl^-) contents in leaves were affected by the elevated salinity levels. However, *A. julibrissin, P. granatum* ‘Wonderful’, and *S. japonica* were able to maintain less Na^+ content in their leaf tissue across all treatments. Gene expression results supported that *P. granatum* ‘Wonderful’ exhibited an early up-regulation of sodium/hydrogen antiporter (*NHX1*) and salt overly sensitive (*SOS2*) genes in leaves and late up-regulation of high-affinity potassium transporter (*HKT1*) in roots in response to salinity stress. In conclusion, landscape plants exhibited different responses to salinity stress, *A. julibrissin, S. japonica, S. ×utahensis* ‘Torrey’, and *P. granatum* ‘Wonderful’ were relatively tolerant, while *A. uva-ursi, C. montanus* ‘Coy’, and two penstemons were relatively sensitive.

(242 pages)

PUBLIC ABSTRACT

Morphological and Physio-Biochemical Responses and Gene Expression Analyses of Landscape Plants under Salinity Stress

Asmita Paudel

Soil salinity is a significant global issue that adversely impacts the growth and development of landscape plants. One of the effective strategies to prevent salinity damage to landscape plants is to cultivate species that are tolerant to the prevailing salinity levels. Salinity tolerance varies among plant species and cultivars. Therefore, this research aimed to investigate the salinity tolerance of nine landscape plants [*Albizia julibrissin* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), *Penstemon barbatus* 'Novapenblu' (rock candy blue® penstemon), *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue), *Punica granatum* 'Wonderful' (pomegranate), *Shepherdia* ×*utahensis* 'Torrey' (hybrid buffaloberry), and *Sophora japonica* (Japanese pagoda tree)] and determine their responses to salinity stress. These landscape plants were tested for salinity tolerance in four separate greenhouse experiments. The effects of salinity levels ranging from electrical conductivity (EC) of 1.0 to 10.0 $dS \cdot m^{-1}$ were investigated. During the 8-week experiments, minimal to no foliar salt damage, such as leaf tip burn, leaf burn, or necrosis, was observed on *A. julibrissin*, *P. granatum* 'Wonderful', *S. japonica*, and *S.* ×*utahensis* 'Torrey'. Whereas *A. uva-ursi* and *C. montanus* 'Coy' were dead when irrigated with saline solution at an

EC of 10.0 $dS \cdot m^{-1}$. Two penstemon species had severe foliar salt damage or were dead when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Elevated salinity reduced the shoot dry weight and photosynthesis of all plants. Furthermore, sodium (Na^+) and chloride (Cl^-) contents in plant tissues were affected by the elevated salinity levels. Chloride accumulation was greater in leaves than in stems or roots. However, Na^+ accumulation was greater in roots compared to that in stems and leaves. *Albizia julibrissin, P. granatum* 'Wonderful', and *S. japonica* were able to maintain less Na^+ content in their leaf tissue across all treatments. In conclusion, landscape plants exhibited different responses to salinity stress, *A. julibrissin, S. japonica, S. ×utahensis* 'Torrey', and *P. granatum* 'Wonderful' were relatively tolerant, while *A. uva-ursi, C. montanus* 'Coy', and two penstemons were relatively sensitive.

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CHAPTER I

INTRODUCTION

Soil salinity is one of the major problems, impacting approximately 10% of all land and 50% of irrigated land worldwide (Guo et al. 2015; Wang et al. 2015). The origins of soil salinity are diverse, involving climatic, soil-related, and anthropogenic factors, such as temperature, evaporation, soil leaching, seawater influx, and plant cultivation practices (Corwin 2020). In arid and semi-arid regions, high temperatures during the summer cause severe evaporation losses, leading to the accumulation of substantial salt deposits on the soil surface. However, the issue is not confined to arid regions but is also prevalent in sub-humid and humid areas, particularly in coastal zones. Soil salinity can result from various sources, including road-deicing salts, irrigation water, excessive use of fertilizers and manure, or inherently sodic soils. Saline soils mainly consist of sodium chloride (NaCl), although they may contain other types of salts, such as calcium sulfate ($CaSO_4$), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), sodium carbonate (Na_2CO_3), and sodium sulfate (Na_2SO_4) (Munns and Tester 2008).

The utilization of saline water for irrigation is identified as a contributing factor to soil salinity in urban landscapes (Gorji et al. 2015). In the United States, many states are increasingly turning to reclaimed water for landscape irrigation as a measure to conserve potable water resources. Reclaimed water, derived from various sources such as residences, educational institutions, workplaces, medical centers, and industrial facilities, has undergone disinfection and purification processes to eliminate certain contaminants, including nutrients and pathogens (Toor and Lusk 2010). In California, 18% of reclaimed

water is used for landscape irrigation (Water Recycling Funding Program 2015). The utilization of reclaimed water for plant growth offers several advantages, such as water conservation, preservation of nutrients and organic matter, energy conservation, and environmental protection (Skimina 1992). However, it is important to note that reclaimed water, despite its benefits, can have an elevated salt content, potentially contributing to soil salinity issues (Khurram and Miyamoto 2005).

Soil salinity can be quantified by measuring the electrical conductivity (EC) of a saturated soil paste extract (Amacher et al. 2000). Within the root zone, salt accumulation occurs through two primary mechanisms: the rise of a shallow water table and the retention of salts in the soil due to inadequate leaching. A soil is considered saline when the EC of the soil solution reaches 4.0 $dS \cdot m^{-1}$ (Acosta-Motos et al. 2017). Saline soils contain elevated concentrations of soluble salts, adversely affecting plant growth and development, often resulting in salinity stress (Liu et al. 2020).

Responses of Plants to Salinity Stress

Plants are classified according to their ability to thrive in saline environments, with some designated as salt-tolerant ‘halophytes’ and others as salt-sensitive ‘glycophytes’ (Himabindu et al. 2016). Halophytes, such as *Rhizophora mangle* (red mangrove), thrive in saline environments, whereas glycophytes may experience growth reduction and can even perish under high salinity levels. The majority of plant species are glycophytes, which are sensitive to salt and susceptible to damage by elevated salinity, although there is variation among them (Greenway and Munns 1980; Xiong and Zhu 2002). Soil salinity affects plants in various ways, including stunted shoot growth,

reduced leaf area, foliar salt damage, alterations in gas exchange, nutritional disorder, and biochemical changes.

Plant growth and visual quality

Plant growth can be assessed through measurements such as shoot elongation, root elongation, leaf area expansion, and shoot biomass. The initial impact of salinity stress on plants is a reduction in growth rate, occurring in two phases (Munns 2005; Munns and Tester 2008). Phase I, known as the osmotic phase, results from the external salt. During this phase, the salt concentration near rootzone increases, leading to a decrease in the amount of water that plants utilize, consequently resulting in a significant reduction of shoot growth. Phase II, identified as the ion-specific phase, results from internal salt accumulation (Munns and Tester 2008). This phase involves a salt-specific or ion-excess effect of salinity, where the rate of death of old leaves surpasses the rate of new leaf production, thereby reducing the plant's overall growth rate.

Growth reduction has been observed in many landscape plants in response to salinity stress (Chen et al. 2017; Liu et al. 2020; Wang et al. 2019a). For example, *Cornus alba* (Tatarian dogwood) experienced a 50.8% reduction in plant height and a 55.2% decrease in shoot dry weight after being irrigated with a saline solution at an EC of 5.0 $dS \cdot m^{-1}$ for 8 weeks (Liu et al. 2020).

For agriculture production, the most critical trait for salinity tolerance is growth or yield. However, for ornamental plants, aesthetic quality is an important trait. The aesthetic appearance of plants is one of the primary focuses when screening ornamental plants for salinity tolerance (Niu and Cabrera 2010; Veatch-Blohm et al. 2014). Four herbaceous perennial ornamentals, *Sedum rupestre* (angelina), *Sedum telephium* (autumn

joy), *Sedum reflexum* 'Blue Spruce' (stonecrop), and *Evolvulus glomeratus* (blue daze) showed a reduction in their growth in response to salinity stress (Hooks and Niu 2019). However, foliar salt damage was not severe in any of the species throughout the 8-week experiment when irrigated with a saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Therefore, these perennials are still recommended for landscapes facing moderate salinity problem. On the other hand, *Penstemon ×mexicali* 'Red Rocks' (red rocks penstemon) demonstrated relatively low tolerance to salinity stress at 3000 $mg \cdot L^{-1}$ (~ 4.7 $dS \cdot m^{-1}$) NaCl, as it exhibited sharp declines in visual quality (Zollinger et al. 2007).

Photosynthetic parameters

Salinity stress gradually induces a decrease in photosynthetic activity. In the short term, stomatal limitations caused by dehydration can affect photosynthesis and stomatal conductance, leading to a reduction in carbon assimilation (Garcia-Caparros and Lao 2018). Over the long term, the accumulation of high concentrations of sodium (Na^+) and/or chloride (Cl^-) in leaves, coupled with decreases in chlorophyll and carotenoid concentrations, inhibits the photosynthesis (Acosta-Motos et al. 2017; Zhang et al. 2000). The impact of salinity stress on photosynthesis can also result from decreased carbon dioxide (CO_2) availability or oxidative stress arising from the imposition of multiple stresses (Chaves et al. 2009). For instance, stomatal closure leads to an internal reduction of CO_2 and a decrease in the activity of enzymes (Chaves et al. 2009), thus limiting carboxylation and reducing the net photosynthetic rate. The negative impact of salinity on plant photosynthesis has been previously reported in various ornamental plants (Sun et al. 2015; Wang et al. 2019a). Similarly, there was decrease in photosynthesis and stomatal conductance in *Penstemon palmeri* (palmer penstemon) with increasing salinity levels in

the saline solution (Zollinger et al. 2007). Additionally, the closure of stomata reduces water loss through transpiration, which impacts both light absorption and energy conversion processes and leads to changes in chloroplast activity (Chaves et al. 2011).

Nutritional imbalances

Nutrients play a crucial role in the structure, metabolism, and osmoregulation of plant cells. Salinity can disrupt nutrient availability, competitive uptake, and transport or partitioning within the plant, leading to nutrient imbalances. Additionally, physiological inactivation of nutrients during salinity stress increases the internal requirement of plants for essential elements (Grattan and Grieve 1999). As salinity stress occurs, the presence of Na^+ and Cl^- ions increases in the growing medium and plant tissue, resulting in visual damage such as leaf tip and marginal burn, negatively influencing aesthetic value (Cassaniti et al. 2009). Sodium toxicity causes leaf burn, scorch, and dead tissue along the leaf margins, beginning with the oldest leaves. As the severity increases, the drying effect extends toward the center of the leaf until the entire tissue is dead (Garcia-Caparros and Lao 2018). On the other hand, damage caused by Cl^- toxicity begins at the tip of older leaves and progresses backward as the severity increases (Cassaniti et al. 2013).

The accumulation of Na^+ and Cl^- may compete with essential nutrients such as nitrogen (N), phosphorus (P), potassium (K^+), and calcium (Ca^{2+}), leading to nutrient deficiencies in plants (Garcia-Caparros and Lao 2018; Yildiz et al. 2020). Under salinity stress, N absorption is primarily hindered due to the antagonism between Cl^- and nitrate (NO_3^-) (Munns and Gilliham 2015), while P availability is reduced because of the antagonism between Cl^- and dihydrogen phosphate [$(H_2PO_4)^-$] (Parihar et al. 2015). Furthermore, the excessive presence of Na^+ at the root surface affects K^+ nutrition. As

Na^+ and K^+ have similar chemical nature, Na^+ can inhibit the K^+ uptake by the roots (Jouyban 2012). Potassium has a crucial role in maintaining cell turgor, membrane potential, and enzyme activities. Therefore, K^+ deficiency leads to growth inhibition. Likewise, increasing salt concentration in irrigation water caused an increase in Na^+ and Cl^- and a reduction of K^+ in *Clematis fruticosa* (Mangolian gold clematis), *Epilobium septentrionale* (northern willowherb), and *Tetraneuris acaulis* var. *arizonica* (Arizona four-nerve daisy) (Paudel et al. 2019).

Oxidative stress

Salinity is linked to oxidative stress because of the generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen (Hernandez et al. 2001; Isayenkov 2012). These reactive oxygen species interrupt vital cellular functions in plants, causing damage to cellular components such as proteins, lipids, and DNA (Gupta and Huang 2014). The overproduction of ROS in chloroplast during oxidative stress reduces the efficiency of the photosynthetic electron transport and induces lipid peroxidation of the plasma membrane.

Salinity Tolerance Strategies

Some plants are inherently tolerant to salinity stress, exhibiting various adaptation mechanisms, including morphological, physiological, biochemical, and molecular changes (Acosta-Motos et al. 2017). These plants can successfully grow and complete their life cycle in substrates rich in soluble salts. Three key strategies employed by plants to survive in saline environments include osmotic adjustment, salt/ion exclusion, and the ability to tolerate high concentration of ions.

Osmotic adjustment

In stressful conditions, plants must maintain their internal water potential below that of the soil to facilitate water uptake and sustain cell turgor (Tester and Davenport 2003). The ability to preserve leaf growth and stomatal conductance, coupled with the synthesis of compatible solutes, allows plants to effectively cope with the stress imposed by accumulated salts (Sharma et al. 2016). Importantly, the accumulation of osmolytes or compatible solutes in the cytoplasm is a major salt tolerance mechanism of halophytes (Flowers 2004). These solutes include mannitol, glycine betaine, proline, polyols, sugar alcohols, and soluble sugars (Chinnusamy et al. 2005). Glycine betaine stabilizes quaternary structures of proteins and the highly ordered states of membranes. Mannitol acts as a free-radical scavenger. Similarly, proline accumulation is a recognized adaptive response in plants against salinity stress conditions (Amini et al. 2015). Its major roles include osmotic adjustment, protection of enzymes and membranes, and acting as a reservoir of energy and nitrogen. Proline accumulation might indicate salt tolerance, as the increase in proline content is positively correlated with the level of salt tolerance (Kaur and Asthir 2015). Osmolyte profiles may vary among species depending on the specific salinity stress. For example, some halophytic species accumulate sucrose as a compatible solute, while others synthesize proline (Van Zelm et al. 2020). These compounds, known as osmoprotectants, reduce osmotic potential, helping to restore and maintain the potential gradient between the plant cell and the external soil solution.

However, the production of osmolytes comes at a metabolic cost, potentially limiting plant growth by consuming substantial amounts of carbon that could otherwise be used for growth (Flowers and Colmer 2005). This trade-off is exemplified by the

reduction in leaf area and flower weight observed in *Calendula officinalis* (marigold), despite an increase in proline content (Adamipour et al. 2019). The elevated proline content, however, conferred salinity tolerance in *C. officinalis* under saline conditions below 150 mM NaCl (Adamipour et al. 2019). On the other hand, salt-tolerant plants, such as halophytes, employ an alternative strategy by accumulating inorganic ions to reduce osmotic potential (Guo et al. 2022). This involves storing ions like Na^+ and Cl^- mainly in the vacuole, where they can be utilized for osmotic adjustment of the plant cell (Chen and Jiang 2010). The strategy of accumulating inorganic ions consumes less energy compared to the synthesis of organic substances.

Salt exclusion

Salt-tolerant ornamental plants usually exhibit lower Na^+ and Cl^- contents in their leaves compared with salt-sensitive plants (Wu et al. 2016). In many plant species grown under salinity, Na^+ tends to reach toxic levels before Cl^- (Munns and Tester 2008). Sodium exclusion is a prevalent salinity adaptation mechanism found in glycophytes (Greenway and Munns 1980). The regulation of sodium uptake and transport across plasma membranes and tonoplast is a crucial factor determining the plant cell's response to saline condition. Salt-tolerant plants often exhibit reduced sodium uptake or restricted transport from roots to shoots (Munns 2002). Sodium uptake is controlled by blocking Na^+ influx into the root, enhancing Na^+ efflux at the root, or reducing Na^+ transport to shoots and its distribution to roots and root-stem junctions (Chen et al. 2018). For example, in strawberry plants, Na^+ is primarily excluded from the leaf tissue (Saied et al. 2005). Similar observations were made in *Malvaviscus arboreus* var. *drummondii* (Turk's cap) and *Tamarix ramosissima* (salt cedars) (Sookbirsingh et al. 2010; Sun et al. 2015).

Pomegranate plants were tolerant to saline solution up to an EC of 15.0 $dS \cdot m^{-1}$, showcasing their capability to restrict either the uptake or transport of Na^+ and Cl^- to leaves, thereby minimizing salt damage (Sun et al. 2018). Additionally, the salt overly-sensitive-1 (SOS1) gene aids plants in exporting Na^+ back to the growth medium or apoplastic spaces, preventing its cytosolic accumulation (Calzone et al. 2021). Furthermore, the expression of the high-affinity K^+ transporter (HKT) gene family facilitates the reabsorption of Na^+ from the xylem, circulating it in the phloem, and preventing the accumulation of Na^+ in aboveground plant tissues (Apse and Blumwald 2007; Calzone et al. 2021).

Tolerance of high concentration of ions

Some plants exhibit tolerance to the accumulation of Na^+ and Cl^- in their shoot tissues (Munns and Tester 2008). This tolerance involves the compartmentalization of Na^+ and Cl^- at cellular and intracellular levels, particularly within mesophyll cells of the leaf, to prevent toxic concentrations within the cytoplasm (Munns and Tester 2008). This mechanism is instrumental in preventing damage to older leaves by mitigating the toxic effects of accumulated salts (Sharma et al. 2016). To maintain low Na^+ levels, plants employ Na^+/H^+ antiporters, which remove Na^+ from the cytoplasm by transporting it in exchange for H^+. Sodium is transported to the apoplast and the vacuole through plasma membrane-localized and the vacuole-localized Na^+/H^+ antiporters, respectively (Zhao et al. 2021). The compartmentation of Na^+ in vacuoles is achieved by tonoplast Na^+/H^+ antiporters belonging to the Na^+/H^+ exchanger (NHX) family (Munns and Tester 2008). For example, plants such as *C. fruticosa*, *Gazania rigen* (treasure flower), *T. acaulis* var. *arizonica*, and *S. reflexum* have demonstrated the ability to tolerate high internal

concentrations of Na^+ and/or Cl^- in their tissues (Hooks and Niu 2019; Niu and Rodriguez 2006; Paudel et al. 2019).

Mitigation of Salinity Stress in Plants

There are several approaches to alleviate the salinity stress in plants, including selecting salt-tolerant species, leaching salts with additional irrigation water, utilizing the proper growing medium, monitoring irrigation water quality, using nano technology, and applying soil amendments such as arbuscular mycorrhizal fungi (AMF), calcium, potassium, silicon (Si), or vermicompost.

Selecting salt-tolerant plants

Selecting salt-tolerant species is a pivotal strategy in mitigating salinity stress. Recent studies have yielded valuable insights into the relative salt tolerance of various ornamental plants. For example, when ten herbaceous perennials and groundcovers were grown in raised beds and drip-irrigated with saline solutions, *Achillea millefolium* (common yarrow), *Gaillardia aristata* (great blanket flower), *Lantana ×hybrida* 'New Gold'(lantana), *Lonicera japonica* (shrub verbenas), and *Rosmarinus officinalis* 'Huntington Carpet' (rosemary) exhibited no observable foliar salt damage up to an EC of 5.4 $dS \cdot m^{-1}$ (Niu et al. 2007).

In field studies, other factors such as temperature, light intensity, humidity, and wind speed can also influence plant response to salinity (Niu et al. 2007; Zollinger et al. 2007). Greenhouse screening techniques are widely employed for fast and effective identification of salinity tolerance. Physiological and molecular mechanisms, including sodium exclusion, osmotic adjustment, and ion compartmentalization, contribute to

salinity tolerance. Ornamental grasses and wildflowers, such as *Eragrostis spectabilis* (purple love grass)*, Miscanthus sinensis* 'Gracillimus' (maiden grass), *Panicum virgatum* 'Northwind' (switchgrass), and *Schizachyrium scoparium* (little bluestem), and *Ratibida columnaris* (Mexican hat), exhibited tolerance to saline solution irrigation in various studies (Niu et al. 2012; Wang et al. 2019b). Furthermore, 22 *Punica granatum* (pomegranate) cultivars were reported to be highly tolerant to saline solution irrigation up to an EC of 15.0 $dS \cdot m^{-1}$ (Sun et al. 2018).

Leaching salts

Leaching salts from the soil by applying excess water is an effective measure for the reclamation of saline soil. The removal of salt from the soil profile is more effective with frequent irrigation but shorter intervals (Bauder et al. 2004). Maintaining elevated soil moisture levels between irrigation events helps decrease the concentration of salts in the root zone, thereby reducing the salinity hazard. The amount of water required for leaching depends on both the salt concentration in the irrigation water and the plant's tolerance to it. Similarly, the effectiveness of leaching depends on various factors, including the physical properties of the soil, the degree of salinity, and the required quantity of water for leaching. Monitoring salinity levels in the soil profile during leaching is imperative to determine when to cease water application, ensuring that the soil salinity levels reach safe levels for optimal plant growth. For the restoration of saline soils, irrigation water is applied beyond the plant's requirements throughout the growing season (Devkota et al. 2015). This excess water, defined as the leaching fraction, increases in conjunction with the growing season as the plant's root system expands (Cuevas et al. 2019). While leaching salts with additional water can provide benefits, it

also comes with drawbacks, such as the production of lower-quality drainage water, loss of nutrients or pesticides, and water wastage (Bauder et al. 2004; Beltran 1999). Additionally, it is essential to maintain an appropriate leaching fraction throughout the season to prevent subsequent resalinization of the topsoil layer (Cuevas et al. 2019).

Proper growing medium

Currently, various soilless substrates are utilized in horticulture to produce ornamental plants. A shallow soilless substrate can present more challenges regarding salinity compared with field soils, primarily due to the limited root zone volume and high water-holding capacity (Narvaez-Ortiz et al. 2018). Previous studies have established that the properties of the growing substrate play a crucial role in determining the plants response to salinity stress (Martinez and Clark 2009). Peat, characterized by its low salt content, is the most popular substrate component, mainly used in tree nurseries. Similarly, a soilless medium with good drainage contributes to enhanced aeration in the root zone, mitigating the effects of salinity stress on plants. Substrates such as sand, known for their excellent drainage ability, can be particularly beneficial in this regard (Fussy and Papenbrock 2022). Moreover, substrates that incorporate a higher proportion of plant bark can potentially help reduce salinity problems. A plant bark-based substrate is known for its low water-holding capacity and high bulk density, which also reduces the potential for over-watering.

Monitoring irrigation water quality

The quality of irrigation water varies across different regions or locations, depending on groundwater extraction methods, utilization, and rainfall intensity (Zaman et al. 2018). Regular monitoring of irrigation water for changes in salt content is crucial

for mitigating salinity stress in plants, especially in arid and semiarid regions where ground water or secondary water (untreated, unfiltered water) is commonly used for nursery production. Salinity in irrigation water can cause salt damage to sensitive species, particularly during certain months when salt levels may be higher. For example, in Utah, Cache Valley Nursery experienced relatively higher salinity levels in its irrigation water in Feb 2021 (Fig. 1-1). There are some basic criteria for evaluating water quality for irrigation purposes including EC, Na^+ content, and Cl^- content (Zaman et al. 2018). Regular monitoring of the salinity levels of irrigation water can help growers identify problems and determine mitigation strategies.

Nano technology

Recent studies have highlighted the potential of nanotechnology in improving salinity tolerance. Engineered nanoparticles (NPs), characterized by their ultra-small particle size and unique physicochemical properties, offer a promising avenue for alleviating salinity stress in plants. The application of these NPs has demonstrated improvements in plant growth, regulation of carbohydrate and protein synthesis, and the enhancement of antioxidant enzyme activities, including catalase, under salinity stress and thus aids in reducing levels of stress-induced ROS in plants (Etesami et al. 2021; Wu et al. 2018). For example, the use of nanoparticle cerium oxide (CeO_2) has been shown to enhance salinity stress tolerance in *Arabidopsis* and *Gossypium hirsutum* (cotton) (Liu et al. 2021; Wu et al. 2018). Additionally, the application of nano-silicon dioxide (nSiO2) has been reported to improve the growth rate and productivity of strawberry plants under salinity stress conditions (Avestan et al. 2019).

Soil amendments

Arbuscular mycorrhizal fungi, forming symbiotic relationships with the roots of 80% of land plants (Smith and Read 2008), play a crucial role in enhancing nutrient uptake and transfer (Ruiz-Lozano et al. 2012). This symbiosis aids plants in efficiently managing salinity stress by improving nutrient acquisition and water uptake, maintaining osmotic balance, stimulating antioxidant activities, enhancing photosynthetic efficiency, and modulating phytohormone profile (Evelin et al. 2012; Khalloufi et al. 2017; Ruiz-Lozano et al. 2012). Numerous studies have reported that mycorrhizal plants exhibit better growth than non-AMF plants under salinity stress, with examples including *Elaeagnus angustifolia* (Russian olive) (Chang et al. 2018) and *Chrysanthemum morifolium* (chrysanthemums) (Wang et al. 2018).

Application of Ca^{2+} can reduce stress injury in plants by increasing cell wall strength, maintaining plasma membrane integrity, and supporting mineral nutrition and water transport (Palta 1996; Pathak et al. 2020). Ca^{2+} also restricts Na^{+} entry through non-selective cation channels and inhibits K^{+} loss from cells by K^{+} efflux channels (Shabala et al. 2016). Application of Ca^{2+} to *Limonium stocksii* (marsh-rosemary) has enhanced plant biomass by improving water balance, reducing Na^{+} entry, and maintaining membrane integrity (Ahmed et al. 2021).

Under salinity stress, external K^{+} plays a key role in maintaining K^{+} homeostasis, thereby improving plant growth (Abbasi et al. 2015; Chakraborty et al. 2016). The exogenous application of nutrients, either through the root zone or as a foliar spray, can overcome salt-induced nutritional deficiencies (Akram and Ashraf 2011). Supplementary K^{+} ion application to the growth medium can alleviate the salt-induced reductions in K^{+} uptake and translocation in sunflower (Delgado and Sachez-Raya 1999). Similarly, foliar

application of K^+ ion, along with P, mitigated the deleterious effects of salinity stress on growth and yield of strawberries (Kaya et al. 2001). Therefore, potassium fertilizer application can effectively improve the salinity tolerance of plants.

Silicon is known for enhancing quantitative and qualitative plant traits, especially under environmental stresses such as salinity, drought, and heavy metal toxicity (Etesami and Jeang 2018; Wu et al. 2015). Silicon content varies among plant species due to differences in their Si absorption capabilities (Ma and Yamaji 2008). Silicon regulates root growth and architecture (Kim et al. 2014; Zhu et al. 2015), improves shoot growth, and maintains a high photosynthetic rate in salt-stressed plants (Coskun et al. 2019; Yin et al. 2013; Zargar et al. 2019). Furthermore, the application of potassium silicate has shown beneficial effects on the growth and quality of cut flowers, such as *Rosa hybrida* 'Pinocchio' (miniature rose) in rockwool culture systems (Hwang et al. 2005) and enhance the flower quality of hydroponically grown *Gerbera jamesonii* (gerbera) (Savvas et al. 2002).

The use of vermicompost is a promising eco-friendly technique for converting various types of waste, acting as reservoirs of environmental contaminants (Yuvaraj et al. 2021). Vermicompost positively affects soil structure, and its application, or derivatives thereof, has been reported to enhance plant salinity tolerance (Ruiz-Lau et al. 2020). Rich in microbial diversity, including fungi, bacteria, yeasts, actinomycetes, and algae, vermicompost produces growth regulators such as auxins, gibberellins, and cytokinins, all of which potentially benefit plant growth and development (Ruiz-Lau et al. 2020). In addition, humic substances in vermicompost increase the availability of beneficial nutrients such as N, P, K, and zinc (Zn). In a study by Adamipour et al. (2019), the

application of vermicompost improved the morpho-physiological parameters and mineral nutrient uptake in *Calendula officinalis* (marigold) under salinity conditions.

Exploring Landscape Plants for Salinity Tolerance

Salinity tolerance differs among species with distinct mechanisms to cope with adverse impacts of salinity stress (Munns and Tester 2008). Therefore, it is necessary to perform additional research to investigate the salinity tolerance of landscape plants. Despite the values in landscaping with *Albizia julibrissin* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), *Penstemon barbatus* 'Novapenblu' (rock candy blue® penstemon), *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue), *Punica granatum* 'Wonderful' (pomegranate), *Shepherdia*×*utahensis* 'Torrey' (hybrid buffaloberry), and *Sophora japonica* (Japanese pagoda tree), research-based information is limited regarding their salinity tolerance. Therefore, this dissertation research was conducted to investigate their responses to salinity stress and identify plants that are tolerant to salt for use in urban landscapes.

Research Objectives

1. To quantify the morphological and physiological responses of two woody ornamental plants [*Albizia julibrissin* (mimosa tree) and *Sophora japonica* (Japanese pagoda tree)] to saline water irrigation.
2. To measure the morphological and physiological responses, mineral nutrient status, and proline content of four Utah native plants [*Arctostaphylos uva-ursi*

(kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* ‘Coy’ (alder-leaf mountain mahogany), and *Shepherdia* ×*utahensis* ‘Torrey’ (hybrid buffaloberry)] irrigated with saline water.

3. To quantify the effects of salinity stress on the morphological, physiological, and biochemical responses and mineral nutrient of two penstemon species [*Penstemon barbatus* ‘Novapenblu’ (rock candy blue® penstemon) and *Penstemon strictus* ‘Rocky Mountain’ (rocky mountain beardtongue)].
4. To investigate the effects of various salinity levels on the growth, gas exchange, mineral nutrients, and transporter gene expression in *Punica granatum* ‘Wonderful’ (pomegranate).

Research Hypotheses

1. *Albizia julibrissin* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* ‘Coy’ (alder-leaf mountain mahogany), *Penstemon barbatus* ‘Novapenblu’ (rock candy blue® penstemon), *Penstemon strictus* ‘Rocky Mountain’ (rocky mountain beardtongue), *Punica granatum* ‘Wonderful’ (pomegranate), *Shepherdia* ×*utahensis* ‘Torrey’ (hybrid buffaloberry) and *Sophora japonica* (Japanese pagoda tree) irrigated with higher salinity levels exhibit wilting, discoloration, and foliar salt damage and have decreased plant growth.
2. Net photosynthetic rate of *A. julibrissin*, *A. uva-ursi*, *C. ledifolius*, *C. montanus* ‘Coy’, *P. barbatus*, *P. strictus*, *P. granatum* ‘Wonderful’, *S.* ×*utahensis* ‘Torrey’, and *S. japonica*, decreases with increasing salinity levels in the irrigation water.

3. Mineral nutrient contents in tissue of *A. julibrissin, A. uva-ursi, C. ledifolius, C. montanus* ‘Coy’, *P. barbatus, P. strictus, P. granatum* ‘Wonderful’, *S.* ×*utahensis* ‘Torrey’, and *S. japonica* changes with the exposed salinity levels.
4. *Arctostaphylos uva-ursi, C. ledifolius, C. montanus* ‘Coy’, *P. barbatus, P. strictus,* and *S.* ×*utahensis* ‘Torrey’ cultivated under saline condition have different proline contents in leaf tissues.
5. Expression of catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen antiporter (*NHX1*), and salt overly sensitive (*SOS1*, *SOS2*) genes of *P. granatum* ‘Wonderful’ changes in response to salinity stress.

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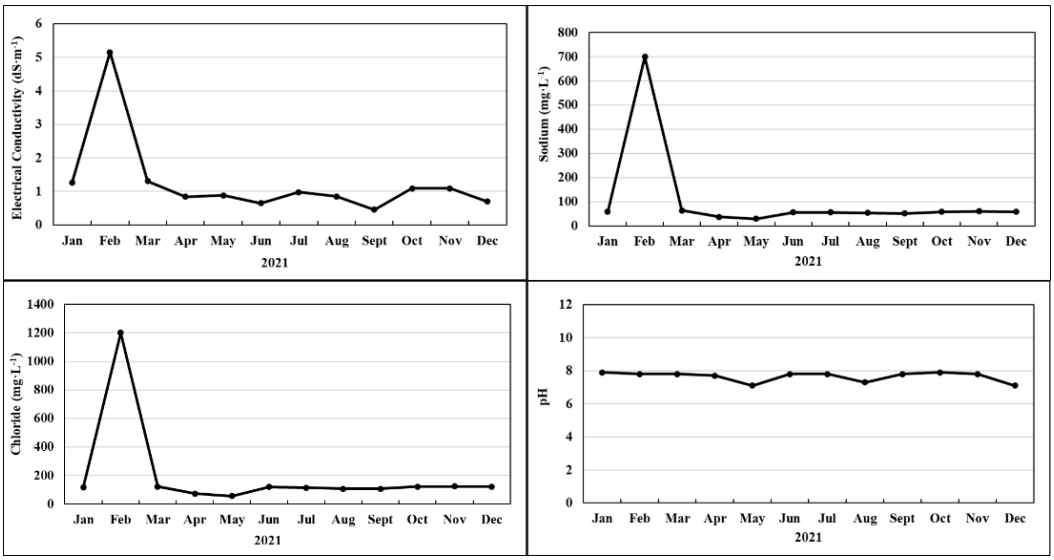

Fig. 1-1. Electrical conductivity, sodium and chloride contents, and pH of irrigation water samples from Cache Valley Nursery (Hyrum, UT) in 2021.

CHAPTER II

GROWTH, GAS EXCHANGE, AND MINERAL NUTRIENTS OF *ALBIZIA JULIBRISSIN* AND *SOPHORA JAPONICA* IRRIGATED WITH SALINE WATER[1]

Abstract

Albizia julibrissin (mimosa tree) and *Sophora japonica* (Japanese pagoda tree) are drought-tolerant landscape plants; however, salinity responses of these two species are not well documented. The objective of this study was to investigate the morphological and physiological responses of these two species to three salinity levels in greenhouse conditions. Two studies were conducted in the summer/early fall of 2020 and the spring of 2021. In 2020, uniform plants were irrigated weekly for the first 2 weeks and every other day for the following 3 weeks with a nutrient solution at an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$ as a control or saline solution at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$. In 2021, plants were irrigated weekly for 8 weeks with the same treatment solutions as described previously. *Albizia julibrissin* and *S. japonica* survived in both experiments with minimal foliar salt damage (leaf burn or necrosis). Irrigation water at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ reduced plant height and dry weight (DW) of both species. In the fall experiment, *A. julibrissin* irrigated with a saline solution at an EC of 10.0 $dS \cdot m^{-1}$ had the highest reduction in plant height (61%) compared with control. *Albizia julibrissin* and *S. japonica* irrigated with a saline solution at an EC of 10.0 $dS \cdot m^{-1}$ had 52% and 47% reductions in

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shoot DW compared with control, respectively. In the spring experiment, compared with the control, there were 72% and 45% reductions in height of *A. julibrissin* and *S. japonica*, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. In addition, compared with the control, *A. julibrissin* and *S. japonica* had 58% and 64% reductions in shoot DW, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Increasing salinity levels in the irrigation water also reduced leaf greenness [Soil Plant Analysis Development (SPAD)], leaf net photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of both species. Furthermore, sodium (Na^+) and chloride (Cl^-) concentrations in leaves were affected by elevated salinity levels in the irrigation water. Visual score, P_n, g_s, and E negatively correlated to Na^+ and Cl^- concentrations in leaves. But Cl^- accumulation had more impact on the growth of *A. julibrissin* and *S. japonica*. In summary, both species were tolerant to saline solution irrigation up to 5.0 $dS \cdot m^{-1}$ and moderately tolerant to saline solution irrigation up to 10.0 $dS \cdot m^{-1}$.

Introduction

Urban landscaping cleans air, water, and soil, and reduce greenhouse gas emissions. Aesthetically appealing landscapes are important elements of high-quality living environments in urban areas. Despite its importance, landscapes are facing soil salinity problems due to road de-icing salts, poor-quality irrigation water, excessive fertilizer use, or inherently sodic soil. Irrigation with saline water is one of the important causes of soil salinity in urban landscapes (Gorji et al., 2015). Many states in the United States are using reclaimed water for landscape irrigation. Florida is using 56% of its

reclaimed water to irrigate lawns in municipal parks, schools, and golf courses (Toor and Lusk, 2010). Similarly, 18% of reclaimed water in California is used for landscape irrigation (Water Recycling Funding Program, 2015). Using reclaimed water to irrigate landscape plants can help conserve a huge amount of potable water; however, being rich in salts, roughly two to three times higher than potable water, reclaimed water leads to soil salinity (Khurram and Miyamoto, 2005).

Salinity impedes plants growth all over the world, especially in arid and semi-arid regions. High soil salinity decreases water potential, reduces water availability to plants, and causes stunted plant growth along with foliar injuries such as leaf burn, scorch, necrosis, and premature defoliation (Munns, 2002; Niu and Cabrera, 2010). In addition, salinity can disturb plant metabolic functions, including internal solute balance, nutrient uptake, water relations, and photosynthesis (Grattan and Grieve, 1999). Salinity can inhibit plant growth in two phases. First, water or osmotic stress leads to a rapid growth reduction because of salts present in the soil. The second phase of growth reduction takes time to develop because of excessive salts accumulation in the plant (Greenway and Munns, 1980).

Nutritional disorders caused by saline conditions can have adverse effects on plant performance by affecting nutrient availability, competitive uptake, and transport or partition within plants (Grattan and Grieve, 1999). An optimum concentration of nutrients is required for proper plant growth and development. Concentrations below or above the optimal range cause nutrient deficiency or ion toxicity, thus affecting plant growth (Munns, 2002). Nutrient uptake by plants is directly affected by salinity, for example, sodium (Na^+) reduces potassium (K^+) uptake and calcium (Ca^+) availability, and chloride

(Cl^-) reduces nitrate (NO_3^-) uptake. On the other hand, less foliar salt injury and growth reduction are observed in salt-tolerant plants grown in saline conditions (Cai et al., 2014). Plants tolerate salinity through ion exclusion, maximizing Na^+ efflux from roots, maintaining a high cytosolic potassium to sodium (K^+/Na^+) ratio, or accumulation of compatible solutes (Tester and Davenport, 2003). Plant species or cultivars have different responses to salinity stress. Therefore, it is crucially important to know salinity tolerance mechanisms and to screen more productive crops considering the future state of climate change.

In the United States, more than 16 million of deciduous flowering trees are sold annually with an estimated sale value of $376 million (U.S. Department of Agriculture, 2015). *A. julibrissin* (mimosa tree) and *S. japonica* (Japanese pagoda tree) are widely planted ornamentals in the United States. *A. julibrissin* is a fast-growing, deciduous tree. It is a small to medium-sized tree with a vase shape and height of 6 to 12 m. It has compound leaves with tiny leaflets with a frond-like appearance. It produces fluffy, pink flower heads that bloom throughout the summer. Leaves close when touched and at night (Missouri botanical garden, 2021a). The bark and flowers of *A. julibrissin* are used as a medicinal herb (Chen and Hsieh, 2010; Kokila et al., 2013). *A. julibrissin* is distributed in the Northeast, and southern portions of the Midwest, South Central, and Southeast. *S. japonica* is a medium to large deciduous tree, 15 to 23 m tall. It has attractive compound foliage and fragrant flowers (Missouri botanical garden, 2021b). Dried flowers and buds of *S. japonica* are used as a medicinal herb (Chen and Hsieh, 2010). *S. japonica* is often found in humid temperate regions of the United States. Both *A. julibrissin* and *S. japonica* are drought tolerant (Gilman and Watson, 1993; Wood, 2006).

Plants in the genus *Albizia* and *Sophora* have been studied regarding their salinity tolerance. Miah (2013) investigated the effects of salts on seed germination, survival rate, and growth performance of *Albizia procera* (white siris) and *Albizia lebbeck* (woman's tongue) and suggested that *A. procera* is tolerant to salinity and can be planted in coastal areas. However, *A. lebbeck* can grow in less saline zones. In addition, Mo et al. (2011) reported that *S. japonica* is more tolerant to salinity stress than *A. julibrissin*. However, the authors did not investigate the gas exchange traits, that is, leaf net photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (E) during their study (Mo et al., 2011). Likewise, *Sophora secundiflora* (Texas mountain laurel) was observed to be a tolerant plant when irrigated with saline solution at an EC of 6.0 $dS \cdot m^{-1}$ (Niu et al., 2010)· However, Miyamoto (2008) listed *S. secundiflora* and *S. japonica* trees as salt-sensitive and A. *julibrissin* as moderately sensitive to salinity stress. Lee et al. (2015) reported that *A. julibrissin* and *S. japonica* had a survival rate of more than 90% and had good tree vigor when grown in salt-affected areas. In addition, McFarland et al. (2014) listed *A. julibrissin* and *S. japonica* as moderately tolerant and moderately sensitive to salinity stress, respectively. The varied responses reported for these species urge further research.

Despite the landscape values of *A. julibrissin* and *S. japonica*, research-based information is not clear regarding the salinity tolerance of these landscape trees. Therefore, it is necessary to perform additional research to investigate their responses to salinity stress and identify salt-tolerant plants for landscape use. It has been reported that the response of plants to salinity stress can have seasonal variations (Niu and Rodriguez, 2006). In this research, two separate studies were conducted to determine the

morphological and physiological responses of *A. julibrissin* and *S. japonica* to salinity stress in different seasons and durations.

Materials and Methods

Plant materials and culture. Two experiments were conducted in this study: from 3 Aug. to 16 Sept. 2020 and 10 Mar. to 3 May 2021. Experiments were conducted at the Utah State University (USU) Research Greenhouse in Logan, UT (lat. 41° 45’ 28” N, long. 111° 48’ 48” W, elevation 1409 m). For simplicity, the two experiments are referred to as fall and spring experiments. Seeds of *A. julibrissin* and *S. japonica* were scarified by dipping in 98.1% sulfuric acid (Fisher Chemical, Ottawa, ON) for 30 min to break their exogenous physical dormancy. Seeds after scarification were germinated in trays with moist perlite (Expanded Perlite; Malad City, ID) and sphagnum peat moss (SunGro Horticulture, Agawam, MA) at volumetric ratio of 2:1. Trays were placed in the greenhouse and covered with a plastic cover until seeds germinated. The temperature of the greenhouse was maintained at 20 ºC. Seedlings were transplanted into 3.9-L injection-molded, polypropylene containers (PC1D-4, Nursery Supplies, Orange, CA) filled with Metro-Mix 820 (Canadian Sphagnum peat moss, 35-45% composted pine bark, coir, coarse perlite, and dolomitic limestone; SunGro Horticulture, Agawam, MA). Seedlings of *A. julibrissin* and *S. japonica* were 16.0 ± 3.6 cm (mean ± SD) and 56.1 ± 11.2 cm tall in the fall experiment and 7.6 ± 1.3 cm and 27.8 ± 6.6 cm tall in the spring experiment, respectively. Seedlings in the fall experiment were ≈8 months old and those in the spring experiment were 2 to 4 months old before transplanting. Plants were kept in the research greenhouse, and tap water was applied. During fall experiment, greenhouse

temperature was maintained at 25.5 ± 0.5 ^{o}C (mean ± SD) during the day and 24.1 ± 1.0 ^{o}C at night. During spring experiment, greenhouse temperature was maintained at 25.0 ± 0.5 ^{o}C during the day and 21.5 ± 0.4 ^{o}C at night. Daily light integrals (DLI) inside the greenhouse were 32.6 ± 5.0 and 27.4 ± 8.7 mol·m^{-2}·d^{-1}, during the fall and spring experiment, respectively. Light intensities were recorded using a heated silicon chip pyranometer (SP-230; Apogee Instruments, Logan UT) mounted to a weather station at the Greenville research farm, nearly 1000 m away from the research greenhouse. A light transmission rate of 68% was used to calculate the DLI inside the greenhouse. Supplemental light at 211 ± 67.7 µmol·m^{-2}·s^{-1}, measured with a Quantum flux meter (MQ-200X, serial # 1006, Apogee Instruments, Logan, UT), was provided using 1000-W high-pressure sodium lamps at plant canopy level from 600 to 2200 $_{HR}$ when light intensity inside the greenhouse was less than 500 µmol·m^{-2}·s^{-1}.

Salinity treatments. Two salinity treatments were tested on *A. julibrissin* and *S. japonica* that included irrigation water at ECs of 5.0 and 10.0 dS·m^{-1}. The control group received only a nutrient solution at an EC of 1.2 dS·m^{-1}· Uniform plants were selected and randomly assigned to the treatments. The nutrient (control) solution was prepared in a 100-L tank by adding 0.8 g·L^{-1} 15N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special; ICL Specialty Fertilizers, Dublin, OH) to the tap water. The saline solutions of EC of 5.0 and 10.0 dS·m^{-1} were prepared using sodium chloride (NaCl; Fisher Scientific, Waltham, MA) and dihydrate calcium chloride ($CaCl_2·2H_2O$; Hi Valley Chemical, Centerville, UT) at a molar ratio of 2:1 to the nutrient solution (Table 2-1). The initial pH of treatment solutions was adjusted to 6.0 to 6.5 using 1 mol·L^{-1} nitric acid (Fisher Chemical, Fair Lawn, NJ) as needed. The sodium adsorption ratio (SAR) and

elemental analysis were confirmed by the USU Analytical Laboratory and these values are presented in Table 2-1. For the fall experiment, a 5-week study, 1000 ml treatment solutions per pot were applied manually once per week for the first 2 weeks and every other day thereafter (12 irrigation events). Plants in this study were growing vigorously and consumed more water, therefore, irrigation frequency increased. For the spring experiment, an 8-week study, 1000 ml of treatment solutions were applied manually to each plant weekly (eight irrigation events). The leaching fraction was targeted to ≈25%. In-between treatments, plants were watered with additional 250 to 500 mL of distilled water, as necessary, to avoid drought conditions.

Leachate and substrate EC. Leachate EC was determined using the pour-through method described by Cavins et al. (2008) using an EC meter (LAQUA Twin; Horiba, Kyoto, Japan). In brief, at least 30 minutes after every irrigation, a saucer was placed under the container and 100 ml of distilled water was poured from the top surface. Afterwards, EC was measured from the leachate. One plant per treatment per species was chosen for measurement. Substrate EC was measured using the saturated paste method explained by Gavlak et al. (2005) with some modifications. In brief, the pots containing soilless media were left to dry in the greenhouse for 2 weeks after harvest. A sample (10 g) was taken from the substrate at the top 5-cm surface as salts moved upward during the drying process. Then, 100 ml of deionized water was added to the substrate sample in a flask to make a paste. All samples were stored overnight at room temperature after covering the flasks with parafilm (American National Can, Menasha, WI) and EC measurements were taken.

Visual quality. A visual score of 0 to 5 was assigned to each plant at the end of the experiment to assess foliar salt damage. Visual score was assigned as 0 = dead, 1 = severe foliar damage (>90% leaves with burnt edges or necrosis), 2 = moderate foliar damage (90% to 50%), 3 = slight foliar damage (50% to 10%), 4 = good quality with minimal foliar damage (<10%), and 5 = excellent without foliar damage (Sun et al., 2015). Plant growth parameters were not considered while assigning the visual score.

Growth parameters. Plant height (centimeters) were recorded at the beginning and end of the experiment. Height was recorded from the surface of the growing medium to the top of the plants. Increase in plant height was calculated as the difference between the initial height and final height. At harvest, leaf area (square centimeters) was measured using a leaf area meter (LI-3100; LI-COR Biosciences, Lincoln, NE). In addition, shoot dry weight (DW) (stem DW + leaf DW) and root DW of plants were measured after being dried in an oven at 60 ºC for 1 week.

Chlorophyll content and gas exchange. Relative chlorophyll content (or leaf greenness) of all plants was recorded using a chlorophyll meter [Soil Plant Analysis Development (SPAD)-502; Minolta Camera, Osaka, Japan] before harvest. Eight mature leaves from each plant were measured, and the averaged value was recorded. Leaf P_n, g_s, and E of plants in each treatment were measured 4 d before harvest using a portable photosynthesis system with an automatic universal PLC3 universal leaf cuvette (CIRAS-3; PP Systems, Amesbury, MA) or LI-6800 photosynthesis system (LI-COR Biosciences) for the fall and spring experiment, respectively. Fully expanded, healthy leaves without damage were used for the gas exchange measurements. Environmental conditions in the cuvette were controlled at 25 ºC, 1000 µmol·m^{-2}·s^{-1} photosynthetic photon flux and 400

$\mu mol \cdot mol^{-1}$ carbon dioxide concentration. Data were recorded once the environmental conditions and gas exchange parameters in the cuvette became stable. All plants were watered 1 day before measurements to avoid water stress.

Mineral analyses. In the spring experiment, four dried plants per species per treatment were selected randomly and each was ground with a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ) and allowed to pass through 1-mm-mesh screen. The powder samples were analyzed at the USU Analytical Laboratories for mineral contents. In brief, the concentration of chloride (Cl^-) were quantified using 2% acetic acid, and sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), sulfur (S), zinc (Zn^{2+}), and manganese (Mn^{2+}) using nitric/hydrogen peroxide following the protocol described in Gavlak et al. (2005). The Cl^- concentration was determined by ion-selective electrode using a Flow Injection Analysis and Ion Chromatograph System (QuikChem 8000; Lachat Instrument, Loveland, CO) and reported on a dry plant basis ($mg \cdot g^{-1}$). For Na^+, Ca^{2+}, K^+, Mg^{2+}, S, Zn^{2+}, and Mn^{2+}, 0.5 g of powder samples and 6 ml of nitric acid (HNO_3) were added into a digestion tube that was then placed in a digestion block for 10 minutes at 80 °C and subsequently cooled for 2 min. A total of 2 mL of 30% hydrogen peroxide (H_2O_2) was added into the digestion tube that was placed again in the digestion block at 130 °C for 1 h. Mixing using a vortex stirrer was performed followed by cooling and diluting. Then the digestion tube was cooled at room temperature, and the contents of the digestion tube were transferred into a 25-mL volumetric flask. The digest was analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometer (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA) and reported on a dry plant basis ($mg \cdot g^{-1}$).

Experimental design and data analyses. The experiment was a randomized complete block design with two species, three treatments, and 10 replicates. An experimental unit consisted of one pot containing one plant. Analysis of variance was conducted to test the effects of saline solution irrigation on plant growth, gas exchange parameters, and mineral nutrient concentrations. Log transformation was done for all data except for mineral analysis, substrate EC, and visual score data. Means separation among treatments was adjusted using Tukey's method for multiplicity at $\alpha = 0.05$. Correlation analyses were carried out for Na^+, Cl^- concentrations, and the K^+:Na^+ ratio in plant tissue compared with the visual scores and gas exchange parameters. All statistical analyses were conducted using SAS (Version 14.1, SAS Institute, Cary, NC) with PROC MIXED procedure.

Results and Discussion

Leachate and substrate EC. Leachate EC increased over the time of saline solutions irrigation (Fig.2-1). In the fall experiment, leachate EC ranged from 0.7 to 1.2 $dS \cdot m^{-1}$ when a nutrient solution at an EC of 1.2 $dS \cdot m^{-1}$ was applied. Irrigation with saline solution at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ increased leachate EC from 2.2 to 7.1 and 4.4 to 12.9 $dS \cdot m^{-1}$, respectively. In the spring experiment, leachate EC ranged from 1.2 to 2.1, 3.7 to 11.0, and 5.7 to 19.0 $dS \cdot m^{-1}$ when irrigated with a nutrient solution at an EC of 1.2 $dS \cdot m^{-1}$ and saline solutions at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$, respectively. In both experiments, substrate EC increased with increasing salinity levels of irrigation water (Fig. 2). In the fall experiment, substrate EC was 3.5 $dS \cdot m^{-1}$ for both *A. julibrissin* and *S. japonica* after irrigation with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ for 5 weeks; saline

solution irrigation at an EC of 10 $dS \cdot m^{-1}$ further increased the substrate EC to 8.5 and 10.5 $dS \cdot m^{-1}$ for *A. julibrissin* and *S. japonica*, respectively (Fig. 2). In the spring experiment, substrate EC was 3.8 and 4.6 $dS \cdot m^{-1}$ for *A. julibrissin* and *S. japonica*, respectively, after irrigation with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ for 8 weeks. Saline solution at an EC of 10 $dS \cdot m^{-1}$ further increased the substrate EC to 9.7 and 10.6 $dS \cdot m^{-1}$ for *A. julibrissin* and *S. japonica*, respectively (Fig. 2-2). Evaluation of leachate and substrate EC from both experiments indicated the effects of saline solution irrigation on the EC of the root zone. Similarly, Wu et al. (2016) and Xing et al. (2021) reported that leachate and substrate EC increased with saline water irrigation over time as salts accumulated in the substrate. Salt accumulation is a potential problem when poor-quality saline water is used for landscape irrigation. Therefore, best management practices including monitoring water quality, increasing leachate fraction, and using tolerant species should be adopted to limit salinity stress in plants.

Visual quality. Salinity stress causes plant foliar damage like leaf burn, necrosis, and/or discoloration (Paudel et al., 2019; Sun and Palmer, 2018). Saline solution irrigation had significant effects on the visual score of both species in the fall and spring experiments ($P = 0.006$ and $P < 0.0001$, respectively, Table 2-2). There were no interactive effects for visual score between salinity treatment and species in the fall experiment ($P = 0.06$), but significant interactive effects were observed in the spring experiment ($P = 0.02$, Table 2-2). All *A. julibrissin* and *S. japonica* plants survived regardless of treatment (data not shown). No foliar damage was observed on plants irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ in both experiments, but both species had minimal foliar salt damage when exposed to the saline solution at an EC of 10.0

$dS \cdot m^{-1}$ in the spring experiment (Fig. 2-3). The visual score of *A. julibrissin* was 4.7 and 3.9 when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ in the fall and spring experiments, respectively. *S. japonica* exhibited no foliar salt damage in the fall experiment and had a visual score of 4.3 in the spring experiment when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. In this study, the experiment conducted in the fall had relatively less foliar damage than in the spring. Although the number of irrigation events was greater in the fall experiment, plants were treated for a longer time in the spring experiment. In addition, seedlings used in the fall experiment were older compared with those in the spring experiment. Salt injury was observed on old leaves in the lower canopy only; however, new leaves in the upper canopy were unaffected.

Foliar salt damage is problematic for landscape plants (Veatch-Blohm et al., 2014); therefore, the aesthetic appearance of plants is one of the primary focuses when screening landscape plants for salt tolerance (Niu and Cabrera, 2010; Veatch-Blohm et al., 2014). It is important to select plants that maintain good visual quality in the landscape that are affected by salinity (Niu and Rodriguez, 2006; Wahome et al., 2001). In this study, *A. julibrissin* and *S. japonica* exhibited no foliar damage on plants irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ but minimal foliar salt damage when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. In line with our results, Niu et al. (2010) reported that *S. secundiflora* had no foliar salt injury when irrigated with saline solutions at ECs of 3.0 and 6.0 $dS \cdot m^{-1}$. Based on visual quality alone, *A. julibrissin* and *S. japonica* can be good candidates for growing in salt-prone landscapes.

Growth parameters. Plant height and leaf area were affected by saline solution irrigation in both experiments ($P < 0.0001$, Table 2-2). In the fall experiment, the plant

height of *A. julibrissin* decreased by 38% compared with the control when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Table 2-3). *A. julibrissin* and *S. japonica* were 61% and 50% shorter than those in control, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, in the spring experiment, *A. julibrissin* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ was 72% shorter than those in control. *Sophora japonica* irrigated with saline solutions at ECs of 5.0 and 10 $dS \cdot m^{-1}$ were 30% and 45% shorter than those in control. On the other hand, it has been reported that the plant height of *S. secundiflora* was unaffected when irrigated with a saline solution containing NaCl, magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), and calcium chloride ($CaCl_2$) at an EC of 6.0 $dS \cdot m^{-1}$ for the first 4 months (Niu et al., 2010). However, *S. secundiflora* plants were shorter after irrigation for 6 months. This contrast may be due to the application of saline solution at different concentrations and compositions and different duration of saline solution irrigation. In the current research, saline solutions containing NaCl and $CaCl_2$ were applied for 5 and 8 weeks in the fall and spring experiments, respectively. In addition, plant species may have different responses to salinity stress.

Albizia julibrissin and *S. japonica* had no significant reduction in leaf area when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ in both experiments (Table 2-3). In the fall experiment, *A. julibrissin* and *S. japonica* had 41% and 36% reductions in leaf area, respectively, compared with the control when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ (Table 2-3). In the spring experiment, there were 35% and 44% reductions in leaf area for *A. julibrissin* and *S. japonica*, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. In addition, leaf area varied with species in both fall and

spring experiments (Tables 2-2 and 2-3). Previous studies have also reported reduced leaf area with increasing salt concentrations in irrigation solution (Niu et al., 2012; Paudel et al., 2019; Sun et al., 2018a). Likewise, the leaf area of pomegranate cultivars decreased when irrigated with saline groundwater at an EC of 6.0 $dS \cdot m^{-1}$ (El-Khawaga et al., 2013). This is because salinity-induced water deficit causes leaf senescence and reduces leaf expansion, thereby leading to decreased leaf area (Muchate et al., 2016; Munns and Tester, 2008).

Furthermore, leaf DW, stem DW, and shoot DW of both species decreased with increasing salinity levels in the irrigation water in both experiments ($P < 0.0001$, Table 2-2, Fig. 2-4). Compared with the control, the leaf DW and stem DW decreased by 37% to 48% and 56% to 60%, respectively, for both species irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ in the fall experiment. In addition, there were 53% to 58% and 68% to 71% leaf DW and stem DW reductions, respectively, for both species in the spring experiment. Likewise, shoot DW of *S. secundiflora* was 25% and 46% less when irrigated with saline solutions at ECs of 3.0 and 6.0 $dS \cdot m^{-1}$ for 6 months, respectively, compared with the control (Niu et al., 2010). In addition, root DW of *A. julibrissin* and *S. japonica* decreased significantly with increasing salinity levels in the irrigation water in the fall ($P = 0.002$) and spring experiments ($P < 0.0001$, Table 2-2, Fig. 2-4). Root is the first tissue to perceive salinity stress, therefore, plays an important role in plant development. However, the effect of salinity stress on roots of *A. julibrissin* and *S. japonica* has not been reported previously. Salinity stress appears to stimulate the transition from cell division to elongation and suppress root meristem activity (West et al., 2004). In line with our results, it has been reported that declining root DW is common

for landscape plants when exposed to salinity stress (Acosta-Motos et al., 2015; Hooks and Niu, 2019). For example, root DW of rose (*Rosa ×fortuniana*, *Rosa multiflora*, and *Rosa odorata*) rootstocks when irrigated with saline solutions at ECs of 1.6, 3.0, 6.0, and 9.0 $dS \cdot m^{-1}$ decreased linearly with increasing salinity levels in the irrigation water (Niu et al., 2008).

Leaf greenness (SPAD reading) and gas exchange. Saline solution irrigation affected SPAD readings of *A. julibrissin* and *S. japonica* in the fall and spring experiments ($P = 0.01$ and $P < 0.0001$, respectively, Table 2-2). There were no interactive effects between salinity treatment and species (Table 2-2). In addition, SPAD readings varied with species in both fall ($P < 0.0001$) and spring ($P = 0.0001$) experiments. In the fall experiment, there was a 15% reduction in the SPAD reading of *A. julibrissin* irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ compared with the control, but not statistically different. However, saline solution at an EC of 10.0 $dS \cdot m^{-1}$ reduced the SPAD reading of *A. julibrissin* by 17%. Saline solution at an EC of 5.0 or 10.0 $dS \cdot m^{-1}$ did not impact SPAD readings of *S. japonica* (Table 2-4). In the spring experiment, compared with the control, saline solution at an EC of 5.0 $dS \cdot m^{-1}$ reduced the SPAD reading of *S. japonica* by 19%. In addition, SPAD readings of both species decreased by 16% to 22% when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, various studies reported that SPAD readings reduced with increasing salt concentrations in irrigation water (Chen et al., 2019; Liu et al., 2017; Sun et al., 2015). For example, SPAD readings of *Physocarpus opulifolius* (ninebark) were reduced by 19% when irrigated with saline solution at an EC of 6.5 $dS \cdot m^{-1}$ compared with the control (Chen et al., 2019). These results consistently

indicate that salinity stress causes chlorophyll degradation and decreases chlorophyll content (Santos, 2004).

In the fall experiment, saline solution irrigation had significant effects on P_n ($P < 0.0001$), g_s ($P = 0.02$), and E ($P = 0.05$) (Table 2-2). P_n of *A. julibrissin* irrigated with saline solutions at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ decreased by 38% and 45% compared with the control, respectively (Table 2-4). In *A. julibrissin*, compared with the control, saline solution at an EC of 10.0 $dS \cdot m^{-1}$ reduced g_s by 56% (Table 2-5). Although E of *A. julibrissin* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ decreased by 31% compared with control, it was not statistically significant. Likewise, compared with the control, *S. japonica* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ had a 58% reduction in P_n (Table 2-4). However, there was no significant change in the g_s and E of *S. japonica* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ compared with the control (Table 5).

In the spring experiment, saline solution irrigation significantly affected P_n ($P < 0.0001$), g_s ($P < 0.0001$), and E ($P < 0.0001$) (Table 2-2). In *A. julibrissin*, compared with the control, saline solution at an EC of 5.0 $dS \cdot m^{-1}$ reduced P_n, g_s, and E by 44%, 53%, and 48%, respectively (Tables 2-4 and 2-5). Similarly, saline solution at an EC of 10.0 $dS \cdot m^{-1}$ reduced P_n, g_s, and E of *A. julibrissin* by 72%, 73%, and 70%, respectively. *Sophora japonica* irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ had 49% reduction in P_n compared with the control. Likewise, compared with the control, *S. japonica* irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ had 66%, 75%, and 71% reductions in P_n, g_s, and E, respectively. In both experiments, gas exchange parameters were reduced at higher salinity levels. This is similar to the report by Niu et al. (2010) that leaf P_n and

g_s of *S. secundiflora* were lower when irrigated with 3.0 or 6.0 $dS \cdot m^{-1}$ compared with the control. However, different results were observed in the fall and spring experiments. These results may indicate that plant photosynthetic parameters depend on the environmental factors and plant growth stage. In addition, the use of two different instruments may have some influence on those parameters.

Salinity stress may harm plants' photosynthetic apparatus and reduce photosystem II efficiency, which inhibits plant photosynthesis (Sharma et al., 2012; Taiz et al., 2015). Saline conditions also create water deficits that lead to stomatal closure and ultimately decrease transpiration (Wang et al., 2019). Salinity induced stomatal closure reduces internal CO_2 concentration and decreases enzyme activity involved in carboxylation, such as Ribulose-1,5- biphosphate carboxylase oxygenase (Chaves et al., 2009), thus reducing net photosynthetic rate. In addition, a decrease in leaf area and chlorophyll content may reduce photosynthesis under high salinity stress (Sharma et al., 2012). Reduced photosynthesis eventually impairs plant growth (Menezes et al., 2017; Odjegba and Chukwunwike, 2012). Furthermore, a plant can experience growth reduction due to the diversion of energy from growth to the homeostasis of salinity stress (Atkin and Macherel, 2009).

Mineral nutrients. Sodium and Cl^- concentrations in the leaf tissue of *A. julibrissin* and *S. japonica* were significantly affected by both elevated salinity levels and plant species interactively (Table 2-6). In this study, the NaCl concentration of *A. julibrissin* increased almost six times, from 0.04 to 0.26 $mg \cdot g^{-1}$, as the EC of saline solutions increased from 1.2 to 10.0 $dS \cdot m^{-1}$ (Table 2-6). However, there was no difference in the Na^+ concentration among treatments for *S. japonica*. Furthermore, Na^+

concentrations in the leaf tissue of *A. julibrissin* and *S. japonica* were less than 1 $mg \cdot g^{-1}$. Salt-tolerant ornamental plants may accumulate less Na^+ in their leaves compared with salt-sensitive plants (Wu et al., 2016) because sodium uptake is reduced, and/or restricted transport of Na^+ from roots to shoots occurs for salt-tolerant plants (Munns, 2002). Similarly, the Na^+ concentration of *Punica granatum* (pomegranate) was also less than 1 $mg \cdot g^{-1}$ when irrigated with saline solutions up to EC of 15.0 $dS \cdot m^{-1}$ for 7 weeks (Sun et al., 2018b). On the other hand, *S. secundiflora* irrigated for 6 months with saline solution at an EC of 6.0 $dS \cdot m^{-1}$ had 8.5 $mg \cdot g^{-1}$ Na^+ ions in leaves, which is 15 times greater than the control (Niu et al., 2010). This contrast may be due to longer irrigation time and different species. Compared with the control, Cl^- concentration increased by 17 and 32 times for *A. julibrissin* and 14 and 25 times for *S. japonica* when plants were irrigated with saline solutions at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$, respectively. Similarly, *S. secundiflora* accumulated ≈18.0 ·of Cl^- in its leaves when irrigated for 6 months with saline solution at an EC of 6.0 $dS \cdot m^{-1}$, which was increased by three times compared with the control (Niu et al., 2010). These results indicate that *A. julibrissin* and *S. japonica* accumulated many fewer Na^+ ions than Cl^- ions in their leaf tissue.

In this study, minimal foliar salt damage was observed, but significant negative correlations between visual score and Na^+ and Cl^- concentrations were obtained (Fig. 2-5). Similarly, it has been reported that *Rosa chinensis* 'Major' (China rose) and *Rosa rubiginosa* (sweet brier) accumulated Cl^- ions to toxic levels that are responsible for leaf necrosis (Wahome et al., 2001). More importantly, Cl^- is a beneficial micronutrient ion that helps in photosynthesis, osmoregulation, turgor regulation, and plant growth (Chen et al., 2016; Flowers, 1988; Homann, 1987). However, high concentrations in plant tissue

can turn Cl^- from nutrient to toxicant (Geilfus, 2018). In addition, increasing Na^+ and Cl^- concentrations in plant leaves can cause ion toxicity and reduce photosynthesis (Taiz et al., 2015). In this study, there were more Cl^- ions than Na^+ ions in leaf tissue. Negative correlations between photosynthesis and Na^+ ($P = 0.01$; $r^2 = 0.25$) and Cl^- ($P < 0.0001$; $r^2 = 0.62$) were observed (Fig. 2-5). Therefore, the inhibition of photosynthesis may be more related to Cl^- accumulation. Similarly, negative correlations between Na^+ and Cl^- concentrations and g_s and E were also observed (Fig. 2-5).

Calcium concentration in the leaf tissue was affected by both salt treatment and plant species interactively (Table 6). In the present study, $CaCl_2$ was used to reduce the deficiency of Ca^{2+} ions and provide osmoprotection by its additive role with NaCl (Jaleel et al., 2007). Although $CaCl_2$ was added to prepare the saline solution, compared with the control, only an ≈2-time increment of the Ca^{2+} concentration in leaf tissue was observed. It has previously been reported that calcium transport and mobility to plant parts are reduced by salinity stress (Grattan and Grieve, 1999). Compared with the control, there was a 21% increment in the K^+ concentration of *A. julibrissin* when irrigated with saline solutions at an EC of 10.0 $dS \cdot m^{-1}$ (Table 2-6). However, researchers have reported that there is a decline in K^+ concentration in plant tissue when plants are exposed to salinity stress (Grattan and Grieve, 1999). There was also an increase in K^+ content in the tissue of *Acacia auriculiformis* (northern black wattle) with increasing soil salinity (Patel et al., 2010). *A. julibrissin* might have the ability to transport K^+ against the Na^+ gradient, which leads to an increase in leaf K^+ concentration (Grattan and Grieve, 1999). On the other hand, there was no change in the leaf K^+ concentration of *S. japonica*. As salinity levels increased in the irrigation water, the K^+:Na^+ ratio in the leaf tissue decreased in both

species (P = 0.0003, Table 2-6). In line with our results, it has been reported that salinity stress decreased the K^+:Na^+ ratio in plants (Gomez-Bellot et al., 2015; Guo et al., 2020). External Na^+ often inhibits K^+ uptake and hence high cytosolic K^+:Na^+ ratios are the key salt tolerance trait in plants (Assaha et al., 2017; Shabala and Pottosin, 2014). In addition, the K^+:Na^+ ratio had positive correlations with the visual score, P_n, g_s, and E (Fig. 5). In the present study, Mg^{2+} and S content in the leaf tissue of *A. julibrissin* increased in response to elevated salinity levels in irrigation water (Table 2-6). In addition, Zn^{2+} and Mn^{2+} contents in the leaf tissue of *A. julibrissin* and *S. japonica* increased in response to elevated salinity levels in irrigation water. Similarly, salinity stress increased Zn^{2+} and Mn^{2+} concentrations in *A. auriculiformis* (Patel et al., 2010). Magnesium and Mn^{2+} play important roles in the biosynthesis of chlorophyll and successful growth of plants under normal or stressful conditions (FarhangiAbriz and Ghassemi-Golezani, 2021). Similarly, S is an integral part of several important compounds in plants, such as vitamins, coenzymes, and phytohormones (Li et al., 2020). In addition, Zn^{2+} reduces excessive Na^+ uptake under saline conditions by affecting the structural integrity and permeability of cell membrane (Tolay, 2021). However, Yildiz et al. (2020) reported that Na^+ and Cl^- ions compete with nutrients and lead to nutrient deficiency in plants. Therefore, an increase in the concentration of these nutrients with increasing salinity levels might be the strategy of these species to survive in saline conditions.

In conclusion, *A. julibrissin* had only minimal foliar salt damage at higher EC levels and *S. japonica* had no foliar salt damage. Saline solution irrigation reduced plant growth of *A. julibrissin* and *S. japonica* as indicated by plant height, leaf area, and DW in both experiments. Salinity stress also reduced plant photosynthesis and caused Cl^- uptake

and accumulation. However, Na^+ uptake and accumulation are less pronounced compared with Cl^-. *Albizia julibrissin* and *S. japonica* are probably capable of restricting either the uptake or transport of Na^+ and tolerating high concentrations of Cl^- in the leaf tissue while maintaining good aesthetic quality. Therefore, both species are suitable for landscape use in salt-affected areas.

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Table 2-1. The mineral contents, sodium adsorption ratio (SAR), and electrical conductivity (EC) of nutrient and saline solution used in the study.

Item [z]	Nutrient solution [y]	Saline solution [x]	
		5.0 $dS \cdot m^{-1}$	10.0 $dS \cdot m^{-1}$
Ca^{2+} ($mg \cdot L^{-1}$)	102.3	455.5	965.1
Mg^{2+} ($mg \cdot L^{-1}$)	33.9	28.6	29.4
Na^{+} ($mg \cdot L^{-1}$)	2.2	369.9	876.7
SO_4^{2-} ($mg \cdot L^{-1}$)	8.6	9.0	10.2
Cl^{-} ($mg \cdot L^{-1}$)	3.0	1290.0	3150.0
B ($mg \cdot L^{-1}$)	0.16	0.18	0.16
SAR	0.05	4.53	7.57
Adjusted SAR	0.08	11.16	20.57
EC ($dS \cdot m^{-1}$)	1.2 ± 0.1	5.1 ± 0.1	10.1 ± 0.1

[z] Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), sulphate (SO_4^{2-}), chloride (Cl^{-}), and boron (B) ions.

[y] The nutrient solution at an EC of 1.2 $dS \cdot m^{-1}$ was made by mixing 0.8 $g \cdot L^{-1}$ 15N-2.2P-12.5K water-soluble fertilizer (Peter Excel 15-5-15 Ca-Mag Special) in tap water.

[x] Sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) were used to prepare saline solution. The nutrient solution was supplemented with NaCl at 0.92 $g \cdot L^{-1}$ and $CaCl_2 \cdot 2H_2O$ at 1.17 $g \cdot L^{-1}$ to obtain the saline solution at an EC of 5.0 $dS \cdot m^{-1}$, while 2.27 $g \cdot L^{-1}$ NaCl and 2.88 $g \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$ was added to nutrient solution to make the saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

Table 2-2. A summary of analysis of variance for the effects of salinity treatment and their interactions with species on visual score (VS), plant height (Ht), leaf area (LA), leaf dry weight (DW), stem DW, shoot DW (leaf DW + stem DW), root DW, leaf greenness [Soil Plant Analysis Development (SPAD) reading], net photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse[z].

Source	Analysis of variance										
	VS	Ht	LA	Leaf DW	Stem DW	Shoot DW	Root DW	SPAD	P_n	g_s	E
Fall 2020											
Species	NS	NS	****	**	****	*	NS	****	****	****	****
Treatment	**	****	****	****	****	****	**	*	****	*	*
Species * Treatment	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS
Spring 2021											
Species	NS	****	*	***	***	NS	NS	***	*	NS	NS
Treatment	****	****	****	****	****	****	****	****	****	****	****
Species * Treatment	*	**	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution.

NS, *, **, *** , **** Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001 respectively.

Table 2-3. Plant height and leaf area of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse.[z]

		Albizia julibrissin			***Sophora japonica***		
		Control	**EC 5**	**EC 10**	**Control**	**EC 5**	**EC 10**
Height (cm)	**Fall 2020**	68.7 a[y]	42.3 b	27.1 b	60.5 a	35.2 ab	30.5 b
	Spring 2021	34.5 a	23.7 a	9.5 b	51.3 a	36.1 b	28.1 b
Leaf area	**Fall 2020**	1064 a	846 ab	625 b	1583 a	1273 ab	1010 b
(cm^2)	**Spring 2021**	671 a	742 a	434 b	1017 a	790 ab	574 b

[z]Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution.

[y]Means with the same lowercase letters within a row and species are not significantly different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 2-4. Leaf greenness [Soil Plant Analysis Development (SPAD) reading] and net photosynthesis rate (P_n) of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse.[z]

		Albizia julibrissin			*Sophora japonica*		
		Control	EC 5	EC 10	Control	EC 5	EC 10
SPAD	Fall 2020	39.3 a[y]	33.3 ab	32.5 b	50.4 a	50.4 a	44.4 a
	Spring 2021	44.7 a	40.4 ab	37.7 b	54.7 a	44.2 b	42.7 b
P_n ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Fall 2020	15.2 a	9.4 b	8.4 b	7.7 a	6.1 a	3.2 b
	Spring 2021	15.4 a	8.7 b	4.3 c	11.6 a	5.9 b	4.0 c

[z]Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution.

[y]Means with same lowercase letters within a row and species are not significantly different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 2-5. Stomatal conductance (g_s) and transpiration rate (E) of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse.[z]

		Albizia julibrissin			*Sophora japonica*		
		Control	EC 5	EC 10	Control	EC 5	EC 10
g_s ($mmol \cdot m^{-2} \cdot s^{-1}$)	Fall 2020	455.2 a[y]	177.0 ab	200.4 b	95.0 a	112.0 a	57.4 a
	Spring 2021	124.9 a	58.5 b	33.6 b	118.6 a	70.0 a	29.2 b
E ($mmol \cdot m^{-2} \cdot s^{-1}$)	Fall 2020	8.5 a	5.0 a	5.9 a	3.9 a	3.7 a	2.3 a
	Spring 2021	2.3 a	1.2 b	0.7 b	2.1 a	1.3 a	0.6 b

[z]Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution.

[y]Means with same lowercase letters within a row and species are not significantly different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 2-6. Leaf mineral ion concentrations and potassium-to-sodium (K^+:Na^+) ratio of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 dS·m^{-1}; control] or saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] in a greenhouse.[z]

Species	Treatment	Ion concn (mg·g^{-1})								
		Na^+	Cl^-	Ca^{2+}	K^+	K^+:Na^+	Mg^{2+}	S	Zn^{2+}	Mn^{2+}
Albizia julibrissin	**Control**	0.04 b[y]	2.04 c	8.44 c	15.32 b	422.25 a	2.51 b	1.62 b	0.03 b	0.02 c
	EC5	0.08 b	35.93 b	19.28 b	17.26 ab	261.29 ab	3.41 a	2.01 ab	0.04 ab	0.04 b
	EC10	0.26 a	66.48 a	27.77 a	18.52 a	86.81 b	3.49 a	2.39 a	0.05 a	0.07 a
Sophora japonica	**Control**	0.04 a	1.81 c	9.72 b	22.95 a	654.17 a	2.00 b	2.25 a	0.03 b	0.01 b
	EC5	0.07 a	27.60 b	21.04 a	24.45 a	393.39 b	2.72 a	2.31 a	0.04 ab	0.04 a
	EC10	0.08 a	46.48 a	19.65 a	25.06 a	356.52 b	2.11 b	2.25 a	0.04 a	0.04 a
Species		*	****	*	****	***	****	*	*	***
Treatment		***	****	****	*	***	***	*	***	***
Species*Treatment		**	***	****	NS	NS	*	*	NS	****

[z] Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2·2H_2O$) to the nutrient solution. Leaf samples from the experiment in Spring 2021 were used for mineral analyses. Sodium (Na^+), chloride (Cl^-), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), sulfur (S), zinc (Zn^{2+}), and manganese (Mn^{2+}) ions.

[y] Means with same lowercase letters within a column and species are not significantly different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

[NS], *, **, ***, **** Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

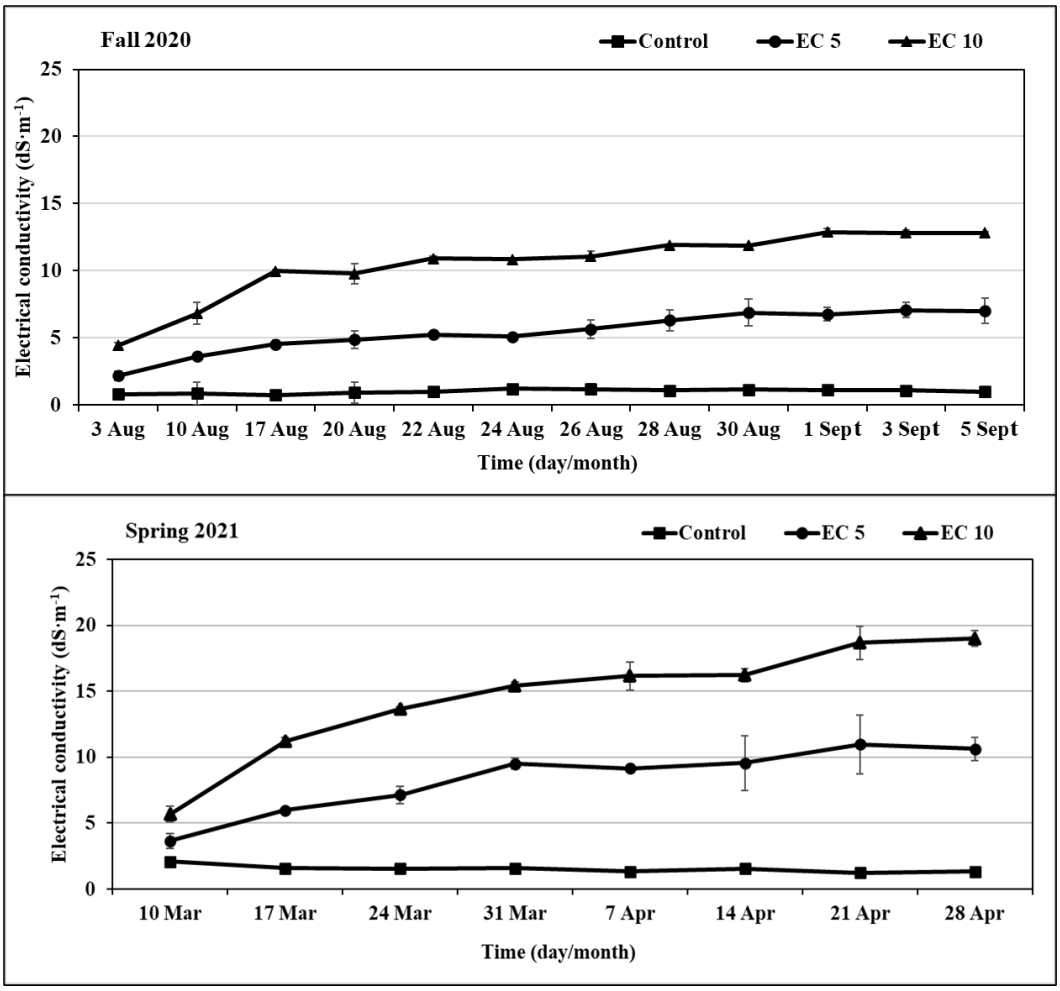

Fig. 2-1. Electrical conductivity (EC) of leachate solution collected after irrigating *Albizia julibrissin* and *Sophora japonica* with a nutrient solution (EC = 1.2 $dS \cdot m^{-1}$; control) or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] over the course of experiment in Fall 2020 and Spring 2021. Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution. Treatment solutions were applied from 3 Aug. to 5 Sept. 2020 (12 irrigation events) and 10 Mar. to 28 Apr. 2021 (8 irrigation events) in Fall 2020 and Spring 2021, respectively. Vertical bars represent standard errors of two measurements.

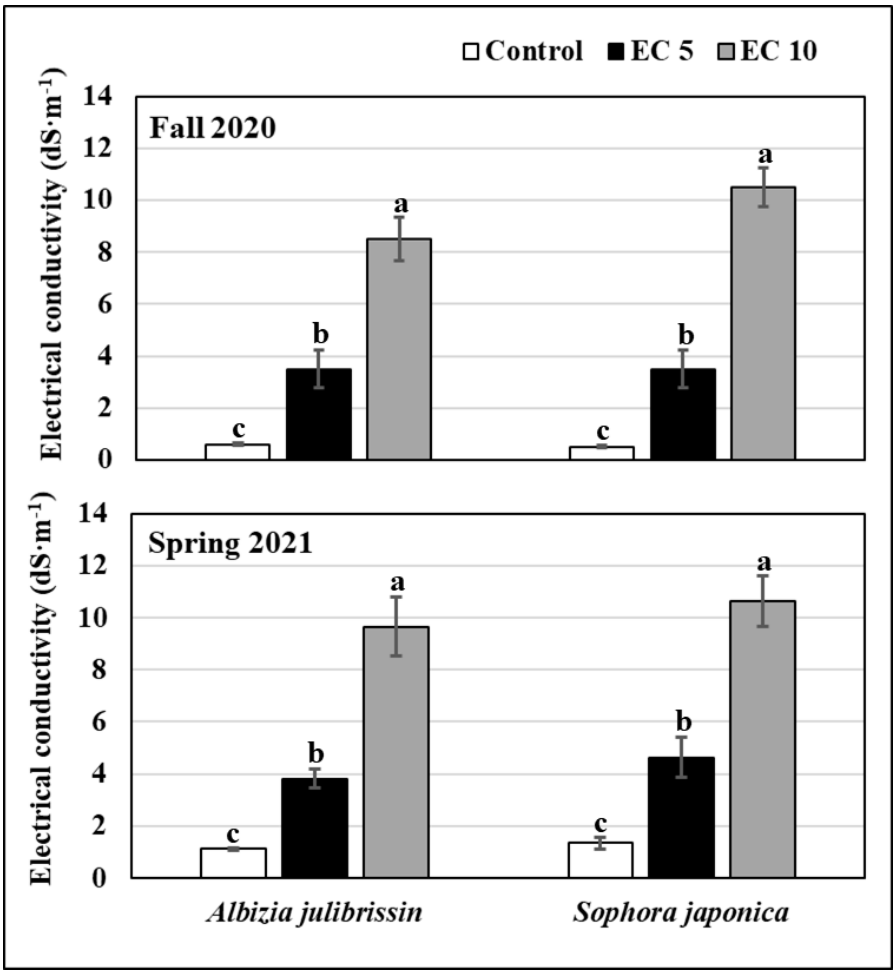

Fig. 2-2. Electrical conductivity (EC) of soil extraction for *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution (EC = 1.2 dS·m^{-1}; control) or a saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] over the course of experiment in Fall 2020 and Spring 2021. Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution. Treatment solutions were applied from 3 Aug. to 5 Sept. 2020 (12 irrigation events) and 10 Mar. to 28 Apr. 2021 (eight irrigation events) in Fall 2020 and Spring 2021, respectively. Vertical bars represent standard errors of five measurements. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$.

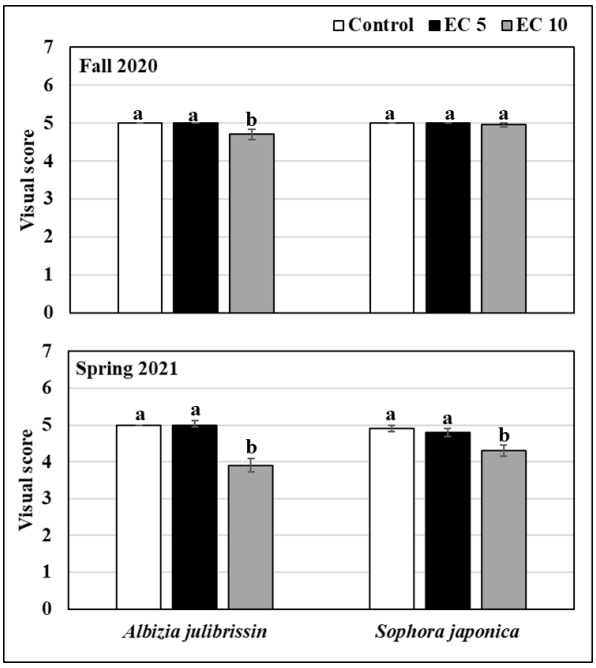

Fig. 2-3. Visual score of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] over the course of experiment in Fall 2020 and Spring 2021. Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution. Visual score reference scale: 0 = dead; 1 = severe foliar damage (>90% leaves with burnt edges or necrosis); 2 = moderate foliar damage (90% to 50%); 3 = slight foliar damage (50% to 10%); 4 = good quality with minimal foliar damage (< 10%); 5 = excellent without foliar damage. Treatment solutions were applied from 3 Aug. to 5 Sept. 2020 (12 irrigation events) and 10 Mar. to 28 Apr. 2021 (eight irrigation events) in Fall 2020 and Spring 2021, respectively. Vertical bars represent standard errors of 10 measurements. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$.

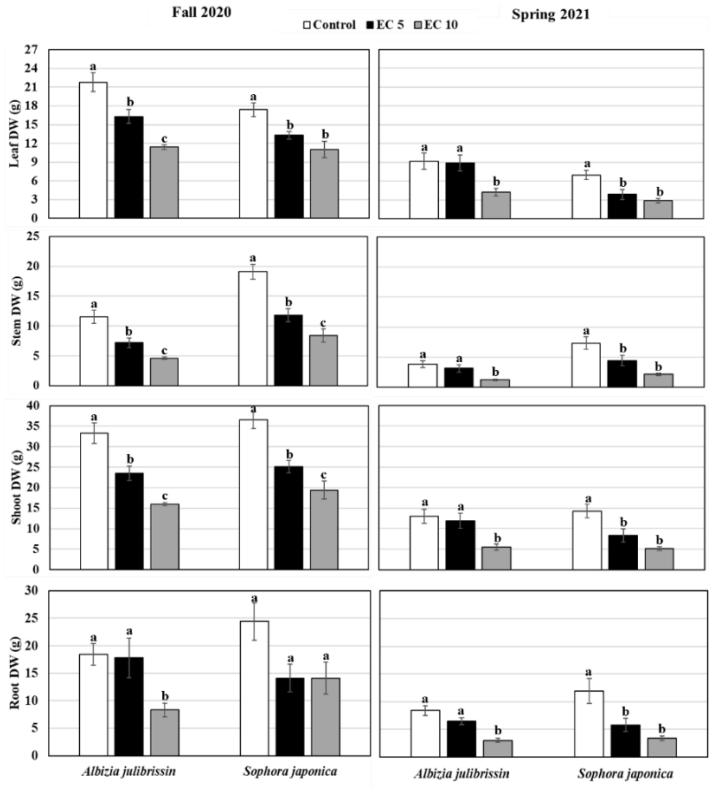

Fig. 2-4. Leaf dry weight (DW), stem DW, shoot DW (stem + leaf DW) and root DW of *Albizia julibrissin* and *Sophora japonica* irrigated with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] over the course of experiment in Fall 2020 and Spring 2021. Saline solution was created by adding sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) to the nutrient solution. Treatment solutions were applied from 3 Aug. to 5 Sept. 2020 (12 irrigation events) and 10 Mar. to 28 Apr. 2021 (eight irrigation events) in Fall 2020 and Spring 2021, respectively. Vertical bars represent standard errors of 10 measurements for shoot DW and leaf DW and five measurements for root DW. Same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$.

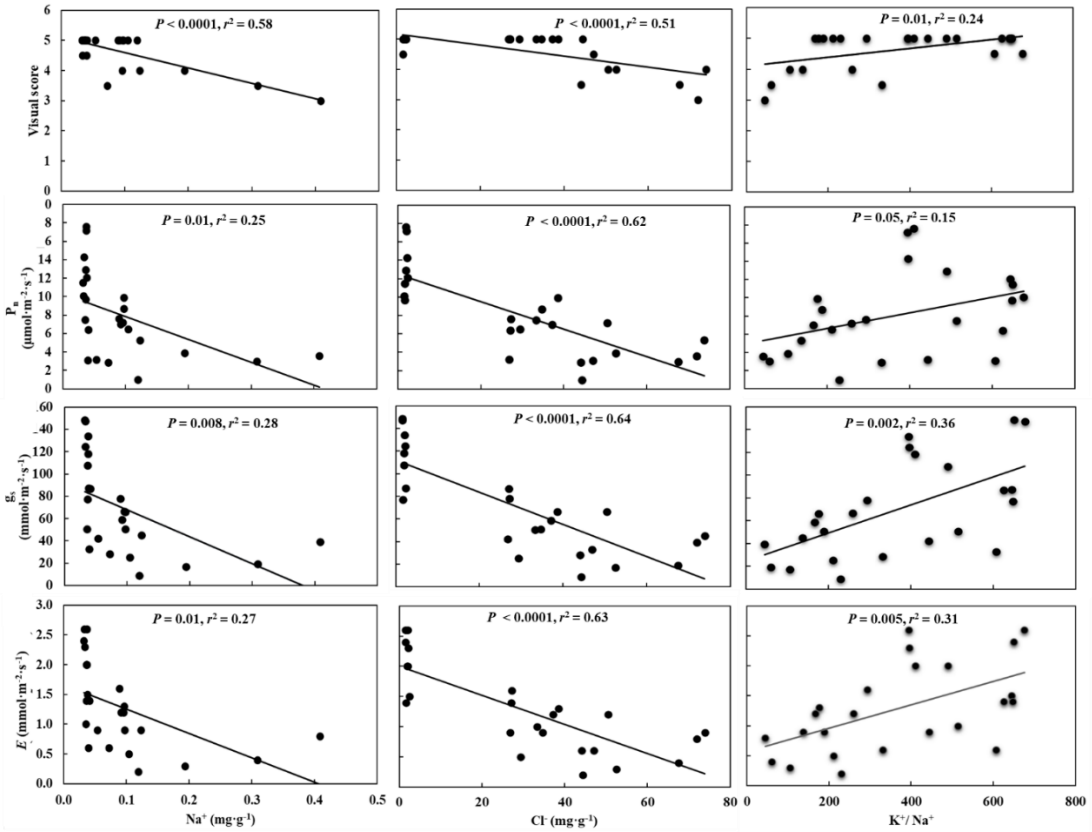

Fig. 2-5. Linear correlation analyses of sodium (Na^+), chloride (Cl^-), potassium-to-sodium ratio (K^+:Na^+) levels in plant tissue compared with the visual score, net photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of *Albizia julibrissin* and *Sophora japonica*. Visual score reference scale: 0 = dead; 1 = severe foliar damage (>90% leaves with burnt edges or necrosis); 2 = moderate foliar damage (90% to 50%); 3 = slight foliar damage (50% to 10%); 4 = good quality with minimal foliar damage (< 10%); 5 = excellent without foliar damage.

CHAPTER III

GROWTH, MORPHOLOGICAL, AND BIOCHEMICAL RESPONSES OF FOUR NATIVE SPECIES TO SALINITY STRESS[2]

Abstract

Native plants are of great value in landscape maintenance. Despite their importance in the landscape, the salt tolerance of most native plants has received little attention. The present research was designed to assess morphological, physiological, and biochemical responses of four Utah-native plants [*Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), and *Shepherdia* ×*utahensis* 'Torrey' (hybrid buffaloberry)] at different salinity levels. Each species was irrigated with a nutrient solution at an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$ (control) or saline solutions at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$ for 8 weeks. The experiment was a randomized complete block design with 10 replications. At 8 weeks after the initiation of the experiment, *A. uva-ursi* and *C. montanus* 'Coy' had slight foliar salt damage with an average visual score of 3.7 (0 = dead, 5 = excellent with no sign of foliar salt damage) when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ and were dead at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, *C. ledifolius* had an average visual score of 3.2 when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. However, almost no foliar salt damage was observed on *S.* ×*utahensis* 'Torrey' during the experimental period. In addition, the shoot dry weight of all species

[2] Paudel A, Sun Y. 2023. Growth, morphological, and biochemical responses of four native species to salinity stress. HortScience 58(6):651-659. https://doi.org/10.21273/HORTSCI17044-23.

was reduced with elevated salinity levels in the irrigation water. Salinity stress also reduced gas exchange rates of plants and affected their mineral content. Proline accumulated in the leaves of native plants but was species-dependent. In conclusion, *S.* ×*utahensis* 'Torrey' was tolerant to salinity stress followed by *C. ledifolius*; *A. uva-ursi* and *C. montanus* 'Coy' were sensitive to salinity stress.

Introduction

Salinity in both irrigation water and soil is one of the major abiotic factors responsible for soil degradation. Nearly 6% of all lands worldwide are affected by salinity (Munns 2005). Salinity stress in plants is caused by excessive amounts of water-soluble salts. Some of the most common deleterious salts in soil include sodium sulphate (Na_2SO_4), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), sodium bicarbonates ($NaHCO_3$), sodium carbonate (Na_2CO_3), potassium sulphate (K_2SO_4), calcium sulphate ($CaSO_4$), magnesium sulphate ($MgSO_4$), and magnesium chloride ($MgCl_2$) (Sazzad 2007). With salinity-affected areas and ever-increasing competition for potable water, planting salt-tolerant ornamental plants has become a sustainable strategy for urban landscape development.

In a saline environment, morphological and physiological processes in plants are disturbed, leading to an inhibition of growth (Alvarez and Sanchez-Blanco 2014). High concentrations of salts in soil or water affect stomatal conductance, photosynthesis, and ion balance in plants (Navarro et al. 2008). In addition, when sodium (Na^+) and chloride (Cl^-) are present in the soil, they can interfere with enzymatic transporters and disrupt the uptake of nutrients such as potassium (K^+) (Tester and Davenport 2003). If accumulated

and not compartmentalized in vacuoles, Na^+ and Cl^- become metabolically toxic, causing leaf damage, nutritional disorders, stunted growth, and reduction in photosynthesis (Shannon and Grieve 1999; Zhang et al. 2014).

Plant salinity tolerance is the ability to tolerate high salt concentrations in the root zone without adverse effects (Shannon and Grieve 1999). Salinity tolerance differs among species with different mechanisms to cope with the detrimental effects of salinity stress (Munns and Tester 2008). Salt-tolerant ornamental plants may accumulate less Na^+ and Cl^- in their leaves when compared with salt-sensitive plants (Munns 2002). Sodium uptake is usually reduced or transporting sodium from roots to shoots is restricted in salt-tolerant plants (Munns 2002). On the other hand, some plants can tolerate accumulated Na^+ and Cl^- in shoot tissue (Munns and Tester 2008). There is a compartmentalization of Na^+ and Cl^- at cellular and intracellular levels to avoid the toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf (Munns and Tester 2008). Similarly, osmotic adjustment is an important adaptation of plants to salinity, as it helps to maintain cell turgor and volume. Osmolytes or compatible solutes in the cytoplasm are among the major compounds for halophytes to tolerate salt stress (Flowers 2004). Compatible solutes include compounds such as proline, betaine, polyols, sugar alcohols, and soluble sugars (Chinnusamy et al. 2005).

Native plants occur naturally in a region without direct or indirect human actions. Native plants are of great value in low-water landscapes (Rupp and Wheaton 2014). The use of native plants has gained popularity in ecological landscape design, green building construction, and urban habitat development. Consumers are increasing their interest in natural landscapes and showing a willingness to pay a premium price for native plant

products (McCoy 2011). However, limited information exists on salinity stress responses of native plants.

In this study, we compared the salinity tolerance of four Utah-native plants, *A. uva-ursi* (kinnikinnick), *C. ledifolius* (curl-leaf mountain mahogany), *C. montanus* 'Coy' (alder-leaf mountain mahogany), and *S.* ×*utahensis* 'Torrey' (hybrid buffaloberry). *Arctostaphylos uva-ursi* is a drought-tolerant and winter-hardy evergreen plant and found as a pioneer plant on disturbed sites (Wood et al. 2013). It is a groundcover adaptable to infertile soils and requires very little maintenance once established. *Cercocarpus ledifolius* is an evergreen shrub or small tree that is adapted to low-water landscapes (Rupp and Wheaton 2014). *Cercocarpus montanus* 'Coy' is a dwarf evergreen cultivar with nitrogen-fixing ability and low water demand (Paudel et al. 2020). *Shepherdia* ×*utahensis* 'Torrey' is an interspecific hybrid of *Shepherdia argentea* (silver buffaloberry) and *Shepherdia rotundifolia* (roundleaf buffaloberry) (Sriladda et al. 2016). *Shepherdia* ×*utahensis* 'Torrey' is a nitrogen-fixing plant that tolerates disturbed soil and drought stress (Chen et al. 2021; Sriladda et al. 2016). Limited research has been conducted regarding the salinity tolerance of these four plant species. Young et al. (2012) investigated the effect of NaCl at concentrations of 10, 30, 70, and 140 mM (~ 0.9, 2.7, 5.1, and 10.2 $dS \cdot m^{-1}$) on the survival and growth of *A. uva-ursi* and claimed that it can tolerate up to 70 mM NaCl (~ 5.1 $dS \cdot m^{-1}$). In addition, Qin et al. (2010) reported that *S. argentea* subjected to 200, 400, and 600 mM NaCl solutions (~ 14.6, 29.2, and 43.8 $dS \cdot m^{-1}$) is tolerant to salinity levels tested in this study. Further research is required to understand the salinity stress responses of these native plants and select tolerant species for salt-affected landscapes.

Materials and Methods

Plant materials and growth condition. This study was conducted at the Utah State University (USU) Research Greenhouse in Logan, UT, USA (lat. 41°45'28"N, long. 111°48'48"W, elevation 1409 m). Native plants, *A. uva-ursi*, *C. ledifolius*, *C. montanus* 'Coy', and *S. ×utahensis* 'Torrey' in 3.8-L injection molded polypropylene containers (No. 1, Nursery Supplies, Orange, CA, USA) were used in this study. *A. uva-ursi* was purchased from J&J Nursery and Garden Center (Layton, UT, USA). *C. montanus* 'Coy', and *S. ×utahensis* 'Torrey' were vegetatively propagated via cuttings and grown for eight months. *C. ledifolius* seedlings were collected from the USU campus in Jun 2019 and grown for 2 years. The plants were transplanted into 7.6-L injection molded polypropylene containers (No. 2B; Nursery Supplies) filled with Metro-Mix® 820 (Canadian Sphagnum peat moss, 35-45% composted pine bark, coir, coarse perlite, and dolomitic limestone; SunGro Horticulture, Agawam, MA, USA) on 09 Jun 2021. The plants were kept in the research greenhouse. Logan City potable water [EC = 0.35 ± 0.01 $dS \cdot m^{-1}$; pH = 7.7 ± 0.2, mean ± standard deviation] was applied when needed and water-soluble fertilizer (Peters Excel 15-5-15 Cal-Mag Special; ICL Specialty Fertilizers, Dublin, OH, USA) was applied twice before the treatments. Before treatments started, *C. montanus* 'Coy' and *S.×utahensis* 'Torrey' were pruned to 30 cm high. *A. uva-ursi* and *C. ledifolius* were 23.2 ± 5.5 and 20.9 ± 5.8 cm high, respectively. The experiment started on 23 Aug 2021 and terminated on 23 Oct 2021. The mean air temperature inside the greenhouse was maintained at 25.9 ± 0.3 ºC during the day and 22.0 ± 0.3 ºC at night. Daily light integral (DLI) inside the greenhouse was 26.6 ± 3.6 $mol \cdot m^{-2} \cdot d^{-1}$. When light intensity inside the greenhouse was less than 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, supplemental light at

225.5 ± 86.5 µmol·m^{-2}·s^{-1}, measured using a Quantum Flux Meter (MQ-200X, serial # 1006, Apogee Instruments, Logan, UT, USA), was provided using 1000-W high-pressure sodium lamps at plant canopy level from 600 to 2200 HR.

Treatments. Two salinity treatments were subjected to *A. uva-ursi*, *C. ledifolius*, *C. montanus* 'Coy', and *S. ×utahensis* 'Torrey' that included irrigation solutions at an EC of 5.0 or 10.0 dS·m^{-1}. The control group received only a nutrient solution at an EC of 1.2 dS·m^{-1}. Uniform plants were selected and randomly assigned to the treatments. The nutrient (control) solution was prepared by adding 0.8 g·L^{-1}15N-2.2P-12.5K water-soluble fertilizer to potable water in a 100-L tank. The saline solution treatments at ECs of 5.0 and 10.0 dS·m^{-1} were prepared using sodium chloride (NaCl; Fisher Scientific, Waltham, MA, USA) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$; Hi Valley Chemical, Centerville, UT, USA) at a molar ratio of 2:1 to the nutrient solution (Table 3-1). Calcium chloride was added to reduce salinity-induced calcium deficiency (Guo et al. 2021). The initial pH of treatment solutions was adjusted to 6.0-6.5 using 88% potassium hydroxide pellets (Sigma-Aldrich, St. Louis, MO, USA) or 1M nitric acid (Fisher Chemical, Fair Lawn, NJ, USA) as necessary. The sodium adsorption ratio (SAR) and elemental analysis were confirmed by the USU Analytical Laboratory (Table 3-1). Treatment solutions, 1,200 ml per pot, were applied manually once per week for 8 weeks. The leaching fraction was targeted to ~25%. In-between treatments, plants were watered with an additional 250-500 mL of distilled water, as necessary, to avoid the confounding effect of drought conditions.

Leachate and substrate EC. Leachate EC was measured weekly following the pour-through method described by Cavins et al. (2008) using an EC meter (LAQUA

Twin, Horiba, Kyoto, Japan). Briefly, a saucer was placed under the container at least 30 minutes after each irrigation treatment and 100 ml of distilled water was poured from the top surface. Afterward, EC was measured from the leachate. Substrate EC was measured using the saturated paste method explained by Gavlak et al. (2005) with minor modifications. In brief, the pots containing soilless media were left to dry in the greenhouse after harvest. A 10 g sample of the substrate was taken from the top 5 cm surface, as salts move upward during the drying process. Then, 100 ml of deionized water was added to the substrate sample in a flask to make a paste. All samples in the flask were covered with Parafilm® (American National Can™, Menasha, WI, USA), stored overnight at room temperature, and EC measurements were taken the following day.

Survival rate and visual quality. Dead plants were recorded at the end of the experiment and the survival rate was calculated. A visual score of 0 to 5 was assigned for each plant weekly to assess foliar salt damage without considering plant size. A visual score was assigned as 0 = dead (plants died because of salinity stress), 1 = severe foliar damage (> 90% burnt leaves, tip burn, or necrosis), 2 = moderate foliar damage (90% to 50%), 3 = slight foliar damage (50% to 10%), 4 = good quality with minimal foliar damage (< 10%), and 5 = excellent without foliar damage (Sun et al. 2015).

Growth parameters. The number of shoots was recorded for each plant at the beginning and end of the experiment. Shoots longer than 5 cm were included in the count. At harvest, leaf area was measured using a leaf area meter (LI-3100; LI-COR® Biosciences, Lincoln, NE, USA). In addition, the shoot dry weight and root dry weight of plants were obtained by drying the samples in an oven at 60 ºC for 1 week.

Gas exchange. Net photosynthesis rate (P_n), transpiration rate (*E*), and stomatal conductance (g_s) of the native plants in each treatment were measured 2 d before harvest using a portable LI-6800 photosynthesis system (LI-COR® Biosciences). Fully expanded, healthy leaves without damage were used. All measurements were taken within a range of 1000 and 1400 HR on sunny days. Environmental conditions in the cuvette were controlled at 25 ºC, 1000 µmol·m^{-2}·s^{-1} photosynthetic photon flux (895.5 µmol·m^{-2}·s^{-1} red and 99.5 µmol·m^{-2}·s^{-1} blue) and 400 µmol·mol^{-1} carbon dioxide concentration. All plants were watered sufficiently 1 d before the measurements to avoid water stress.

Mineral analyses. Four plants of each native plant species were selected randomly from each treatment and leaf samples were ground with a grinder (Model 80393; Hamilton Beach, VA, USA). The chloride (Cl^-) analysis was performed using a chloride analyzer (Model 926, Nelson-Jameson, Marshfield, WI, USA) and reported on a dry plant basis (mg·g^{-1}). Briefly, 0.3 g of powdered leaf samples was extracted in 15 mL of 2% acetic acid (Fisher Chemical) in a conical tube placed on a platform shaker (Innova 2100; New Brunswick Scientific, Edison, NJ, USA) for 30 min and allowed to stand for 60 minutes. The extracted solution was filtered and retained for further analysis. Solution (500 µl) was added to the acid buffer (Nelson-Jameson) and Cl^- content was quantified. Furthermore, powder samples were analyzed at the USU Analytical Laboratories for other mineral contents. In brief, sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), manganese (Mn^{2+}), sulphur (S), phosphorus (P), iron (Fe), and zinc (Zn^{2+}) contents were quantified using nitric/hydrogen peroxide following the protocol described in Gavlak et al. (2005). A total of 0.5 g of powder samples and 6 mL of nitric acid (HNO_3) were added into a digestion tube followed by incubation in a digestion block

for 10 minutes at 80 °C and subsequently cooled for 2 min. A total of 2 ml of 30% hydrogen peroxide (H_2O_2) was added into the digestion tube and then incubated again in the digestion block at 130 °C for 1 h. Tubes were placed in a vortex stirrer for mixing, cooled down, and diluted to the final volume. Then the digestion tube was cooled at room temperature, and the contents of the digestion tube were transferred into a 25-mL volumetric flask. The digest was analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA, USA) and reported on a dry plant basis ($mg \cdot g^{-1}$).

Proline. Proline in the leaves was estimated by the acid-ninhydrin method (Bates et al. 1973; Claussen 2005; Rakesh et al. 2021). Leaf samples were directly collected in liquid nitrogen on 28 Sep 2021 (after the sixth irrigation event) and stored at -80 ºC until further use. The leaf samples (0.1 g) were ground in 5 mL of 3% sulfosalicylic acid (Spectrum Chemical, Gardena, CA, USA) and centrifuged at 5000 rpm for 5 min at room temperature using a benchtop centrifuge (Spectrafuge^{TM} Labnet 6C Centrifuge, Edison, NJ). Then 200 µl of supernatant, 200 µl of glacial acetic acid (Fisher Chemical), and 200 µl of acid ninhydrin (Sigma-Aldrich) were mixed in a tube and incubated in a boiling water bath at 95 ºC for 1 hour. After 1 h, tubes were immediately placed in an ice-bath to arrest the reaction. Thereafter, 400 µl of toluene (Fisher Chemical, Ottawa, ON, Canada) was added to each tube, vortexed well, and allowed to settle for 10 minutes. The upper pinkish color layer was separated and 200 µl of the sample was pipetted into the well of a micro-plate reader (Cellstar, F-bottom, BMG LabTech, Cary, NC, USA). Absorbance at 520 nm was recorded using a spectrophotometer (Spectra max M2; Molecular Devices,

San Jose, CA, USA). Proline (L-Proline, St. Louis, MO, USA) was used to generate a standard curve that was used to estimate the proline content in the samples.

Experimental design and data analyses. The experiment was conducted in a randomized complete block design with four species, three treatments, and 10 replicates. An experimental unit consisted of one pot containing one plant. An analysis of variance was conducted to test the effect of saline solution irrigation and species on plant growth, gas exchange, and mineral nutrients. All data were subjected to log transformation. Because of different growth habits of each species, means separation among treatments was adjusted using Tukey's method for multiplicity at $\alpha = 0.05$. In addition, mean separation among species was performed for visual score and proline content. Correlation analyses were carried out for Na^+ and Cl^- contents, and K^+/Na^+ ratio in plant tissue was compared with visual scores and gas exchange parameters. All statistical analyses were conducted using SAS (Version 14.1, SAS Institute, Cary, NC, USA) with PROC MIXED procedure.

Results

Visual quality and Survival rate. A. uva-ursi irrigated with saline solution at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ started showing foliar salt damage (necrosis and burnt leaves) at 5 and 4 weeks after treatment initiation, respectively (data not shown). *C. ledifolius* at an EC of 10.0 $dS \cdot m^{-1}$ exhibited foliar salt damage (tip burn and burnt leaves) at 4 weeks after treatment initiation (data not shown). Moreover, *C. montanus* 'Coy' started showing foliar salt damage (tip burn and burnt leaves) at 6 and 4 weeks after treatment initiation (data not shown) when irrigated with saline solution at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$,

respectively. Saline solution irrigation had significant effects on the visual score of native plants at 8 weeks and there were significant interactive effects between species and treatment ($P < 0.0001$, Tables 3-2 and 3-3). At 8 weeks, all four species survived when they were irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Table 3-3). *C. ledifolius* and *S. ×utahensis* 'Torrey' plants also survived with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, but *A. uva-ursi* and *C. montanus* 'Coy' plants were dead. *A. uva-ursi* had visible foliar salt damage with an averaged visual score of 3.7 when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Table 3-3, Fig. 3-1). *C. ledifolius* had minimal to no foliar salt damage when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ but plants at an EC of 10.0 $dS \cdot m^{-1}$ had foliar salt damage with an averaged visual score of 3.2. Similarly, *C. montanus* 'Coy' had averaged visual score of 3.6 when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$. However, *S. ×utahensis* 'Torrey' was healthy without foliar salt damage when plants were irrigated with saline solution at an EC of 5.0 and 10.0 $dS \cdot m^{-1}$ throughout the experiment.

Plant growth. The number of shoots and leaf area varied with treatments and species ($P < 0.0001$, Table 3-2). Compared with the control, the number of shoots was reduced by 26% and 37% for *A. uva-ursi* and *C. montanus* 'Coy' treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$, respectively (Table 3-4). Similarly, *S. ×utahensis* 'Torrey' treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ had a 32% reduction in the number of shoots. In addition, the leaf area of *A. uva-ursi* treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ was 52% less than the control. *C. montanus* 'Coy' had 26% less leaf area than control plants when treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ but was not different. *C. ledifolius* had 44% less leaf area than control plants when treated with

saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Although the leaf area of *S. ×utahensis* 'Torrey' tended to decrease with saline solutions, there were no differences among treatments.

Shoot dry weight varied with treatments and species ($P < 0.01$, Table 3-2). Compared with control, although there were no significant differences, there was a trend of reduced shoot dry weight of *A. uva-ursi* and *C. montanus* 'Coy' treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ and *C. ledifolius* treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ (Table 3-5). More replications might be needed to improve the statistical power of the analysis and show significant differences. In addition, the shoot dry weight of *S. ×utahensis* 'Torrey' was reduced by 32% compared with the control when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, root dry weight differed among species but was not affected by salinity treatments (Tables 3-2 and 3-5).

These results indicate that plants experienced significant salinity stresses which attribute to the salts accumulated in the soilless growing substrate. Leachate EC or substrate EC is an indirect or direct way to measure salinity levels in soil and growing substrate. In this study, leachate EC increased over time with saline solution irrigation (Fig. 3-2). The highest leachate EC was 1.9, 11.3, and 17.3 $dS \cdot m^{-1}$ for the control or saline solutions at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$, respectively, during the experiment. Similarly, the higher the salinity of irrigation water, the more salts accumulated in the substrate (Fig. 3-3). By the end of the experiment, average ECs of the substrate were 7.1 ± 2.4 and 15.1 ± 1.0 $dS \cdot m^{-1}$ when irrigated with saline solution at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$, respectively, reflecting salt accumulation in soilless media.

Gas exchange. With the increase of salinity levels in irrigation water, the net photosynthesis rate (P_n) of four native plants decreased ($P < 0.0001$, Table 3-2, Fig. 3-4).

In addition, P_n varied with species and had interactive effects between treatment and species ($P < 0.0001$). Net photosynthesis rate of *A. uva-ursi* and *C. montanus* 'Coy' decreased from 3.8 and 9.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 2.2 and 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$, respectively. Similarly, P_n decreased from 11.1 and 16.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 0.6 and 4.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *C. ledifolius* and *S. ×utahensis* 'Torrey', respectively, when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

Transpiration rate (*E*) decreased as salinity levels in the irrigation water increased for *C. ledifolius* and *C. montanus* 'Coy' (Fig. 3-4). However, *E* was not significantly reduced for *S. ×utahensis* 'Torrey'. The stomatal conductance (g_s) also decreased with increasing salinity levels. The g_s of *C. montanus* 'Coy' decreased from 125.5 to 20.9 $mmol \cdot m^{-2} \cdot s^{-1}$ when treated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$. Similarly, g_s decreased from 119.4 and 170 $mmol \cdot m^{-2} \cdot s^{-1}$ to 18.4 and 25 $mmol \cdot m^{-2} \cdot s^{-1}$ for *C. ledifolius* and *S. ×utahensis* 'Torrey', respectively, when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

Mineral contents. For all four native plants, leaf Na^+ content was lower than Cl^- content ($P < 0.0001$) but varied with species. Salinity treatments significantly increased Na^+ contents in the leaves of native plants ($P < 0.0001$, Table 3-6). The highest level of Na^+ at 8.3 $mg \cdot g^{-1}$, 35 times higher than that in the control, was found in the leaves of *A. uva-ursi* when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, Na^+ content in the leaves of *C. ledifolius*, *C. montanus* 'Coy', and *S. ×utahensis* 'Torrey' when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ wasd 4.1, 5.5, and 5.2 $mg \cdot g^{-1}$, respectively, which increased by 81, 78, and 21 times compared to the control. Furthermore, there was an increase in Cl^- content with increasing salinity levels ($P < 0.0001$, Table 3-6). The

highest level of Cl^- at 51.9 $mg \cdot g^{-1}$, which was 44 times higher when compared with the control, was found in the leaves of *C. montanus* 'Coy' when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

Calcium content in leaves of the native plants was significantly affected by salinity ($P < 0.0001$, Table 3-6); however, increase in Ca^{2+}content was less pronounced when compared with Na^+ and Cl^- contents. Compared with the control, there was less than 2 times increment of the Ca^{2+} content in the leaf tissue when plants were treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Salinity treatments had no effects on K^+ content in the leaves of native plants, except *S. ×utahensis* 'Torrey', of which the K^+ content decreased when they were irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, compared with the control and those with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Table 3-6). However, salinity stress dramatically decreased the K^+/Na^+ ratio in all plants ($P < 0.0001$). Similarly, Mg^{2+} content increased with increasing salinity levels in the irrigation water for *A. uva-ursi*, *C. ledifolius*, and *C. montanus* 'Coy'. Manganese content increased in *A. uva-ursi*, *C. montanus* 'Coy', and *S. ×utahensis* 'Torrey' at higher EC levels. Elevated salinity led to a slight decrease in S content of *C. ledifolius*, *C. monatnus* 'Coy', and *S. ×utahensis* 'Torrey' (data not shown). However, the P, Fe, and Zn^{2+} contents of native plants did not vary among salinity treatments tested in this experiment (data not shown).

Proline content. Leaf proline content observed in the experiment was mostly species-dependent ($P < 0.0001$, Tables 3-2 and 3-7). *C. montanus* 'Coy' had the highest proline content of 16.2 $\mu mol \cdot g^{-1}$ when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ without differences among treatments. The proline content of *S. ×utahensis* 'Torrey'

was the highest when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ compared with the lower salinity treatments.

Discussion

Landscape plant species have different abilities to tolerate salts in irrigation water. It is therefore necessary to evaluate and distinguish them for salt tolerance. In this study, four Utah-native plants with potential landscape use were investigated to determine their salinity tolerance. The salinity levels tested in this research were above 4.0 $dS \cdot m^{-1}$, which is reported to cause soil salinity problems and affect plant productivity and quality (Chinnusamy et al. 2005; Shrivastava and Kumar 2015).

In the present study, several parameters were studied to evaluate the salinity tolerance of Utah-native plants. Aesthetic value is an important component when screening ornamental plants for salt tolerance, as foliar salt damage is problematic for many landscape plants (Cassaniti et al. 2012; Niu and Cabrera 2010; Veatch-Blohm et al. 2014). Researchers use visual ratings to compare relative salt tolerance among plant species (Cameron et al. 2004; Fox et al. 2005; Sun et al. 2015). Leaf burn and necrosis were observed on *A. uva-ursi*, *C. ledifolius* and *C. montanus* 'Coy', but not on *S. ×utahensis* 'Torrey', which corresponds to the increasing salinity levels (Tables 3-3 and 3-8). According to these results, *S. ×utahensis* 'Torrey' was the most salt-tolerant species followed by *C. ledifolius*, whereas *A. uva-ursi* and *C. montanus* 'Coy' performed similarly and were relatively salt sensitive. Similarly, Young et al. (2012) reported that *A. uva-ursi* became more brittle and drier with increasing NaCl concentration in irrigation

water. *Shepherdia argentea* was described as highly tolerant to salinity, as it survived at the salinity level of 600 mM (~ 43.8 $dS \cdot m^{-1}$) for at least 30 d (Qin et al. 2010).

Salinity stress is a critical factor that affects plant growth and metabolism. In the present study, salinity stress depressed plant growth and biomass, and affected survival of the native plants. Salt accumulation leads to leaf necrosis and senescence, which decreases the supply of carbohydrates and/or growth hormones to meristematic parts and inhibits plant growth (Acosta-Motos et al. 2017). Furthermore, leaf area was reduced with increasing salinity levels in irrigation solution in previous studies (Niu et al. 2012; Paudel et al. 2019; Sun et al. 2018), and in *A. uva-ursi*, *C. ledifolius*, and *C. montanus* 'Coy' in this current study. There was no difference in the leaf area of *S. ×utahensis* 'Torrey' among salinity treatments. In contrast, the leaf area of *S. argentea* significantly reduced at all tested salinity levels with 200, 400, and 600 mM NaCl solutions (~ 14.6, 29.2, and 43.8 $dS \cdot m^{-1}$) (Qin et al. 2010). In our study, NaCl and $CaCl_2$ were used to prepare saline solution, but only NaCl was used in the study conducted by Qin et al. (2010). It is also possible that salt tolerance may have increased in the hybrid *S. ×utahensis* 'Torrey' compared with the parent *S. argentea*. In previous studies, hybrids were observed to be more salt tolerant than parents (Koonce et al. 2020; Zeng et al. 2015).

Biomass changes are parameters normally used to determine plant tolerance to salinity (Bastias et al. 2004; Gama et al. 2007). Plant growth and dry matter accumulation are often reduced in ornamental species under salinity stress (Alvarez et al. 2012; Cassaniti et al. 2012); however, these changes vary among species. In this study, there was a decreasing trend in the shoot dry weight of all four species but relatively lower

reductions in root dry weight for *C. ledifolius*, *C. montanus* ‘Coy’, and *S. ×utahensis* ‘Torrey’. This reflects that plants spend more photosynthetic energy on root production under salinity stress to maintain a relatively high water relation (Cheeseman 1988; Iqbal 2005).

During the experiment, leachate ECs from the substrate and EC of the substrate increased throughout the duration. Similarly, Paudel et al. (2019) and Xing et al. (2021) reported that leachate and substrate EC increased with saline water irrigation over time. Salt accumulation in the soilless substrate mainly depends on irrigation leaching fraction, salinity of irrigation water, irrigation frequency and amount, and substrate properties (Martinez and Clark 2009; Sharma and Minhas 2005). In field conditions, salt concentration in the soil can vary due to evaporation, irrigation water quality, rising water tables, rainfall, and soil properties (Munns and Tester 2008; Shrivastava and Kumar 2015).

Plants under salinity stress have reduced photosynthetic rates, which are mainly due to reductions in water potential. Accumulation of high Na^+ and/or Cl^- ions also inhibits P_n and directly interferes with plant growth (Zhang et al. 2014). In the present study, P_n of the native plants was reduced in response to salinity and negatively correlated with Na^+ and Cl^- contents in the leaf tissue ($P = 0.006$ and $P < 0.0001$, respectively, Table 3-8). Likewise, P_n of *S. argentea* was reduced when irrigated with saline solution of 600 mM (~43.8 $dS \cdot m^{-1}$) NaCl (Qin et al. 2010). Furthermore, P_n was positively correlated with the visual scores of native plants ($P = 0.0002$, Table 3-8). *S. ×utahensis* ‘Torrey’, the most salt tolerant among the four native plants, and has higher P_n than the other three species, which suggests the more tolerant species tended to have higher P_n than the more

sensitive ones (Dong et al. 2019). According to our results, the increasing salinity levels decreased *E* and g_s of four native plants. This finding is consistent with a study for *S. argentea*, which had reductions in P_n, *E,* and g_s with the increase of salinity (Qin et al. 2010). It is believed that salinity-induced impairment in stomatal movement causes the reduction in *E* and g_s (Orzechowska et al. 2021). Limiting transpiration is an effective mechanism for plants using water efficiently, which further reduces the uptake of harmful salt ions (Hasegawa et al. 2000).

Nutrients have a role in the structure, metabolism, and osmoregulation of plant cells. Salinity disorders may result from nutrient availability, competitive uptake, transport, or partition within the plant. Sodium and Cl^- contents increased in leaves, stems, and roots of several ornamental plants treated with saline solutions (Alvarez et al. 2012; Paudel et al. 2020). In this study, Na^+ and Cl^- contents increased in the leaves of four native plants with increasing salinity levels. A negative correlation between visual score and Na^+ and Cl^- content was also observed ($P < 0.0001$; Table 3-8). The Na^+ and Cl^- contents were highest in *A. uva-ursi* and *C. montanus* 'Coy', respectively, which might be responsible for their foliar injury. This suggests that *A. uva-ursi* and *C. montanus* 'Coy' might exhibit a low ability to exclude these ions from shoots and a low tolerance for Na^+ and/or Cl^- accumulation. The rapid increase of ions in the cell walls or cytoplasm when vacuoles can no longer sequester incoming salts causes salt injury in leaves (Acosta-Motos et al. 2017). In a saline environment, tolerating high salt concentrations in the upper parts of plants, restricting entry through the roots, and limiting transport to the shoots are important mechanisms that allow plants to survive

under saline conditions (Colmer et al. 2005; Murillo-Amador et al. 2006). In this study, *S.* ×*utahensis* 'Torrey' show tolerance to higher Na^+ and Cl^- content in the leaves.

Calcium helps in maintaining membrane integrity and ion-transport regulation and remediates the adverse effects of salinity on plants (Martinez-Ballesta et al. 2006; Nedjimi and Daoud 2009). Calcium uptake is generally disturbed under saline conditions (Alam et al. 2001), which leads to calcium deficiency similar to many horticultural crops under nonsaline conditions (Grattan and Grieve 1999). Reduced K^+ content in the roots due to salinity can be restored to adequate levels by an additional supply of Ca^{2+}, as it protects cell membranes from the adverse effects of Na^+ and minimizes the leakage of cytosolic K^+ (Tuna et al. 2007). Therefore, an adequate supply of Ca^{2+} in the solutions is important to control the severity of ion toxicities in the plants that are susceptible to NaCl injury (Qadir et al. 2001). In this study, $CaCl_2$ was added while preparing the saline solution (Table 3-1). Plants had higher Ca^{2+} content in leaf tissue under elevated salinity conditions.

Potassium has an important role in plant growth and development, and in maintaining cell turgor and membrane potential. In plants, K^+ is the major cation that counterbalances the negative charge of anions and plays an important role in the activation of enzymes responsible for metabolism, synthesis of proteins and carbohydrates, and regulation of stomatal movement (Rahneshan et al. 2018). The uptake of K^+ ions was not changed with increasing salinity levels during this experiment. However, the decrease in the K^+/Na^+ ratio with the increase in salinity levels suggests that Na^+ ions were transported in greater proportion to K^+ ions in leaves. Sodium competes with K^+ uptake through Na^+-K^+ cotransporters under salinity stress, as they

have a similar chemistry (Jouyban 2012; Zhu 2003). A high cytosolic K^+/Na^+ ratio is essential for normal cellular functions in plants. Results from the current study suggest that there were no effective mechanisms in tested native plants to control the net uptake of Na^+ to leaf tissue.

Magnesium greatly contributes to the processes in chloroplasts including photosynthesis, where chlorophyll-bound Mg^{2+} accounts for 6% to 25% of the total Mg^{2+} content (Luczak et al. 2021). Furthermore, manganese is an essential element that acts as an enzyme cofactor or as a metal with catalytic activity in biological clusters (Andresen et al. 2018). It has been observed that salinity induces Mg^{2+} deficiency and affects plant growth (Khan et al. 2000). Conversely, Mg^{2+} and Mn^{2+} contents in the leaf tissue of four native plants remained the same or increased with increasing salinity levels (Table 3-6). Accumulation of these nutrients might be one of the strategies for these species to thrive in saline conditions. In addition, no effect on P, Fe, and Zn^{2+} content indicates that salinity stress was not imposing deficiency of these nutrients in these native plants.

Osmotic adjustment is another mechanism in plants for tolerating salinity stress. Solute accumulation helps plants to tolerate salinity by reducing the cellular solute potential (Hasegawa et al. 2000). Proline has a role in pH adjustment in the cytosol protecting cell membranes and proteins and brings reactive oxygen species into a normal range (Behzadi Rad et al. 2021). Proline is also known as a source of carbon and nitrogen for plant recovery after stress. In this study, the amount of proline in leaves remained similar for *A. uva-ursi*, *C. ledifolius*, and *C. montanus* ‘Coy’ in response to salinity stress; however, the amount of proline in *S. ×utahensis* ‘Torrey’ increased at high salinity levels. The high levels of proline in *S. ×utahensis* ‘Torrey’ may explain its higher tolerance to

salinity compared with the other species. Many studies have demonstrated that higher proline content was observed in salt-tolerant than salt-sensitive species (Kumar et al. 2010; Mansour and Ali 2017). The reallocation of energy resources from cumulative growth to maintenance processes such as ion compartmentation and synthesis of proline could have contributed to a reduced biomass of *S. ×utahensis* 'Torrey' at high salinity levels.

Conclusions

Four Utah-native plants tested in this study showed some variations in response to salinity stress. *A. uva-ursi* and *C. montanus* 'Coy' had severe foliar salt damage and *C. ledifolius* had moderate to slight foliar salt damage at elevated salinity levels. However, *S. ×utahensis* 'Torrey' had no foliar salt damage. Saline solution irrigation reduced the growth and biomass of all four native species. Photosynthesis, E, and g_s of native plants also decreased after saline solution irrigation. Furthermore, salinity stress caused Na^+ and Cl^- uptake and accumulation. In addition, more proline was accumulated in leaves of *S. ×utahensis* 'Torrey' as a possible protective metabolic adaptation to prevent leaf tissue from damage under high salinity. Based on research results, *S. ×utahensis* 'Torrey' was considered salt tolerant, *C. ledifolius* was moderately salt tolerant, and *A. uva-ursi* and *C. montanus* 'Coy' were salt sensitive.

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Table 3-1. The mineral content, sodium adsorption ratio (SAR), and electrical conductivity (EC) of nutrient and saline solutions used to irrigate container-grown plants native to Utah.

Item[i]	Nutrient solution[ii]	Saline solution[iii]	
		5.0 $dS \cdot m^{-1}$	10.0 $dS \cdot m^{-1}$
Ca^{2+} ($mg \cdot L^{-1}$)	95.90	561.40	1,140.00
Mg^{2+} ($mg \cdot L^{-1}$)	37.60	32.00	30.00
Na^{+} ($mg \cdot L^{-1}$)	3.30	450.90	1,029.00
SO_4^{2-} ($mg \cdot L^{-1}$)	13.10	14.30	16.40
Cl^{-} ($mg \cdot L^{-1}$)	4.40	1,380.00	3,160.00
B ($mg \cdot L^{-1}$)	0.17	0.19	0.19
SAR	0.07	5.00	8.20
Adjusted SAR	0.13	11.67	21.33
EC ($dS \cdot m^{-1}$)	1.21 ± 0.03[iv]	5.09 ± 0.09	10.15 ± 0.10

[i]Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), sulphate (SO_4^{2-}), chloride (Cl^{-}), and boron (B).

[ii] The nutrient solution at an EC of 1.2 $dS \cdot m^{-1}$ was made by mixing 0.8 $g \cdot L^{-1}$ 15N-2.2P-12.5K water-soluble fertilizer (Peter Excel 15-5-15 Ca-Mag Special) in potable water.

[iii] Sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) were used to prepare the saline solution. The nutrient solution was supplemented with NaCl at 0.92 $g \cdot L^{-1}$ and $CaCl_2 \cdot 2H_2O$ at 1.17 $g \cdot L^{-1}$ to obtain the saline solution at an EC of 5.0 $dS \cdot m^{-1}$, and 2.27 $g \cdot L^{-1}$ NaCl and 2.88 $g \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$ was added to the nutrient solution to make the saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

[iv]Mean ± standard deviation.

Table 3-2. A summary of analysis of variance for the effects of treatments and their interactions with plant species on visual score (VS), number of shoots, leaf area (LA), shoot dry weight (DW), root DW, net photosynthesis rate (P_n), transpiration rate (E), stomatal conductance (g_s) and proline content of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$, control] or saline solution (EC = 5.0 $dS \cdot m^{-1}$ or 10.0 $dS \cdot m^{-1}$) in a greenhouse for 8 weeks.

Source	Analysis of variance								
	VS	No. of Shoots	LA	Shoot DW	Root DW	P_n	E	g_s	Proline
Plant	****[i]	****	****	****	***	****	NS	NS	****
Treatment	****	****	****	**	NS	****	***	****	*
Plant * Treatment	****	NS	*	NS	NS	****	NS	NS	*

[i] NS, *, **, ***, ****: Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

Table 3-3. Visual score and survival rate of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$, control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse for 8 weeks.

Plants	Visual score (0-5) [i]			Survival (%)		
	Control	EC 5	EC 10	Control	EC 5	EC 10
Arctostaphylos uva-ursi	5 a[ii]A[iii]	3.7 bB	0 cC	100	100	0
Cercocarpus ledifolius	5 aA	4.9 aA	3.2 bB	100	100	100
Cercocarpus montanus 'Coy'	5 aA	3.6 bB	0 cC	100	100	0
Shepherdia ×utahensis 'Torrey'	5 aA	5 aA	4.9 aA	100	100	100

[i] 0 = dead (plants died because of salinity stress), 1 = severe foliar damage (>90% burnt leaves, tip burn, or necrosis), 2 = moderate foliar damage (90% to 50%), 3 = slight foliar damage (50% to 10%), 4 = good quality with minimal foliar damage (<10%), and 5 = excellent without foliar damage (Sun et al. 2015).

[ii] Means with same lowercase letters within species are not different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

[iii] Means with same uppercase letters within column are not different among species by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 3-4. Number of shoots and leaf area of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 dS·m^{-1}, control] or saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] in a greenhouse for 8 weeks.

Plants	No. of shoots			Leaf area (cm^2)		
	Control	EC 5	EC 10	Control	EC 5	EC 10
Arctostaphylos uva-ursi	105.2 a[i]	77.8 b	-[ii]	1648 a	791 b	-
Cercocarpus ledifolius	8.4 a	7.1 a	6.7 a	180 a	173 a	101 b
Cercocarpus montanus 'Coy'	10.7 a	6.7 b	-	285 a	211 a	-
Shepherdia ×utahensis 'Torrey'	19.8 a	16.6 ab	13.4 b	1559 a	1230 a	1028 a

[i] Means with same lowercase letters within species and dependent variable are not different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

[ii] *A. uva-ursi* and *C. montanus* 'Coy' were dead when treated with saline solution at an EC of 10 dS·m^{-1}.

Table 3-5. Shoot and root dry weight of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$, control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse for 8 weeks.

Plant	Shoot dry wt (g)			Root dry wt (g)		
	Control	EC 5	EC 10	Control	EC 5	EC 10
Arctostaphylos uva-ursi	52.4 a[i]	34.6 b	-[ii]	11.5 a	8.9 a	-
Cercocarpus ledifolius	10.1 a	11.1 a	8.5 a	5.3 a	5.6 a	4.9 a
Cercocarpus montanus 'Coy'	17.0 a	14.1 a	-	6.7 a	6.2 a	-
Shepherdia ×utahensis 'Torrey'	37.4 a	28.1 ab	25.6 b	12.8 a	10.9 a	11.5 a

[i] Means with same lowercase letters within species and dependent variable are not different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

[ii] *A. uva-ursi* and *C. montanus* 'Coy' were dead when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$.

Table 3-6. Leaf mineral content and potassium to sodium (K^+/Na^+) ratio of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 dS·m^{-1}, control] or saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] in a greenhouse for 8 weeks.

Plant	Treatment	Ion content (mg·g^{-1})[i]						
		Na^+	Cl^-	Ca^{2+}	K^+	K^+/Na^+	Mg^{2+}	Mn^{2+}
Arctostaphylos uva-ursi	Control	0.23 c[ii]	0.95 b	8.34 c	10.27 a	44.83 a	2.06 b	0.03 b
	EC5	4.80 b	22.49 a	11.9 b	9.83 a	2.05 b	2.48 ab	0.05 a
	EC10	8.32 a	22.94 a	16.81 a	10.95 a	1.32 b	3.04 a	0.07 a
Cercocarpus ledifolius	Control	0.05 c	0.88 c	8.70 b	12.47 a	272.80 a	2.49 b	0.02 a
	EC5	0.39 b	6.56 b	10.33 b	10.79 a	27.89 b	2.72 b	0.03 a
	EC10	4.11 a	47.13 a	15.30 a	11.95 a	2.91 c	3.45 a	0.03 a
Cercocarpus montanus 'Coy'	Control	0.07 c	1.16 c	11.55 b	13.79 a	189.34 a	2.11 b	0.04 b
	EC5	0.42 b	23.77 b	18.65 a	15.33 a	36.29 b	2.89 a	0.06 a
	EC10	5.54 a	51.89 a	21.88 a	14.43 a	2.61 c	3.13 a	0.08 a
Shepherdia × *utahensis* 'Torrey'	Control	0.24 c	1.54 c	11.64 b	21.33 a	88.62 a	5.69 a	0.07 b
	EC5	1.92 b	6.65 b	19.00 a	20.74 a	10.78 b	6.36 a	0.12 a
	EC10	5.17 a	29.11 a	22.05 a	17.29 b	3.34 c	5.21 a	0.11 a
Plant		****[iii]	**	****	****	****	****	****
Treatment		****	****	****	NS	****	****	****
Plant*Treatment		****	****	NS	*	****	**	NS

[i] Sodium (Na^+), chloride (Cl^-), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), and manganese (Mn^{2+}) ions.
[ii] Means with same lowercase letters within a column and species are not different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.
[iii] NS, *, **, ***, **** Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

Table 3-7. Proline content in leaves of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 dS·m^{-1}, control] or saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] in a greenhouse for 8 weeks.[i]

Plant	Proline content (µmol·g^{-1})		
	Control	EC 5	EC 10
Arctostaphylos uva-ursi	0.6 a[ii]B[iii]	0.4 aB	0.3 aC
Cercocarpus ledifolius	1.3 aB	2.6 aA	2.2 aB
Cercocarpus montanus 'Coy'	10.3 aA	7.3 aA	16.2 aA
Shepherdia ×utahensis 'Torrey'	0.7 bB	2.6 bA	15.1 aA

[i] Leaves were harvested after the sixth irrigation event for proline estimation.

[ii] Means with same lowercase letters within species are not different among treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

[iii] Means with same uppercase letters within a column are not different among species by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 3-8. Correlation probability (upper triangular) and coefficients (lower triangular) for sodium (Na^+), chloride (Cl^-), potassium to sodium ratio (K^+/ Na^+), visual score (VS), net photosynthesis rate (P_n), transpiration rate (E), and stomatal conductance (g_s) of container-grown plants native to Utah after irrigating with a nutrient solution [electrical conductivity (EC) = 1.2 $dS \cdot m^{-1}$; control] or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse for 8 weeks.

	Na^+	Cl^-	K^+/ Na^+	VS	P_n	E	g_s
Na^+		<0.0001	<0.0001	<0.0001	0.0058	NS[i]	NS
Cl^-	0.7914		0.0002	<0.0001	<0.0001	0.0123	0.0157
K^+/ Na^+	-0.5842	-0.5668		0.0023	0.0046	NS	NS
VS	-0.7821	-0.7622	0.4305		0.0002	NS	NS
P_n	-0.4341	-0.6440	0.4439	0.5550		NS	NS
E	-0.1709	-0.3973	0.1836	0.1864	0.1823		<0.0001
g_s	-0.1505	-0.3844	0.1470	0.1751	0.2196	0.9913	

[i] NS = nonsignificant.

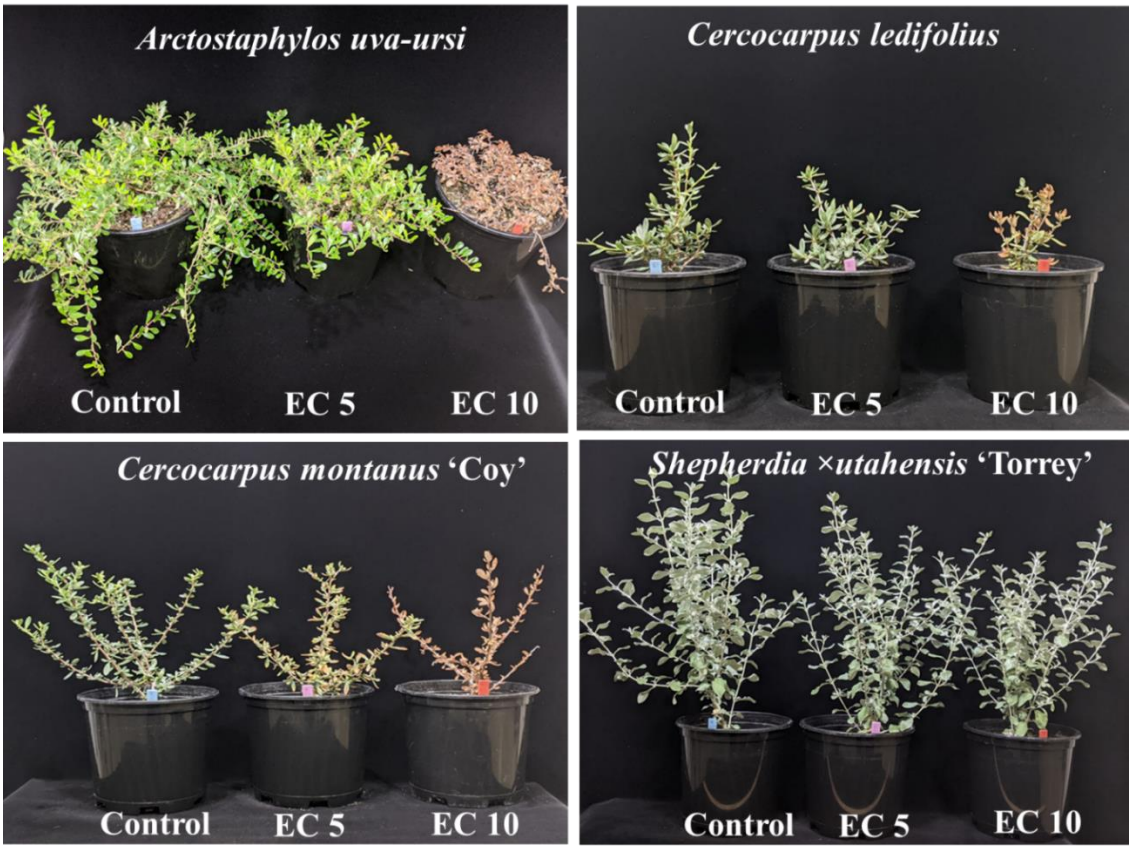

Fig. 3-1. Photos of representative container-grown plants native to Utah after irrigating with a nutrient solution (EC = 1.2 $dS \cdot m^{-1}$; control) or a saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse for 8 weeks.

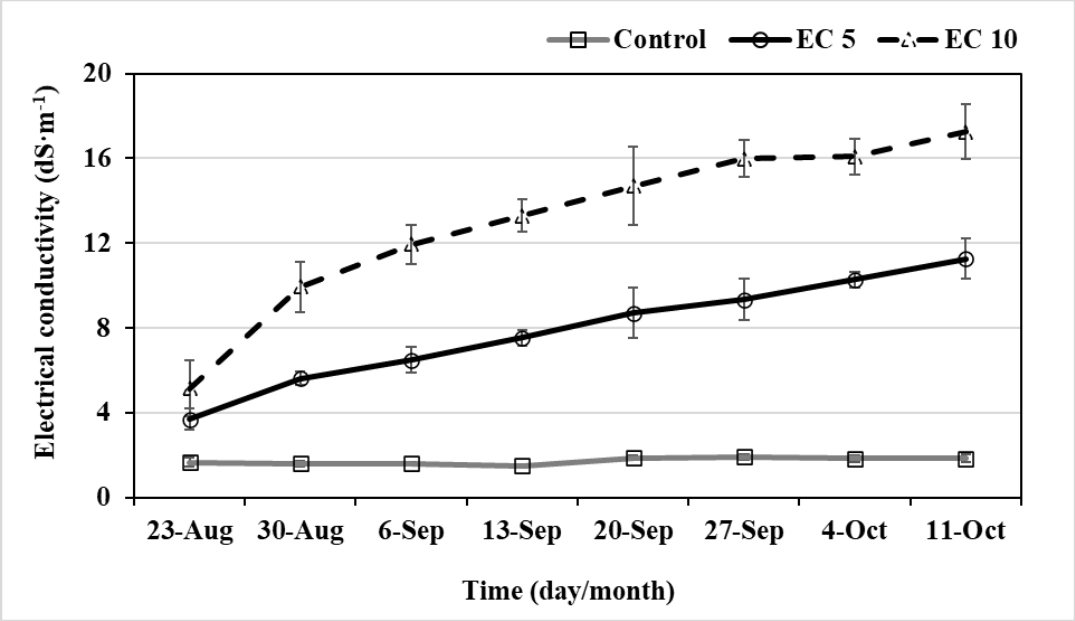

Fig. 3-2. Electrical conductivity (EC) of leachate solution collected after irrigating container-grown plants native to Utah with a nutrient solution (EC = 1.2 $dS \cdot m^{-1}$, control) or saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] over the course of the experiment. Vertical bars represent standard errors of four measurements.

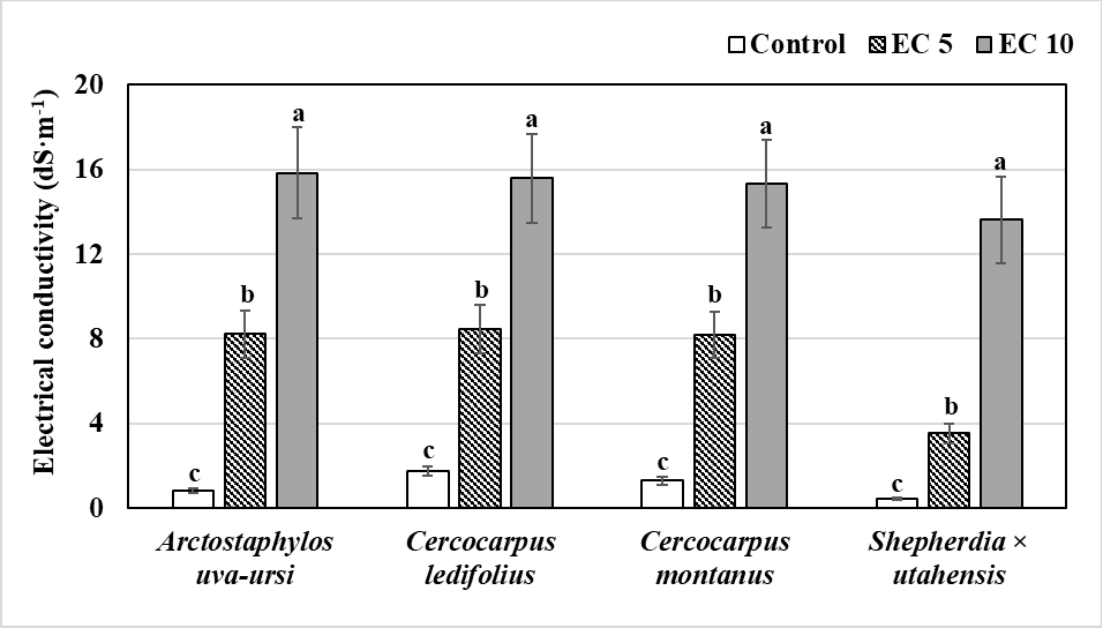

Fig. 3-3. Electrical conductivity (EC) of soil extraction from container-grown plants native to Utah after irrigating with a nutrient solution (EC = 1.2 $dS \cdot m^{-1}$, control) or a saline solution [EC = 5.0 $dS \cdot m^{-1}$ (EC 5) or 10.0 $dS \cdot m^{-1}$ (EC 10)] in a greenhouse for 8 weeks. Vertical bars represent standard errors of five measurements. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$.

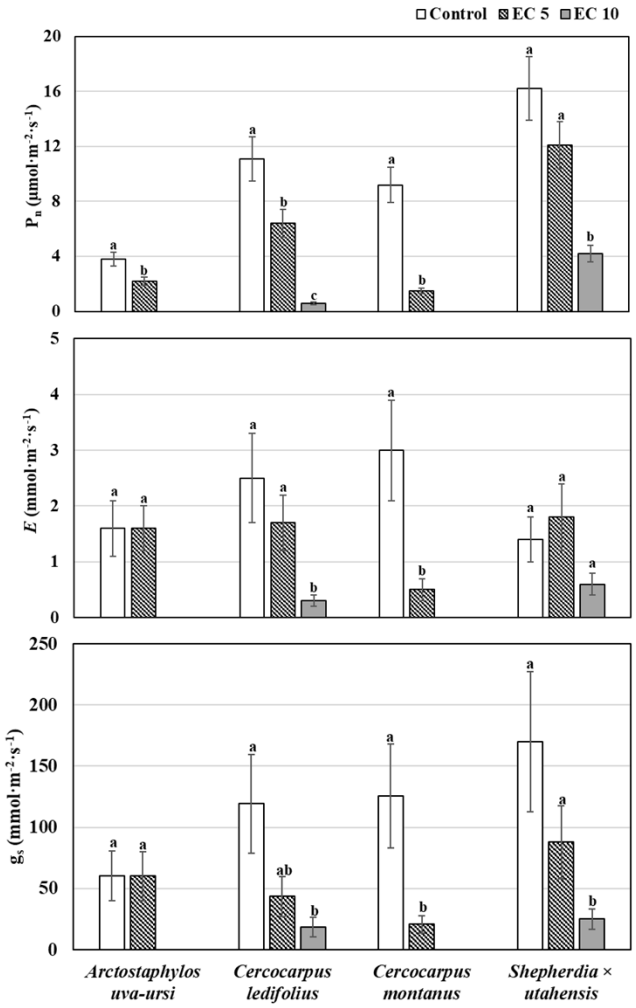

Fig. 3-4. Net photosynthesis rate (P_n), transpiration rate (E), and stomatal conductance (g_s) of container-grown plants native to Utah after irrigating with a nutrient solution (EC = 1.2 dS·m^{-1}, control) or saline solution [EC = 5.0 dS·m^{-1} (EC 5) or 10.0 dS·m^{-1} (EC 10)] in a greenhouse for 8 weeks. Vertical bars represent standard errors of five measurements. The same letters above column bars within species represent no significance between/among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$. *Arctostaphylos uva-ursi* and *Cercocarpus montanus* 'Coy' died when treated with saline solution at an EC of 10.0 dS·m^{-1} so gas exchange data were not taken.

CHAPTER IV

EFFECT OF SALT STRESS ON THE GROWTH, PHYSIOLOGY, AND MINERAL NUTRIENTS OF TWO PENSTEMON SPECIES[3]

Abstract

Penstemons are a diverse group of flowering plants valued for their ability to enhance the visual appearance of urban landscapes. *Penstemon barbatus* (Cav.) Roth 'Novapenblu' (rock candy blue® penstemon) and *Penstemon strictus* Benth 'Rocky Mountain' (rocky mountain beardtongue) are widely utilized in landscapes, but their tolerance to soil salinity remains poorly understood. This study aimed to investigate the effects of salinity levels at electrical conductivities (ECs) of 1.0 (nutrient solution), 2.5, 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$ on two penstemons (*P. barbatus* and *P. strictus*). Penstemons were irrigated with nutrient or saline solution for 8 weeks and various growth and physiological data were recorded before harvest. Salinity stress degraded the visual quality of penstemon species and led to a reduction in the growth rate and biomass production. Leaf burn and necrosis were observed in penstemons because of salinity stress. The visual score of *P. barbatus* and *P. strictus* decreased with increasing EC levels in the saline solution. When irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$, *P. barbatus* and *P. strictus* had severe-to-moderate foliar salt damage with average visual scores of 1.7 and 2.5, respectively (0 = dead plant; 5 = excellent plant without any foliar damage). The two penstemon species

[3] Paudel A, Sun Y. 2024. Effect of salt stress on the growth, physiology, and mineral nutrients of two penstemon species. HortScience. 59(2):209-219. https://doi.org/10.21273/HORTSCI17409-23.

had severe foliar salt damage or were dead when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. There were 87% and 92% decreases in the leaf area of *P. barbatus* and *P. strictus*, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ compared with those in the control. Although not statistically significant, there were 7% to 18% decreases in shoot dry weight of *P. barbatus* when irrigated with saline solutions at ECs of 2.5 to 10.0 $dS \cdot m^{-1}$, compared with control. However, *P. strictus* displayed declines of 13% to 31% in shoot dry weight as the salinity levels of the irrigation solution increased. As the salinity levels increased, net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration (E) rates decreased. Furthermore, sodium (Na^+) and chloride (Cl^-) contents of *P. barbatus* and *P. strictus* increased with the increase in salinity levels of the treatment solution. Consequently, *P. barbatus* and *P. strictus* demonstrated sensitivity to salinity stress at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$. This study provides important insights for their effective utilization in landscaping practices within saline-prone areas.

Introduction

Ornamental plants play a significant role in the horticultural industry because they are widely used in landscaping to create visually appealing outdoor environments. Traditionally, homeowners have used good-quality water to irrigate landscape plants because of the primary importance of their external appearance. However, landscape plants consume a substantial amount of water. The water requirement for producing 1 kg of dry matter in ornamental plants ranges from 100 kg to 350 kg, depending on plant species, cultivation system, and growing environment (Fornes et al. 2007).

As the population and agricultural production increase, there is a growing competition for good-quality water. Although recycled water can be used to irrigate landscape plants, it often contains higher salt content, which can lead to soil salinity (Carter and Grieve 2008). Recycled or reclaimed water is being used to irrigate landscape plants in many parts of the world, which can be a significant factor contributing to soil salinity in urban landscapes (Gorji et al. 2015). Ornamental plants are sold in potted containers filled with substrates such as peat moss and grown under field conditions (Reid and Jiang 2012). Whether grown in pots or in landscapes, ornamental plants are influenced by soil salinity and irrigation water quality (Garcia-Caparros and Lao 2018).

The presence of excessive salts reduces the availability of water to plants by decreasing the soil water potential. As a result, plants experience limited access to water, hindering essential physiological processes such as nutrient uptake, photosynthesis, and cellular expansion (Munns 2002; Zhang et al. 2013). Excessive salt levels disrupt the ionic balance and impose osmotic stress on plants, resulting in severe damage to their morphology, biomass, and biochemical processes (Rahneshan et al. 2018; Zhang et al. 2013). Soil salinity leads to increased sodium (Na^+) and chloride (Cl^-) contents in plants, which affect the normal ionic activities in plants (Singh et al. 2014).

Plants have developed various strategies to combat these challenges, including osmotic adjustment, compartmentalization, which helps store excess Na^+ in the vacuole, and the synthesis of osmolytes (Queiros et al. 2009; Rahneshan et al. 2018; Silva et al. 2015). Osmolytes, such as proline, protect plant cells, aiding in osmotic adjustment and increasing salinity tolerance (Rahneshan et al. 2018). Additionally, high salt levels can affect the metabolism of sensitive plants and cause the accumulation of toxic ions,

disrupting normal cellular processes (Munns and Tester 2008). The effects of salinity in various ornamental plants have been previously studied. For example, *Sedum telephium* (autumn joy) and *Sedum reflexum* (blue spruce) were considered relatively salt-tolerant, whereas *Sedum rupestre* (angelina) and *Evolvulus glomeratus* (blue daze) were found to be less tolerant (Hooks and Niu 2019). Similarly, *Tetraneuris acaulis* cultivar arizonica (arizona four-nerve daisy) was reported as a salt-tolerant species (Paudel et al. 2019).

Among the diverse array of ornamental plants, penstemons stand out as one of the most attractive native flowers of North America, with high aesthetic importance in urban landscape, leading to their increasing popularity. Penstemon represents America's largest endemic genus within the Plantaginaceae family, encompassing more than 270 species (Kramer 2009). These plants are commonly used in gardens because of their showy flowers during spring and summer (Lattier 2016) and have been used in ecological restoration efforts (Howe et al. 2006). Most penstemon species are drought-tolerant and thrive in well-drained soils (Kratsch 2011). In the United States, the annual sales of potted penstemon for garden and landscape uses are estimated at $3.2 million (U.S. Department of Agriculture 2020).

Penstemon species are listed as herbaceous plants that have medium tolerance to saline soil (Jull 2009). Previous research of the salinity tolerance of penstemons is limited. Niu and Rodriguez (2006) investigated the salt tolerance of *Penstemon eatonii* A. Gray (firecracker penstemon), *Penstemon pseudospectabilis* M.E. Jones (desert beardtongue), and *Penstemon strictus* Benth. up to an electrical conductivity (EC) of 12.0 $dS \cdot m^{-1}$ and found that these plants exhibited low salt tolerance. Niu and Rodriguez (2006) studied the effect of salinity stress on plant growth, osmotic potential, and mineral

nutrient content. However, they did not investigate the gas exchange rate of penstemons, which has been recorded in this present study. Similarly, Zollinger et al. (2007) reported that *Penstemon palmeri* A. Gray (palmer penstemon) showed intermediate levels of salt tolerance, whereas *Penstemon* ×*mexicali* 'Red Rocks' (red rocks penstemon) was relatively intolerant to salinity stress at 3000 $mg \cdot L^{-1}$ (~ 4.7 $dS \cdot m^{-1}$). Zollinger et al. (2007) tested penstemons up to 5000 $mg \cdot L^{-1}$ (~ 6.3 $dS \cdot m^{-1}$). However, during the present study, penstemons were tested for salinity levels up to an EC of 10.0 $dS \cdot m^{-1}$. The present study aimed to access the morphological and physiological responses of two penstemon species, namely *Penstemon barbatus* (Cav.) Roth 'Novapenblu' (rock candy blue® penstemon) and *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue), under salt stress. By examining their response to elevated salt levels, this research contributes to our understanding of the salt tolerance of penstemon species and inform their potential use in landscape and gardening practices. We hypothesized that *P. barbatus* and *P. strictus* irrigated with higher salinity levels exhibit foliar salt damage, decreased plant growth, and altered plant physiological status.

Materials and methods

To assess the salinity tolerance of penstemons across varying salinity levels, a greenhouse study was conducted. The experiment was focused on investigating the morphological, physiological, and biochemical attributes of the penstemons under controlled conditions.

Plant materials and culture conditions. The experiment was conducted at the Utah State University (USU) Research Greenhouse in Logan, UT, USA. *Penstemon*

barbatus (Cav.) Roth 'Novapenblu' (rock candy blue® penstemon) and *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue) in 2.8-L injection molded polypropylene containers (Pro-Cal ™; South Gate, CA, USA) were purchased from Perennial Favorites (Layton, UT, USA). The plants were transplanted into 7.6-L injection molded polypropylene containers (No. 2B; Nursery Supplies, Orange, CA, USA) filled with a soilless growing medium (Metro-Mix® 820; Canadian sphagnum peat moss, 35 to 45% composted pine bark, coir, coarse perlite, and dolomitic limestone; SunGro Horticulture, Agawam, MA, USA) on 2 May 2022. Logan City potable water [EC = 0.35 ± 0.01 $dS \cdot m^{-1}$; pH = 7.7 ± 0.2, mean ± *SD*] was applied to plants when needed. Penstemons were pruned to 12 cm tall, and flowers were removed. Uniform plants that were free from any visible signs of stress or disease were then selected for the salinity study. A shadecloth (60%) was placed at the top of the greenhouse during the research period. The experiment started on 16 Jun 2022 and ended on 12 Aug 2022. Plants were grown in the greenhouse with day temperatures of 26.2 ± 0.5 ºC and night temperatures of 22.8 ± 0.6 ºC and daily light integral of 13.0 ± 2.9 $mol \cdot m^{-2} \cdot d^{-1}$.

Treatments. This study exposed penstemons to either a nutrient or a saline solution at ECs of 1.0, 2.5, 5.0, 7.5, or 10.0 $dS \cdot m^{-1}$. The nutrient (control) solution was prepared in a 100-L tank by adding 0.8 $g \cdot L^{-1}$ 15N-2.2P-12.5K water-soluble fertilizer (Peters Excel 15-5-15 Cal-Mag Special; ICL Specialty Fertilizers, Dublin, OH, USA) to reverse osmosis water. The saline solutions were prepared by adding sodium chloride (NaCl; Fisher Scientific, Waltham, MA, USA) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$; Hi Valley Chemical, Centerville, UT, USA) to the nutrient solution at a molar ratio of 2:1 (Table 4-1). The initial pH of the treatment solutions was adjusted to

6.0 to 6.5 using 88% potassium hydroxide pellets (Sigma-Aldrich, St. Louis, MO, USA) or 1M nitric acid (Fisher Chemical, Fair Lawn, NJ, USA) as needed. The EC, sodium adsorption ratio (SAR), adjusted SAR (Lesch and Suarez 2009), and elemental analysis results were confirmed by the Utah State University Analytical Laboratory (Logan, UT, USA) (Table 4-1). Treatment solutions of 1200 mL per pot were manually applied weekly, and 30% of the leachate volume was maintained. The treatment solutions were applied using a beaker through the top of the pot in the morning of each week. Plants were irrigated with 500 to 600 mL of reverse osmosis water when the top (~ 1 cm) soilless medium was dry to avoid the confounding effects of drought.

Leachate and substrate EC. The EC of the leachate was measured by the pour-through method as described by Cavins et al. (2008) using an EC meter (LAQUA Twin; Horiba, Kyoto, Japan) after applying the treatment solution. When the leachate EC was greater than that of the treatment solution, the substrate was washed with reverse osmosis water to maintain similar EC levels in the substrate over time. A single plant was chosen for the measurement of leachate EC in each treatment for each species. After harvest, the substrate EC was determined using the saturated paste extraction method with some changes (Gavlak et al. 2005) after the substrate was left to dry for 2 weeks. Five plants were chosen for measurement in each treatment. The leachate and substrate EC data were pooled across the species because there were no differences observed between species.

Visual quality. A visual score of 0 to 5 was assigned to each plant biweekly based on the percentage of leaves with burnt leaves or necrosis (Sun et al. 2015) (Table 4-2). A score of 0 indicated that the plant was dead. A score of 1 indicated severe foliar damage (>90%). A score of 2 indicated moderate foliar damage (51%-90%). A score of 3

indicated slight foliar damage (10% to 50%). A score of 4 indicated good quality with minimal foliar damage (<10%). A score of 5 indicated excellent without any foliar damage. The plant growth was not considered while determining the visual score.

Growth parameters and plant harvest. Plant heights (centimeters) were recorded at the beginning and end of the experiment. At harvest, the area (square centimeters) of all leaves was measured for all the surviving plants using a leaf area meter (LI-3100; LI-COR® Biosciences, Lincoln, NE, USA). Additionally, the shoot dry weight (DW) (stem DW + leaf DW) and the root DW of plants were determined by drying the plants for 1 week at 60 ºC.

Chlorophyll content, chlorophyll fluorescence, and leaf gas exchange. Relative chlorophyll content (or leaf greenness) of all plants was determined using a chlorophyll meter [Soil Plant Analysis Development (SPAD)-502; Minolta Camera, Osaka, Japan] before harvest. Eight mature leaves from each plant were measured, and the average value was recorded.

The maximum photochemical quantum yield of photosystem II (PSII) [$F_v/F_m = (F_m-F_o)/F_m$] was measured on dark-adapted leaves using a chlorophyll fluorometer (PEA version 12.1; Hansatech Instrument Ltd., Norfolk, UK), where F_o denotes the minimum fluorescence at low-modulated light and F_m denotes the maximum fluorescence signal at saturating light. Six plants for each species and treatment were used for measurement. Firstly, leaves were adapted in the dark for at least 30 min using leaf clips (diameter, 4mm) (Hansatech Instrument Ltd.). Measurements were performed in the middle of fully developed leaves on both species. Additionally, the performance index (PI_{abs}) for energy conservation from photons absorbed by PSII was recorded.

Leaf gas exchange of five plants for each species and treatment was measured using a portable photosynthesis system (CIRAS-3; PP Systems, Amesbury, MA, USA) with an automatic universal leaf cuvette (PLC3; PP Systems). The leaf net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of plants in each treatment were recorded based on the measured carbon dioxide (CO_2) and water vapor (H_2O) exchange on the youngest fully emerged leaves. All plants were watered 1 d before measurements to avoid water stress condition.

Mineral analyses. Dried penstemon leaves were ground with a stainless-steel Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and allowed to pass through a 1-mm mesh screen. The powder samples were analyzed at the Utah State University Analytical Laboratories for mineral contents. In brief, the concentrations of Na^+, calcium (Ca^{2+}), potassium (K^+), boron (B), magnesium (Mg^{2+}), phosphorus (P), zinc (Zn^{2+}), and manganese (Mn^{2+}) were determined using nitric/hydrogen peroxide following the protocol described in Gavlak et al. (2005). The concentration of Cl^- was measured using a Flow Injection Analysis and Ion Chromatograph System (QuikChem 8000; Lachat Instrument, Loveland, CO, USA) and expressed on a dry plant basis ($mg \cdot g^{-1}$). To determine the levels of Na^+, Ca^{2+}, K^+, B, Mg^{2+}, P, Zn^{2+}, and Mn^{2+}, 0.5 g of powdered samples were mixed with 6 mL of nitric acid (HNO_3) in a digestion tube, which was then subjected to a digestion block for 10 min at 80 °C, followed by cooling for 2 min. A total of 2 mL of 30% hydrogen peroxide (H_2O_2) was added into the digestion tube that was placed again in the digestion block at 130 °C for 1 h. The digestion tubes were mixed using a vortex stirrer. Then, the digestion tube was cooled at room temperature, and the contents of the digestion tube were transferred into a 25-mL volumetric flask. The digest

was analyzed using an inductively coupled plasma-optical emission spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA, USA) and reported based on DW ($mg \cdot g^{-1}$).

Proline estimation. The acid-ninhydrin method was used for the quantification of proline in penstemons (Bates et al. 1973; Claussen 2005; Rakesh et al. 2021). In brief, leaf samples collected after the sixth irrigation event were frozen in liquid nitrogen and subsequently stored at -80 ºC until use. Leaf samples (0.2 g) were ground in 5 ml of 3% sulfosalicylic acid (Spectrum Chemical, Gardena, CA, USA) and centrifuged for 5 min at room temperature using a benchtop centrifuge (Spectrafuge^{TM} Labnet 6C Centrifuge; The Laboratory Depot, Dawsonville, GA, USA) with 5000 g_n. After centrifugation, 200 µL of supernatant, 200 µL of glacial acetic acid (Fisher Chemical, Fair Lawn, NJ, USA), and 200 µL of acid ninhydrin (Sigma-Aldrich, St. Louis, MO, USA) were combined in a tube and incubated in a boiling water bath at 95 ºC for 1 h. After 1h, tubes were immediately placed in an ice-bath to arrest the reaction. Thereafter, 400 µL of toluene (Fisher Chemical, Colonnade Road, Ottawa, ON, USA) was added to each tube, and the mixture was vortexed and left to settle for 10 min. The upper layer of 200 µL of the resulting solution was pipetted to a microplate reader (Greiner bio-one; Cellstar, F-bottom, Monroe, NC, USA). Absorbance was recorded using a spectrophotometer (Spectra max M2; Molecular Devices, CA, USA) at 520 nm. Proline (L-Proline, St. Louis, MO, USA) was taken as standard, and a graph was plotted to estimate the proline content in the samples. The concentration of proline was calculated as follows:

$\mu mol \cdot g^{-1}$ tissue = μg proline$\cdot mL^{-1}$ * mL toluene/115.5 * $25 \cdot g^{-1}$ sample

Experimental design and statistical analysis. The experiment was conducted using a randomized complete block design with 10 replications, encompassing five treatments and two species. Each experimental unit consisted of one pot containing a single plant. Statistical analyses were conducted using SAS (version 14.1; SAS Institute, Cary, NC, USA) with PROC MIXED procedure. An analysis of variance (ANOVA) was performed to evaluate the effects of saline solution irrigation and species on various plant characteristics, including growth, gas exchange, and mineral nutrients. To normalize the data, logarithmic transformation was applied for all response variables to improve model performance. Dead plants were excluded from data analysis for all parameters except for the visual score. Because of the diverse growth habits of each species, means separation among treatments was adjusted using Tukey's method for multiplicity at $\alpha = 0.05$. Furthermore, means separation between species was conducted for proline content. To explore relationships, correlation analyses were performed between visual scores, Na^+ and Cl^- contents, and K^+/Na^+ and Ca^{2+}/Na^+ ratios. Correlation analyses of gas exchange parameters, Na^+ and Cl^- contents, and K^+/Na^+ and Ca^{2+}/Na^+ ratios were performed. Additionally, linear and quadratic trend analyses of the plant growth data were performed.

Results

During this study, we delve into the effects of salinity stress on *P. barbatus* and *P. strictus*, with a focus on visual quality, plant growth, and physiological parameters. Understanding these responses is critical to formulating strategies to mitigate the adverse effects of salinity stress on urban landscapes.

Visual score and plant growth parameters. Salt damage was observed on penstemon species at higher salinity levels, mainly in the form of burnt leaves and necrosis (Fig. 4-1). After the second irrigation event, visual scores were affected by salinity ($P < 0.0001$) (Table 4-3), and an interaction was observed between species × salinity ($P = 0.004$). The average visual score of *P. barbatus* at an EC of 10.0 $dS \cdot m^{-1}$ was 4.3, which was lower than that of other treatments (Fig. 4-2). After the fourth irrigation event, visual scores were affected by salinity ($P < 0.0001$), and an interaction was observed between species × salinity ($P = 0.002$) (Table 4-3). *Penstemon barbatus* had an average visual score of 3.7 when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$ (Fig. 4-2). However, minimal to no foliar salt damage was observed in *P. strictus* for up to 4 weeks. When irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, the visual scores were 3.0 and 3.7 for *P. barbatus* and *P. strictus*, respectively. After the sixth irrigation event, visual scores were affected by salinity ($P < 0.0001$), and an interaction was observed between species × salinity ($P < 0.0001$) (Table 4-3). *Penstemon barbatus* and *P. strictus* had average visual scores of 1.6 and 3.4, respectively, when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$ (Fig. 4-2). Visual scores were 1.2 and 1.6, respectively, when *P. barbatus* and *P. strictus* were irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. When irrigated with saline solution at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$, one *P. barbatus* plant in each treatment died. Finally, after the eighth irrigation event, salinity affected the visual score ($P < 0.0001$), and the interaction between species × salinity was insignificant (Table 4-3). Visual scores were 3.2 and 4.0 for *P. barbatus* and *P. strictus*, respectively, when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Fig. 4-2). *Penstemon barbatus* and *P. strictus* had average visual scores of 1.7 and 2.5, respectively,

when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$. Similarly, visual scores were 1.4 and 1.3, respectively, when *P. barbatus* and *P. strictus* were irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Furthermore, two *P. barbatus* died when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$. At an EC of 10.0 $dS \cdot m^{-1}$, three *P. barbatus* and five *P. strictus* plants were dead.

After 8 weeks of growing under different saline solutions, the two penstemon species exhibited differences in terms of growth measurements. Saline solution irrigation significantly impacted the height of penstemon plants ($P < 0.0001$) (Table 4-3), leading to an 84% to 94 % reduction in the height of *P. barbatus* at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$ compared with the control (Table 4-4). However, there was no notable difference in plant height of *P. strictus* among treatments. Furthermore, leaf area varied significantly with species and salinity ($P < 0.0001$) (Table 4-3). The leaf area of *P. barbatus* and *P. strictus* decreased linearly with increasing EC levels in the saline solution ($P < 0.0001$) (Table 4-4). In addition, leaf area of *P. strictus* decreased quadratically ($P < 0.0001$). Compared with the control, there was an 87% reduction in the leaf area of *P. barbatus* when irrigated with saline solutions at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$. Similarly, a 69% to 92% reduction in the leaf area of *P. strictus* was observed when irrigated with saline solutions at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$. Furthermore, although it is not statistically significant, there was a 12% to 39% reduction in the leaf area of *P. barbatus* and *P. strictus* when irrigated with saline solutions at ECs of 2.5 and 5.0 $dS \cdot m^{-1}$.

Shoot dry weight was impacted by salinity ($P = 0.003$) (Table 4-3). The shoot dry weight of *P. strictus* decreased linearly with increasing EC levels in the saline solution ($P = 0.02$) (Table 4-5). Compared with the control, although not significant, there were 7%,

18%, 7%, and 12% decreases in shoot DW of *P. barbatus* when irrigated with saline solutions at ECs of 2.5, 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$, respectively. Similarly, shoot DW of *P. strictus* decreased by 13%, 13%, 30%, and 31%, respectively, when irrigated with saline solutions at ECs of 2.5, 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$. Furthermore, root DW was significantly impacted by salinity ($P = 0.004$) (Table 4-3). Root DW of *P. strictus* decreased linearly ($P = 0.01$) and quadratically ($P = 0.047$) with increasing EC levels in the saline solution (Table 4-5). There were 45% to 50% decreases in root DW of *P. barbatus* when irrigated with saline solution at ECs of 5.0 to 10.0 $dS \cdot m^{-1}$ compared with the control. Similarly, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, there was a 50% reduction in root DW of *P. strictus*.

The visual quality and growth data suggest that the penstemons underwent salinity stresses, which can be attributed to the accumulation of salts in the substrate. The EC of the leachate solution remained consistent throughout the experiment (Fig. 4-3). In addition, the pH of the leachate solution during the experiment was 6.6 ± 0.4. However, the EC of the substrate increased with increasing EC level of saline solution (Fig. 4-4). The average ECs of the substrate were 11.5, 13.4, and 15.2 $dS \cdot m^{-1}$ when irrigated with saline solution at ECs of 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$, respectively.

Relative chlorophyll content, chlorophyll fluorescence, and gas exchange. The relative chlorophyll content (SPAD reading) of two penstemon species varied with species and salinity ($P < 0.0001$) (Table 4-3). There were 28% and 26% reductions in the SPAD readings of *P. barbatus* and *P. strictus*, respectively, at an EC of 5.0 $dS \cdot m^{-1}$, compared with those of the control (Fig. 4-5). Likewise, 71% and 40% reductions in the SPAD readings were observed in *P. barbatus* and *P. strictus*, respectively, when irrigated

with saline solution at an EC of 7.5 $dS \cdot m^{-1}$. In addition, F_v/F_m and PI_{abs} readings varied with salinity levels ($P < 0.0001$) (Table 4-3). *Penstemon barbatus* had significant reductions in both F_v/F_m and PI_{abs} when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$, but readings were similar for *P. strictus* among treatments.

The net photosynthetic rate (P_n) of two penstemon species decreased with increasing salinity levels of the solution ($P < 0.0001$) (Table 4-3). In addition, P_n varied with species ($P = 0.0024$) and showed interactive effects between species and salinity levels ($P = 0.0477$). *Penstemon barbatus* had a P_n of 13.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when irrigated with saline solution at an EC of 2.5 $dS \cdot m^{-1}$ (Fig. 4-6). The net photosynthetic rate of *P. barbatus* decreased from 18.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 7.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$. Similarly, P_n of *P. strictus* decreased from 20.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 12.6 and 9.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when irrigated with saline solutions at ECs of 5.0 and 7.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

The stomatal conductance (g_s) also decreased with increasing salinity levels ($P < 0.0001$) (Table 4-3). The g_s of *P. barbatus* decreased from 731.3 to 252.3 $mmol \cdot m^{-2} \cdot s^{-1}$ when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (Fig. 4-6). Similarly, g_s decreased from 783.7 to 174.9 $mmol \cdot m^{-2} \cdot s^{-1}$ for *P. strictus* when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$. The transpiration rate (E) decreased as salinity levels in the solution increased for two penstemon species ($P < 0.0001$, Table 4-3). The E of *P. barbatus* decreased from 10.3 to 5.4 $mmol \cdot m^{-2} \cdot s^{-1}$ when irrigated with saline solution at an EC of 5.0 $dS \cdot m^{-1}$ compared with the control (Fig. 4-6). Similarly, E decreased from 10.4 to 4.8 $mmol \cdot m^{-2} \cdot s^{-1}$ for *P. strictus* when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$.

Mineral nutrients. Leaves accumulated significant number of anions and cations, particularly Na^+ and Cl^- ($P < 0.0001$) (Table 4-6). There was a significant effect of species for Na^+ accumulation in leaves ($P < 0.0001$), but not for Cl^-. After 8 weeks of irrigation, the Na^+ content of control plants was 0.05 $mg \cdot g^{-1}$ for *P. barbatus* and 0.03 $mg \cdot g^{-1}$ for *P. strictus*. However, Na^+ content increased to 5.02 $mg \cdot g^{-1}$ for *P. barbatus* and 2.80 $mg \cdot g^{-1}$ for *P. strictus* when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, which were 99-times and 92-times greater than their respective controls. Similarly, control plants had Cl^- contents of 2.3 and 3.0 $mg \cdot g^{-1}$ for *P. barbatus* and *P. strictus*, respectively. However, the Cl^- content increased to 51.1 and 53.5 $mg \cdot g^{-1}$ for *P. barbatus* and *P. strictus*, respectively, when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$; these were 21-times and 17-times greater than that of their respective control.

A low increment observed in the Ca^{2+} content with increasing salinity levels in the solution, but the content varied with both species and salinity ($P < 0.0001$) (Table 4-6). After 8 weeks of irrigation, Ca^{2+} content increased from 20.6 to 32.5 $mg \cdot g^{-1}$ for *P. barbatus* and 11.7 to 25.5 $mg \cdot g^{-1}$ for *P. strictus* when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$ compared with control plants.

The reduction in K^+ content was observed in leaves with increasing salinity levels in the solution ($P = 0.04$) (Table 4-6). *Penstemon strictus* had 10.9%, 18.4%, 8.8%, and 23.4% reductions in the K^+ content when irrigated with saline solutions at ECs of 2.5, 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$, respectively, compared with the control. Similarly, there was 15.3%, 9.7%, 2.7%, and 10.8% reductions in K^+ content of *P. barbatus* leaves when irrigated with saline solutions at ECs of 2.5, 5.0, 7.5, and 10.0 $dS \cdot m^{-1}$, respectively, compared with the control. However, these reductions were not statistically significant.

Furthermore, the K^+/ Na^+ and Ca^{2+}/ Na^+ ratios in penstemon leaves also varied with species and salinity ($P < 0.0001$) (Table 4-6). As salinity levels increased in the solutions, a decrease in both ratios was observed. The K^+/Na^+ ratio was 112-times and 138-times greater in control for *P. barbatus* and *P. strictus*, respectively, than that in those irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$. Similarly, the Ca^{2+}/Na^+ ratio was 63-times and 48-times greater in control for *P. barbatus* and *P. strictus* than that in those irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, respectively.

In addition, the B content decreased with increasing salinity levels in the solutions for *P. barbatus* and *P. strictus* ($P < 0.0001$) (Table 4-7). The Mg^{2+} content remained similar among treatments but varied with species. Furthermore, saline solutions had a significant impact on the P, Zn^{2+}, and Mn^{2+} content of penstemons.

Proline content

The leaf proline content observed in the experiment was mostly species-dependent ($P < 0.0001$) (Table 4-3). There was no difference among salinity levels in the proline content of *P. barbatus* and *P. strictus* (Table 4-8). However, a greater proline content was observed in *P. strictus* when compared with that in *P. barbatus*. *Penstemon strictus* had the highest proline content of 1.2 $\mu mol \cdot g^{-1}$ when irrigated with saline solution at an EC of 7.5 $dS \cdot m^{-1}$.

Discussion

Salinity stress can severely impact the overall appearance of plants, making visual quality assessments crucial when evaluating the aesthetic appeal and market value of ornamental plants in landscaping projects and horticultural industries. Visual quality

considers factors such as flowers, foliage color, texture, shape, and form. The present data revealed the detrimental effects of salinity on the visual quality of *P. barbatus* and *P. strictus* caused by leaf burn and necrosis. The results indicated that *P. barbatus* experienced more foliar salt damage and was more susceptible to saline water irrigation than *P. strictus*. Similarly, *P. eatonii*, *P. pseudospectabilis*, and *P. strictus* had salt injury with leaf necrosis and browning when they were irrigated with saline solutions prepared using NaCl, magnesium sulfate ($MgSO_4$), and $CaCl_2$ for 12 weeks (Niu and Rodriguez 2006). In addition, *P. ×mexicali* exhibited sharp declines in visual quality as salinity levels increased from 3000 to 5000 $mg \cdot L^{-1}$ (~4.7 to 6.3 $dS \cdot m^{-1}$) (Zollinger et al. 2007). Likewise, severe leaf burns and wilting were observed in *P. palmeri* when exposed to saline solution greater than 3000 $mg \cdot L^{-1}$ (~4.7 $dS \cdot m^{-1}$) (Zollinger et al. 2007). In this study, Zollinger et al. (2007) irrigated *P. ×mexicali* and *P. ×palmeri* with a saline solution containing $CaCl_2$ and NaCl at a molar ratio of 2:1.

During this study, we observed salt injury on the leaves of *P. barbatus* and *P. strictus* as the salinity levels in the solution increased, but no mortality was observed at less than 7.5 $dS \cdot m^{-1}$. However, Niu and Rodriguez (2006) reported that *P. strictus* did not survive when irrigated with saline solution at an EC of 3.2 $dS \cdot m^{-1}$ or greater. This difference in observations could be attributed to variations in climate, saline solutions, growing substrates, and irrigation procedures. Saline solutions with various compositions can lead to different responses in plants, influencing nutrient uptake, osmotic regulation, and plant health (Nebauer et al. 2013). Niu and Rodriguez (2006) used saline solutions prepared with NaCl, $MgSO_4$, and $CaCl_2$, whereas the present study only used NaCl and $CaCl_2$ for the saline solution. Moreover, local climate conditions and growing substrate

properties have been found to influence the response to salinity stress in previous studies (Costello et al. 2003; Fox et al. 2005). Microclimate conditions like temperature and humidity in a greenhouse play an important role in plant physiology and growth patterns.

In addition to its visual effects, salinity stress can significantly impede plant growth. In this study, the plant heights of both *P. barbatus* and *P. strictus* were affected by salinity stress. Exposure of ornamental plants to saline conditions can lead to decreased plant growth, which may have the benefit of achieving more compact plant sizes. However, it is essential to consider that maintaining compact plant size may require continued irrigation with saline water, potentially posing harm to other plants in the landscape. Additionally, salinity stress impacts the expansion of cells in young leaves, resulting in a decrease in leaf area (Munns and Tester 2008). Reduced leaf growth is the earliest response of glycophytes when exposed to salinity stress (Munns and Termaat 1986). Plants reduce leaf size to minimize water loss by transpiration, which allows conservation of soil moisture and prevents an increase in salt concentration in the soil (Munns and Tester 2008). In the case of *P. barbatus* and *P. strictus*, an increase in EC levels in the saline solution corresponded to a reduction in leaf area. Likewise, the decrease in leaf area was noted in numerous plants as the concentration of saline water increased (Paudel et al. 2019; Sun et al. 2020; Wu et al. 2016).

Plant biomass is one of the most direct indicators of salinity tolerance. Acosta-Motos et al. (2017) reported that the decrease in shoot DW of plants exposed to salinity stress is primarily attributed to the development of smaller and fewer leaves, and stunted plant growth. In this study, there was a significant reduction in shoot DW of *P. strictus*. Similarly, shoot DW of *P.* ×*mexicali* and *P. palmeri* decreased with increasing salinity

levels in the solution (Zollinger et al. 2007). Additionally, Niu et al. (2010) found that *Angelonia angustifolia* (angelonia) cultivars in the Plantaginaceae family exhibited a 25% and 50% reduction in shoot DW when irrigated with saline solution at ECs of 5.1 and 7.4 $dS \cdot m^{-1}$, respectively, compared with those at an EC of 2.8 $dS \cdot m^{-1}$ (Niu et al. 2010). Plant roots are highly susceptible to salinity because they are in direct contact with salts, impacting their ability to absorb water, their water use efficiency, and other physiological processes (Sanchez-Blanco et al. 2014). In this study, there was a decreasing trend in the root DW of penstemons, reflecting the inhibition of root growth caused by osmotic and toxic effects of high salinity (Banon et al. 2012). Inhibition of root growth may have been caused by the reduced capacity of the shoot to deliver nutrients to the roots, which can affect plant development and survival (Munns and Termaat 1986).

The negative effects on the visual quality and stunted growth of *P. barbatus* and *P. strictus* may be attributed to the salts accumulated in the growing substrate, which can directly affect plant health. This accumulation of salts in the substrate can be evaluated through the pour-through method as described by Cavins et al. (2008) or the saturated paste extraction method described by Gavlak et al. (2005). During the experiment, although leachate ECs from the substrate remained similar throughout the duration of each treatment, there was a significant difference among the treatments. The EC of the substrate was greater than that of the corresponding saline solution, indicating the presence of salt accumulation in the growing substrate.

The results of the study revealed significant variations in the SPAD readings of *P. barbatus* and *P. strictus* under different salinity levels. These findings align with those of previous research, where increasing levels of salinity in the solution caused reductions in

the SPAD readings of several landscape plants (Liu et al. 2017; Niu et al. 2007a). Interestingly, the present study also demonstrated species-specific responses to salinity stress. The SPAD readings of *P. barbatus* exhibited a greater sensitivity to salinity compared with *P. strictus*. This was evident from the larger reductions in SPAD readings for *P. barbatus* at an EC of 7.5 $dS \cdot m^{-1}$. Furthermore, the measurements of chlorophyll fluorescence parameters (F_v/F_m and PI_{abs}) provided insights into the photosynthetic efficiency of the penstemon species. The significant reduction in F_v/F_m and PI_{abs} for *P. barbatus* with increasing salinity levels in the solution indicates compromised photosynthetic activity. The F_v/F_m parameter is of crucial importance because it signifies the effectiveness of the light reaction and is extensively used for studying the effects of stress on plants. Salinity stress obstructs the process of electron transfer from the primary acceptor to the secondary acceptor in the PS II, which may have led to a reduction in F_v/F_m (Shu et al. 2012).

Salinity stress can have significant effects on plant growth because it disrupts several essential physiological processes. These processes may encompass interference with photosynthesis, osmoregulation, and mineral supply to the aerial part (Negrao et al. 2017). The photosynthetic apparatus of plants may be harmed, and plant photosynthesis can be inhibited under salinity stress (Taiz et al. 2015). Exposing plants to high levels of soil salinity can disrupt their water balance, resulting in water moving out of plant cells and into the surrounding soil (Munns and Tester 2008). This, in turn, can lead to dehydration and reduction in P_n. Additionally, high levels of salt can also cause damage to chloroplasts, which are responsible for P_n, and interfere with the process of photosynthesis itself. Furthermore, the buildup of excessive amounts of Na^+ and Cl^- ions

can impede P_n, as noted by Zhang et al. (2014). Moreover, salinity stress can also impact g_s, which refers to the opening and closing of the stomata. When plants are exposed to high levels of salinity, the stomata may close to conserve water, reducing the uptake of carbon dioxide (CO_2) for P_n and the release of oxygen (O_2). In the present study, P_n and g_s of the penstemons were reduced in response to salinity stress. Similarly, there were decreases in the P_n and g_s rates in *P. palmeri* with increasing salinity levels in the solution (Zollinger et al. 2007). To mitigate the negative impacts of salinity stress on P_n and g_s, several strategies can be employed. For instance, the application of silicon (Si) can improve the shoot growth and net photosynthetic rates. Research indicated that Si may play a crucial role in sustaining high photosynthetic rates in plants under salt stress conditions (Coskun et al. 2019; Zargar et al. 2019). Furthermore, applying arbuscular mycorrhizal fungi (AMF) has been found to help maintain osmotic balance, exhibit a greater g_s, and improve the overall photosynthetic efficiency of plants (Evelin et al. 2009). In addition, saline conditions may create a water deficit in plants, leading to stomatal closure and, ultimately, a decrease in *E* (Wang et al. 2019). Likewise, two penstemon species in this study exhibited reductions in *E* in response to salinity stress.

The nutritional status of a plant is influenced by salinity stress through a complicated network of interactions, which may involve a reduction in the uptake and/or transportation of nutrients from roots to shoots (Munns and Tester 2008). Although low Na^+ levels can be beneficial to plants in some situations, moderate and high levels are harmful to most plants (Maathuis 2014). Furthermore, plants take-up Cl^- ions, which are important for stabilizing membrane potential and regulating pH and turgor (Marschner 2012). Although plants generally require only small amounts of Cl^-, deficiencies are rare.

However, excessive Na^+ and Cl^- ions in plants can lead to toxicity. Under the salinity treatments in this study, the Na^+ and Cl^- contents of penstemon leaves in both species were dramatically increased. This suggests an accumulation of these ions in the plant tissues caused by salinity stress. Importantly, a negative correlation between visual score and Na^+ and Cl^- contents was observed ($P < 0.0001$) (Fig. 4-7). The visual score provides an indication of the foliar salt damage, and the negative correlation suggests that higher Na^+ and Cl^- contents are associated with greater visual damage. In addition to the visual damage, the reduction in P_n observed in the penstemons under salinity stress was also negatively correlated with the Na^+ and Cl^- contents in the leaf tissue ($P \leq 0.009$) (Fig. 4-8). Similarly, leaf Na^+ and Cl^- concentration in *A. angustifolia* increased as salinity levels of irrigation water increased (Niu et al. 2010). The increasing ion concentration in the leaves of penstemons could have caused foliar salt damage.

Calcium plays a crucial role in maintaining the integrity of cell plasma membranes in roots and restricting the toxic effect of Na^+ (Gucci and Tattini 1997; Rengel 1992). It also serves as a secondary messenger in regulating signal transduction pathways for abiotic stress response and in promoting K^+/Na^+ selectivity (Rengel 1992). Similarly, the leaf Ca^+ concentration has been reported to increase with increasing salinity in many ornamental plant species (Paudel et al. 2019; Sun et al. 2015; Wu et al. 2016). Consistent with these findings, the present study also observed an increase in leaf Ca^+ concentration, likely caused by the use of $CaCl_2$ in the preparation of the saline solution (Table 1). A positive correlation was observed between visual score and K^+/Na^+ and Ca^{2+}/Na^+ ratio ($P \leq 0.05$) (Fig. 4-7). The increment in Ca^{2+} content was low in penstemons when compared with the Na^+ and Cl^- increments. These findings highlight

the role of Ca^{2+} in mitigating the toxic effects of Na^{+} and its involvement in maintaining ion selectivity and osmotic adjustment in response to salinity stress (Rengel 1992). The positive correlation between the visual score and Ca^{2+}/Na^{+} ratio further supports the significance of Ca^{2+} in minimizing the harmful effects of salinity. Enhancing Ca^{2+} availability to plants under salinity stress is crucial for maintaining normal physiological processes in plants. In our case, calcium chloride was added to the solution so that plants could presumably access more Ca^{2+}. To increase calcium uptake in plants, boosting water flow to the roots is an option, but it is not ideal because of limited freshwater availability (Yang et al. 2012). Increasing the cation exchange capacity of soil can be another strategy to make more Ca^{+} available to plants (Mengel 2023).

In plants, K^{+} serves as the primary cation that counterbalances anions and activates enzymes for metabolic processes, protein and carbohydrate synthesis, and the regulation of stomatal movement (Rahneshan et al. 2018). In the present study, the K^{+} contents remained similar for *P. barbatus* and slightly reduced for *P. strictus* under salinity stress. However, the K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratio decreased with increasing salinity levels for both penstemon species. This suggests that the high concentration of Na^{+} present in saline soils may have replaced the essential K^{+} and Ca^{2+} ions in the penstemons, which are crucial for proper growth and development. Furthermore, P_n of the penstemons was reduced in response to salinity and positively correlated with K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios ($P \leq 0.02$) (Fig. 4-8). Similarly, g_s and E of *P. strictus* were also influenced by K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios ($P \leq 0.01$) (Fig. 4-8). These findings suggest that the imbalance caused by the replacement of K^{+} and Ca^{2+} with Na^{+} in the penstemons negatively affected their physiological processes.

Boron plays an important role in several physiological processes in plants, including cell wall structure, root elongation, shoot growth, membrane integrity, and reproduction (Broadley et al. 2012). Boron deficiency can lead to inhibition of root elongation and shoot meristematic growth (Broadley et al. 2012). In this study, it was observed that B content was reduced in two penstemons species in response to salinity stress, which also may have contributed to the reduction in the growth of shoots and roots.

Magnesium is a component of chlorophyll and is required for photosynthesis and protein synthesis, while P is crucial for nucleic acids and carbohydrate transfer in leaf cells (Hawkesford et al. 2012). Zinc contributes to detoxification of superoxide radicals, membrane integrity, protein synthesis, and phytohormone production (Broadley et al. 2012). Manganese activates enzymes involved in detoxification and lignin synthesis (Broadley et al. 2012). In this study, Mg^{2+} content remained consistent in both penstemon species, but the P content decreased in *P. strictus* under higher salinity levels. Plant type and growing conditions play an important role in P accumulation (Grattan and Grieve 1999). Moreover, Zn^{2+} content increased in *P. strictus* and Mn^{2+} content increased in both species, suggesting a potential role in detoxification of superoxide radicals (Broadley et al. 2012).

Some plants can tolerate salinity stress through osmotic adjustment, which involves the accumulation of solutes that help reduce cellular solute potential and maintain water uptake (Hasegawa et al. 2000). Proline is one such solute that can assist plants in adjusting the pH in the cytosol, thereby protecting cellular membranes and proteins from damage during salinity stress (Behzadi Rad et al. 2021). However, in this

study, the proline content in the leaves varied between the two penstemon species while remaining similar across all treatments. This suggests that proline may not play a significant role in the salinity stress responses for these penstemon species within the context of this study. The variations in proline content observed could be attributed to inherent genetic differences or physiological adaptations between the species.

Conclusions

Two penstemon species tested during this study showed variations in response to salinity stress. Saline solution irrigation reduced the growth and biomass of both penstemon species. The net photosynthetic rate, g_s and E of penstemons were negatively impacted by salinity stress. Furthermore, the Na^+ and Cl^- uptake increased, and the K^+ and Ca^{2+} uptake was also affected. Based on the findings of this study, *P. barbatus* and *P. strictus* are sensitive to salinity levels at ECs of 7.5 and 10.0 $dS \cdot m^{-1}$. These results have implications for the cultivation and management of these penstemon species in saline environments, thus highlighting the need for appropriate irrigation strategies to mitigate the negative effects of salinity stress on their growth and physiological processes.

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Table 4-1. Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), sulphate (SO_4^{2-}), chloride (Cl^{-}), and boron (B) contents, sodium adsorption ratio (SAR), adjusted SAR, and electrical conductivity (EC) of nutrient and saline solutions used to irrigate penstemon plants.

Item	**Nutrient Solution**[i]	**Saline solution**[ii]			
		2.5 dS·m^{-1}	**5.0 dS·m^{-1}**	**7.5 dS·m^{-1}**	**10.0 dS·m^{-1}**
NaCl	-	30.9	91.7	145.0	226.5
$CaCl_2 \cdot 2H_2O$	-	39.5	116.7	183.0	280.4
Ca^{2+} (mg·L^{-1})	48.1	189.7	448.6	723.3	960.7
Mg^{2+} (mg·L^{-1})	17.8	18.9	18.6	14.2	15.4
Na^{+} (mg·L^{-1})	1.3	140.8	374.0	638.1	876.1
SO_4^{2-} (mg·L^{-1})	2.7	3.1	3.5	5.5	5.3
Cl^{-} (mg·L^{-1})	1.1	428.0	1360.0	2280.0	3050.0
B (mg·L^{-1})	0.2	0.2	0.2	0.2	0.2
SAR	0.04	2.6	4.7	6.4	7.7
Adjusted SAR	0.1	3.6	7.7	11.8	14.6
EC (dS·m^{-1})	1.0 ± 0.1	2.5 ± 0.1	5.1 ± 0.1	7.6 ± 0.2	10.1 ± 0.2

[i] The nutrient solution at an electrical conductivity (EC) of 1.0 dS·m^{-1} was made by mixing 0.8 g·L^{-1} 15N-2.2P-12.5K water-soluble fertilizer (Peter Excel 15-5-15 Ca-Mag Special) in reverse osmosis water.

[ii] Sodium chloride (NaCl) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) were added at a molar ratio of 2:1 to the nutrient solution to prepare the saline solution.

Table 4-2. Visual score of penstemon plants in response to salinity stress.

Visual score	Salt damage[i]	Percentage of foliar salt damage (%)
0	Dead plants because of salinity stress	100
1	Severe foliar salt damage	>90
2	Moderate foliar salt damage	51-90
3	Slight foliar salt damage	10-50
4	Minimal foliar salt damage	<10
5	No foliar salt damage	0

[i]Burn and necrosis symptoms of penstemon leaves.

Table 4-3. Summary of the analysis of variance of the effects of species, treatments, and their interactions on visual score (VS) of *Penstemon barbatus* and *P. strictus* after irrigating with a nutrient solution [electrical conductivity (EC) = 1.0 $dS \cdot m^{-1}$] or saline solution [EC = 2.5, 5.0, 7.5 or 10.0 $dS \cdot m^{-1}$] for a period of 2, 4, 6, and 8 weeks, as well as on the increase in plant height (Ht), leaf area (LA), shoot dry weight (DW), root DW, chlorophyll content [Soil Plant Analysis Development (SPAD)], chlorophyll fluorescence parameters (F_v/F_m and PI_{abs}), net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and proline content after irrigating for a period of 8 weeks in a greenhouse.

	Analysis of variance														
Source	**VS (2)**	**VS (4)**	**VS (6)**	**VS (8)**	**Ht**	**LA**	**Shoot DW**	**Root DW**	**SPAD**	**F_v/F_m**	**PI_{abs}**	**P_n**	**g_s**	***E***	**Proline**
Species (S)	**[i]	***	***	*	NS	****	****	****	****	NS	NS	**	NS	NS	****
Treatment (T)	****	****	****	****	****	****	**	**	****	****	****	****	****	****	NS
S * T	**	**	****	NS	****	**	NS	NS	****	**	**	*	NS	NS	NS

[i]NS, *, **, ***, ****: Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

Table 4-4. Increase in the plant height (ht) and leaf area of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse.

EC ($dS \cdot m^{-1}$)	**Plant ht (cm)**		**Leaf area (cm^2)**	
	P. barbatus	***P. strictus***	***P. barbatus***	***P. strictus***
1.0	12.6 a[i]	4.2 a	991 a	1832 a
2.5	9.8 a	5.2 a	775 a	1614 a
5.0	10.6 a	5.9 a	610 a	1361 a
7.5	0.7 b	4.0 a	132 b	576 b
10.0	2.0 b	2.1 a	128 b	145 c
Linear	NS[ii]	NS	<0.0001	<0.0001
Quadratic	NS	NS	NS	<0.0001

[i]The mean values within a column for each species followed by the same letters are not significantly different at $\alpha = 0.05$ according to Tukey's method for multiplicity.

[ii]NS, not significant at $P < 0.05$.

Table 4-5. Dry weights (DWs) of shoots and roots of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse.

	Shoot DW (g)		**Root DW (g)**	
EC ($dS \cdot m^{-1}$)	***P. barbatus***	***P. strictus***	***P. barbatus***	***P. strictus***
1.0	23.2 a[i]	31.4 a	21.0 ab	54.2 ab
2.5	21.6 a	27.2 ab	25.5 a	47.8 ab
5.0	19.1 a	27.2 ab	11.6 b	55.2 a
7.5	21.6 a	21.9 b	10.6 b	41.2 ab
10.0	20.4 a	21.6 b	11.6 b	26.9 b
Linear	NS[ii]	0.02	NS	0.01
Quadratic	NS	NS	NS	0.047

[i] The mean values within a column for each species followed by the same letters are not significantly different at $\alpha = 0.05$ according to Tukey's method for multiplicity.

[ii]NS, not significant at $P < 0.05$.

Table 4-6. Contents of sodium (Na^+), chloride (Cl^-), calcium (Ca^{2+}), potassium (K^+), K^+/ Na^+ ratio, and Ca^{2+}/ Na^+ ratio in leaves of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse.

		Ion content ($mg \cdot g^{-1}$)					
Species	**EC ($dS \cdot m^{-1}$)**	**Na^+**	**Cl^-**	**Ca^{2+}**	**K^+**	**K^+/ Na^+**	**Ca^{2+}/ Na^+**
P. barbatus	**1.0**	0.05 d[i]	2.30 c	20.61 b	22.48 a	452.85 a	415.04 a
	2.5	0.41 c	12.03 b	23.61 b	19.05 a	45.97 b	56.97 b
	5.0	1.79 b	34.64 a	28.20 a	20.29 a	11.32 c	15.73 c
	7.5	4.48 a	47.58 a	31.45 a	21.87 a	4.88 d	7.02 d
	10.0	5.02 a	51.08 a	32.51 a	20.04 a	3.99 d	6.47 d
P. strictus	**1.0**	0.03 e	3.00 c	11.72 d	24.63 a	938.43 a	446.46 a
	2.5	0.14 d	11.57 b	13.97 c	21.95 ab	154.19 b	98.15 b
	5.0	0.50 c	32.50 a	17.93 b	20.11 ab	40.2 c	35.82 c
	7.5	1.34 b	47.36 a	23.10 a	22.46 ab	16.75 d	17.22 cd
	10.0	2.80 a	53.49 a	25.45 a	18.87 b	6.74 e	9.1 d
Species (S)		****[ii]	NS	****	NS	****	****
Treatment (T)		****	****	****	*	****	****
S * T		NS	NS	**	NS	NS	NS

[i] The mean values within a column for each species followed by the same letters are not significantly different at $\alpha = 0.05$ according to Tukey's method for multiplicity.

[ii] NS, *, **, ****: not significant or significant at $P < 0.05$, 0.01, or 0.0001, respectively.

Table 4-7. Contents of boron (B), magnesium (Mg^{2+}), phosphorus (P), zinc (Zn^{2+}), and manganese (Mn^{2+}) in leaves of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse.

		Ion content ($mg \cdot g^{-1}$)				
Species	**EC ($dS \cdot m^{-1}$)**	**B**	**Mg^{2+}**	**P**	**Zn^{2+}**	**Mn^{2+}**
P. barbatus	**1.0**	0.03 a[i]	4.57 a	3.19 a	0.02 a	0.03 b
	2.5	0.03 ab	4.44 a	3.11 a	0.02 a	0.04 ab
	5.0	0.02 bc	4.57 a	3.24 a	0.02 a	0.04 ab
	7.5	0.02 bc	4.11 a	3.27 a	0.02 a	0.05 a
	10.0	0.02 c	4.10 a	2.79 a	0.02 a	0.05 a
P. strictus	**1.0**	0.02 a	4.52 a	4.86 a	0.02 b	0.03 bc
	2.5	0.02 a	4.98 a	4.07 ab	0.03 ab	0.02 c
	5.0	0.02 bc	5.10 a	3.96 ab	0.03 a	0.04 ab
	7.5	0.02 b	5.15 a	3.85 b	0.03 ab	0.04 ab
	10.0	0.02 c	5.13 a	3.46 b	0.02 ab	0.06 a
Species (S)		****[ii]	****	****	*	NS
Treatment (T)		****	NS	**	**	***
S*T		NS	NS	NS	NS	NS

[i] The mean values within a column for each species followed by the same letters are not significantly different at $\alpha = 0.05$ according to Tukey's method for multiplicity.
[ii] NS, *, **, ***, ****: not significant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

Table 4-8. Proline contents in leaves of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solutions with varying EC levels ranging from 2.5 to 7.5 $dS \cdot m^{-1}$ in a greenhouse.[i]

Species	**Proline content ($\mu mol \cdot g^{-1}$)**			
	1.0	2.5	5.0	7.5
P. barbatus	0.30 a[ii]A[iii]	0.20 aB	0.21 aB	0.23 aB
P. strictus	0.64 aA	1.02 aA	0.60 aA	1.22 aA

[i] Leaves were harvested after the sixth irrigation event. Because of foliar damage observed in penstemons when irrigated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$, proline estimation was not performed.

[ii] The mean values within a row followed by the same lowercase letters are not significantly different among treatments at $\alpha = 0.05$ according to Tukey's method for multiplicity.

[iii] The mean values within a column followed by the same uppercase letters are not significantly different between species at $\alpha = 0.05$ according to Tukey's method for multiplicity.

Fig. 4-1. Photos of representative penstemons after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) and saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse.

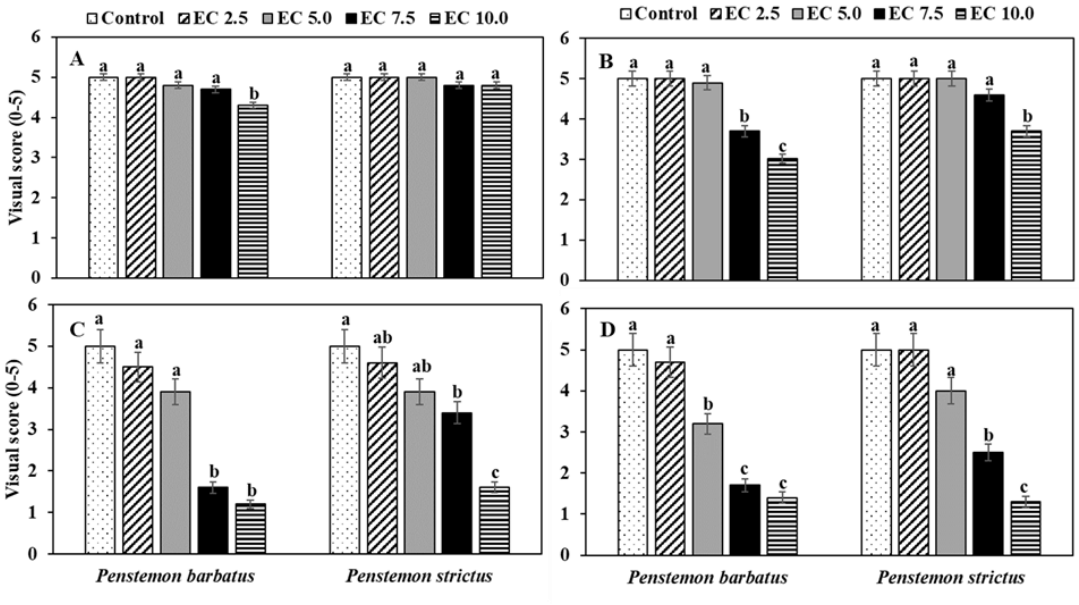

Fig. 4-2. Visual score of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) and saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period 2 (A), 4 (B), 6 (C), and 8 (D) weeks in a greenhouse. Vertical bars represent *SE*s of 10 plants. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$. Visual scores: 0 = dead plant due to salinity stress; 1 = severe foliar damage (burnt leaves and necrosis, >90%); 2 = moderate foliar damage (51% -90%); 3 = slight foliar damage (10%-50%); 4 = good quality with minimal foliar damage (<10%); and 5 = excellent without foliar damage (Sun et al. 2015).

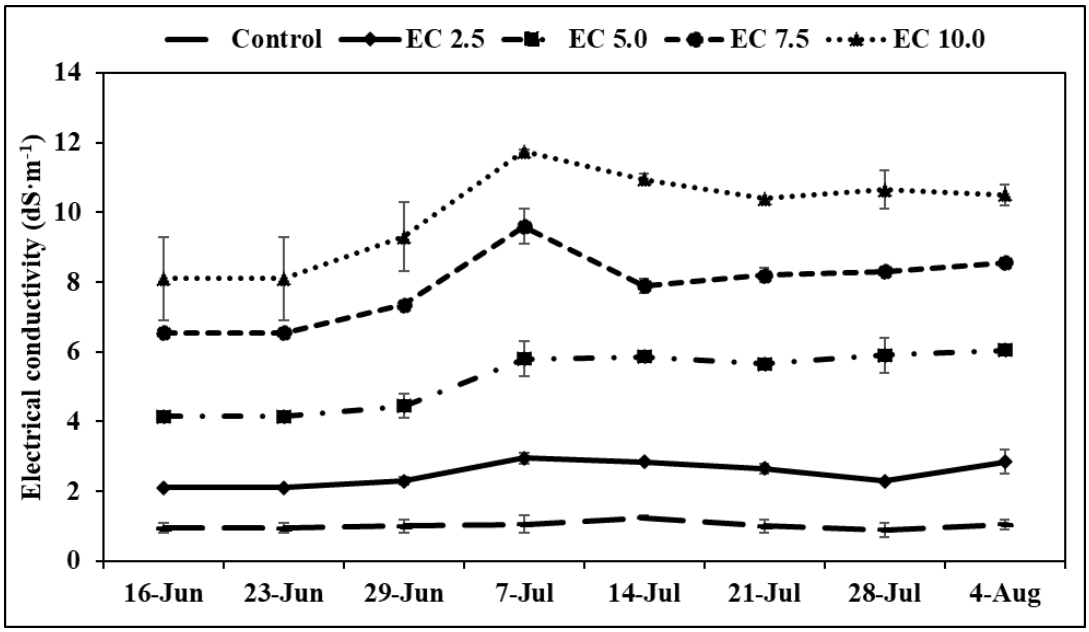

Fig. 4-3. Electrical conductivity (EC) of leachate solution over the course of the experiment collected after *Penstemon barbatus* and *P. strictus* were irrigated with a nutrient solution at an EC of 1.0 $dS \cdot m^{-1}$ (control) and saline solutions with varying EC levels ranging from 2.5 to 10.0 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse. Vertical bars represent *SEs* of two measurements.

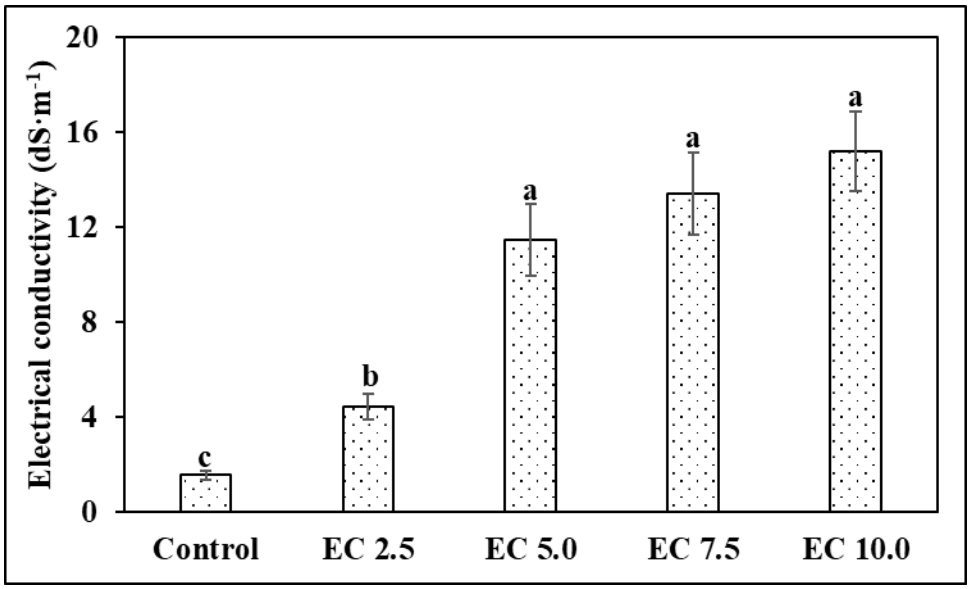

Fig. 4-4. Electrical conductivity (EC) of soil extraction from *Penstemon barbatus* and *P. strictus* irrigated with a nutrient solution at an EC of 1.0 dS·m^{-1} (control) and saline solutions with varying EC levels ranging from 2.5 to 10.0 dS·m^{-1} for a period of 8 weeks in a greenhouse. Vertical bars represent standard errors of five measurements.

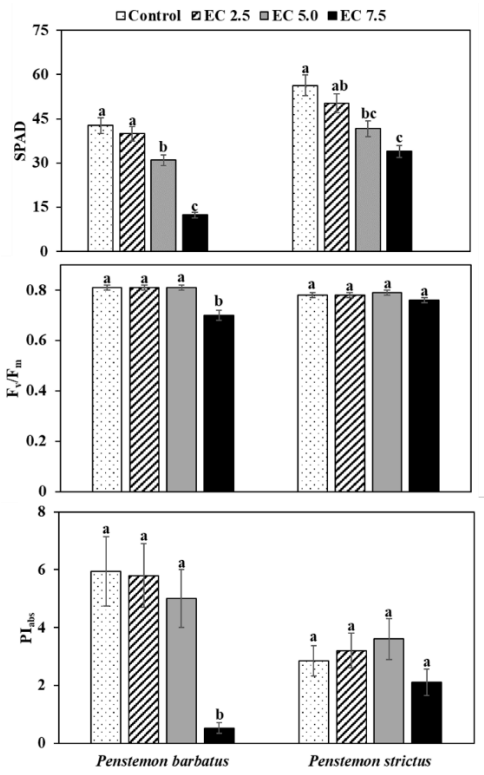

Fig. 4-5. Chlorophyll content [Soil Plant Analysis Development (SPAD)], chlorophyll fluorescence parameters (F_v/F_m, and PI_{abs}) of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) and saline solutions with varying EC levels ranging from 2.5 to 7.5 $dS \cdot m^{-1}$ for a period of 8 weeks in a greenhouse. Vertical bars represent standard errors of 10 measurements for SPAD and six measurements for F_v/F_m, and PI_{abs}. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$. Penstemons were dead or had severe foliar salt damage when treated with saline solution at an EC of 10.0 $dS \cdot m^{-1}$; therefore, the chlorophyll content and fluorescence data were not taken.

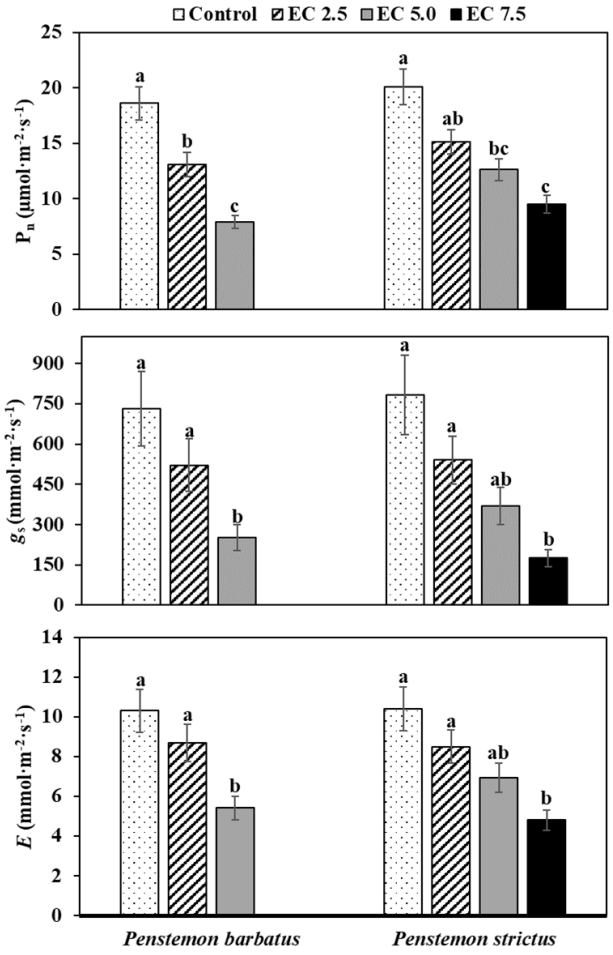

Fig. 4-6. Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) of *Penstemon barbatus* and *P. strictus* after irrigation with a nutrient solution at an electrical conductivity (EC) of 1.0 dS·m^{-1} (control) and saline solutions with varying EC levels ranging from 2.5 to 7.5 dS·m^{-1} for a period of 8 weeks in a greenhouse. Vertical bars represent *SEs* of five measurements. The same letters above column bars within species represent no significance among treatments as determined by Tukey's method for multiplicity at $\alpha = 0.05$. Gas exchange data of *P. barbatus* at ECs of 7.5 and 10.0 dS·m^{-1} and *P. strictus* at an EC of 10.0 dS·m^{-1} were not measured as plants had severe foliar salt damage or were dead.

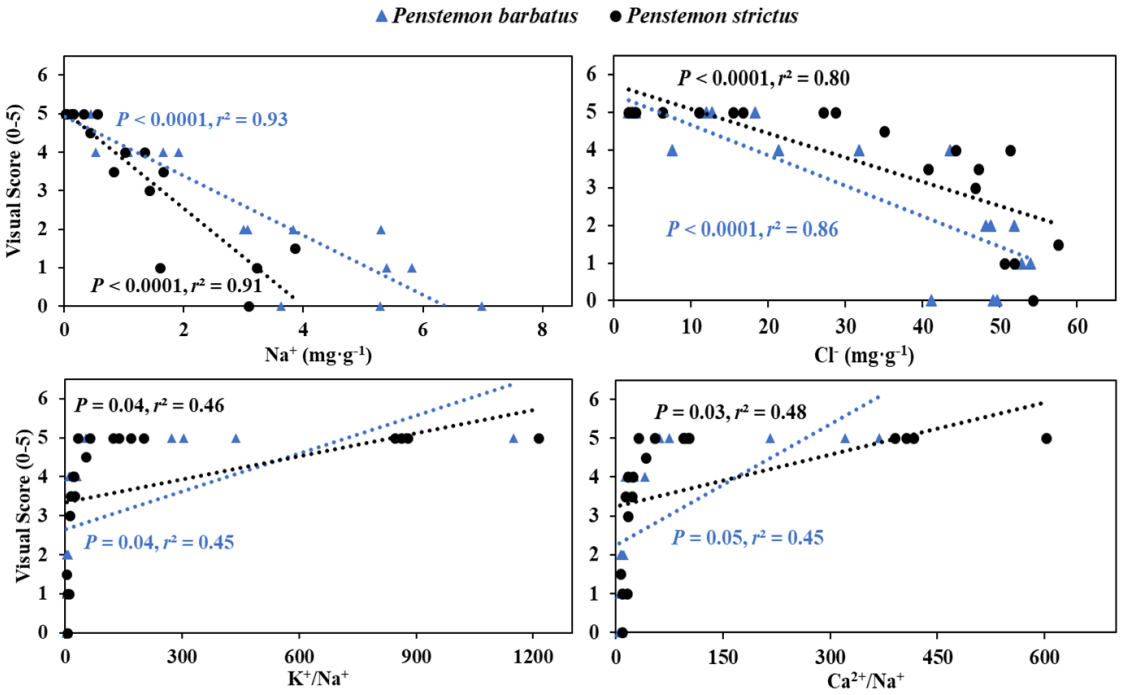

Fig. 4-7. Correlation analyses between the sodium (Na^+), chloride (Cl^-), potassium-to-sodium ratio (K^+/Na^+), calcium-to-sodium ratio (Ca^{2+}/Na^+), and visual score of *Penstemon barbatus* and *P. strictus*. Visual score: 0 = dead plant due to salinity stress; 1 = severe foliar damage (burnt leaves and necrosis, >90%); 2 = moderate foliar damage (51%-90%); 3 = slight foliar damage (10%-50%); 4 = good quality with minimal foliar damage (<10%); and 5 = excellent without foliar damage (Sun et al. 2015).

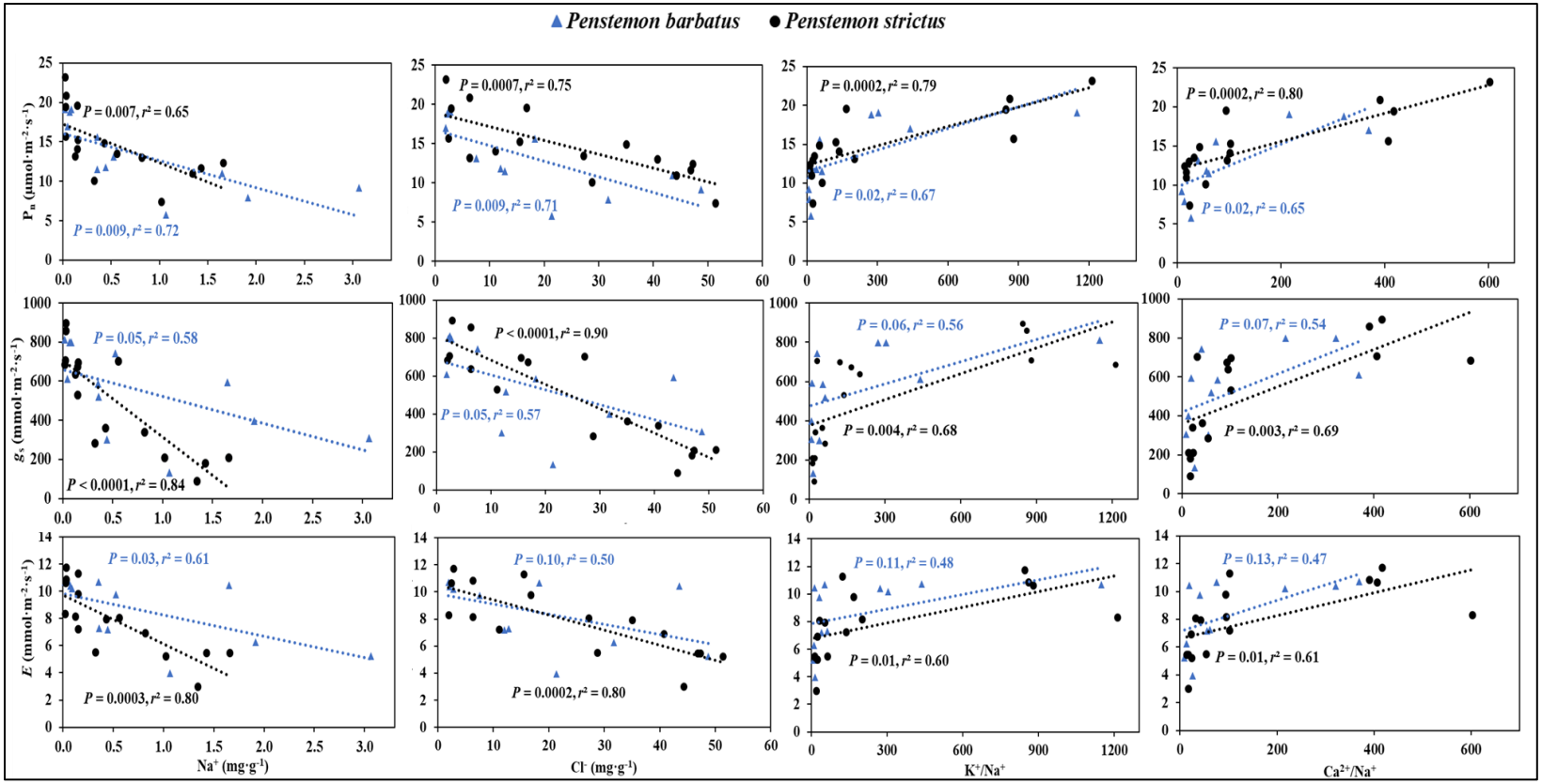

Fig. 4-8. Correlation analyses between the sodium (Na^+), chloride (Cl^-), potassium-to-sodium ratio ($K^+/Na^{+)}$, calcium-to-sodium ratio (Ca^{2+}/Na^+), net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of *Penstemon barbatus* and *P. strictus*.

CHAPTER V

EXPLORING GROWTH, PHYSIOLOGICAL, AND MOLECULAR RESPONSES TO SALINITY IN *PUNICA GRANATUM* 'WONDERFUL'

Abstract

Soil salinity poses a significant environmental challenge that impacts the growth of landscape plants globally. Tolerance to salinity differs among species, each possessing unique mechanisms to mitigate the negative effects of stress. Therefore, it is important to explore the responses of landscape plants to salinity tolerance. This study aimed to understand the impacts of varying salinity levels on the growth, gas exchange, biochemical processes, mineral nutrients, and gene expression in *Punica granatum* 'Wonderful' (pomegranate). Plants were irrigated weekly with a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ as the control, or with a saline solution at an EC of 5.0 or 10.0 $dS \cdot m^{-1}$ for 8 weeks. Throughout the entire experimental period, the plants exhibited no foliar salt damage. Similarly, plant height was unaffected by salinity stress. However, elevated salinity levels led to reduction in shoot dry weight. In addition, the net photosynthetic rate (P_n) was reduced at higher salinity levels. As the salinity levels of treatment solution increased, the chloride (Cl^-) concentration in leaves was elevated. Chloride accumulation was greater in leaves than in stems or roots. However, sodium (Na^+) accumulation was greater in roots compared to that in stems and leaves. The Na^+ content in leaf tissue in all treatments was less than 1 $mg \cdot g^{-1}$. Gene expression results revealed that *P. granatum* 'Wonderful' exhibited an early up-regulation of sodium/hydrogen exchanger (*NHX1*) and salt overly sensitive (*SOS2*) genes in the leaves and late up-regulation of high-affinity potassium transporter (*HKT1*) gene in the roots in

response to salinity stress. These results suggest that *P. granatum* 'Wonderful' is sufficiently tolerant to salinity level up to the EC of 10.0 $dS \cdot m^{-1}$ with slight growth reduction.

Introduction

Salinity is a major environmental challenge limiting plant productivity, particularly in arid and semi-arid regions (Ashraf and Harris 2004). The harmful effects of salinity depend on various factors, including plant species, climatic conditions, and soil conditions (Tang et al. 2015). Plants are classified as salt-tolerant 'halophytes' and salt-sensitive 'glycophytes' based on their ability to thrive in saline conditions (Himabindu et al. 2016). The majority of plant species are glycophytes and can be easily damaged by high salinity, although their responses vary (Greenway and Munns 1980; Xiong and Zhu 2002).

The presence of sodium chloride (NaCl) in the soil leads to elevated sodium (Na^+) and chloride (Cl^-) levels within plants, causing osmotic and ionic stresses (Munns and Tester 2008). Initially, plants face osmotic stress upon exposure to saline conditions, which can affect their growth. Furthermore, ionic toxicity occurs when salt content increase in plant tissues, disrupting turgor, photosynthesis, nutrient uptake, enzymatic processes, and protein synthesis (Maathuis and Amtmann 1999; Parihar et al. 2015). In addition, plants subjected to salinity stress experience oxidative damage through an increase in reactive oxygen species (ROS), including singlet oxygen (O_2), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^-) (Miller et al. 2010).

The overproduction of ROS reduces the efficiency of photosynthetic electron transport and induces lipid peroxidation of plasma membrane (Hasanuzzaman et al. 2021).

Some plants can tolerate salinity stress, but the mechanisms of tolerance vary among species and even within cultivars of the same species. The response to salinity tolerance in a plant comprises various factors that depend on complex physiological interactions (Grattan and Grieve 1999; Munns 2002). Throughout their life cycle, plants employ different strategies to cope with salinity stress. They adapt by managing increased vacuolar Na^+ levels through the maintenance of cellular ion balance and the accumulation of osmotic-adjustment compounds such as soluble sugars and amino acids (Flowers and Colmer 2015), as well as by activating antioxidant systems that protect them from damage caused by ROS (Pang and Wang 2008).

Plant salinity tolerance is a quantitative trait controlled by multiple genes (Kaundal et al. 2022; Munns and Tester 2008; Volkov 2015). When plants are exposed to salinity stress, the concentration of ions such as Na^+ and Cl^- in the cytosol increases. Sodium, if accumulated in the cytosol, can be toxic. Sodium can move symplastically into an adjacent cell through plasmodesmata and efflux back into the cell wall or be transported into the vacuole. The efflux of Na^+ occurs through plasma membrane antiporters such as salt overly sensitive (SOS) genes (Zhu 2003). Similarly, compartmentalization in the vacuole occurs through Na^+/H^+ antiporters, such as NHX (Blumwald et al. 2000). In addition, mechanisms contributing to salt tolerance include the retrieval of Na^+ from the xylem (Apse and Blumwald 2007; Maathuis et al. 2014) and its circulation through the phloem to counteract the excessive accumulation of Na^+ in

aboveground tissues. These processes are regulated by the high-affinity potassium transporter (HKT) gene family.

Pomegranate (*Punica granatum*) is an important fruit crop cultivated in various climatic regions, including subtemperate, subtropical, and tropical areas (Verma et al. 2010). The fruit offers nutritional benefits and has medicinal uses (Teixeira da Silva et al. 2013). In addition, pomegranates have the potential to be cultivated in landscapes as an ornamental plant. In many states of the United States, pomegranate trees are cultivated commercially and grown in residential landscapes. The pomegranate cultivar 'Wonderful' comprises more than 90-95% of commercially grown pomegranates in the U.S. (Chater et al. 2018).

Furthermore, a few studies have identified *P. granatum* 'Wonderful' as a salt-tolerant cultivar (Abdeen and Mancy 2018; Calzone et al. 2020; Dichala et al. 2022; Sun et al. 2018; Sun et al. 2024). Understanding the mechanisms of salt tolerance is important for enhancing or breeding more resilient cultivars. However, these mechanisms have not yet been adequately understood in pomegranates (Dichala et al. 2022). This research aimed to determine the effects of salinity stress on the growth, physiological, and molecular characteristics of *P. granatum* 'Wonderful', to better understand the mechanisms of salt tolerance, and to provide a reference for pomegranate cultivation in saline areas.

Materials and Methods

Plant materials, and growth conditions. Punica granatum 'Wonderful' hardwood cuttings were received from Marcelino Nursery (Tornillo, TX, USA) on 27 Jan 2021 and

stored in a refrigerator at 4 ºC until propagation. Fresh slanted cuts were made at the base of the cuttings, and wounding was performed by scraping the bark on one side (~ 2 cm). The cuttings were dipped in double distilled water and then in talc-based formulation of 3000 mg·L^{-1} indole-3-butyric acid (IBA; Hormodin® 2; OHP, Inc., Mainland, PA, USA) and stuck in a rooting medium containing perlite (Expanded Perlite; Malad City, ID, USA) and peatmoss (SunGro Horticulture, Agawam, MA, USA) in a 2:1 volumetric ratio within yellow UV-stabilized cone-tainers (3.8 cm × 21.0 cm; SC10U, Stuewe & Sons, Tangent, OR, USA). These cuttings were placed on the bench with the intermittent mist system, controlled at 80 vapor pressure deficit (VPD) units using a Water Plus VPD mist controller (Phytotronics, Inc., Earth City, MO, USA). The rooted cuttings were transferred to a polyethylene greenhouse on 13 Aug 2021. Rooted cuttings were overwintered in a controlled environment maintained at 0 ºC, which slows down their growth and induces dormant state. On 11 May 2022, these rooted cuttings were transplanted to 3.9-L injection-molded, polypropylene containers (PC1D-4, Nursery Supplies, Orange, CA, USA) filled with a soilless growing medium (Metro-Mix® 820; Canadian sphagnum peat moss, 35-45% composted pine bark, coir, coarse perlite, and dolomitic limestone; SunGro Horticulture, Agawam, MA, USA), and placed in the Utah Agricultural Experiment Station's research greenhouse located in Logan, UT, USA (lat. 41° 45’ 28” N, long. 111° 48’ 48” W, elevation 1409 m). Water with a pH of 7.7 ± 0.2 (mean ± SD) and an electrical conductivity (EC) of 0.35 ± 0.01 dS·m^{-1} from Logan City was used to irrigate plants as needed. In addition, 15N-2.2P-12.5K water-soluble fertilizer (Peters Excel 15-5-15 Cal-Mag Special; ICL Specialty Fertilizers, Dublin, OH, USA) was applied twice at a one-month interval.

On 7 Jul 2022, plants were transplanted into 7.6-L injection molded polypropylene containers (No. 2B, Nursery Supplies, Orange, CA, USA) filled with the soilless growing medium (Metro-Mix® 820; SunGro Horticulture). Furthermore, plants were pruned to a uniform height of 30 cm on 12 Jul 2022. A shade cloth (60%) was placed at the top of the greenhouse during the research period. The experiment was initiated on 21 Jul and ended on 16 Sep 2022. The greenhouse temperature was maintained at 26.02 ± 0.30 ºC and 22.61 ± 0.52 ºC during the day and at night, respectively. The daily light integral (DLI) inside the greenhouse was 10.87 ± 3.87 $mol \cdot m^{-2} \cdot d^{-1}$ recorded using a full-spectrum quantum sensor (SQ-500-SS; Apogee Instruments Logan, UT, USA).

Experimental Treatments. *Punica granatum* 'Wonderful' was irrigated with nutrient or saline solutions at an electrical conductivity (EC) of 1.0 (control), 5.0, or 10.0 $dS \cdot m^{-1}$ (Paudel et al. 2024). The nutrient solution was prepared by adding 0.8 $g \cdot L^{-1}$ 15N-2.2P-12.5K water-soluble fertilizer to reverse osmosis water in a 100-L tank. Saline solutions were prepared using sodium chloride (NaCl; Fisher Scientific, Waltham, MA, USA) and dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$; Hi Valley Chemical, Centerville, UT, USA) in a molar ratio of 2:1 to the nutrient solution. The pH of the treatment solutions was adjusted to 5.6-6.5 using 88% potassium hydroxide pellets (Sigma-Aldrich, St. Louis, MO, USA) or 1M nitric acid (Fisher Chemical, Fair Lawn, NJ, USA). Each plant was irrigated once a week with 1000 ml of treatment solution for the first 3 weeks and 1500 ml for the subsequent 5 weeks. The first treatment application was performed on 21 Jul 2022, and the eighth treatment application was performed on 8 Sep 2022. Plants

were watered with an additional 500 to 600 ml of reverse osmosis water when the top (~ 1 cm) substrate was dry to prevent any potential complications from drought.

Leachate EC. After half hour of treatment application, leachate was collected using a pour-through method reported by Cavins et al. (2008), and the EC of the leachate was measured using an EC meter (LAQUA Twin, Horiba, Kyoto, Japan). When the EC of the leachate exceeded that of the treatment solution, the substrate was rinsed with reverse osmosis water to maintain consistent EC levels in the substrate throughout the experimental period. Two plants from each treatment group were selected to measure the leachate EC.

Growth analysis and plant harvest. Plant heights (centimeters) were recorded at the start and end of the experimental period. Half of the plants were destructively harvested on 16 Sep 2022, 1 week after the eighth treatment application (~8 weeks after initiation of experiment). After harvest, the leaf area (square centimeter) was recorded using a leaf area meter (LI-3100; LI-COR® Biosciences, Lincoln, NE, USA). Furthermore, the dry weight (g) of the shoots (stem and leaf) and roots were determined after being dried in an oven at 60 ºC for 1 week.

Photosynthetic parameters. The relative chlorophyll content was determined for all plants using a chlorophyll meter [Soil Plant Analysis Development (SPAD)-502; Minolta Camera, Osaka, Japan] before harvest. Eight mature leaves from each plant were measured, and the average value was recorded. Leaf gas exchange of five plants for each treatment were measured using a portable photosynthesis system (CIRAS-3; PP Systems, Amesbury, MA, USA) with an automatic universal leaf cuvette (PLC3; PP Systems). The parameters assessed during the leaf gas exchange measurements included net

photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), water use efficiency (WUE), and vapor pressure deficit (VPD). All plants were adequately watered one day prior to the measurements to prevent any potential water-induced stress conditions.

Mineral analyses. Dried *P. granatum* 'Wonderful' leaves, stems, and roots were finely grounded using a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and allowed to pass through a 1-mm-mesh screen. The powdered samples were subjected to mineral analysis at the USU Analytical Laboratories. The concentration of cations and various minerals including Na^+, calcium (Ca^{2+}), potassium (K^+), manganese (Mn^{2+}), phosphorus (P), sulfur (S), and zinc (Zn^{2+}) were determined using a method involving nitric/hydrogen peroxide, following the protocol reported in Gavlak et al. (2005). The concentration of chloride (Cl^-) was measured using a Flow Injection Analysis and Ion Chromatograph System (QuikChem 8000; Lachat Instrument, Loveland, CO, USA) and results were reported based on the dry weight of the plant material ($mg \cdot g^{-1}$). To ascertain the levels of Na^+, Ca^{2+}, K^+, Mn^{2+}, P, S, and Zn^{2+}, 0.5 g of powdered samples were combined with 6 ml of nitric acid (HNO_3) within a digestion tube. This mixture was then subjected to a digestion block, heated at 80 °C for 10 min, followed by cooling for 2 min. Subsequently, 2 ml of 30% hydrogen peroxide (H_2O_2) was added to the digestion tube, which was then placed back into the digestion block at 130 °C for 1 hour. The contents of the digestion tubes were thoroughly mixed using a vortex stirrer. After cooling to room temperature, the contents were transferred into a 25-ml volumetric flask. The resulting digest was analyzed using an Inductively Coupled Plasma-Optical Emission

Spectrometry (iCAP 6300 ICP-AES; Thermo Scientific, Waltham, MA, USA) and reported on the dry weight basis ($mg \cdot g^{-1}$).

Electrolyte leakage. For measurement of electrolyte leakage, the youngest fully expanded leaves of *P. granatum* 'Wonderful' were collected after 6 days of eighth treatment application. Leaves were collected from four plants in each treatment, with two leaves being taken from each plant. A total of 10 leaf discs (from two leaves) per plant were prepared using a single hole paper punch and placed into 50 ml centrifuge tube (VWR, Aurora, CO, USA). The tubes were then filled with 20 ml of deionized water and placed on a platform shaker (Innova 2100 Platform Shaker; New Brunswick Scientific, NJ, USA) for 20 hours at 150 rpm. After 20 hours, the EC of each sample was measured. Once the initial measurements were taken, samples were autoclaved (Sterivap 669, MMM Group, Munchen, Germany) for 15 minutes at 121 °C. The EC was measured again once the samples were cooled down to room temperature. The initial EC measurement before the autoclave cycle measured only the electrolytes that had leaked from salinity stress. On the other hand, the EC measurement after the autoclave cycle measured all the electrolytes from the ruptured cells. The electrolyte leakage was calculated using formula:

Electrolyte leakage = [(Before/After) x 100]

Before: measurement of EC obtained prior to the autoclave cycle

After: measurement of EC obtained after the autoclave cycle

Quantitative Reverse Transcription-PCR (qRT-PCR). Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was conducted to identify differentially expressed genes in *P. granatum* 'Wonderful'. Leaf and root tissues (100 mg)

were collected in liquid nitrogen and later stored at -80 ºC until used. Tissues samples were collected three times: 48 hours after the first treatment, 24 hours after the second treatment (after the first week), and 24 hours after the fifth treatment application (after the fourth week). For simplicity, the latter two collections are referred to as the end of first week and fourth week when discussed further. The extraction of total ribonucleic acid (RNA) from the leaf and root tissues was achieved using an RNA Kit (RNeasy® Plant Mini Kit; QIAGEN Sciences, MD, USA). A NanoDrop™ Spectrophotometer (Nanodrop 2000; Thermo Scientific, MA, USA) was utilized to determine the RNA concentration at 260 nm. Furthermore, deoxyribonucleic acid (DNA) free RNA was synthesized using TURBO DNA-free TM Kit (Thermo Scientific, Vilnius, Lithuania) following the manufacturer's instructions.

For the initial identification, catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen exchanger 1 (*NHX1*), and salt overly sensitive (*SOS1*, *SOS2*) genes were tested. Actin 7 (*ACT7*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used as endogenous reference genes. To design qRT-PCR primers, representative protein sequences of *Arabidopsis thaliana* (arabidopsis) were retrieved from Arabidopsis Information Resource (TAIR) database and used as query sequences in tBLASTn for translated nucleotide databases and searching the pomegranate genomic database. The corresponding mRNA sequence was derived from the coding sequence for each gene and was used to design PCR primers. For PCR amplicons, primers were designed from these sequences using the Integrated DNA Technologies (IDT) online software with following criteria: 18-20 bp primers size, primers melting temperature of 55-63 ºC, and primers GC content of 30-60% (Table 5-1). The specificity of the resulting

primer pair sequence was checked using BLAST analysis. The PCRs were performed using a 7500 Real-Time PCR System (CFX Connect™ Real-Time System, BIO-RAD, CA, USA). Normalized relative expressions of *CAT*, *HKT1*, *NHX1*, *SOS1*, and *SOS2* genes were studied. The reference genes were used on one plate for each time point, with plates prepared simultaneously.

Experimental design and statistical analysis

The experiment in greenhouse was conducted in a randomized complete block design with 3 treatments and 10 replications. The experimental unit consisted of one pot containing a single plant. Statistical analyses were conducted using SAS (version 3.81; SAS Institute, Cary, NC, USA) with PROC MIXED procedure. An analysis of variance was performed to evaluate the effects of salinity stress on various plant characteristics, including growth, biochemical process, gas exchange, mineral nutrients, and gene expression. Logarithmic transformation was applied for gas exchange parameters, mineral nutrients, and gene expression data to normalize the data. Trend analyses were conducted for plant growth and gas exchange parameters to test the relationship between plant responses and salinity levels. Mean separation among treatments was adjusted using Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

Results

Leachate EC

Leachate EC was used as an indirect method of measuring salinity levels in growing substrates. In this study, the EC of the leachate solution remained relatively consistent throughout the experiment (Fig. 5-1). The highest leachate EC for the nutrient

solution treatment of 1.0 $dS \cdot m^{-1}$ was 1.5 $dS \cdot m^{-1}$, and for saline solutions of 5.0 and 10.0 $dS \cdot m^{-1}$, it was 7.7 and 12.5 $dS \cdot m^{-1}$, respectively.

Morphological performance

No foliar salt damage was observed in *P. granatum* 'Wonderful' plants during the experimental period. No difference in height was observed for *P. granatum* 'Wonderful' when treated with a nutrient solution at an EC of 1.0 $dS \cdot m^{-1}$ (control) or saline solutions at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ (Table 5-2). This indicates that the salinity treatments had no significant effect on the height of *P. granatum* 'Wonderful'. However, plants treated with treatment solution at an EC of 10.0 $dS \cdot m^{-1}$ showed a significant decrease in leaf area compared to those treated with an EC of 1.0 $dS \cdot m^{-1}$ (control) or 5.0 $dS \cdot m^{-1}$ ($P = 0.0006$). A quadratic reduction in the leaf area was observed with increasing EC of the treatment solution ($P = 0.0003$). The leaf area decreased from 1457 to 1076 cm^2 per plant as the EC in the treatment solutions was increased from 1.0 to 10.0 $dS \cdot m^{-1}$. Additionally, the shoot dry weight exhibited a significant reduction in response to salinity stress ($P = 0.02$), decreasing from 34.7 to 25.9 g per plant as the salinity level in the treatment solution was increased from 1.0 to 10.0 $dS \cdot m^{-1}$. A quadratic reduction in the shoot dry weight was observed with the increasing EC of the treatment solution ($P = 0.005$). In contrast, the root dry weight remained consistent when treated with either nutrient or saline solutions.

Relative chlorophyll content and gas exchange

The relative chlorophyll content of *P. granatum* 'Wonderful', measured using a SPAD meter, was reduced linearly with the increasing EC of the treatment solution ($P = 0.01$) (Table 5-3). It was highest at 59.8 when plants were treated with the nutrient solution. In contrast, SPAD value was reduced to 56.2 when plants were treated with the

saline solution at an EC of 5.0 $dS \cdot m^{-1}$. In addition, SPAD value was 57.8 at an EC of 10.0 $dS \cdot m^{-1}$.

There was a significant difference observed for P_n ($P < 0.0001$) and g_s ($P < 0.0001$) in *P. granatum* 'Wonderful' plants among treatments (Table 5-3). At harvest, the P_n and g_s both decreased linearly and quadratically with the increasing EC of the treatment solution (both P values ≤ 0.009). Net photosynthetic rates were measured at 18.0, 16.2, and 14.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, when treated with nutrient and saline solutions at ECs of 1.0, 5.0, and 10.0 $dS \cdot m^{-1}$. Furthermore, g_s values were recorded at 435.8, 194.8, and 133.3 $mmol \cdot m^{-2} \cdot s^{-1}$, respectively, when treated with nutrient and saline solutions at ECs of 1.0, 5.0, and 10.0 $dS \cdot m^{-1}$. Similarly, there was a significant difference observed for E in *P. granatum* 'Wonderful' plants among treatments (Table 5-3) ($P = 0.004$). At harvest, E decreased linearly and quadratically as EC of the treatment solution increased (both P values ≤ 0.01). On the other hand, WUE of plants did not show significant differences among treatments. However, a significant difference observed for VPD among treatments ($P < 0.0001$). Similarly, the VPD increased linearly and quadratically with increasing salinity levels in the treatment solution (both P values ≤ 0.003). Leaf VPD was increased from 2.2 to 3.1 kPa with increasing salinity levels in the treatments.

Mineral contents

In *P. granatum* 'Wonderful', there were significant differences observed in both Na^+ ($P < 0.0001$) and Cl^- ($P < 0.0001$) contents across the treatments and plant parts. When treatment solution at an EC of 1.0 $dS \cdot m^{-1}$ was applied, the leaf Na^+ content was 0.02 $mg \cdot g^{-1}$, while Cl^-, Ca^{2+}, and K^+ content was 5.7, 15.4, and 24.4 $mg \cdot g^{-1}$, respectively

(Fig. 5-2). As salinity levels increased, there was an increment in both Na^+ and Cl^- contents, with values reaching 0.11 mg·g^{-1} and 13.7 mg·g^{-1} at 5.0 dS·m^{-1}, and 0.25 mg·g^{-1} and 18.4 mg·g^{-1} at 10.0 dS·m^{-1}, respectively. However, the Ca^{2+} and K^+ contents in the leaves remained relatively stable across the treatments. In the stems, Na^+ content increased from 0.04 mg·g^{-1} at an EC of 1.0 dS·m^{-1} to 1.01 mg·g^{-1} at an EC of 10.0 dS·m^{-1}. Similarly, Cl^- content increased from 4.1 mg·g^{-1} to 7.8 mg·g^{-1} at an EC of 5.0 dS·m^{-1} but was not significantly different among the treatments. Similarly, Ca^{2+} and K^+ contents in the stems remained relatively constant across the treatments. The Na^+ content in roots showed increasing trends, which increased 6 and 8 times when treatment solution at ECs of 5.0 and 10.0 dS·m^{-1} was applied to plants compared with control. Chloride, Ca^{2+}, and K^+ content was similar in roots of *P. granatum* 'Wonderful' across the treatments.

The accumulation of Mn^{2+}, P, S, and Zn^{2+} varied in the leaves, stems, and roots of *P. granatum* 'Wonderful' (all P values < 0.0001). Similarly, content of Mn^{2+}, P, S, and Zn^{2+} were different among treatments (all P values ≤ 0.01; Fig. 5-3). In the leaves, Mn^{2+} content was highest at 0.07 mg·g^{-1} when treated with saline solution at an EC of 10.0 dS·m^{-1}. However, P content was highest at 4.6 mg·g^{-1} when treated with nutrient solution. Furthermore, S content in leaves were similar among treatments, and Zn^{2+} content was highest at 0.03 mg·g^{-1}, when treated with a saline solution at an EC of 10.0 dS·m^{-1}. The Mn^{2+}, P, and Zn^{2+} contents in stems were higher at an EC of 10.0 dS·m^{-1}. In the roots, P and S content were similar among treatments. The Mn^{2+} content was highest in roots at ECs of 5.0 and 10.0 dS·m^{-1}, measuring 0.02 mg·g^{-1}. In addition, Zn^{2+} content was highest in roots at ECs of 5.0 and 10.0 dS·m^{-1}, measuring 0.03 mg·g^{-1}.

Electrolyte leakage

The electrolyte leakage from the leaves of pomegranate remained stable across all the treatments (Table 5-4). The electrolyte leakage observed after 6 days of eighth treatment application was 18-19%.

Gene expression

In the leaves of *P. granatum* 'Wonderful', 48 hours after the initial treatment application the expression of *CAT* was higher at an EC of 5.0 $dS \cdot m^{-1}$ when compared with 1.0 $dS \cdot m^{-1}$ (control). By the end of the first week, the expression of *CAT* was higher at an EC of 10.0 $dS \cdot m^{-1}$ but was not statistically different (Fig. 5-4; Fig. 5-5). The expression of *CAT* remained similar among all treatments by the end of the fourth week. Furthermore, the expression of *HKT1* showed variation among treatments at different time points, but the differences were not statistically significant. On the other hand, *NHX1* expression was significantly upregulated at an EC of 10.0 $dS \cdot m^{-1}$ 48 hours after the initial treatment application, but expression levels did not vary by the end of the first week and the fourth week. *SOS1* expression showed no difference among treatments both 48 hours after the initial treatment application and by the end of the first week. However, by the end of the fourth week, the expression of *SOS1* was downregulated at an EC of 10.0 $dS \cdot m^{-1}$ when compared with 1.0 $dS \cdot m^{-1}$ and 5.0 $dS \cdot m^{-1}$. Furthermore, after 48 hours of initial treatment application, the expression of *SOS2* was significantly upregulated at an EC of 10.0 $dS \cdot m^{-1}$ when compared with 1.0 $dS \cdot m^{-1}$ and 5.0 $dS \cdot m^{-1}$. However, the expression of *SOS2* at the end of fourth week follows the opposite pattern but not significantly different. In addition, the expression of *SOS2* showed no difference among treatments at the end of first week.

In the roots of *P. granatum* ‘Wonderful’, the expression of *CAT* remained consistent after 48 hours of first treatment application and by the end of first week (Fig. 5-6; Fig. 5-7). By the end of fourth week, the expression of *CAT* was upregulated at an EC of 10.0 $dS \cdot m^{-1}$ when compared with 1.0 $dS \cdot m^{-1}$ and 5.0 $dS \cdot m^{-1}$. After 48 hours of first treatment application, the expression of *HKT1* were similar among treatments. Furthermore, the expression of *HKT1* was upregulated at EC of 10.0 $dS \cdot m^{-1}$ by the end of first and fourth week when compared with 1.0 $dS \cdot m^{-1}$. In addition, lower expression of *HKT1* in all treatments was observed by the end of first week. On the other hand, the expression of *NHX1* and *SOS1* were downregulated at an EC of 10.0 $dS \cdot m^{-1}$ after 48 hours of first treatment application. The maximum expression of *NHX1* was observed by the end of fourth week. Similarly, the expression of *SOS2* were not significantly different among treatments at all time points.

Discussion

The *P. granatum* ‘Wonderful’ plants exhibited no visible foliar salt damage throughout the entire experimental period. *Punica granatum* ‘Wonderful’ may possess the ability to tolerate salinity stress without showing visible signs of salt damage. Plant growth parameters are important indicators used to assess a plant’s ability to survive in a saline environment. Interestingly, there was no significant difference in height observed among *P. granatum* ‘Wonderful’ plants subjected to either nutrient solution or saline solution. This implies that salinity stress did not have a substantial impact on the pomegranate height, aligning with previous research findings indicating that salinity treatment did not inhibit the plant height of the *P. granatum* ‘Wonderful’ (Sun et al. 2018;

Sun et al. 2024). In contrast, another study reported that the shoot length of *P. granatum* 'Wonderful' seedlings decreased with increasing salinity levels in the treatment solution (Abdeen and Mancy 2018). This difference could be attributed to variations in climate, growing substrates, and saline solutions used. Abdeen and Mancy (2018) used sandy soil with compost as a growing substrate and natural saline water as treatments at salinity levels of 1500, 3000, and 4500 $mg \cdot L^{-1}$ (~ 2.3, 4.7, and 5.6 $dS \cdot m^{-1}$, respectively).

Furthermore, in this study, leaf area and shoot dry weight of *P. granatum* 'Wonderful' plants were significantly reduced with increasing salinity levels, which is consistent with previous findings (Abdeen and Mancy 2018; El-Khawaga et al. 2013; Sun et al. 2018). The reduction in plant growth due to salinity stress is primarily attributed to the excessive buildup of salts in the root zone, which subsequently impacts water absorption (Munns 2002). However, the dry weight of roots showed no significant changes among treatments. Similarly, Liu et al. (2020) reported no reduction in the root dry weight of *P. granatum* 'Taishanhong' up to 300 mM (~22.0 $dS \cdot m^{-1}$) of NaCl.

Salinity stress reduces photosynthesis through mechanisms such as increased stomatal sensitivity or a decrease in chlorophyll content (Arif et al. 2020; Betzen et al. 2019). When initially exposed to salinity, plants experience water deficit due to osmotic stress, leading to the closure of stomata in response to factors like decreased leaf turgor, a high vapor pressure deficit in the atmosphere, or chemical signals from the roots (Liu et al. 2011). This reduces the supply of CO_2 to Rubisco, resulting in a decrease in P_n. In this study, salinity stress had a significant impact on the P_n and SPAD of *P. granatum* 'Wonderful'.

Furthermore, a significant reduction in g_s and E was observed in *P. granatum* 'Wonderful' due to salinity stress. This decline in g_s may have resulted in a lower concentration of intercellular CO_2, thereby reducing the activity of various enzymes, limiting carboxylation, and reducing the overall photosynthetic rate (Chaves et al. 2009). Similarly, Sun et al. (2018) reported reductions in P_n and g_s of *P. granatum* 'Wonderful' under salinity stress. Furthermore, reduction in E serves as an adaptive mechanism for plants, helping to minimize the uptake of harmful salts (Hasegawa et al. 2000). In this study, leaf WUE showed no significant difference among treatments throughout the experiment. In contrast, an increase in VPD was observed in *P. granatum* 'Wonderful' leaves due to salinity stress. This rise in VPD indicates elevated forces exerted on the plant, from leaves to roots, ultimately subjecting the plants to stress (Koverda 2020).

Abundant Na^+ and Cl^- are the primary minerals for causing detrimental effects in plants when subjected to salinity stress. These ions primarily influence important metabolic processes like photosynthesis in leaves and nutrient uptake in roots (Teakle and Tyerman 2010; White and Broadley 2001). In response to the excessive Na^+ and Cl^-, plants employ three fundamental strategies in their roots: (i) adjusting osmotic potential, (ii) excluding Na^+ and Cl^- from stems and leaves, and (iii) developing tissue tolerance to the accumulation of Na^+ and Cl^- (Apse and Blumwald 2007; Munns and Tester 2008). In this study, no significant differences in Na^+ content were observed in the leaves of *P. granatum* 'Wonderful' among the treatments. However, differences in Na^+ accumulation were observed in stems and roots. The highest Na^+ content at 1.61 $mg \cdot g^{-1}$ was observed in roots after the application of treatment at an EC of 10.0 $dS \cdot m^{-1}$ for 8 weeks. One important mechanism contributing to salt tolerance is the management of Na^+ absorption

from the roots and its distribution within the plant to prevent harmful accumulation of Na^+ in the shoots (Tester and Davenport 2003). The *P. granatum* 'Wonderful' plants in this study may have such mechanisms, which help in maintaining low shoot Na^+ levels. These findings align with previous research, indicating that *P. granatum* 'Wonderful' plants effectively maintained low shoot Na^+ content under salinity stress (Calzone et al. 2021; Sun et al. 2018).

The Cl^- content was increased in leaves of *P. granatum* 'Wonderful' but remained similar in stems and roots. Chloride accumulation in the leaves was over two-fold higher than that of Na^+. The minimal Cl^- content required for plant growth usually ranges from 0.2 to 0.4 $mg \cdot g^{-1}$, depending on the specific plant species. Some species have demonstrated the capacity to tolerate significantly higher levels of Cl^- (Colmenero-Flores et al. 2019). Similarly, in this study, *P. granatum* 'Wonderful' exhibited tolerance to Cl^- content up to 18.4 $mg \cdot g^{-1}$ without showing any symptoms of salt damage.

The Ca^{2+} and K^+ content of *P. granatum* 'Wonderful' were not affected by the salinity stress in this study. Interestingly, the accumulation of Ca^{2+} and K^+ was notably higher in the leaves, showing a two-fold increase compared to the levels observed in the stems and roots. This indicates a distinct distribution pattern of these minerals within the plant. During salinity stress, Na^+ competes with K^+ for uptake through Na^+-K^+ cotransporters due to their similar chemical properties (Jouyban 2012; Zhu 2003). The results from this study suggest that *P. granatum* 'Wonderful' plants possess effective mechanisms for uptaking K^+ into leaf tissue. Potassium is an essential mineral nutrient for optimal plant growth and development, and it plays a crucial role in maintaining cell

turgor, osmoregulation, leaf stomata movements, and enzyme activation (Shabala and Pottosin 2014).

There was a noticeable reduction in P content in the leaves. Similarly, it has been previously reported that P content significantly decreased with increasing salinity levels in pomegranates (Abdeen and Mancy 2018; Kulkarni et al. 2007). The reduction of P content in plant tissue during saline condition may be due to the presence of Cl^- in salinity treatments which can interfere with P absorption (Abdeen and Mancy 2018). Furthermore, in the current research, Mn^{2+}, S, and Zn^{2+} content were similar or increased at higher salinity levels, which could be one of the response mechanisms to counteract the effects of increased salinity, since it is an essential micronutrient that plays a crucial role in various physiological processes.

The consistent electrolyte leakage levels suggest the integrity of the cell membranes remained largely intact during salinity stress. The plant's ability to maintain membrane integrity could be an important factor in its tolerance to salinity stress.

Salinity stress responses include the activation of sensing and signaling pathways, followed by transcriptional reprogramming, which results in the activation of mechanisms for cellular homeostasis, ROS detoxification, osmoprotection, and ion homeostasis (Nefissi Ouertani et al. 2021). The differential expression of several genes has been reported to enhance salinity tolerance in the plants. According to our results, expression of *CAT* in roots by the end of fourth week was higher at an EC of 10.0 $dS \cdot m^{-1}$ when compared with the control. Elevated levels of *CAT* activity, one of the enzymes for scavenging ROS, were observed in pomegranate plants experiencing stress. This

indicates that *CAT* may have played a beneficial role in regulating the amount of ROS within cells under saline conditions (Pourghayoumi et al. 2017).

Genes involved in maintaining K^+ and Na^+ homeostasis in higher plants are considered candidates for genetic manipulation (Munns 2005). For example, *HKT1* is important for regulating Na^+ and K^+ homeostasis. However, in this study the expression levels of *HKT1* in leaves of *P. granatum* 'Wonderful' were not significantly different among treatments. Similarly, in previous research, Calzone et al. (2021) discussed that *HKT1* was not identified as being involved in Na^+ recirculation in *P. granatum* 'Wonderful' leaves. However, the expression of *HKT1* in the roots of *P. granatum* 'Wonderful' increased at an EC of 10.0 $dS \cdot m^{-1}$ by the end of first and fourth week. *HKT1* may have played a role in retrieving Na^+ from xylem and bringing it back to the roots, thereby inhibiting its movement to the shoot. Similarly, in response to salinity stress, *HKT1* was mainly expressed in roots of the halophytic grass *Puccinellia tenuiflora* (forage grass) (Zhang et al. 2017). In addition, the roots of *Prunus persica* 'Nemaguard' (almond) showed expression of *HKT1* under salinity stress (Kaundal et al. 2019). The upregulation of the *HKT1* gene in roots may have contributed to ion homeostasis in plants (Zhang et al. 2017).

In addition, ion transport can occur actively through symporters and antiporters, which transport ions against an electrochemical gradient (Tester and Davenport 2003). The NHX family of antiporters (Na^+/H^+ exchangers) are selective for Na^+. *NHX1* plays a role in transporting Na^+ into the vacuole. In this study, expression of *NHX1* after 48 hours of the first treatment application was upregulated in leaves at an EC of 10.0 $dS \cdot m^{-1}$ when compared to those at ECs of 1.0 and 5.0 $dS \cdot m^{-1}$. However, the lack of significant changes

in *NHX1* expression in leaves by the end of first and fourth week indicates a potential adaptation or acclimation of the plant to prolonged salinity stress. It is possible that other regulatory mechanisms come into play over time, mitigating the need for continued upregulation of *NHX1*. Similarly, *NHX1* expression was downregulated in roots at an EC of 10.0 $dS \cdot m^{-1}$ suggest its minimal role in salinity tolerance in this study; however, it helps sequester the ions in the vacuole in the early stages in leaves. Similarly, the expression level of *NHX1* in shoots was higher than in roots in *P. tenuiflora* (Zhang et al. 2017). A similar trend was observed in *Gossypium hirsutum* (cotton) and *Dendranthema morifolium* (chrysanthemum) (Wu et al. 2004; Zhang et al. 2012).

Under saline conditions, in the presence of high levels of external Na^+, Na^+ efflux from plant cells is an active process (Apse and Blumwald 2007). The SOS1 Na^+/H^+ antiporter plays an important role in Na^+ exclusion and overall plant salt tolerance (Saibi and Brini 2021). In this study, expression of *SOS1* in leaves was unaffected after 48 hours and 1 week but was downregulated in higher salinity level by the end of fourth week. This suggests that *SOS1* may not play an immediate role in the early response to salinity stress. The downregulation after 4 weeks could signify a potential adaptation strategy employed by the plant to mitigate the effects of prolonged exposure to high salinity. In current research, the expression of *SOS1* was downregulated in higher salinity level in roots after 48 hours of first treatment application and unaffected at other time points. Similarly, Calzone et al. (2021) reported that salinity treatments never upregulated *SOS1* expression in roots of *P. granatum* 'Wonderful'.

SOS2 acts as a multifunctional regulator in plant salt tolerance, particularly modulating Na^+ extrusion, a crucial component of salt tolerance (Kaundal et al. 2022;

Yang et al. 2015). In this study, the expression of *SOS2* in *P. granatum* 'Wonderful' leaves was upregulated in higher salinity levels after 48 hours of first treatment application. The rapid upregulation of *SOS2* indicates its role in the initial defense against salinity stress. In accordance with the less Na^+ content, results in this work indicate that upregulation of *SOS2* along with *NHX1* in leaves, and *HKT1* in roots might have promoted the efflux and accumulation of Na^+ in roots and thereby influencing its distribution to leaves. However, no changes in expression level of SOS2 were observed in the roots at ECs of 5.0 and 10.0 $dS \cdot m^{-1}$ when compared with control. These findings suggest that additional regulatory mechanisms also play an important role in salinity tolerance of *P. granatum* 'Wonderful'.

Conclusions

Foliar salt damage was not observed in *P. granatum* 'Wonderful' during the experimental period, which suggests a certain level of salinity tolerance in the pomegranate plants. Plants irrigated with saline solutions showed minimal adverse effects on growth. In addition, gas exchange parameters exhibited significant variations among salinity treatments, indicating altered physiological responses that could have potentially impacted growth reduction. The analysis of Na^+, Cl^-, and K^+ contents suggested efficient regulation of ion uptake and balance by *P. granatum* 'Wonderful' plants under saline conditions. Additionally, the study highlights changes in the expression of genes (*CAT*, *HKT1, NHX1*, *SOS1*, and *SOS2*) in the leaves and roots of *P. granatum* 'Wonderful', indicating their roles for salinity tolerance. *HKT1* in the roots, along with *NHX1* and *SOS2* in the leaves, work together to maintain the Na^+ level in roots and protect the

leaves from Na^+ toxicity. *Punica granatum* ‘Wonderful’ exhibits tolerance to salinity levels up to an EC of 10.0 $dS \cdot m^{-1}$ with slight reduction in growth. Further research to understand specific adaptation mechanisms will be crucial for the sustainable growth and productivity of pomegranate plants in saline environments.

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Table 5-1. Actin 7 (*ACT7*), catalase (*CAT*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), high-affinity potassium transporter 1 (*HKT1*), sodium/hydrogen exchanger 1 (*NHX1*), salt overly sensitive 1 (*SOS1*), and salt overly sensitive 2 (*SOS2*) genes, along with locus tags and accession IDs for *Arabidopsis thaliana* and *Punica granatum*, respectively, and forward and reverse primer (written in 5'-3') for the assay used in this study.

Gene	***A. thaliana***	***P. granatum***	**Primer Forward**	**Primer Reverse**	**Amplicon length**
ACT7[i]	AT5G09810	MTKT01000281	CGGTCGTACAACTGGTATT	GTGAACATGTACCCTCTCTC	168
CAT	AT1G20630	MTKT01004609	CCTGAGTGGAAGCTGTTTA	CCTGAGTGGAAGCTGTTTA	114
GAPDH[i]	AT1G16300	MTKT01002214	GAAGCAGCGGCAGTATTA	CATGGGTGGAGTCGTATTT	165
HKT1	AT4G10310	MTKT01001080	TGGGAACGTCGGATTTAC	CTTCAGCCTCCCAAAGAA	151
NHX1	AT5G27150	MTKT01006319	AAAGGAGCAGTCTCAGTTT	CCCTTCATCAGCAGTAGTAG	155
SOS1	AT2G01980	MTKT01000544	TCACACAGCTTGTCGTATC	AGGACTTGCTGGCTAAAC	152
SOS2	AT5G35410	XM_031529626	GAAGAGCACCACGTAACA	CATTTGCTGGGCGTTTAG	160

[i] Reference genes.

Table 5-2. Plant height, leaf area, shoot dry weight (DW), and root DW of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solution at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$ for 8 weeks in a greenhouse.

EC ($dS \cdot m^{-1}$)	**Height (cm)**	**Leaf area (cm^2)**	**Shoot DW (g)**	**Root DW (g)**
1.0	60.4 a[i]	1457.3 a	34.7 a	14.0 a
5.0	48.9 a	1534.1 a	34.5 a	11.7 a
10.0	55.6 a	1076.1 b	25.9 b	10.2 a
Treatment	NS[ii]	$P = 0.0006$	$P = 0.02$	NS
Linear	NS	NS	NS	NS
Quadratic	NS	$P = 0.0003$	$P = 0.005$	NS

[i] Means with same lowercase letters within a column are not different among treatments by Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

[ii]NS: Non significant.

Table 5-3. Leaf chlorophyll content [Soil Plant Analysis Development (SPAD)], net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), water use efficiency (WUE), and vapor pressure deficit (VPD) of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solution at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$ for 8 weeks in a greenhouse.

EC ($dS \cdot m^{-1}$)	**SPAD**	**P_n ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**g_s ($mmol \cdot m^{-2} \cdot s^{-1}$)**	**E ($mmol \cdot m^{-2} \cdot s^{-1}$)**	**WUE ($\mu mol \cdot mmol^{-1}$)**	**VPD (kPa)**
1.0	59.8 a[i]	18.0 a	435.8 a	8.2 a	2.2 a	2.2 c
5.0	56.2 b	16.2 b	194.8 b	5.8 b	2.8 a	2.7 b
10.0	57.8 ab	14.5 c	133.3 b	4.9 b	2.9 a	3.1 a
Treatment	$P = 0.05$	$P < 0.0001$	$P < 0.0001$	$P = 0.004$	NS[ii]	$P < 0.0001$
Linear	$P = 0.01$	$P = 0.009$	$P < 0.0001$	$P = 0.01$	NS	$P = 0.003$
Quadratic	NS	$P < 0.0001$	$P < 0.0001$	$P = 0.007$	NS	$P < 0.0001$

[i] Means with same lowercase letters within a column are not different among treatments by Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

[ii]NS: Non significant.

Table 5-4. Electrolyte leakage of *Punica granatum* 'Wonderful' subjected to nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ or saline solution at ECs of 5.0 or 10.0 $dS \cdot m^{-1}$ for 8 weeks in a greenhouse.

EC ($dS \cdot m^{-1}$)	**Electrolyte leakage** (%)
1.0	18.8 a
5.0	18.3 a
10.0	19.0 a
Treatment	NS

[i] Mean values within a column followed by the same letters are not significantly different at $\alpha = 0.05$ by Tukey-Kramer method for multiplicity.

[ii] NS: Nonsignificant.

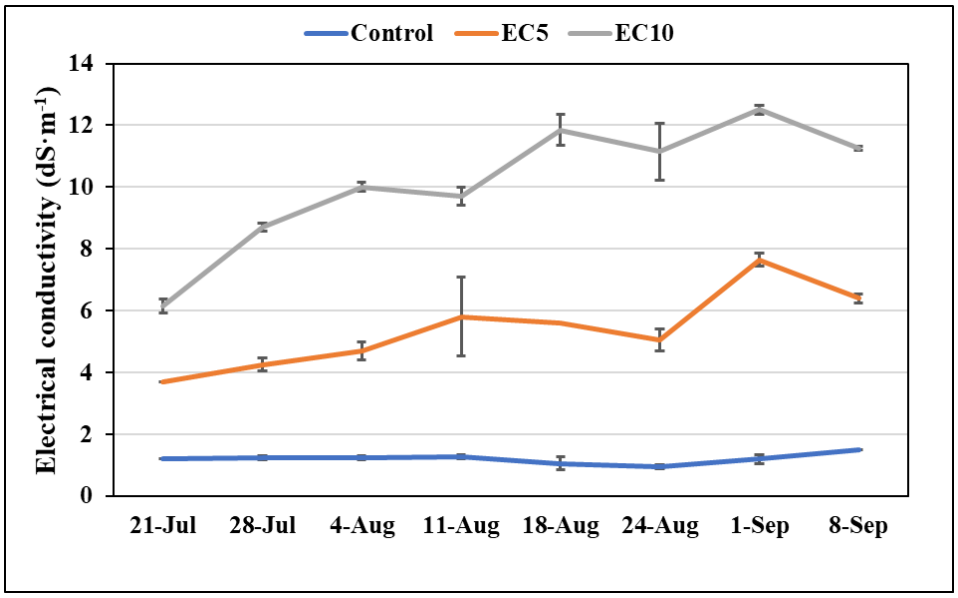

Fig. 5-1. Electrical conductivity (EC) of leachate solution over the course of the experiment when *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 dS·m^{-1} (control) or saline solution at an EC of 5.0 dS·m^{-1} (EC5) or 10.0 dS·m^{-1} (EC10) for 8 weeks in a greenhouse. Vertical bars represent standard errors of two measurements.

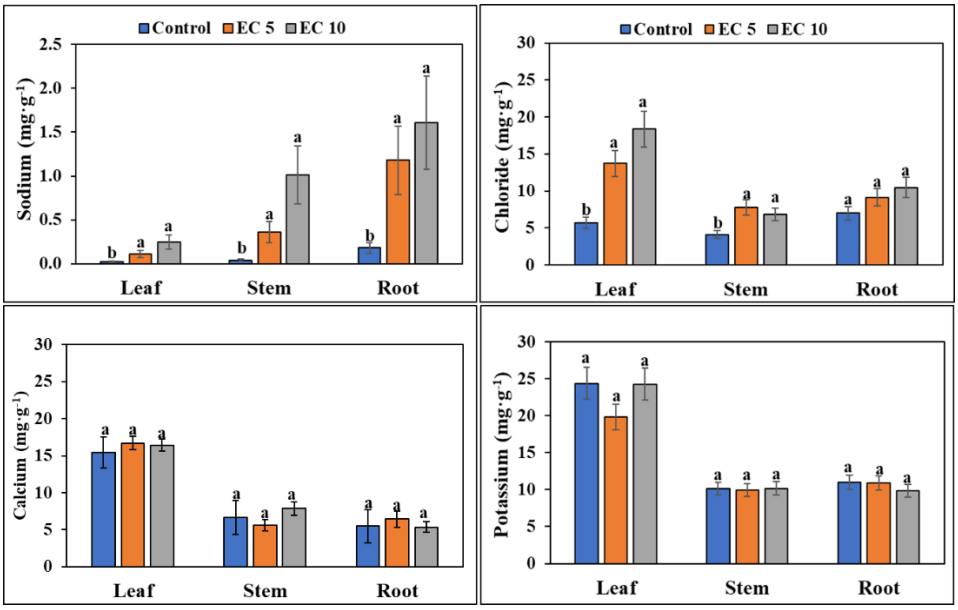

Fig. 5-2. Contents of sodium, chloride, calcium, and potassium in the leaf, stem, and root of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. Vertical bars represent standard errors of four measurements.

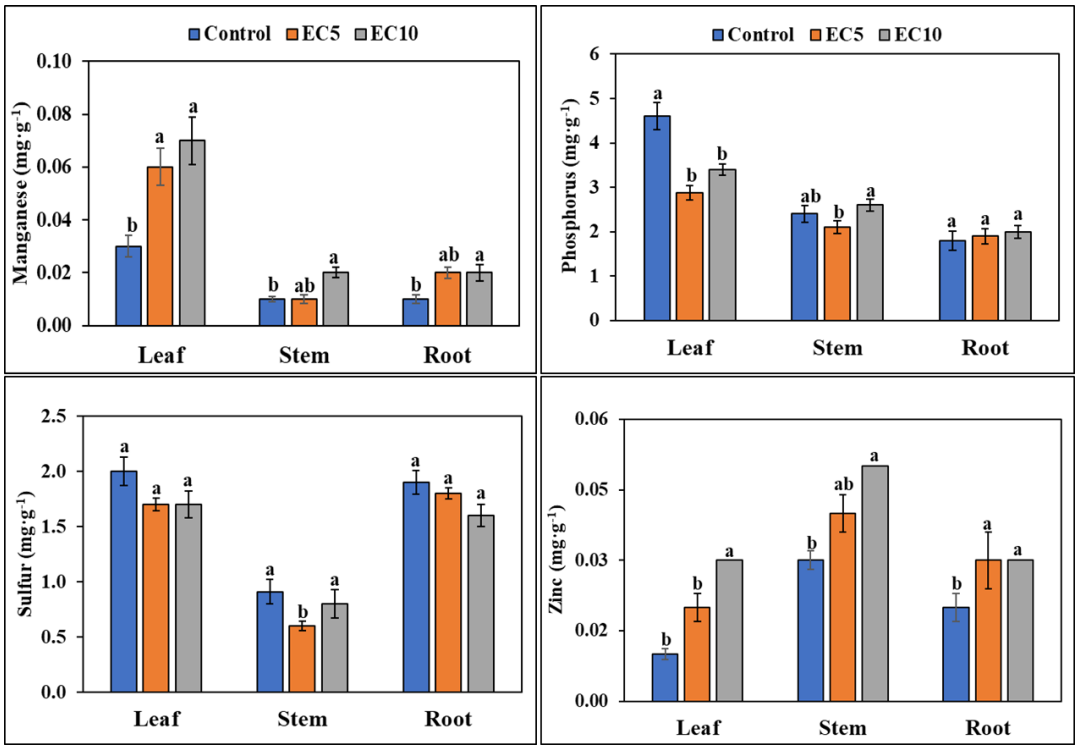

Fig. 5-3. Contents of manganese, phosphorus, sulfur, and zinc in the leaf, stem, and root of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. Vertical bars represent standard errors of four measurements.

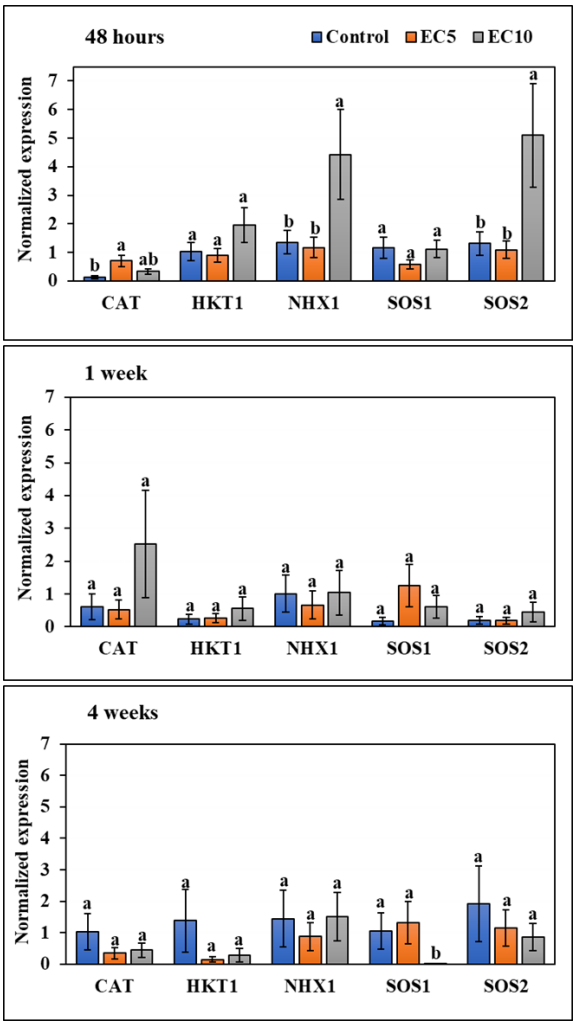

Fig. 5-4. The expression levels of catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen exchanger (*NHX1*), and salt overly sensitive (*SOS1* and *SOS2*) genes in *Punica granatum* 'Wonderful' leaves subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. The analysis was performed at 48 hours and at the end of first (1 week) and fourth week (4 weeks) following the initial treatment application. Vertical bars represent standard errors of three to six measurements.

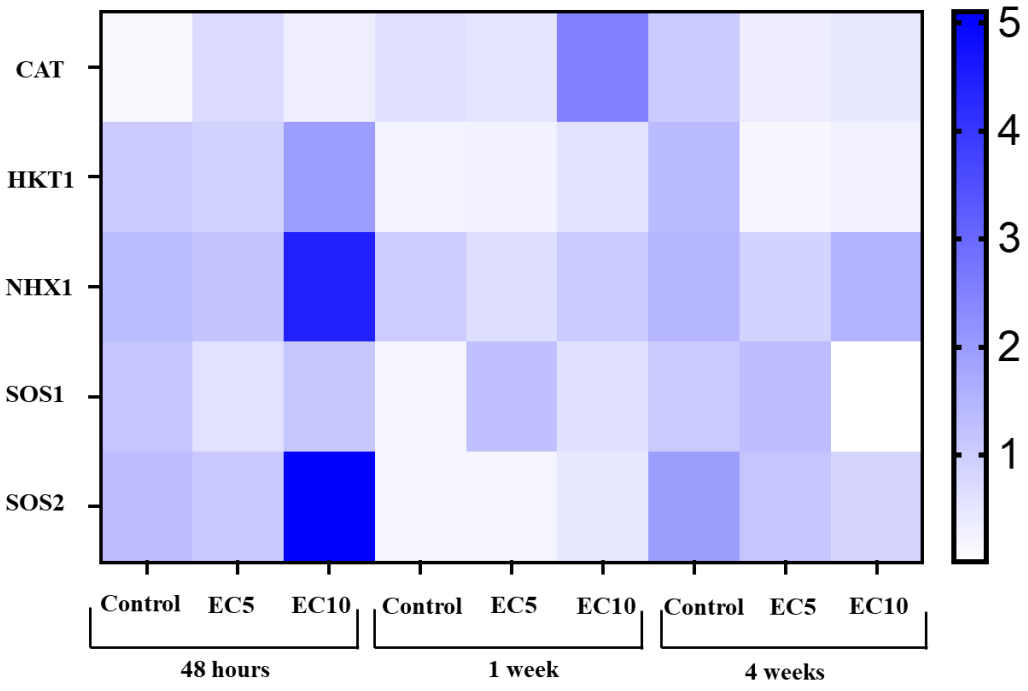

Fig. 5-5. Heat map depicting fold change of genes [catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen exchanger (*NHX1*), and salt overly sensitive (*SOS1* and *SOS2*)] in leaves of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. The analysis was performed at 48 hours and at the end of first (1 week) and fourth week (4 weeks) following the initial treatment application.

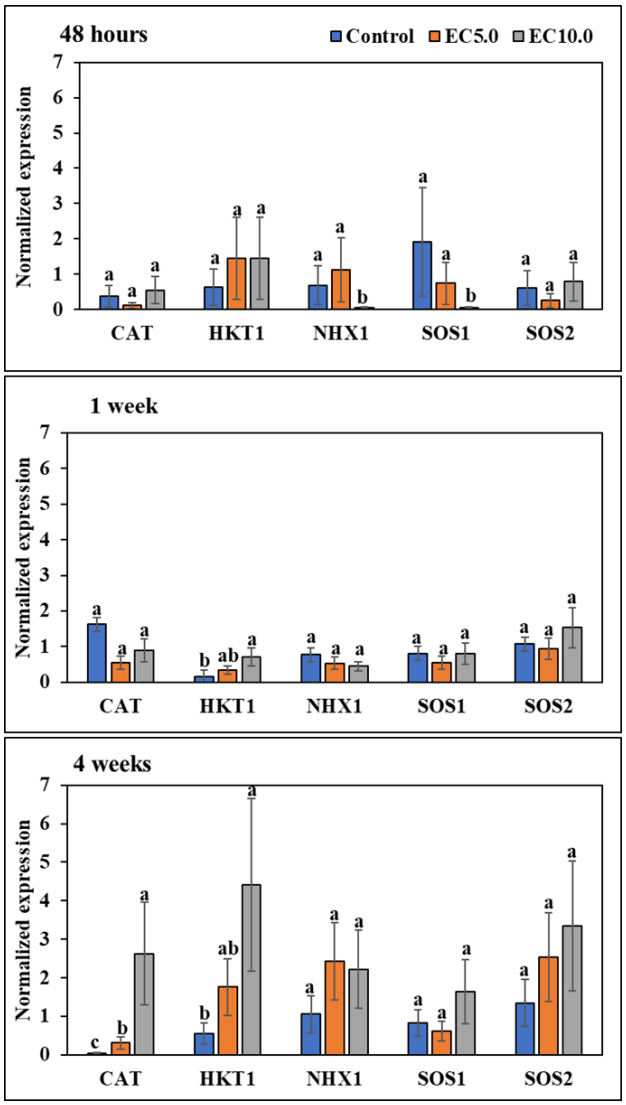

Fig. 5-6. The expression levels of catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen exchanger (*NHX1*), and salt overly sensitive (*SOS1* and *SOS2*) genes in *Punica granatum* 'Wonderful' roots subjected to a nutrient solution with electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. The analysis was performed at 48 hours and at the end of first (1 week) and fourth week (4 weeks) following the initial treatment application. Vertical bars represent standard errors of three to six measurements.

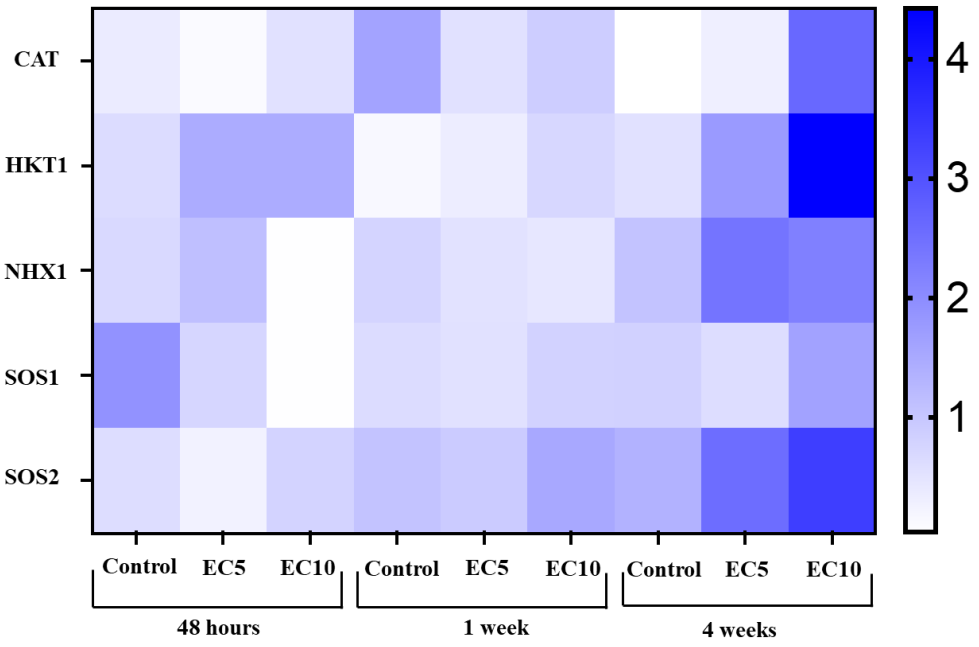

Fig. 5-7. Heat map depicting fold change of genes [catalase (*CAT*), high-affinity potassium transporter (*HKT1*), sodium/hydrogen exchanger (*NHX1*), and salt overly sensitive (*SOS1* and *SOS2*)] in roots of *Punica granatum* 'Wonderful' subjected to a nutrient solution at an electrical conductivity (EC) of 1.0 $dS \cdot m^{-1}$ (control) or saline solution at an EC of 5.0 $dS \cdot m^{-1}$ (EC5) or 10.0 $dS \cdot m^{-1}$ (EC10) for 8 weeks in a greenhouse. The analysis was performed at 48 hours and at the end of first (1 week) and fourth week (4 weeks) following the initial treatment application.

CHAPTER VI

CONCLUSIONS

In areas affected by high salinity and where saline water is used for landscape irrigation, it is essential to conduct research on the selection of salt-tolerant landscape plants. This dissertation evaluates the salinity tolerance of nine landscape plants: *Albizia julibrissin* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), *Penstemon barbatus* 'Novapenblu' (rock candy blue® penstemon), *P. strictus* 'Rocky Mountain' (rocky mountain beardtongue), *Punica granatum* 'Wonderful' (pomegranate), *Shepherdia* ×*utahensis* 'Torrey' (hybrid buffaloberry), and *Sophora japonica* (Japanese pagoda tree). According to the findings, the landscape plants showed some variations in response to salinity stress.

In *A. julibrissin*, minimal foliar salt damage was observed, but no foliar salt damage was noticed in *S. japonica*. Salinity stress resulted in decreased growth of *A. julibrissin* and *S. japonica*. Salinity stress also reduced plant photosynthesis and caused chloride (Cl^-) uptake and accumulation. However, sodium (Na^+) uptake and accumulation were lesser. Both *A. julibrissin* and *S. japonica* may possess mechanisms to restrict either the uptake or transport of Na^+ and can tolerate elevated levels of Cl^- in their leaf tissue. These species demonstrate an ability to manage salt levels without significant foliar salt damage, making them suitable for landscape use in salt-affected areas.

Arctostaphylos uva-ursi, *C. montanus* 'Coy', and *C. ledifolius* exhibited moderate to severe foliar salt damage at elevated salinity levels. However, *S.* ×*utahensis* 'Torrey' showed no foliar salt damage. Salinity stress led to reduction in growth and biomass in all

four native species. Moreover, salinity stress caused Na^+ and Cl^- uptake and accumulation. In addition, more proline was accumulated in leaves of *S. ×utahensis* 'Torrey' as a possible protective metabolic adaptation to prevent leaf tissue from damage under high salinity. *Shepherdia ×utahensis* 'Torrey' can be considered salt tolerant, *C. ledifolius* moderately salt tolerant, and *A. uva-ursi* and *C. montanus* 'Coy' as salt sensitive species.

Similarly, salinity stress led to foliar salt damage in *P. barbatus* and *P. strictus*, alongside a reduction in growth and biomass. Furthermore, the uptake of Na^+ and Cl^- increased, and the uptake of potassium (K^+) and calcium (Ca^{2+}) was also affected. According to the findings of a current study, *P. barbatus* and *P. strictus* are sensitive to salinity levels at electrical conductivities (ECs) of 7.5 and 10.0 $dS \cdot m^{-1}$. Therefore, *P. barbatus* and *P. strictus* are not suitable to be grown in areas affected by salt.

No signs of salt damage were observed on the leaves of the *P. granatum* 'Wonderful' throughout the experiment. Plants irrigated with saline solutions showed minimal negative effect on their growth. Moreover, gas exchange parameters exhibited significant variations among salinity treatments, indicating altered physiological responses that may have potentially impacted growth reduction. The analysis of Na^+ and Cl^- contents suggested efficient regulation of ion uptake and balance by *P. granatum* 'Wonderful' plants under saline conditions. Furthermore, sodium/hydrogen exchanger 1 (*NHX1*) and salt overly sensitive (*SOS2*) genes in leaves and high-affinity potassium transporter (*HKT1*) gene in roots could play important roles in salinity tolerance. Therefore, *P. granatum* 'Wonderful' exhibits tolerance to salinity levels up to an EC of 10.0 $dS \cdot m^{-1}$ and is suitable for landscape use in salt-affected areas.

CURRICULUM VITAE

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RESEARCH INTERESTS

Plant physiology

I have profound professional interest in plant physiology, driven by a fascination with the complex mechanisms and processes that govern plant growth, development, and responses to environmental stimuli. Throughout my academic and professional journey, I have acquired a solid foundation in plant physiology and conducted research to unravel the complexities of plant biology.

Horticultural crop production

My interest in horticultural crop production stems from my fascination with the intricate relationship between plants and their environment. I am particularly drawn to the challenges and rewards associated with cultivating high-quality crops that meet market demands while minimizing environmental impact.

Plant propagation

I am passionate about contributing to the advancement of plant propagation techniques and their practical applications. I thrive in dynamic and collaborative environments, working closely with teams to develop propagation protocols, troubleshoot challenges, and implement innovative approaches to maximize plant quality and quantity.

EDUCATION

May 2024 **Ph.D., Plant Science**

Utah State University (USU), Logan, UT

Dissertation title: Morphological and physio-biochemical responses and gene expression analyses of landscape plants under salinity stress.

Advisor: Dr. Youping Sun

December 2020 **M.S., Plant Science**

USU, Logan, UT

Dissertation title: Propagation of two Utah native plants: *Ceanothus velutinus and Cercocarpus montanus*.

Advisor: Dr. Youping Sun

May 2016 **B.S., Agriculture**

Institute of Agriculture and Animal Science (IAAS), Nepal

PROFESSIONAL EXPERIENCE

2021-Present **Ph.D. Research Assistant**, USU, Logan, UT

- Screened landscape plants [*Albizia julibrissi* (mimosa tree), *Arctostaphylos uva-ursi* (kinnikinnick), *Cercocarpus ledifolius* (curl-leaf mountain mahogany), *Cercocarpus montanus* 'Coy' (alder-leaf mountain mahogany), *Penstemon barbatus* 'Novapenblu' (rock candy blue® penstemon) and *Penstemon strictus* 'Rocky Mountain' (rocky mountain beardtongue), *Punica granatum* 'Wonderful' (pomegranate), *Shepherdia* ×*utahensis* 'Torrey' (hybrid buffaloberry) and *Sophora japonica* (Japanese pagoda tree)] for salinity tolerance and understood the mechanisms involved.
- Investigated the effects of different salinity levels on the plant growth, gas exchange, and mineral nutrients.
- Investigated the effects of salinity stress on biochemistry and transporter gene expression levels of plants.
- Studied plant growth and nodulation of *Ceanothus velutinus* (snowbrush ceanothus). Inoculated seedlings with native soil containing *Frankia* bacteria and evaluated them in three soilless substrates (calcined clay, peatmoss, and perlite).
- Grafted pinyon pine for early nut production. A grafting technique was used to propagate superior-producing *Pinus monophylla* (single-leaf pinyon pine) accessions by side-wedge grafting on *Pinus edulis* (two-leaf pinyon pine) seedlings. Grafting success was more than 70%.
- Propagation of a witches' broom selection of *Pinus edulis* (two-leaf pinyon pine). Side-wedge grafting technique was used to propagate the new selection.
- Developed efficient cutting propagation protocols for maximum production of native plants [*Ceanothus prostratus* (prostrate ceanothus), *Ericameria nauseosa* (rabbitbrush), *Paxistima myrsinites* (mountain lover) etc.].

- Helped in monitoring groundwater quality and developing best water management practices for sustainable green industry production in collaboration with six nurseries.
- Helped in data collection of USU climate ready landscape plant trials. Determined aesthetic qualities of landscape plants under different irrigation treatments.

2018-2020 **M.S. Research Assistant**, USU, Logan, UT

- Developed vegetative propagation protocol(s) for Utah native plants [*Ceanothus velutinus* (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf mountain mahogany)].
- Conducted cutting propagation and micropropagation experiments and identified best methods.
- Developed seed propagation protocol(s) for Utah native plants.
- Conducted research on selecting salt-tolerant ornamental plants [*Aquilegia barnebyi* (oil shale columbine), *Clematis fruticosa* (Mongolian gold clematis), *Epilobium septentrionale* (northern willowherb), and *Tetraneuris acaulis* var. *arizonica* (Arizona four-nerve daisy) etc.] for landscape use.

2017-2018 **Homestead Food Production Marketing Officer (HFPMO)**, USAID, Rukum, Nepal

- Directed technical support team for the implementation of production to income activities.
- Improved farmers economic status by training them on growing vegetables and raising poultry.
- Collaborated closely with district team, marketing specialist based in Kathmandu, Nepal and provided necessary support to partner Non-Governmental Organization (NGO).

2017 **Homestead Food Production Training Officer,** USAID, Kapilvastu, Nepal

- Trained farmers on homestead food production to enhance their nutrition levels in coordination with stakeholders and the technical project team.
- Mobilized the community and monitored project inputs.

2017 **Internship, SEAN Seed Service Centre Ltd.**, Kathmandu, Nepal

- Involved in laboratory activities related to seed quality assurance, field inspection of seed crops, seed processing and packing.

2017 **Undergraduate Researcher**, Tribhuvan University, Nepal

- Investigated the efficacy of different concentrations of *Metarhizium anisopliae* (Metsch.) Sorokin against white grub in lab conditions in Chitwan, Nepal.

TEACHING EXPERIENCE

Teaching Assistant for Plant Propagation class, USU, Fall 2019, 2020, 2021, 2022, 2023.

Overview: Taught lab classes, prepared for labs, graded lab quizzes, and provided guest lecture about plant hormones.

Teaching Assistant for Plant Stress Physiology class, USU, Spring 2020, 2021, 2023.

Overview: Taught lab classes and prepared experiments for labs.

Supervised Undergraduate Research Assistant (Macie Sanders) in plant propagation and greenhouse research work. Summer 2021, Fall 2021, Spring 2022, Fall 2022, and Spring 2023.

Supervised Undergraduate Summer Intern (Hannah Limas) in micropropagation (tissue culture) of *Punica granatum* (pomegranate). Summer 2022.

Supervised Undergraduate Summer Intern (Danielle Manybeads) on micropropagation of *Ceanothus velutinus* (snowbrush ceanothus). Summer 2020.

Supervised Undergraduate Research Assistants (Nathan Snow, Julie Hershkowitz, and Riley Hunter) on general tissue culture techniques. Spring 2019, Fall 2019, Fall 2020, and Spring 2021.

Trained high school students on general techniques of tissue culture. Biotechnology Summer Academy, July 12-14, 2023, taught plant tissue culture to Grace Thomas, Henry Shahan, and Otter Kulman.

Trained high school students on general techniques of tissue culture. Biotechnology Summer Academy, June 29-30, 2022, taught plant tissue culture to Mahina Vogt, Christina Zhang, and Kamel Pini.

PUBLICATIONS

<u>Referred Journal Articles</u>

1. Response of *Punica granatum* ‘Wonderful’ to salinity stress. (In preparation)

2. **Paudel A,** Sun Y, Dai X. 2024. Nodulation of snowbrush ceanothus in three soilless substrates. (Submitted to Journal of Environmental Horticulture on February 26, 2024).

3. **Paudel A,** Sun Y, Atanda Oladejo. 2024. Vegetative propagation of *Cercocarpus montanus* ‘Coy’. (Accepted by HortTechnology on September 18, 2023)

4. **Paudel A,** Sun Y. 2024. Effect of salt stress on the growth, physiology, and mineral nutrients of two penstemon species. HortScience. 59(2):209-219. https://doi.org/10.21273/HORTSCI17409-23.

5. **Paudel A**, Sun Y, Rupp LA. 2023. *Cercocarpus ledifolius* var. *intricatus* 'DoubleDown'. HortScience. 58(11):1309-1313. https://doi.org/10.21273/HORTSCI17122-23

6. **Paudel A,** Sun Y. 2023. Growth, morphological, and biochemical responses of four native species to salinity stress. HortScience. 58(6):651-659. https://doi.org/10.21273/HORTSCI17044-23

7. **Paudel A,** Sun Y. 2022. Growth, gas exchange, and mineral nutrients of *Albizia julibrissin* and *Sophora japonica* irrigated with saline water. HortScience. 57(8):841-850. https://doi.org/10.21273/HORTSCI16479-21

8. **Paudel A**, Sun Y, Rupp LA, Carman JG, Love SL. 2021. Vegetative propagation of *Ceanothus velutinus* using stem cuttings. Native Plants Journal. 23(1):123-129. https://doi.org/10.3368/npj.23.1.123

9. **Paudel A**, Sun Y, Rupp LA, Carman J, Love S. 2020. Overcoming seed dormancy in two rocky mountain native shrubs: *Ceanothus velutinus* and *Cercocarpus montanus*. Native Plants Journal. 21(3):353-358. https://doi.org/10.3368/npj.21.3.353

10. **Paudel A**, Sun Y, Rupp LA, Anderson R. 2020. *Cercocarpus montanus* 'USU-CEMO-001': A new Sego Supreme™ plant. HortScience. 55(11):1871-1875. https://doi.org/10.21273/HORTSCI15343-20

11. Sun Y, Chen J, Xing H, **Paudel A**, Niu G, Chappell M. 2020. Growth, visual quality, and morphological responses of 12 *Viburnum* Taxa to saline water irrigation. HortScience. 55(8):1233-1241. https://doi.org/10.21273/HORTSCI14940-20

12. Chen J, Xing H, **Paudel A**, Sun Y, Niu G, Chappell M. 2020. Gas exchange and mineral nutrition of 12 *Viburnum* Taxa irrigated with saline water HortScience. 55(8):1242-1250. https://doi.org/10.21273/HORTSCI14941-20

13. **Paudel A**, Chen J, Sun Y, Wang Y, Anderson R. 2019. Salt tolerance of Sego Supreme™ plants. HortScience. 54(11):2056-2062. https://doi.org/10.21273/HORTSCI14342-19

14. Chen J, Wang Y, **Paudel A**, Sun Y. 2019. Comparing the salt tolerance of the landscape plants using a near-continuous gradient dosing system. HortTechnology. 29(5):611-618. https://doi.org/10.21273/HORTTECH04385-19

15. Bohara JR, Maharjan S, **Paudel A**, Karki K, Bist V, Regmi R, Marahatta S, Kafle L. 2018. Efficacy of different concentration of *Metarhizium anisopliae* (Metsch.) Sorokin against white grub in lab condition in Chitwan Nepal, Journal of Pharmacognosy and Phytochemistry. 149-153.

Bulletins

1. **Paudel A,** Sun Y. 2021. Determining the salt tolerance of two penstemon species using a near-continuous gradient dosing system. American Penstemon Society Bulletin. 80:58-65.

2. **Paudel A**, Chen J, Sun Y. 2020. Determining the salt tolerance of two penstemon species using a near-continuous gradient dosing system. American Penstemon Society Bulletin. 78:30-35.

Abstracts

1. **Paudel A,** Sun Y. 2023. Evaluating two penstemon species for salinity tolerance. HortScience. 58(9): S250.

2. **Paudel A**, Sun Y, Kaundal A. 2023. Response of *Punica granatum* 'wonderful' to salinity stress. HortScience. 58(9): S55-56.

3. Nepal P, Wang Z, **Paudel A**, Sun Y. 2023. Evaluating two penstemon species for salt tolerance. HortScience. 58(9): S299.

4. **Paudel A,** Sun Y. 2022. Responses of Utah native plants to saline water irrigation. HortScience. 57(9): S200.

5. **Paudel A**, Sanders M, Sun Y. 2022. Nodulation of *Ceanothus velutinus*. HortScience. 57(9): S5

6. Chen J, Mathews J, **Paudel A**, Sun Y. 2022. Field trials of 26 ornamental grasses and grass-like plants. HortScience. 57(9): S129.

7. **Paudel A,** Sun Y. 2021. Propagation of single-leaf pinyon pine for pine nut production. HortScience. 56(9): S159-160.

8. **Paudel A**, Sun Y, Harris P, Wytsalucy R, Stewart JR. 2021. Exploration of whether scionwood of single-leaf pinyon pine (*Pinus monophylla*), a promising nut-tree crop native to the Great Basin, USA, can be successfully grafted in the summer and fall. XV World Forestry Congress, Coex, Seoul, Republic of Korea, 2-6 May 2022.

9. **Paudel A,** Sun Y. 2021. Determining the salt tolerance of woody ornamental plants for landscape use. HortScience. 56(9): S195.

10. Sun Y, **Paudel A**, Rupp LA, Carman JG, Love SL. 2021. Developing *Ceanothus velutinus* for nursery production and landscape use. HortScience. 56(9): S237-238.

11. **Paudel A,** Sun Y. 2020. Asexual propagation of *Ceanothus velutinus*. HortScience. 55(9): S34.

12. **Paudel A**, Chen J, Sun Y. 2020. Determining the salt tolerance of two penstemons using a near-continuous gradient dosing system. HortScience. 55(9): S339-340.

13. **Paudel A**, Sun Y, Rupp LA, Carman J, Love SL. 2020. Overcoming seed dormancy in *Ceanothus velutinus* and *Cercocarpus montanus*. HortScience. 55(9): S132-133.

14. Chen J, Xing H, **Paudel A**, Sun Y, Niu G. 2020. Salinity tolerance of twelve viburnum taxa. HortScience. 55(9): S113.

15. Hershkowitz J, Xing H, **Paudel A**, Chen J, Sun Y. 2020. Salinity tolerance of six ornamental grass species. HortScience. 55(9): S113-114.

16. **Paudel A**, Sun Y, Rupp LA, Carman J. 2019. Propagation methods for *Cercocarpus montanus*. 60th Annual International Plant Propagator's Society (IPPS) Annual meeting. Santa Cruz, CA. (**Awarded 1st place**)

17. **Paudel A**, Chen J, Guo S, Wang Y, Sun Y, Rupp LA, Anderson R. 2019. Salt tolerance of Sego Supreme^{TM} plants. HortScience. 54(9) S283.

18. **Paudel A**, Snow N, Sun Y, Rupp LA, Carman J. 2019. Micropropagation of *Cercocarpus montanus*: Stage II. HortScience. 54(9) S198-199.

19. Chen J, Wang Y, **Paudel A**, Sun Y. 2019. Comparing the salt tolerance of three landscape plants using near continuous gradient dosing system. HortScience. 54(9) S283.

PRESENTATIONS

1. **Paudel A**, Sun Y. (2023, December 8). Nodulation of *Ceanothus velutinus*. 2022 Annual Meeting of Western Education/Extension Research Activity (WERA)-1013 group, Intermountain Regional Evaluation and Introduction of Native Plants, Virtual.

2. **Paudel A,** Sun Y. (2023, August 4). Evaluating two penstemon species for salinity tolerance. American Society for Horticultural Science (ASHS), Orlando, FL.

3. **Paudel A**, Sun Y, Kaundal A. (2023, August 1). Response of *Punica granatum* 'wonderful' to salinity stress. ASHS, Orlando, FL.

4. **Paudel A,** Sun Y. (2023, April 12). Evaluating two penstemon species for salinity tolerance. Student Research Symposium, USU, Logan, UT.

5. **Paudel A**, Sun Y. (2022, September 13). Monitoring irrigation water quality and developing best water management practices for nursery production. Center for Water-Efficient Landscaping (CWEL) Virtual Field Day, USU, Logan, UT.

6. **Paudel A,** Sun Y. (2022, August 3). Responses of Utah native plants to saline water irrigation. ASHS, Chicago, IL.

7. **Paudel A**, Sanders M, Sun Y. (2022, July 31). Nodulation of *Ceanothus velutinus*. ASHS, Chicago, IL.

8. **Paudel A,** Sun Y. (2022, July 31). Monitoring groundwater quality and developing best water management practices for sustainable green industry production. ASHS, Chicago, IL.

9. **Paudel A,** Sun Y. (2022, April 5). Responses of Utah native plants to saline water irrigation. Student Research Symposium, USU, Logan, UT.

10. **Paudel A,** Sun Y. (2021, September 21). Pinyon pine grafting: new approach for pine nut production, International Plant Propagator's Society (IPPS) Virtual Meeting.

11. **Paudel A,** Sun Y. (2021, August 8). Determining the salt tolerance of woody ornamental plants for landscape use, ASHS Annual Conference, Denver, CO.

12. **Paudel A,** Sun Y. (2021, August 8). Propagation of single-leaf pinyon pine for pine nut production, ASHS Annual Conference, Denver, CO.

13. **Paudel A.** (2021, March 19). Determining the salt tolerance of woody ornamental plants for landscape use, Virtual Intermountain Sustainability Summit, Weber State University, Ogden, UT.

14. **Paudel A,** Sun Y. (2020, August 10). Asexual propagation of *Ceanothus velutinus*. ASHS Virtual Conference.

15. **Paudel A,** Sun Y, Rupp LA, Carman J, Love SL. (2020, August 11). Overcoming seed dormancy in *Ceanothus velutinus* and *Cercocarpus montanus*. ASHS Virtual Conference.

16. **Paudel A**, Chen J, Sun Y. **(**2020, August 13). Determining the salt tolerance of two penstemons using a near continuous gradient dosing system. ASHS Virtual Conference.

17. **Paudel A**, Sun Y, Rupp LA, Carman J, Love SL. (2020, April 8). Overcoming seed dormancy in *Ceanothus velutinus* and *Cercocarpus montanus*. Student Research Symposium, USU, Logan, UT.

18. **Paudel A**, Sun Y, Rupp LA, Carman J, Love SL. (2020, March 19). Overcoming seed dormancy in *Ceanothus velutinus* and *Cercocarpus montanus*. Annual Intermountain Sustainability Summit. Weber State University Ogden, UT.

19. Paudel A, Sun Y, Rupp LA, Carman J. (2019, September 27). Propagation methods for *Cercocarpus montanus*. 60th International Plant Propagator's Society Annual meeting. Santa Cruz, CA. (Awarded 1st place).

20. **Paudel A**, Chen J, Guo S, Wang Y, Sun Y,Rupp LA, Anderson R. (2019, July 4). Salt tolerance of Sego Supreme™ plants. ASHS Annual Conference, Las Vegas, NV.

21. **Paudel A**, Snow N, Sun Y, Rupp LA, Carman J. (2019, July 23). Micropropagation of *Cercocarpus montanus*: Stage II. ASHS Annual Conference, Las Vegas, NV.

22. **Paudel A**, Chen J, Guo S, Wang Y, Sun Y, Rupp LA, Anderson R. (2019, March 25). Salt tolerance of Sego Supreme™ plants. Research Showcase at the Department of Plants, Soils, & Climate. USU. Logan, UT.

23. **Paudel A**, Chen J, Guo S, Wang Y, Sun Y, Rupp LA, Anderson R. (2019, March 21). Salt tolerance of Sego Supreme™ plants. Annual Intermountain Sustainability Summit. Weber State University, Ogden, UT.

GRANT APPLICATIONS

J. Frank Schmidt Family Charitable Foundation Grant for proposal: Propagation and field trials of a witches' broom selection of *Pinus edulis* (two-leaf pinyon pine). 25 February 2022 – 25 February 2024. Total Project = $5000. (Not funded)

American Penstemon Society Graduate Student Scholarship Grant for proposal: Selecting salt tolerant penstemon plants for landscape use. 1 May 2019 – 30 April 2020. Total Project = $2000.

LEADERSHIP ACTIVITIES

- **Chair,** Propagation Professional Interest Group of American Society for Horticultural Science (ASHS), 2023-2024.
- **Secretary,** Ornamental Plant Breeding Interest Group of ASHS, 2023-2024.
- **Chair-elect,** Propagation Professional Interest Group of ASHS, 2022-2023.
    - Prepared meeting minutes for the business meeting.
    - Helped in preparation of annual report.
- **Secretary**, Propagation Professional Interest Group of ASHS, 2021-2022.

  - Prepared meeting minutes for business meeting.
- **Organized** a social event for the Center for Water Efficient Landscaping (CWEL) group at USU.

PROFESSIONAL AFFILIATIONS

2019 American Penstemon Society (APS)

2019 American Society for Horticultural Science (ASHS)

2019 International Plant Propagator's Society (IPPS)

EXTENSION ACTIVITIES

- Participated and presented research on the **field day** conducted by USU Student Organic Farm, Logan, UT (25 August 2023; 20 participants).

- Helped to organize USU climate ready landscape plant trial **open house** at Greenville Research Farm, Logan, UT: horticultural professionals, master gardeners, and the public were invited to observe and evaluate the performance of selected landscape plants (10 August 2023; 57 participants).

- Delivered a **lecture** on how to propagate native plants to the public at The Green House Nursery, Logan, UT (29 September 2022; 10 participants).

SERVICE ACTIVITIES

- **Moderator** for Water Utilization and Management-Oral Session of ASHS, August 4, 2023, Orlando, FL.
- **Moderator** for Technology Applications in Horticulture/Water Utilization and Management-Poster Session of ASHS, August 3, 2022, Chicago, IL.
- **Moderator** for Propagation-Oral Session of ASHS, August 8, 2021, Denver, Colorado.
- **Reviewer** for two articles for Technology in Horticulture, Maximum Academic Press.

SCHOLARSHIPS, AWARDS & HONORS

2023

- ASHS Annual Conference Student Travel Grant, Orlando, FL. ($500)
- Graduate Student Travel Award from the School of Graduate Studies, USU to attend ASHS Annual Conference. ($100)
- Travel Award from Academic Opportunity Fund, USU to attend ASHS Annual Conference. ($500)

- Open Access Funding from USU Libraries to publish "Effects of salt stress on the growth, physiology, and mineral nutrients of two penstemon species" on HortScience. ($1000)
- AGRI Apogee Instruments-Campbell Scientific Graduate Fellowship, USU. ($2000)
- John Seymour Memorial Scholarship, USU. ($4932)

2022

- ASHS Outstanding Graduate Horticulture Students Award.
- Open Access Funding from USU Libraries to publish "Growth, morphological, and biochemical responses of four native species to salinity stress" on HortScience. ($1000)
- Graduate Student Travel Award from the School of Graduate Studies, USU to attend ASHS Annual Conference. ($300)
- Travel Award from Academic Opportunity Fund, USU to attend ASHS Annual Conference. ($500)
- AGRI Ambassador Ardeshir Zahedi International Endowment Scholarship. USU. ($3000)
- AGRI Elva, Acklam & Arvil L. Stark Scholarship. USU. ($2666)

2021

- Open Access Funding from USU Libraries to publish "Growth, gas exchange, and mineral nutrients of *Albizia julibrissin* and *Sophora japonica* irrigated with saline water" on HortScience. ($1000)
- ASHS Annual Conference Student Travel Grant, Denver, CO. ($500)
- Graduate Student Travel Award from the School of Graduate Studies, USU to attend ASHS Annual Conference. ($200)
- AGRI Ambassador Ardeshir Zahedi International Endowment Scholarship. USU. ($2000)
- AGRI Elva, Acklam & Arvil L. Stark Scholarship. USU. ($5300)

2020

- Open Access Funding from USU Libraries to publish "*Cercocarpus montanus* 'USU-CEMO-001': A new Sego Supreme^{TM} plant" on HortScience. ($1000)
- ASHS Annual Conference Student Travel Grant. ($350) (Conference shifted to virtual format)
- AGRI Ambassador Ardeshir Zahedi International Endowment Scholarship, USU. ($2000)
- AGRI Elva Acklam and Arvil L. Stark Scholarship, USU. ($2205)
- AGRI Apogee Instruments-Campbell Scientific Graduate Fellowship, USU. ($1000)

2019

- First Prize of Poster Presentation for Student Competition from IPPS Annual Meeting. ($500)
- Graduate Student Travel Award from the College of Agriculture and Applied Sciences, USU to attend ASHS Annual Conference. ($300)
- Graduate Student Travel Award from the School of Graduate Studies, USU to attend ASHS Annual Conference. ($200)
- Bruce Briggs Memorial Scholarship to attend the 60th Annual IPPS Annual Meeting. ($1200)
- AGRI Elva Acklam and Arvil L. Stark Scholarship, USU. ($2484)

2012

- The Government of Nepal Scholarship by Tribhuvan University, Kathmandu, Nepal for pursuing B.S. Degree.

SKILLS AND INSTRUMENTS

- Catalase activity test
- Chlorophyll extraction using dimethyl sulphoxide
- CIRAS-3 Portable Photosynthesis System (PP Systems, Amesbury, MA)
- DNA extraction, RNA extraction and cDNA preparation
- Grafting technique
- Li-Cor Portable Area Meter Model Li-3000 (LI-COR® Biosciences, Lincoln, NE)
- LI-6800 Portable Photosynthesis System (LI-COR® Biosciences, Lincoln, NE)
- LI-600 Porometer/Fluorometer (LI-COR® Biosciences, Lincoln, NE)
- Pressure Chamber Instrument (PMS Instrument Company, Albany, OR)
- Primer design, Polymerase chain reactions (PCR), and Quantitative PCR techniques
- Proline extraction using ninhydrin reagent
- R Studio
- SAS Studio (SAS Institute, Cary, NC)
- Spectrophotometer (Spectra max M2, Molecular Devices, San Jose, CA)
- Tissue culture techniques