THESIS

EVALUATION OF SALINITY TOLERANCE OF PINTO BEAN VARIETIES

Submitted by

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ABSTRACT

EVALUATION OF SALINITY TOLERANCE OF PINTO BEAN VARIETIES

Salinity is an abiotic stress restricting agricultural crop production globally, primarily in arid and semi-arid areas. Saline soils are characterized by the accumulation of dissolved salts in the soil solution, which inhibits a plant's ability to absorb water and nutrients. Many crops are affected by high concentrations of salt in the soil. Dry edible pinto beans (*Phaseolus vulgaris*), very important in human nutrition around the world, are sensitive to salinity, and yield losses can occur in saline soils greater than 2 dS/m. The objective of this study was to assess the salinity tolerance of regular and slow darkening pinto bean varieties by evaluating the effect of different salt types on pinto bean germination, growth, and production. This project included three experiments: germination, greenhouse, and field studies. For the first two experiments, six varieties of pinto beans were evaluated: three slow-darkening pinto beans (Gleam, Mystic, Lumen) and three regular pinto beans (Othello, Cowboy, SV6139). In the germination experiment, treatments were arranged in a randomized complete block design with five replications, three saline solutions (NaCl, $CaCl_2$, $MgSO_4.7H_2O$ ($MgSO_4$)), and control (distilled water) at 0.05 M, 0.1 M, and 0.15 M concentrations for each salt. For the greenhouse experiment, saline solutions with the same electrical conductivity (EC) (dS/m), control (distilled water) and the six pinto bean varieties were organized in a Complete Random Design (CRD) with 10 replicates. The field experiment was an observational study where six pinto bean varieties: three slow-darkening pinto beans (Gleam, Mystic, Vibrant) and three regular pinto beans (Othello, Cowboy, SV6139) were planted in a field with a subsurface irrigation system to correlate yield to EC for each variety. The results demonstrated that germination percentage, speed of germination and hypocotyl length decreased as the salt concentrations increased. Othello's vegetative and reproductive parameters were significantly higher compared to the other varieties in the greenhouse under the saline conditions. There was no significant correlation between yield and EC in the field experiment. Results suggest Othello may be a more salt-tolerant pinto bean variety than the other tested varieties.

Keywords: salinity; pulse crop; salt concentration; germination; EC; yield

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1. INTRODUCTION

Pulse crops, also known as grain legumes, belong to the Leguminosae family. They are strictly harvested for their dried seeds. Some of the most cultivated types of pulses include chickpeas (*Cicer arietinum* L.), dry peas (*Pisum Sativum* L.), lentils (*Lens culinaris* Medikus), cowpeas (*Vigna unguiculata* L.) such as black-eyed-peas, and common beans (*Phaseolus vulgaris* L.) such as black, kidney and pinto beans. These particular crops are widely recognized as nutrient-dense foods and play a tremendous role in human nutrition around the world. They are one of the richest sources of dietary fiber and plant protein and combined with their affordable price point and contributions to sustainable food systems, they improve food security and can be considered a prime example of a superfood [1,2]. It has been shown that pulses are vital for sustainable farming systems and environmental regeneration [3]. Introducing grain legumes, especially dry beans, into cropping systems reduces the need for synthetic nitrogen fertilizer while promoting soil health and fertility [3].

Common bean (*Phaseolus vulgaris* L.) has been the most important grain legume, compared to lentils and chickpeas, in human nutrition for more than 300 million of the world's population [4]. It represents a crucial cash crop dryland and irrigated rotation systems in the USA Central High Plains region (Colorado, Nebraska, and Wyoming) [5]. The three main types of common beans grown in Colorado are pinto bean, mayocoba, and light red kidney beans [6]. Pinto represents approximately 75 % of production in comparison to mayocoba and light red kidney, which are about 10 % respectively [7]. However, dry edible pinto beans are susceptible to a phenomenon called postharvest darkening, which affects the color of the seeds after harvest.

Postharvest darkening of the seed coat is a concern for dry bean producers around the world. So many beans, such as pinto, cranberry, and red beans lose their visual quality as they become older, leading to a reduction in consumer preference [8]. Studies have shown that the postharvest darkening phenomenon is caused by some environmental factors such as humidity, elevated temperature, exposure to light [8, 9] as well as crop genetics. To address this issue and increase the market value of pinto beans, several varieties of slow-darkening pinto beans have been developed. These new varieties darken more slowly after harvest than the regular-darkening pinto beans [10]. However, along with the phenomenon of postharvest seed coat darkening, dry edible pinto beans are also sensitive to salinity, and yield losses can occur in saline soils greater than 2 dS/m [11, 12].

Saline soils are characterized by the accumulation of soluble salts in the root zone, which considerably affects a plant's ability to take up water and nutrients [13]. Salinization of soils occurs through natural processes including evaporation of saline ground water, sea water infiltration of coastal ground waters, low precipitation rate, sea water salts in wind and rain, as well as human-induced processes, such as irrigation with marginal water and poor agrotechniques [14,15]. Salinity has a serious impact on seed germination, germination rate, plant growth, and yield components [12]. Soil salinity is a worldwide abiotic stress affecting crop production and yield efficiency, especially in arid and semiarid climates [4,12,16, 17]. More than 30% of the world's food production and nearly one-third of irrigated land are critically affected by salinity [16]. The level of salts in the soil is measured as Electrical Conductivity (EC). Generally, yields of salt sensitive crops are significantly affected when salt levels are 2 to 4 dS/m. Moderately salt tolerant crops are affected by levels of 4 to 5 dS/m, and above 8 dS/m, all crops except only the very tolerant crops are negatively impacted [13]. The unit used to measure EC is called deciSiemens per meter (dS/m). The higher the EC, the lower the crop productivity will be [13].

Most of the salt stresses in nature are from NaCl, especially when there is an excessive concentration of Na^{+1} ion in the soil solution [18]. Many experiments have been conducted on salinity tolerance of pulse crops to sodium chloride [4, 16, 18, 19, 20]. However, in arid regions such as Colorado, other salt compounds such as $CaCl_2$, $MgSO_4$, $CaSO_4$, and $CaCO_3$ can be found in the soil [21]. An estimated 980,000 acres of irrigable land are salt-affected in Colorado [13, 21]. Thus, the main objective of this study was to compare the salinity tolerance between regular and slow darkening pinto bean varieties by evaluating the effect of different salts on bean growth and reproduction. The specific objectives are as follows: (i) Assess the effect of different salt types and salt concentrations on seed germination of pinto beans, (ii) Evaluate the effect of different salts on the growth and reproductive stages of pinto beans, and (iii) to correlate yield component to EC under field conditions.

2. MATERIALS AND METHODS

2.1 GERMINATION EXPERIMENT

The germination experiment was conducted in the Nutrien Building laboratory at Colorado State University (CSU). Six varieties of pinto beans were considered for this assessment: three slow-darkening pinto beans (Gleam, Mystic, Lumen) and three regular pinto beans (Othello, Cowboy, SV6139). In this experiment, three salt compounds (NaCl, $CaCl_2$, $MgSO_4$), at 0.05 M, 0.1 M, and 0.15 M concentrations for each salt were used to prepare saline solutions based on molarity. Distilled water was used as control. Electrical conductivity (EC) measurement was taken after the solution preparation before it was added to the experimental units.

The seeds were washed with distilled water for five minutes before solution application. Five representative seeds per variety were placed on a filter paper (VWR 413, size 9 cm) in a 9-cm diameter petri dish. One mL of distilled water as control and 1 mL of each salt treatment were applied to the filter paper. Then 0.5 mL of the solutions were added overtime when dryness was observed on the filter paper. The petri dishes were sealed with parafilm to prevent evaporation and kept in a humidity chamber at temperature of 20±1°C to stimulate germination conditions. Seeds were considered to have germinated when radicle emergence was observed through the seed coat.

Germinated seeds were counted in each petri dish daily at the same time in the morning for 7 days to determine germination speed (GS). Seven days after initiation of the experiment, the total number of seeds germinated was recorded to calculate germination percentage (GP) and hypocotyl and radicle length (cm) measurements were recorded.

The germination percentage was calculated using the formula below [22, 23]:

$$GP = (NSG \div TNSS) \times 100$$

Where NSG is the number of seeds germinated, and TNSS is the total number of seeds sown.

The following formula was used to calculate GS [22, 23]:

GS= n1/d1+n2/d2+n3/d3+….

Where n1 is the number of seeds germinated on day one after sowing, and d1 the number of days taken for germination from the day of sowing.

The experimental design was a randomized complete block design with five replications. One replicate was conducted at a time to prevent contamination, and to facilitate monitoring. Analysis of variance (ANOVA) was conducted, using RStudio software. Means of each parameter were compared using the Tukey test at $p \leq 0.05$.

2.2 GREENHOUSE EXPERIMENT

The growth and reproductive stage evaluation took place at the Colorado State University Plant Growth Facility. The temperature in the greenhouse ranged from 19°C to 23°C during the day and from 18°C to 21°C at night, with a photoperiod of 16 hours. The experimental soil was collected from the Agricultural Research, Development and Education Center (ARDEC) north of Fort Collins, CO (40°36′36.9″N 104°59′38.2″W). It was a clay soil with low salinity (Table 1), collected from a Garrett loam (fine-loamy, mixed, mesic Pachic Argiustolls) [24]. The soil was air-dried and placed into 15.5 cm x 13.5 cm pots. Each pot was an experimental unit and was filled with 1050 g of soil.

Table 1. Physical and chemical properties of the soil collected from ARDEC.

Parameter	Value
pH	8.1
Soluble salts (dS m^{-1})	0.78
OM (%)	1.6
Nitrate-N (mg kg^{-1})	3.2
Olsen-P (mg kg^{-1})	6

A 1:1 analysis was used to measure the soil pH by shaking the soil with deionized water for two hours prior to assessment [25]. Soluble salts were measured using the modified saturated paste method. Loss on Ignition analysis was used to determine the level of OM (organic matter) content in the soil. The Nitrate-N was determined by Flow Injection Analysis. The level of phosphorus content in the soil was determined by Sodium Bicarbonate Olsen-P extraction [26].

Six varieties of pinto beans were evaluated: three slow-darkening pinto bean varieties (Gleam, Mystic, Lumen) and three regular pinto bean varieties (Othello, Cowboy, SV6139) to assess the influence of salinity on plant growth and reproductive maturity. Three different salt solutions (NaCl, $CaCl_2$, $MgSO_4$) were compared to tap water (0.05 dS/m) (control). The saline solutions with the respective salt concentrations (0.05 M NaCl, 0.1 M $CaCl_2$, 0.15 M $MgSO_4$) (Table 2) were prepared to reach an EC of 5 dS/m for each of the treatments. The saline solution was applied to reach Field Capacity (FC) when 75% of the soil in the pots was dry on the surface. The treatments were applied five times during the entire experiment (14, 17, 22, 29, and 37 days after planting, respectively). The six pinto bean varieties and salt solutions were arranged in a Complete Random Design (CRD) with 10 replicates. Each replication had 24 pots, and 240 pots in total were used for this experiment. Analysis of variance (ANOVA) was conducted, using RStudio software. Means of each parameter were compared using the Tukey test at $p \leq 0.05$.

Table 2. The type and level of salt concentration and electrical conductivity (EC) in the irrigation water treatments.

Salt	Concentration (M)	EC (dS/m)
NaCl	0.05	5.04
$CaCl_2$	0.10	5.01
$MgSO_4$	0.15	5.00

The EC was measured by placing one drop of each saline solution into an EC tester (Oakton ECTestr high conductivity tester, 0 to 19.99 dS/m).

Three representative seeds per variety were sown per pot, and after emergence, pots were thinned to 1 plant/pot. When the first true leaves appeared (6 days after planting), 0.29 g urea per pot and 0.19 g Triple Super Phosphate (TSP) per pot were applied to compensate for the N and P deficiencies in the soil, based on the CSU bean fertilizer recommendations [11], 72 kg N/ha and 44 kg P_2O_5/ha. Four days after fertilizer application, salt treatments were applied 10 days after planting. Plant height, growth stage, chlorophyll level and leaf area index (LAI) were recorded to determine plant growth parameters for the vegetative stage. Flower and pod numbers were recorded at the reproductive stage.

Plant height was measured with a tape measure from the soil surface to the highest leaf tip. A growth stages chart for common beans [27] was used for the growth stage variable to determine the maturity in each pot. At the late vegetative stage, 39 days after planting, photos of the plant canopy were taken from above and uploaded to an image processing software (ImageJ) [28] to calculate the LAI. The chlorophyll level was recorded with a portable chlorophyll meter (SPAD-502, Konica Minolta; Japan) by taking the average of three measurements of the old and young leaves, respectively, at the late vegetative stage, 40 days after planting [29]. Flower numbers were counted when they were completely opened.

At the end of the experiment, 48 days after planting, pod number were measured. The soil was collected from each pot and air-dried. The soil was ground (Grinder General Purpose, MTRP331AB18, Manufactured by Automation Direct Optimation Inc.; China) and passed through a 2 mm sieve for further analysis to determine EC accumulation. EC was measured in a saturated paste extract for each of the samples [25]. The plants were harvested, and fresh biomass for shoot and root were measured separately by weighing all the living plant materials. The samples were stored in paper bags and dried in an oven at 70 °C for 3 days to determine dry biomass of all plant parts. The relative growth was calculated by dividing the variables of plant height 38 days after planting, LAI, dry shoot biomass and chlorophyll level of young and old leaves of each variety by the control for that variety.

2.3 FIELD EXPERIMENT

The field study was conducted on the Subsurface Irrigation Efficiency Project (SIEP) farm near Kersey, Colorado under a subsurface drip irrigation system, on a Nunn clay loam and Colombo clay loam mixed soil type [30]. Six pinto bean varieties: three slow-darkening pinto beans (Gleam, Mystic, Vibrant) and three regular pinto beans (Othello, Cowboy, SV6139) were planted on June 3, 2023, at 5 cm depth, and 247.106 seeds/ha in a 1.78 ha area. Each variety had 36 rows 50.8 cm apart. Each plot was 157.0 m in length and 18.3 m in width.

An EMI survey was carried out using an EM38-MK2 (Geonics Ltd., Mississaugua, Ontario, Canada), TrimbleTM GPS system (Sunnyvale, CA, USA), and Juniper Allegro CX (Logan, UT, USA) for datalogging to identify ideal locations for salinity sampling [31]. This resulted in an EMI survey with paired soil sampling. This survey equipment provided a continuous stream of ECa measurements to an approximate depth of 0.75 m using the horizontal orientation signal (EMh) and to an approximate depth of 1.5 m using the vertical orientation signal (EMv), simultaneously. The Electromagnetic Sampling Analysis and Prediction model (ESAP, ver. 2.35) was used to identify soil sampling locations for laboratory ECe analysis. ESAP uses a response surface sampling design (RSSD) strategy which, in essence, creates a 3-D surface of the ECa measurements and based on the range and variation, selects soil sampling locations that characterize the ECa variation while maximizing the distances between adjacent sampling locations [32]. ESAP-RSSD identified 36 locations, 6 sites per each variety where soil samples were collected using an 8-cm diameter soil auger at 0-5 cm and 0-15 cm, weighing approximately 500 g each. The soil was ground (Grinder General Purpose, MTRP331AB18, Manufactured by Automation Direct Optimation Inc; China) and passed through a 2 mm sieve before ECe analysis. A saturated paste extract analysis was done on all the samples to determine the soil EC [25].

The same soil sampling locations provided by the ESAP model were used to record yield, stand count, and yield components (flower number, pod number, pod weight, seed number, and seed weight) in an area of 1 meter square for each site. Stand count and number of flowers were measured 26 and 67 days after planting, respectively. Yield components (pod number, pod weight, seed number, seed weight) were taken 118 days after planting. The yield components were correlated to the soil EC with a correlation analysis using RStudio software.

3. RESULTS

3.1 GERMINATION EXPERIMENT

3.1.1 GERMINATION PERCENTAGE

Analysis of variance (ANOVA) (Table 3) showed that the types of salt, the pinto bean varieties, and the salt concentrations each significantly ($p \leq 0.05$) influenced the germination percentage of pinto bean seeds (Figure 1). The highest germination percentage means were recorded in the control, $MgSO_4$, and $CaCl_2$ treatments with 70.6%, 69.1%, and 64.22%, respectively. NaCl presented the lowest germination percentage (51.77 %). Regarding germination percentage among varieties, Othello was the variety with the highest germination percentage (80.8%) followed by Gleam (72.4%). The lowest germination percentage was recorded from Cowboy (53.2%) and SV6139 (51.2%). Regarding the germination percentage as influenced by the salt concentrations, the highest germination percentage was recorded at the 0 concentration (70.6%), representing the control treatment, followed by 0.05 M (68%) and 0.1 M (64.2%), respectively. The only salt concentration that was significantly different from control was 0.15 M. Finally, the slow darkening and regular darkening characteristics were not statistically different.

Table 3. ANOVA for germination percentage (GP), germination speed (GS), hypocotyl length (HL) and radicle length (RL) showing main effects and three-factor interactions.

	GP	**GS**	**HL**	**RL**
Factors	**P**	**P**	**P**	**P**
Trt	0.0002	9.751e-06	2.593e-08	5.852e-13
Vrt	3.389e-07	2.115e-07	8.203e-07	0.02445
Conc	0.0004	0.0004	0.0006	3.362e-09
Trt : Vrt	0.4627	0.8571	0.1472	0.1892
Trt : Conc	0.4410	0.7739	0.5849	0.1378
Vrt : Conc	0.8993	0.7419	0.1784	0.3682
Trt : Vrt : Conc	0.8994	0.9913	0.6856	0.13959
Trt	0.0006466	5.033e-05	4.282e-08	6.331e-13
PHD	0.6239857	0.942538	0.003403	0.2716
Conc	0.0010384	0.001163	0.001091	2.524e-09
Trt : PHD	0.3181890	0.558129	0.755084	0.1818
Trt : Conc	0.4730548	0.793625	0.562814	0.1287
PHD : Conc	0.6263894	0.501380	0.286419	0.1912
Trt : PHD : Conc	0.5298861	0.939057	0.315070	0.6784

Trt= treatment (salt type), Vrt= variety, Conc= concentration (salt concentration), PHD= postharvest darkening trait. *p*= *p*-value.

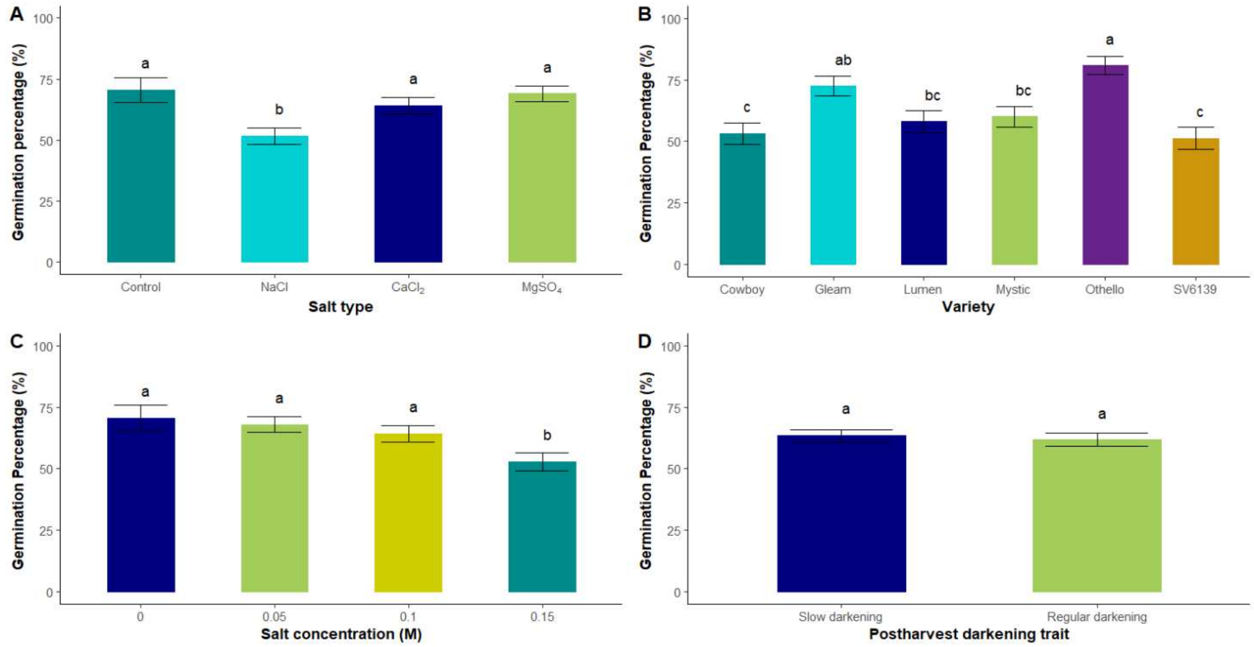

Figure 1. Germination percentage as affected by salt type (A), variety (B), salt concentrations (C) and postharvest darkening characteristics (D). Salt types, varieties, salt concentrations, and postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

3.1.2 SPEED OF GERMINATION

Analysis of variance (ANOVA) (Table 3) showed that salt type, pinto bean variety, and salt concentrations each significantly ($p \leq 0.05$) influenced the germination speed of pinto bean seeds (Figure 2). The highest germination speed means were recorded in the control, $MgSO_4$, and $CaCl_2$ treatments, with 4.04, 3.60, and 3.17 , respectively. NaCl presented the lowest germination speed. Regarding germination speed among varieties, Othello was the variety with the highest germination speed (4.26) followed by Gleam (3.88) and Mystic (3.17). The lowest germination speeds were recorded for Cowboy (2.54), Lumen (2.36) and SV6139 (2.67); however, there was no statistical difference between Cowboy, Lumen, SV6139 and Mystic. With respect to germination speed as affected by salt concentration, the highest germination speed was recorded at concentration 0 M (4.04), representing the control treatment, followed by 0.05 M (3.52) and 0.1 M (3.19), respectively. The lowest germination rate was recorded at the 0.15 M concentration. However, there was no statistical difference between 0 M, 0.05 M and 0.10 M. Finally, the slow darkening and regular darkening characteristics showed no statistical differences.

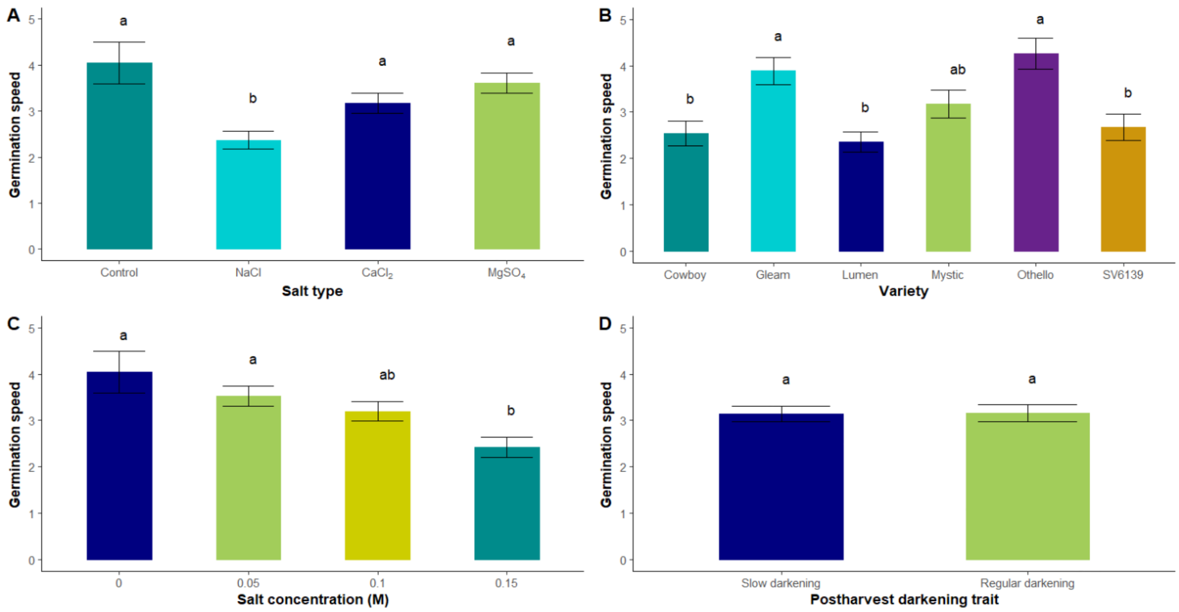

Figure 2. Germination speed as affected by salt type (A), variety (B), salt concentrations (C), and postharvest darkening characteristics (D). Salt types, varieties, salt concentrations, and postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

3.1.3 HYPOCOTYL AND RADICLE LENGTH

The results of the ANOVA analysis (Table 3) indicate statistical differences across salt type, variety, salt concentrations and postharvest darkening phenomenon in hypocotyl and radicle length (Table 4). For the salt types, the highest hypocotyl length was recorded from the control (1.09 cm), followed by $MgSO_4$ (0.98 cm). However, there was no statistical difference between NaCl and $CaCl_2$. Othello was the variety with the longest hypocotyl (1.01 cm). However, there was no significant difference between Cowboy, Gleam, Mystic, Othello and SV6139. The hypocotyl length of Lumen (0.76 cm) was significantly lower than all other varieties. In terms of salt concentrations, the 0.05 M treatment had the greatest hypocotyl length (0.94 cm) after the control (1.09 cm). The postharvest darkening characteristic showed significant differences in hypocotyl length, with regular darkening longer than slow darkening. Most importantly, as concentration increased, hypocotyl length decreased.

For radicle length, the analysis of variance showed no statistical differences among varieties or between postharvest darkening traits (Table 4). However, there was a significant difference between salt types and concentrations. The longest radicle length was recorded in $CaCl_2$ (3.02 cm) followed by the control (2.47 cm). However, there was no significant difference between Control and $CaCl_2$, Control and $MgSO_4$. Regarding the salt concentrations, there was no significant difference between 0 M, 0.05 M and 0.1 M in radicle length.

Table 4. The effect of salt type, pinto bean variety, salt concentration, and slow darkening characteristic on hypocotyl length and radicle length. Means of five replicates with standard error (SE) in parentheses. Salt types, varieties, salt concentrations, and postharvest darkening trait with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test.

Factors	Hypocotyl length	Radicle length
	----------------------cm------------------	
Salt types		
Control	1.09 (0.04) a	2.47 (0.18) ab
NaCl	0.85 (0.02) c	1.61 (0.08) c
$CaCl_2$	0.87 (0.02) c	3.02 (0.17) a
$MgSO_4$	0.98 (0.03) b	2.38 (0.11) b
Variety		
Cowboy	0.92 (0.04) a	2.67 (0.19) a
Gleam	0.94 (0.02) a	2.60 (0.18) a
Lumen	0.76 (0.03) b	2.17 (0.16) a
Mystic	0.95 (0.03) a	2.16 (0.16) a
Othello	1.01 (0.03) a	2.59 (0.16) a
SV6139	0.93 (0.05) a	2.05 (0.17) a
Salt concentrations (M)		
0	1.09 (0.04) a	2.47 (0.18) a
0.05	0.94 (0.03) b	2.77 (0.14) a
0.1	0.92 (0.02) bc	2.53 (0.14) a
0.15	0.83 (0.03) c	1.72 (0.09) b
Postharvest darkening		
Regular darkening	0.96 (0.02) a	2.46 (0.10) a
Slow darkening	0.89 (0.02) b	2.33 (0.09) a

3.2 GREENHOUSE EXPERIMENT

3.2.1 PLANT HEIGHT

The results of the ANOVA analysis (Table 5) indicate statistical differences within salt type, variety, and postharvest darkening traits in plant height at 21, 30 and 38 days after planting (DAP). There was no significant difference among salt types 21 DAP. The highest plant height was recorded from the control at 15.54 cm (Table 6). At 30 DAP, plant height showed statistical difference in salt type. $MgSO_4$ presented the highest plant height (19.05 cm), followed by Control (18.17 cm). However, there was no significant difference between Control, NaCl and $MgSO_4$. The highest plant height at 38 DAP was recorded in Control (22.86 cm) followed by $MgSO_4$ (22.39 cm).

Regarding plant height as affected by pinto bean variety, Othello was slightly higher (16.50 cm) than the other varieties 21 DAP. However, Cowboy and Othello did not present significant difference in plant height at 30 DAP. Othello (21.02 cm) was the variety with the highest plant height followed by Cowboy (18.32 cm). Gleam, Mystic, and Othello were taller than Lumen and SV. Othello was significantly higher than the other varieties with a plant height mean of 26.35 cm at 38 DAP.

Finally, in terms of postharvest darkening characteristic, there was no statistical difference between regular and slow darkening in plant height 21 DAP. The postharvest darkening trait showed significant differences in plant height with regular darkening taller than slow darkening varieties at both 30 and 38 DAP.

Table 5. ANOVA for plant height and growth stage after 21, 30 and 38 days of planting, and LAI showing main effects and two-factor interactions.

	PH21	**PH30**	**PH38**	**GS21**	**GS30**	**GS38**	**LAI**
Factors	**p**	**p**	**p**	**p**	**p**	**p**	**p**
Trt	0.09278	0.01443	< 2.2e-16	0.003867	0.006504	< 2.2e-16	< 2.2e-16
Vrt	3.23e-07	6.236e-06	4.098e-11	4.951e-06	1.191e-12	0.0001063	0.0003052
Trt : Vrt	0.69434	0.14387	0.7372	0.068874	0.759174	0.3011	0.4291503
Trt	0.1364	0.02226	2.75e-15	0.007224	0.01561	<2e-16	< 2.2e-16
PHD	0.7492	0.03161	0.001832	0.066885	1.923e-06	0.1189	0.0001831
Trt: PHD	0.5136	0.26483	0.727503	0.422481	0.74100	0.9897	0.5498264

PH21= plant height 21 days after planting, PH30= plant height 30 days after planting, PH38= plant height 38 days after planting. GS21= growth stage 21 days after planting, GS30= growth stage 30 days after planting, GS38= growth stage 38 days after planting. LAI= Leaf Area Index. Trt= treatment (salt type), Vrt= variety, PHD= postharvest darkening trait. *p*=*p*-value.

Table 6. The effect of salt type, pinto bean variety, and postharvest darkening characteristic on plant height (cm) 21, 30 and 38 days after planting. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	Plant height 21 days after planting	Plant height 30 days after planting	Plant height 38 days after planting
	------------------------------cm------------------------------		
Salt types			
Control	15.5 (0.45) a	18.1 (0.41) ab	22.8 (0.77) a
NaCl	14.9 (0.37) a	16.9 (0.56) ab	11.0 (1.33) b
$CaCl_2$	14.2 (0.45) a	16.6 (0.67) b	14.4 (1.22) b
$MgSO_4$	14.9 (0.32) a	19.0 (0.81) a	22.3 (1.15) a
Variety			
Cowboy	15.0 (0.36) ab	18.3 (0.66) ab	16.7 (1.61) b
Gleam	15.6 (0.37) a	17.4 (0.59) b	16.1 (1.36) b
Lumen	13.5 (0.45) b	16.0 (0.49) b	13.7 (1.39) b
Mystic	15.5 (0.34) a	17.5 (0.62) b	17.9 (1.04) b
Othello	16.5 (0.40) a	21.0 (1.21) a	26.3 (2.01) a
SV6139	13.5 (0.60) b	15.7 (0.66) b	15.2 (1.27) b
Postharvest darkening			
Regular darkening	15.0 (0.28) a	18.3 (0.54) a	19.4 (1.05) a
Slow darkening	15.0 (0.24) a	17.0 (0.33) b	15.9 (0.75) b

3.2.2 GROWTH STAGE

Analysis of variance (ANOVA) (Table 5) indicates statistical differences across salt type, variety, and postharvest darkening traits in growth stage 21, 30 and 38 days after planting (Table 7). The most mature growth stage was recorded in Control (V2) and $MgSO_4$ (V2) 21 DAP. There was no statistical difference between Control and $MgSO_4$ 30 DAP. The result 38 DAP showed that there was no statistical difference between Control (V6) and $MgSO_4$ (V6). However, Control and MgSO4, NaCl and CaCl2 showed no statistical difference. The growth stage from NaCl (V3) was significantly less mature than all other salt types.

Regarding the growth stage of pinto bean varieties, Cowboy (V1) and Lumen (V1) were less mature than the other varieties 21 DAP. However, there was no statistical difference between Cowboy, Gleam and lumen, Gleam, Mystic, Othello and SV6139. Othello was more mature than other varieties 30 DAP. Lumen (V2) was significantly less mature than other varieties. Othello was significantly more mature than the other varieties with a growth stage mean of V6 38 DAP. However, there was no statistical difference between Gleam, Mystic, Othello and SV6139.

Finally, in terms of postharvest darkening characteristic, 21 days after planting, there was no statistical difference between regular and slow darkening in plant height. The regular darkening varieties were more mature than the slow darkening varieties at 30 DAP, but there was no difference at either 21 or 38 DAP.

Table 7. The effect of salt type, pinto bean variety, and slow darkening characteristic on growth stage 21, 30 and 38 days after planting. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test.

Factors	**Growth stage 21 days after planting**	**Growth stage 30 days after planting**	**Growth stage 38 days after planting**
Salt types			
Control	2.0 (0.06) a	3.1 (0.08) a	6.3 (0.13) a
NaCl	1.4(0.07) b	2.6 (0.12) b	2.5 (0.32) c
$CaCl_2$	1.5 (0.08) b	2.7 (0.12) b	4.0 (0.34) b
$MgSO_4$	2.0 (0.07) ab	2.9 (0.11) ab	5.8 (0.20) a
Variety			
Cowboy	1.4 (0.07) b	2.6 (0.10) bc	4.1 (0.40) bc
Gleam	1.5 (0.08) ab	2.7 (0.10) bc	4.6 (0.40) abc
Lumen	1.3 (0.08) b	2.3 (0.10) c	3.9 (0.32) c
Mystic	1.7 (0.08) a	2.7 (0.10) bc	5.3 (0.33) ab
Othello	1.7 (0.08) a	4.0 (0.17) a	5.6 (0.30) a
SV6139	1.7 (0.09) a	3.0 (0.13) b	5.3 (0.50) ab
Postharvest darkening			
Regular darkening	2.0 (0.05) a	3.1 (0.09) a	5.0 (0.23) a
Slow darkening	1.4 (0.05) a	2.6 (0.06) b	4.6 (0.21) a

1= V1 (1st trifoliate leaf unfolded at node 3), 2= V2 (2nd trifoliate leaf unfolded at node 4), 3= V3 (3rd trifoliate leaf unfolded at node 5), 4= V4 (4th trifoliate leaf unfolded at node 6 + branching), 5= V5 (5th trifoliate leaf unfolded at node 7), 6= V6 (6th trifoliate leaf unfolded at node 8).

3.2.3 LEAF AREA INDEX

Analysis of variance (ANOVA) (Table 5) showed that the types of salt, the pinto bean varieties, and the postharvest darkening traits each significantly ($p \leq 0.05$) influenced the LAI (Figure 3), but there were no interactions. For the salt types, the highest LAI was recorded in the Control and $MgSO_4$ with 1.42 and 1.25, respectively. NaCl presented the lowest LAI (0.32). Regarding LAI among varieties, Othello was the variety with the highest LAI (1.15). However, there was no statistical difference between Othello, Cowboy and SV6139. Gleam, Cowboy, Lumen and Mystic were not significantly different from one another. Finally, the postharvest darkening characteristic was statistically different, with Regular darkening higher than Slow darkening.

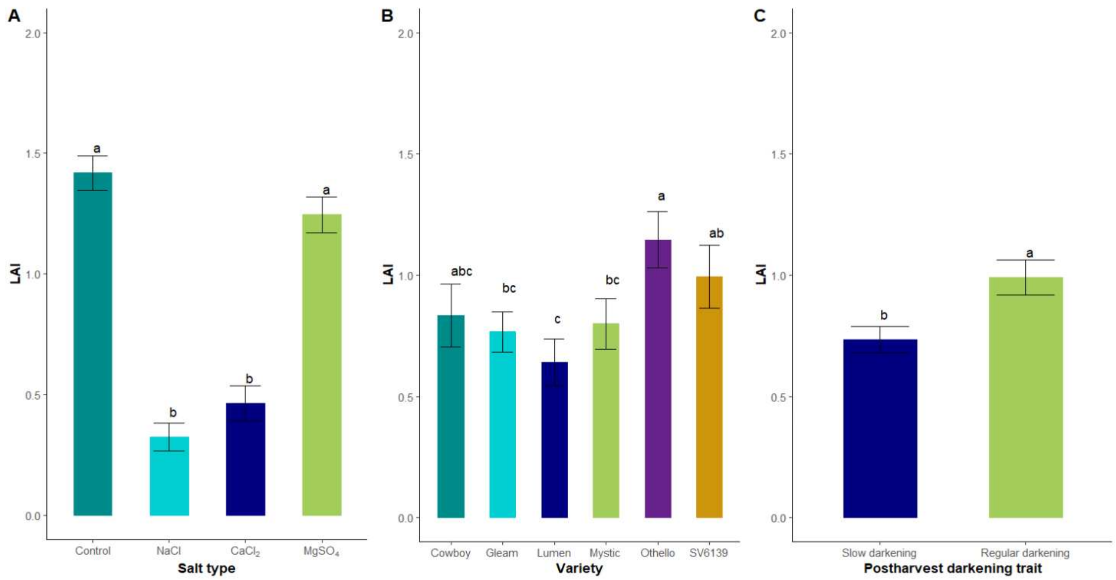

Figure 3. Leaf Area Index (LAI) as affected by salt type (A), variety (B), and postharvest darkening traits (C) 39 days after planting. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test.

3.2.4 CHLOROPHYLL LEVEL FOR YOUNG AND OLD LEAVES

The ANOVA presents evidence that the chlorophyll level of both young and old leaves was influenced by salt type, pinto bean variety, and by the interaction of salt type and variety (Table 8). Therefore, the results are presented across variety within salt type (Table 9). For the chlorophyll level in young leaves, in the control, the highest chlorophyll level in the young leaves was recorded in Othello with 35.3. Nevertheless, the varieties Cowboy, Mystic, Othello and SV6139 did not show statistical differences. Regarding the varieties within NaCl, Othello (26.8) and Mystic (25.7) were higher than other varieties. However, there was no significant difference among Gleam, Lumen, Mystic and Othello. For $CaCl_2$, although Othello (24.0) had the highest chlorophyll level, the varieties were not statistically different. Finally, in $MgSO_4$, the variety with the highest chlorophyll level was Othello (40.4), but there was no significant difference between Cowboy, Othello and SV6139.

Regarding the chlorophyll level in old leaves, in the control, the highest chlorophyll level was recorded in Othello with 41.5, and Lumen presented the lowest chlorophyll level with 31.7. For the NaCl, Mystic (28.3) and Othello (26.0) had the highest chlorophyll levels. However, there was no statistical difference among Mystic, Othello, Gleam and SV6139. In $CaCl_2$, the lowest chlorophyll level was recorded in Cowboy (3.0) followed by Gleam (5.6). Othello had the highest chlorophyll level of 24.8. $MgSO_4$ showed the highest chlorophyll level for all the varieties in old leaves. However, there was no significant difference among varieties. The chlorophyll level was generally higher in old leaves than in young leaves in Control and $MgSO_4$ treatments, but chlorophyll level was generally lower in old leaves in the NaCl and $CaCl_2$ treatments. Finally, analysis of variance shows evidence that postharvest darkening characteristic did not significantly affect chlorophyll level in old leaves (Table 8).

Table 8. ANOVA for chlorophyll level in young and old leaves 40 days after planting showing main effects and two-factor interactions.

	Young Leaves	**Old Leaves**
Factors	**p**	**p**
Trt	< 2.2e-16	< 2.2e-16
Vrt	3.854e-07	1.841e-08
Trt : Vrt	0.003435	0.02213
Trt	< 2.2e-16	<2e-16
PHD	0.138966	0.3102
Trt : PHD	0.003142	0.5706

Trt= treatment (salt type), Vrt= variety, PHD= postharvest darkening trait. *p*= *p*- value.

Table 9. The effect of pinto bean variety within salt types on chlorophyll level of young and old leaves 40 days after planting. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	Young leaves	Old leaves
Salt types (Control)		
Cowboy	31.9 (1.43) ab	34.9 (1.92) ab
Gleam	22.3 (1.37) c	34.3 (1.28) ab
Lumen	24.6 (3.12) bc	31.7 (4.16) b
Mystic	29.1 (1.96) abc	36.3 (1.61) ab
Othello	35.3 (1.29) a	41.5 (0.91) a
SV6139	28.1 (3.45) abc	36.2 (2.32) ab
Salt type (NaCl)		
Cowboy	13.1 (3.36) b	5.9 (3.96) b
Gleam	16.0 (2.21) ab	13.7 (4.78) ab
Lumen	15.9 (1.720 ab	7.9 (4.08) b
Mystic	25.7 (4.07) a	28.3 (3.94) a
Othello	26.8 (2.36) a	26.0 (2.47) a
SV6139	9.3 (2.81) b	12.4 (4.61) ab
Salt type ($CaCl_2$)		
Cowboy	11.4 (3.16) a	3.0 (3.02) c
Gleam	22.4 (3.15) a	5.6 (3.76) bc
Lumen	16.1 (3.34) a	11.3 (4.12) abc
Mystic	22.5 (2.57) a	20.8 (4.82) ab
Othello	24.0 (3.54) a	24.8 (3.17) a
SV6139	17.5 (4.60) a	13.3 (4.93) abc
Salt type ($MgSO_4$)		
Cowboy	33.1 (1.13) ab	42.4 (1.15) a
Gleam	29.6 (2.52) b	35.1 (4.14) a
Lumen	31.0 (1.88) b	39.9 (1.77) a
Mystic	31.8 (2.68) b	40.2 (1.56) a
Othello	40.4 (0.95) a	44.3 (1.47) a
SV6139	37.5 (2.05) ab	39.4 (4.64) a

The result from the analysis of variance shows that the chlorophyll level in young leaves was influenced by postharvest darkening characteristic within each salt type (Table 10), since the interaction between postharvest darkening trait and salt types showed significant differences at $p< 0.05$ (Table 8). In the control and $MgSO_4$ treatments, there was statistical difference in the postharvest darkening trait with regular darkening higher than slow darkening. Regarding NaCl and $CaCl_2$, there was no significant difference between regular and slow darkening pinto varieties.

Table 10. The effect of postharvest darkening traits within salt types on chlorophyll level of young leaves 40 days after planting. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test

Factors	Chlorophyll level
Control	
Regular darkening	31.8 (1.39) a
Slow darkening	25.4 (1.37) b
Salt type (NaCl)	
Regular darkening	16.4 (2.13) a
Slow darkening	19.2 (1.81) a
Salt type ($CaCl_2$)	
Regular darkening	17.6 (2.33) a
Slow darkening	20.3 (1.78) a
Salt type ($MgSO_4$)	
Regular darkening	37.0 (0.99) a
Slow darkening	30.8 (1.34) b

3.2.5 DRY SHOOT BIOMASS

Analysis of variance (ANOVA) (Table 12) showed that the types of salt, the pinto bean varieties, and the postharvest darkening trait each significantly ($p \leq 0.05$) influenced the dry shoot biomass. For the salt types, the highest dry shoot biomass was recorded in $MgSO_4$ and Control with 1.16 g and 1.14 g, respectively (Figure 4). There was no statistical difference between Control and $MgSO_4$ or between NaCl and $CaCl_2$. Regarding dry shoot biomass among varieties, Othello was the variety with the highest dry shoot biomass (1.11 g) followed by Cowboy (1.01 g) and SV6139 (0.99 g), respectively. Finally, the postharvest darkening characteristic was statistically different, with Regular darkening higher than Slow darkening.

Table 12. ANOVA for dry shoot and root biomass, shoot and root water content showing main effects and two-factor interactions.

	Dry Shoot Biomass	Dry Root Biomass	Shoot Water Content	Root Water Content
Factors	**P**	**p**	**p**	**p**
Trt	< 2.2e-16	5.769e-09	0.000258	< 2.2e-16
Vrt	1.492e-07	< 2.2e-16	0.000225	1.006e-07
Trt : Vrt	0.6158	0.001331	0.023047	3.819e-05
Trt	< 2.2e-16	1.412e-06	0.0005061	4.099e-14
PHD	2.826e-07	1.425e-08	0.0969610	0.547908
Trt : PHD	0.05196	0.01268	0.0042066	0.001985

Trt= treatment (salt type), Vrt= variety, PHD= postharvest darkening trait. *p*= *p*-value.

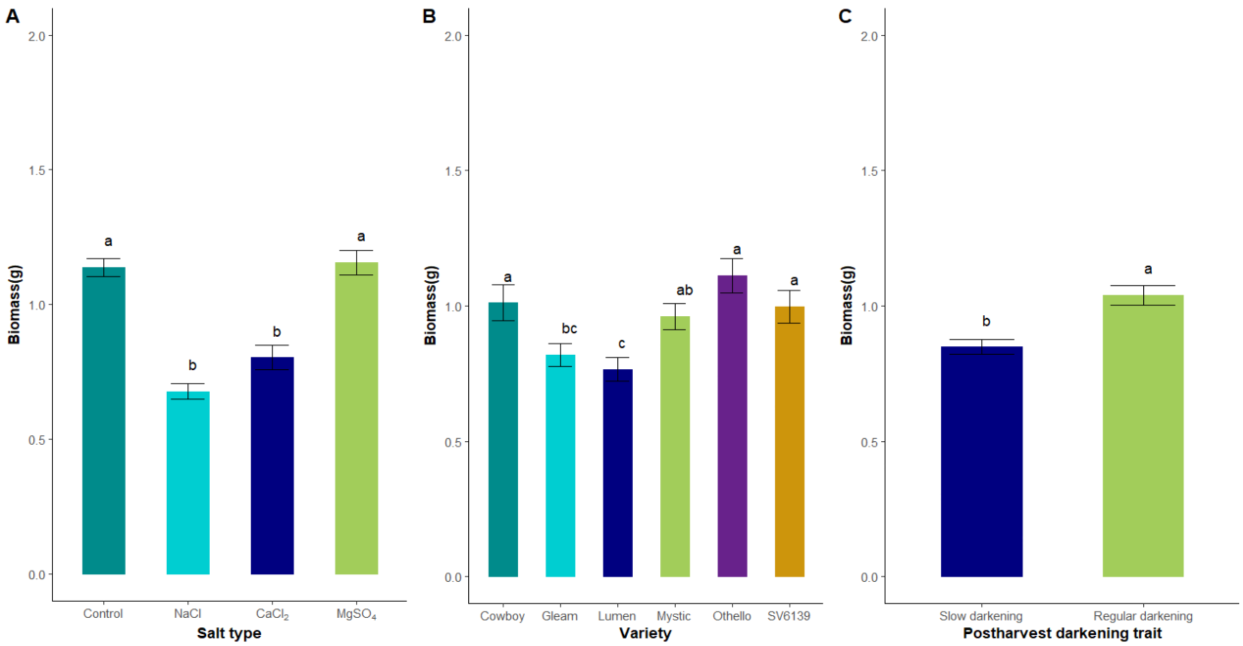

Figure 4. Dry shoot biomass as affected by salt type (A), variety (B), and postharvest darkening traits (C). Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

3.2.6 DRY ROOT BIOMASS, SHOOT AND ROOT WATER CONTENT

The analysis of variance indicates that dry root biomass, and water content of shoots and roots were influenced by pinto bean variety and postharvest darkening characteristic within each salt type, since the interaction between variety and salt types, postharvest darkening and salt types showed significant differences. (Table 12). For dry root biomass, Gleam and Mystic had the highest dry root biomass within each salt type (Table 13). Although Othello had the best performance above ground, it presented the lowest dry root biomass in each salt type. In Control, the postharvest darkening characteristic did not show significant difference for dry root biomass. However, Slow darkening was significantly higher than Regular darkening in NaCl, $CaCl_2$ and $MgSO_4$.

Regarding water content in shoot, no statistical difference was presented in Gleam, Mystic and Othello in Control. In NaCl, there was no significant difference among varieties. Othello had the highest water content in $CaCl_2$, although there was no significant difference between Gleam, Lumen, Mystic and Othello. In $MgSO_4$, Gleam, Lumen, Mystic and Othello were statistically different from Cowboy and

SV6139. For the postharvest darkening characteristic, Slow darkening was statistically higher than Regular darkening in Control and $MgSO_4$. However, no significant difference was presented between Regular and Slow darkening in NaCl or $CaCl_2$.

Finally for the water content in roots, the highest water content was recorded in Gleam, Lumen, Mystic, and Othello roots in the Control. In NaCl, Cowboy and Othello had the higher water content in the roots. However, there was no statistical difference among Cowboy, Gleam, Mystic, Othello and SV6139. In $CaCl_2$, there was no significant difference among varieties. The highest water content in $MgSO_4$ was recorded in Othello, but there was no significant difference among Gleam, Lumen, Mystic and Othello. In general, the result shows that even though Othello had the lowest root biomass, it had higher root water content. The postharvest darkening characteristic was only significant in $MgSO_4$.

Table 13. The effect of pinto bean variety and postharvest darkening trait within salt types on dry root biomass and water content in shoot and root. Means of 10 replicates with significant letters and standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test.

Factors	Dry root biomass	Shoot water content	Root water content
	-----------g----------	--------------------%	--------------------
Variety			
Salt types (Control)			
Cowboy	0.26 (0.02) a	30.68 (4.18) d	46.80 (4.22) c
Gleam	0.26 (0.02) a	69.73 (1.78) a	59.80 (2.99) abc
Lumen	0.16 (0.01) b	46.89 (4.58) bc	71.20 (2.80) a
Mystic	0.26 (0.02) a	62.86 (2.19) a	60.40 (3.49) ab
Othello	0.15 (0.009) b	59.12 (3.05) ab	69.80 (1.97) ab
SV6139	0.25 (0.02) a	42.10 (3.65) cd	56.70 (3.41) bc
Salt types (NaCl)			
Cowboy	0.11 (0.02) c	30.78 (6.66) a	80.90 (2.26) a
Gleam	0.24 (0.03) ab	37.66 (3.59) a	74.95 (2.11) ab
Lumen	0.17 (0.02) bc	29.13 (4.75) a	67.90 (3.54) b
Mystic	0.27 (0.01) a	45.32 (5.24) a	71.53 (4.15) ab
Othello	0.13 (0.008) c	52.51 (4.29) a	80.90 (1.57) a
SV6139	0.15 (0.02) c	41.73 (2.97) a	68.87 (3.60) ab
Salt types ($CaCl_2$)			
Cowboy	0.14 (0.02) b	34.22 (5.15) b	77.40 (3.58) a
Gleam	0.28 (0.02) a	48.15 (8.18) ab	72.53 (2.89) a
Lumen	0.15 (0.02) b	46.49 (6.76) ab	75.70 (1.78) a
Mystic	0.28 (0.04) a	53.03 (5.94) ab	70.98 (4.86) a
Othello	0.14 (0.02) b	62.90 (4.91) a	82.23 (0.80) a
SV6139	0.16 (0.02) b	37.38 (4.34) b	72.63 (1.99) a
Salt types ($MgSO_4$)			
Cowboy	0.28 (0.02) ab	39.17 (5.09) b	51.80 (4.60) c
Gleam	0.33 (0.04) ab	67.91 (6.29) a	68.55 (2.32) ab
Lumen	0.18 (0.02) c	64.27 (3.003) a	70.00 (4.85) ab
Mystic	0.35 (0.03) a	65.07 (3.14) a	61.53 (4.14) abc
Othello	0.17 (0.02) c	63.85 (1.37) a	71.70 (2.55) a
SV6139	0.23 (0.02) bc	31.78 (3.42) b	54.60 (3.23) bc
Postharvest darkening trait			
Salt type (Control)			
Regular darkening	0.22 (0.01) a	43.97 (2.98) b	57.77 (2.55) a
Slow darkening	0.23 (0.01) a	59.82 (2.48) a	63.80 (1.99) a
Salt type (NaCl)			
Regular darkening	0.13 (0.009) b	41.67 (3.18) a	76.89 (1.79) a
Slow darkening	0.23 (0.01) a	26.02 (12.73) a	71.46 (1.96) a
Salt type ($CaCl_2$)			
Regular darkening	0.15 (0.01) b	44.84 (3.59) a	77.42 (1.53) a
Slow darkening	0.24 (0.02) a	49.22 (3.94) a	73.07 (1.94) a
Salt type ($MgSO_4$)			
Regular darkening	0.23 (0.01) b	44.93 (3.25) b	59.37 (2.57) b
Slow darkening	0.28 (0.02) a	65.75 (2.48) a	66.69 (2.29) a

3.2.7 FLOWER NUMBER

The results of the ANOVA analysis (Table 14) showed the influence of salt type, variety, and postharvest darkening traits on flower number at 30 and 38 DAP (Table 15). The salt types presented no significant difference 30 DAP. Control, $CaCl_2$ and $MgSO_4$ showed no significant difference.

Regarding flower number among pinto bean varieties, Othello was significantly different from the other varieties at 30 DAP in that it was the only variety which flowered this early. Flowers were observed in all the varieties 38 DAP. The highest number of flowers was recorded in Othello (2 flowers). However, there was no statistical difference among Gleam, Mystic, Othello and SV6139.

Finally, in terms of postharvest darkening characteristic, regular darkening was significantly higher than slow darkening in flower number at 30 DAP. However, no statistical difference was observed between regular darkening and slow darkening 38 DAP.

Table 14. ANOVA for flower numbers 30 and 38 days after planting, and pod numbers 35 and 47 days after planting showing main effects and two-factor interactions.

	FN30	**FN38**	**PN35**	**PN47**
Factors	P	P	p	p
Trt	0.1726	0.008468	0.01489	< 2.2e-16
Vrt	<2e-16	1.468e-05	< 2e-16	1.411e-07
Trt : Vrt	0.04193	0.486930	4.451e-07	0.5723
Trt	0.3749	0.01524	0.1114	< 2e-16
PHD	3.012e-06	0.34503	5.8e-07	0.009828
Trt : PHD	3.012e-06	0.88342	0.1114	0.62019

FN30= flower number 30 days after planting, FN38= flower number 38 days after planting, PN35= pod number 35 days after planting, PN47= pod number 47 days after planting. Trt= treatment (salt type), Vrt= Variety, PHD= postharvest darkening trait. *p*= *p*-value.

Table 15. The effect of salt type, pinto bean variety, and slow darkening characteristic on flower number. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	Flower number 30 days after planting	Flower number 38 days after planting
Salt types		
Control	0.2 (0.09) a	1.3 (0.16) a
NaCl	0.2 (0.07) a	0.7 (0.20) b
$CaCl_2$	0.1 (0.05) a	1.0 (0.18) ab
$MgSO_4$	0.3 (0.10) a	1.4 (0.18) a
Variety		
Cowboy	0.0 (0.00) b	0.4(0.12) b
Gleam	0.0 (0.00) b	1.5 (0.21) a
Lumen	0.0 (0.00) b	0.4 (0.10) b
Mystic	0 (0.00) b	1.1 (0.24) ab
Othello	1.1 (0.20) a	2.0 (0.30) a
SV6139	0.0 (0.00) b	1.4 (0.24) a
Postharvest darkening		
Regular darkening	0.4 (0.08) a	1.1 (0.13) a
Slow darkening	0.0 (0.00) b	1.0 (0.12) a

3.2.8 POD NUMBER

Analysis of variance (Table 14) showed the influence of salt type, variety, and postharvest darkening traits on pod number at 35 and 47 DAP. The salt types presented the same pod number at 35 DAP (Table 16). However, no significant difference was observed among Control, NaCl and $CaCl_2$, or between $CaCl_2$ and $MgSO_4$. The highest pod number was recorded in $MgSO_4$ (3 pods) followed by Control (2 pods) at 47 DAP. $MgSO_4$ was statistically different from all other salt types.

Regarding the pod number in pinto bean varieties, Othello was the only variety with pods present 35 DAP. By 47 DAP, pods were observed in all the varieties. The highest number of pods was recorded in Othello (3 pods). Othello was statistically different from other varieties.

Finally, in terms of postharvest darkening characteristic, regular darkening was significantly higher than slow darkening in pod number at both 35 and 47 DAP.

Table 16. The effect of salt type, pinto bean variety, and slow darkening characteristic on pod number. Means of 10 replicates with standard error (SE) in parentheses. Salt types, varieties, or postharvest darkening trait with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	**Pod number 35 days after planting**	**Pod number 47 days after planting**
Salt types		
Control	0.2 (0.10) b	2.0 (0.21) b
NaCl	0.2 (0.10) b	0.3 (0.10) c
$CaCl_2$	0.3 (0.12) ab	1.0 (0.21) c
$MgSO_4$	1.0 (0.20) a	3.2 (0.28) a
Variety		
Cowboy	0.0 (0.00) b	1.1 (0.20) b
Gleam	0.0 (0.00) b	1.6 (0.32) b
Lumen	0.0 (0.00) b	1.0 (0.24) b
Mystic	0.0 (0.00) b	1.4 (0.34) b
Othello	2.0 (0.30) a	3.0 (0.33) a
SV6139	0.0 (0.00) b	1.6 (0.31) b
Postharvest darkening		
Regular darkening	1.0 (0.13) a	2.0 (0.18) a
Slow darkening	0.0 (0.00) b	1.3 (0.18) b

3.2.9 EC ANALYSIS OF SATURATED PASTE EXTRACT

The analysis of variance indicates that pinto bean variety had an impact on soil EC within each salt type, since the interaction between variety and salt types showed significant differences in soil EC (Table 17). In general, Control presented the lowest EC followed by $MgSO_4$ compared to the other salt types (Table 18). The highest EC was recorded in NaCl. For the control, SV6139 had the highest EC (1.32 dS/m) compared to the other varieties. The lowest EC was recorded in Othello (0.85 dS/m) followed by Lumen (0.90 dS/m). However, in NaCl, the varieties did not present any statistical difference. Compared to the other varieties, Gleam (5.96 dS/m) was significantly higher than Cowboy (3.71 dS/m) in $CaCl_2$. Mystic (4.08 dS/m), SV6139 (4.05 dS/m) and Cowboy (4.00 dS/m) were significantly higher in $MgSO_4$ compared to Lumen (3.21 dS/m). Finally, there was no statistical difference between postharvest darkening and regular beans within salt type (Table 19).

Table 17. ANOVA for EC (saturated paste extract) showing main effects and two-factor interactions.

	EC
Factors	**p**
Trt	< 2.2e-16
Vrt	0.002533
Trt : Vrt	0.046653
Trt	< 2.2e-16
PHD	4.139e-11
PHD : Trt	0.009573

EC= electrical conductivity. Trt= treatment (salt type), Vrt= variety, PHD= postharvest darkening. p= p-value

Table 18. The effect of pinto bean variety on soil EC (saturated paste extract) within salt types. Means of 10 replicates with significant letters and standard error (SE) in parentheses. Treatments with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	**EC (dS/m)**
Salt type (Control)	
Cowboy	1.06 (0.05) ab
Gleam	1.04 (0.10) ab
Lumen	0.90 (0.05) b
Mystic	1.00 (0.06) ab
Othello	0.85 (0.06) b
SV6139	1.32 (0.12) a
Salt type (NaCl)	
Cowboy	4.06 (0.33) a
Gleam	5.36 (0.39) a
Lumen	5.36 (0.41) a
Mystic	5.10 (0.25) a
Othello	4.69 (0.45) a
SV6139	5.35 (0.56) a
Salt type ($CaCl_2$)	
Cowboy	3.71 (0.25) b
Gleam	5.96 (0.41) a
Lumen	4.44 (0.47) ab
Mystic	4.95 (0.41) ab
Othello	4.60 (0.49) ab
SV6139	5.14 (0.69) ab
Salt type ($MgSO_4$)	
Cowboy	4.00 (0.11) a
Gleam	3.81 (0.26) ab
Lumen	3.21 (0.22) b
Mystic	4.08 (0.16) a
Othello	3.86 (0.15) ab
SV6139	4.05 (0.10) a

Table 19. The effect of postharvest darkening traits on soil EC (saturated paste extract) within salt types. Means of 10 replicates with significant letters and standard error (SE) in parentheses. Treatments with a common letter are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	EC (dS/m)
Control	
Regular darkening	1.08 (0.06) a
Slow darkening	0.98 (0.04) a
Salt type (NaCl)	
Regular darkening	4.70 (0.27) a
Slow darkening	5.28 (0.20) a
Salt type ($CaCl_2$)	
Regular darkening	4.49 (0.31) a
Slow darkening	5.12 (0.27) a
Salt type ($MgSO_4$)	
Regular darkening	3.97 (0.07) a
Slow darkening	3.70 (0.14) a

3.2.10 RELATIVE GROWTH COMPARISONS AMONG VARIETIES

Comparing each variety to its control, the analysis of variance (Table 20) showed the influence of salt type and variety on the relative plant height, LAI, dry shoot biomass and the chlorophyll levels in young and old leaves. The relative plant height, LAI and dry shoot biomass indicated that Othello was significantly higher compared to the other varieties (Figure 5). However, there was no statistically significant difference among Othello, Cowboy, SV6139 in the relative LAI, and Othello, Cowboy, SV6139 and Mystic in the relative dry shoot biomass.

Regarding the relative chlorophyll level in young and old leaves, the ANOVA presented evidence that the relative chlorophyll level of both young and old leaves was influenced by salt type, pinto bean variety, and by the interaction of salt type and variety (Table 20). Overall, Othello was significantly higher than the other varieties in relative chlorophyll level of both young and old leaves within each salt type (Table 21). For the salt types, the relative chlorophyll level of young and old leaves was significantly higher in $MgSO_4$ in general (Table 21).

Table 20. ANOVA for relative plant height, leaf area index (LAI), dry shoot biomass and chlorophyll level in old and young leaves, showing main effects and two-factor interactions.

	Plant Height	**LAI**	**Dry Shoot Biomass**	**Chlorophyll Old Leaves**	**Chlorophyll Young Leaves**
Factors	**p**	**p**	**p**	**p**	**p**
Trt	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
Vrt	7.447e-11	0.0003092	1.926e-07	1.841e-08	3.854e-07
Trt : Vrt	0.7372	0.4291503	0.6158	0.02213	0.003435

Trt= treatment (salt type), Vrt= variety. *p*= *p*-value.

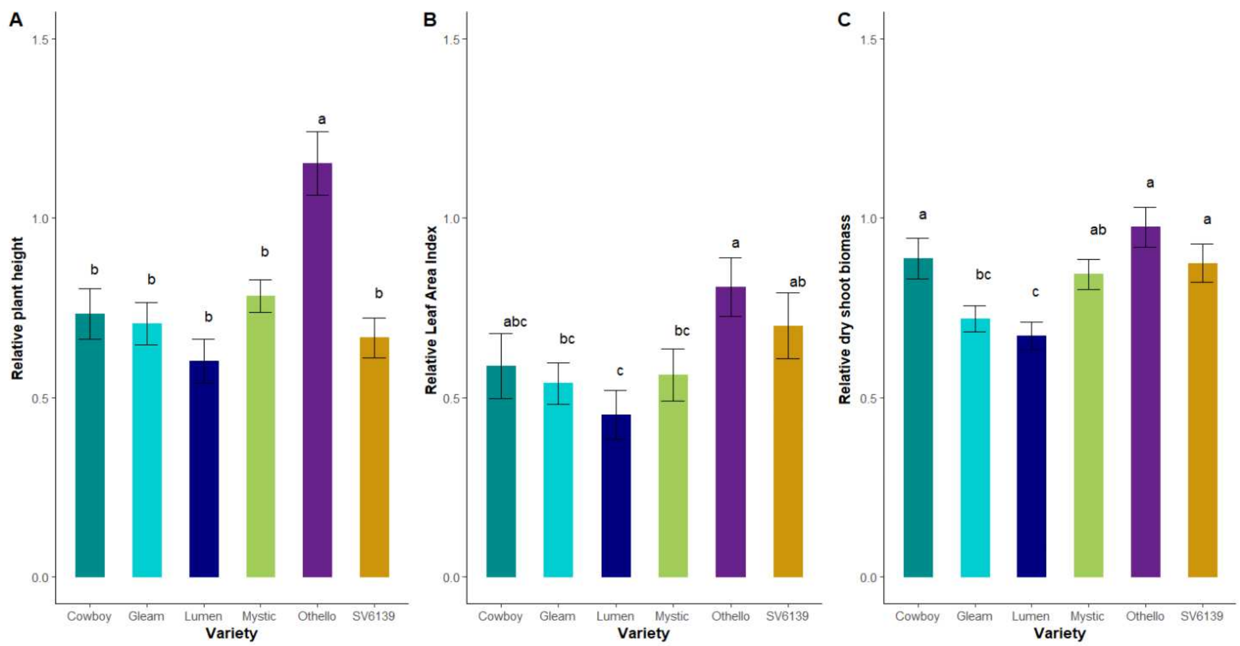

Figure 5. Relative plant height (A), leaf area index (LAI) (B) and dry shoot biomass (C) as affected by variety. Varieties with a common letter are not significantly different (p<0.05) based on Tukey's HSD Test.

Table 21. The relative chlorophyll level of young and old leaves within salt types. Means of 10 replicates with standard error (SE) in parentheses. Varieties with a common letter within salt type are not significantly different ($p<0.05$) based on Tukey's HSD Test.

Factors	Young leaves	Old leaves
Salt types (Control)		
Cowboy	1.12 (0.05) ab	0.97 (0.05) ab
Gleam	0.78 (0.05) c	0.96 (0.04) ab
Lumen	0.86 (0.11) bc	0.88 (0.12) b
Mystic	1.02 (0.07) abc	1.01 (0.04) ab
Othello	1.24 (0.05) a	1.16 (0.03) a
SV6139	0.98 (0.12) abc	1.01 (0.06) ab
Salt type (NaCl)		
Cowboy	0.46 (0.12) b	0.17 (0.11) b
Gleam	0.56 (0.08) ab	0.38 (0.13) ab
Lumen	0.56 (0.06) ab	0.22 (0.11) b
Mystic	0.90 (0.14) a	0.78 (0.11) a
Othello	0.94 (0.08) a	0.73 (0.07) a
SV6139	0.33 (0.09) b	0.35 (0.13) ab
Salt type ($CaCl_2$)		
Cowboy	0.39 (0.11) a	0.08 (0.08) c
Gleam	0.78 (0.11) a	0.16 (0.11) bc
Lumen	0.56 (0.12) a	0.32 (0.11) abc
Mystic	0.78 (0.09) a	0.57 (0.13) ab
Othello	0.84 (0.12) a	0.69 (0.08) a
SV6139	0.61 (0.16) a	0.37 (0.14) abc
Salt type ($MgSO_4$)		
Cowboy	1.16 (0.04) ab	1.18 (0.03) a
Gleam	1.04 (0.09) b	0.98 (0.12) a
Lumen	1.08 (0.06) b	1.11 (0.05) a
Mystic	1.11 (0.09) b	1.12 (0.04) a
Othello	1.42 (0.03) a	1.24 (0.04) a
SV6139	1.31 (0.07) ab	1.09 (0.13) a

3.3 FIELD EXPERIMENT

Regarding the field experiment, the average of EC in the 0-5 cm depth was 1.34 dS/m with a range of 0.99 to 1.88 dS/m, and the EC average in the 0-15 cm depth was 1.97 dS/m with a range of 0.87 to 3.72 dS/m (Table 22). The correlations of the yield, stand count and yield components as a function of EC were not significant (Table 23). These results suggest that these variables were not fundamentally affected by the soil EC. However, Othello tended to have the highest mean yield, stand count, and yield components (Table 24).

Table 22. The range of EC (saturated paste extract) values at two different depths.

Factors	0-5 cm	0-15 cm
EC (dS/m)	0.99-1.88	0.87-3.72

Table 23. The p-values and correlation coefficients (r) of yield, stand count, and yield components (flower number, pod number, pod weight, seed number, seed weight) correlated with the EC values at two different depths.

Factors	EC (0-5 cm)		EC (0-15 cm)	
	p	r	p	r
Yield	0.9872	0.0027	0.3905	-0.1475
Stand count	0.2539	-0.1952	0.0696	0.3059
Flower number	0.3568	-0.1581	0.5477	-0.1035
Pod number	0.8552	0.0315	0.3480	-0.1610
Pod weight	0.9183	0.0177	0.3941	-0.1464
Seed number	0.9040	-0.0208	0.4611	-0.1268
Seed weight	0.9872	0.0027	0.3905	-0.1475

Table 24. Varietal means with standard deviations in parentheses for yield, stand count and yield components (flower number, pod number, pod weight, seed number, seed weight) by variety for the field site.

Variety	Yield (kg/ha)	Stand count	Flower number	Pod number	Pod weight (g)	Seed number	Seed weight (g)
Cowboy	34.2 (18)	19.3 (4.5)	42.5 (7.5)	13.5 (1.2)	6.4 (2.5)	18.2 (6.3)	3.4 (1.8)
Gleam	154.2 (141.8)	24.5 (3.6)	24.5 (23.6)	35.5 (29.3)	22.1 (19.9)	51.0 (48.4)	15.4 (14.1)
Vibrant	204.2 (183.6)	24.2 (4.6)	63.6 (27.03)	53.6 (37.5)	31.5 (27.3)	85.0 (72.3)	20.4 (18.3)
Mystic	439.2 (337.3)	20.6 (7.6)	42.9 (13.8)	67.8 (43.8)	62.5 (46.0)	143.2 (110.8)	43.9 (33.7)
Othello	708.3 (429.3)	27.8 (5.1)	170.2 (55.4)	131.8 (78.4)	96.9 (55.7)	312.0 (197.9)	70.8 (42.9)
SV6139	154.2 (152.7)	19.0 (3.9)	49.3 (10.9)	41.6 (35.2)	25.0 (24.3)	55.8 (57.5)	15.4 (15.2)

4. DISCUSSION

The results from the germination experiment demonstrated reductions in germination percentage and germination speed as salt concentrations increased. Another study on the impact of salinity on seed germination of field pea (*Pisum sativum* L.) indicated that the germination and growth parameters of field pea were significantly affected by higher salt concentrations [33]. Research on the effect of salinity on germination and seed physiology of three bean cultivars (*Phaseolus vulgaris* L.) also documented that the germination percentage, seedling growth and respiration decreased under saline conditions at iso-molar concentration (100 mM) [34]. It has been reported that the exposure to high saline concentrations not only influences germination inhibition but also reduces germination speed and rate [22]. The effect on seed germination under salinity stress may be attributed to delayed water absorption and a decline in the activity of α-amylase, an enzyme associated with starch hydrolysis [35]. This enzyme disintegrates the starch stored in the endosperm into metabolizable sugars that sustain energy to the growing embryo and radicle. Hence, a decrease in α-amylase activity results in a notable reduction in the transfer of sugars, which restrains the embryo's growth and development. Consequently, the seed germination rate is reduced, and the seed germination period is delayed [35, 36, 37, 38, 39, 40]. The α-amylase activity of common bean during germination is an important parameter that could be evaluated in future studies on salt-stress tolerance.

Compared to the control and the other salt types, $CaCl_2$ had longer radicles in the germination study. Other investigations reported that Ca^{2+} plays a tremendous role as regulator of growth and development in plants [41]. It improves root morphogenesis regulation essentially through mediating phytohormone and stress signaling or affecting these signals. Ca^{2+} is crucial in primary root (PR) development. It impacts PR development through auxin accumulation adjustment, transport and signaling, along with the expression of many auxin-related genes. Ca^{2+} also operates as a transducer of auxin signaling throughout PR elongation. It promotes PR growth by influencing brassinosteroid signaling, an important phytohormone for PR development in plants [42, 43, 44, 45] and stimulates PR growth under both normal and stressful conditions. It has been corroborated that Ca^{2+} signaling can induce effects in enabling cell-wall integrity and modulate PR elongation under salt stress [42, 46].

Compared to the other salt types and the Control in the greenhouse study, $MgSO_4$ was significantly higher in the vegetative parameters such as plant height, growth stage, chlorophyll level in old and young leaves, and in the reproductive parameters, flower, and pod numbers. This finding suggests that $MgSO_4$ may generate a possible fertilizer effect. Magnesium (Mg) is an essential element for plant growth and development and plays a crucial role in plant defense mechanisms in abiotic stress conditions. It is very well known as the central atom of the chlorophyll molecule in the light-absorbing complex of chloroplasts and its contribution to photosynthetic fixation of carbon dioxide [47, 48, 49, 50]. Mg is a mobile nutrient [51], and its high phloem mobility allows it to be easily transferred to active growing parts of the plant where it is required for chlorophyll composition, enzyme activation for protein biosynthesis, and phloem export of photosynthates to ensure vegetative and generative growth [47, 52, 53]. A meta-analysis study showed that magnesium fertilization enhances the average yield in crop production by 8.5% [54], and significantly enhanced production of fruits (12.5%), grasses (10.6%), tobacco (9.8%), tubers (9.4%), vegetables (8.9%), cereals (8.2%), oil crops (8.2%), and tea (6.9%) [54]. Another study has indicated that $MgSO_4$ application at 3.0 g/m^2 promoted growth and grain yield of paddy rice. This application rate also improved the qualitative and quantitative production of flowers like rose, china rose (*Rosa chinensis*), marigold and sunflower and vegetables (bitter gourd) [55].

The positive significant effect of $MgSO_4$ on the growth parameters in the greenhouse study could also be induced by the presence of sulfur (S), one of the essential nutrients that is required for the adequate growth and development of plants [56]. Its deficiency affects disease resistance, performance of plants, the nutritional quality of crops and ultimately yield [56, 57]. Plants absorb the anionic form of sulfate (SO_4^{2-}) from soil through the roots and synthesize S-amino acids cysteine and methionine, a fundamental amino acid for cell and DNA functions [56, 57]. Several studies indicated that the scarcity of the sulfur-containing amino acids cysteine and methionine leads to chlorosis and inhibition of protein synthesis, and leaves of S-deficient plants have low chlorophyll contents [58- 63]. A study on the effects of S nutrition on the growth and photosynthesis of rice showed that an increase in the sulfate concentration in the medium up to 0.03 mM resulted in a significant increase in the relative growth rate [63]. Sulfur is a component of numerous protein enzymes that regulate photosynthesis and nitrogen fixation [64]. It has been reported that the greatest levels of S are accumulated in cruciferous plants, less in legumes and root vegetables and the least in grains and grasses [56, 65, 66]. Because of the role of S in N fixation, it is needed at higher levels for legumes like alfalfa and soybeans than for grass hay and corn [64]. Higher yielding fields generally have a higher rate of S removal when compared with lower producing fields [64].

Othello presented the highest germination percentage and germination speed as compared to other varieties in both control and saline conditions. In addition, the greenhouse experiment showed that Othello performed better aboveground than the other varieties. Othello had the highest plant height, growth stage, LAI, shoot biomass and chlorophyll level in young and old leaves. This may be facilitated by the early season maturity trait of Othello. Othello (PI 578268) was developed by USDA-ARS in cooperation with Washington State University and released in 1986 [67]. Othello is an F7 selection from the lineage 'NW-410' Pinto/2/'Victor' Pink/'Aurora' (NW- 410='Pinto UI-114'/'Sutter Pink'; Victor='Red Mexican UI-35'/1/PI 203958/2 UI-35/3/Sutter Pink/4/Aurora). It was identified as GH-215 and tested thoroughly in the Pacific Northwest from 1984 to 1986, in the interregional Cooperative Dry Bean Nursery at different areas in the USA and Canada. Othello equaled or exceeded other pinto cultivars in seed yield, size, and quality [67]. It has very early maturity (70 to 90 days), and the plants are vigorous, short, and adequately rigid [54]. A study on *Bean common mosaic virus* (BCMV) and rust-resistant pinto bean cultivars reported that the mean seed weight for Othello compared to two other varieties UIP35 and UIP40 was higher across 10 locations in the Cooperative Dry Bean Nursery (CDBN) in the United States and Canada in 2015 [68]. Other results in the same study showed that the cultivar Othello (85 d) had early maturity compared to other varieties tested (Blackfoot (86 d), Nez Perce (94 d), Twin Falls (108 d)) in the greenhouse at Kimberly, ID [68].

Although Othello had the greatest relative height, LAI, and shoot biomass under saline conditions, the lowest root biomass was also recorded in Othello. Othello also presented the highest root water content. It appears that Othello may be better adapted to soil with saline conditions than the other varieties. It has been reported in various studies that some plants use the mechanism of osmotic adjustment to maintain water uptake under salinity and drought stress by accumulating large quantities of osmolytes [14, 69, 70, 71]. Two types of osmolytes, organic solutes and inorganic ions, are crucial in osmotic adjustment. Organic solutes, known as compatible solutes, including amino acids, glycerol, sugars, proline, glycine-betaine, polyamines, and other low molecular weight metabolites, serve a function in cells to lower or balance the osmotic potential of intracellular and extracellular ions in resistance to osmotic stresses. Inorganic ions for osmotic adjustment are primarily Na^+, K^+, Ca^{2+}, and Cl^-. Inorganic ions are beneficial in osmotic adjustment through ion transport processes with related ion antiporters and ion channels [14, 69]. Salinity stress induces the synthesis of osmolytes [14, 72]. Some mechanisms of salt tolerance include strategies for ion exclusion from salt-sensitive organs or subcellular compartments/tissues by their exclusion in less sensitive areas such as the root, old leaves, or vacuoles. For this reason, some plants compartmentalize Na^+ into the vacuoles of most tissues to reduce the toxic concentration of Na^+ in the cytosol, thereby protecting the plant from salinity stress [14, 73,74]. Therefore, it is possible that Othello can take up excess salt and store it in less sensitive parts of the plant, such as the roots, which helps Othello to perform better in soil with high salinity.

Comparing the postharvest darkening characteristic, the results indicated that the differences presented in the greenhouse and field experiments between slow darkening and regular darkening pintos were mainly influenced by Othello. Regular darkening varieties were significantly higher in the vegetative and reproductive parameters because of the early maturity trait of Othello. However, in terms of the soil EC, slow darkening and regular darkening were statistically the same. Regarding the germination study, slow and regular darkening traits showed no significant difference for the parameters evaluated, except for the hypocotyl length, which also was impacted by Othello. These findings suggest that the comparison between these two groups of pinto beans did not imply a notable difference because one variety mainly influenced the variables assessed in this study. Therefore, it cannot be assumed that regular darkening pintos are more suitable for saline conditions. Implementing salt-tolerant pinto cultivars along with adequate management practices is a more appropriate recommendation to maintain yield in farming systems affected by salinity.

5. CONCLUSIONS

High salt concentrations affected the germination percentage and speed of the pinto bean varieties evaluated in this study. As concentration increased, germination percentage, germination speed and hypocotyl length decreased. The course of germination was significantly negatively affected by NaCl, and $CaCl_2$ enhanced the radicle length.

Each variety responded differently to saline conditions during their vegetative and reproductive stages. This study showed that salt buildup over time caused a height reduction, especially in soil affected by NaCl and $CaCl_2$. Othello (an early maturing variety) started flowering earlier than all the other varieties, regardless of salt treatment. Flowering and pod number were negatively affected by the presence of NaCl or $CaCl_2$ applications for most varieties. The variables analyzed in this study presented significantly higher performance in the control and $MgSO_4$ treatments than in the Cl salts.

Pinto beans are sensitive to salinity, but the extent of sensitivity varied among cultivars and salt types. Farmers growing beans in saline soils or with saline irrigation water may be able to avoid negative yield impacts by selecting a more salt-tolerant variety. This research demonstrates that Othello not only is an early maturing variety, but also achieved greater development and higher relative height, LAI, and biomass under saline conditions as compared to the other types of pinto bean, possibly by using some mechanisms such as osmotic adjustment and salt exclusion to adapt better to saline soils. It cannot be concluded that regular darkening pinto beans performed better than slow darkening pinto beans under salt stress because the significant differences were primarily impacted by one variety. The field study demonstrates that the yield components were not impacted by the soil EC due to the low EC values at that particular site. The evaluation of nodulation of salinity tolerant legumes in intercropping and rotation systems could be further studied to estimate the ability of specific cultivars to create favorable soil conditions to achieve significant yield improvements in saline soils.

REFERENCES

1. Keshavarz, R.; Didinger, C.; Duncan, A.; Thompson, H. Pulse crops and their key role as staple foods in healthful eating patterns. Fact sheet No. 0.313, 2022; Colorado State University Extension.
2. Didinger, C.; Thompson, H.J. The role of pulses in improving human health: A review. *Legume Science* **2022**, *4*, DOI: 10.1002/leg3.147.
3. Dongyu, Q. Keeping the spotlight on pulses: "roots" for sustainable agriculture and food security. 2023. Available online: https://www.un.org/en/un-chronicle/keeping-spotlight-pulses-%E2%80%9Croots%E2%80%9D-sustainable-agriculture-and-food-security#:~:text=For%20farmers%2C%20pulses%20are%20an,synthetic%20fertilizers%20and%20promote%20biodiversity. (accessed on October 8, 2023).
4. Kazem, G. G.; Neda, N. R.; Saeid, Z. S. Effect of salinity on yield and yield components of pinto bean cultivars. *International Journal of Plant, Animal and Environmental Sciences* **2012**, *2*, 47-51
5. Schwartz, H.F.; and Brick, M.A. Dry bean pest management and production, 3rd ed.; Colorado State University: Fort Collins, CO, USA.
6. Colorado Department of Agriculture. 2023. Colorado beans are a magical ingredient - Colorado proud's January recipe of the month. Available online: https://ag.colorado.gov/press-release/colorado-beans-are-a-magical-ingredient-colorado-prouds-january-recipe-of-the-month (accessed on September 13, 2023)
7. Colorado dry beans. Available online: https://coloradodrybeans.org/colorado-dry-bean-committee/dealers/#:~:text=Colorado%20grows%20many%20types%20of,Black%20beans (accessed on 23 October 2023).
8. Duwadi, K.; Austin, R. S.; Mainali, H. R. Slow darkening of pinto bean seed coat is associated with significant metabolite and transcript differences related to proanthocyanidin biosynthesis. *BMC Genomics* **2018**, *19*, DOI: 10.1186/s12864-018-4550-z.
9. Park, D.; Maga, J. A. Dry bean (*Phaseolus vulgaris*) color stability as influenced by time and moisture content. *Journal of Food Processing and Preservation* **1999**, *23*,443–522

10. Dale H. Slow-darkening pinto beans developed by NDSU, 2020; Available online: https://agupdate.com/farmandranchguide/news/crop/slow-darkening-pinto-beans-developed-by-ndsu/article_f8bf7bf4-d745-11ea-8d06-5fdf555291a3.html (accessed on 15 November 2023).
11. Davis, J. G; Brick, M. A. Fertilizing dry beans. Fact sheet No. 0. 539, 2009; Colorado State University Cooperative Extension.
12. Rasoul, K. K.; Vahid, C.; Mojtaba, I. Effects of salinity stress and drought due to different concentrations of sodium chloride and polyethylene glycol 6000 on germination and seedling growth characteristics of pinto bean (*Phaseolus vulgaris* L.). *Advances in Environmental Biology* **2015**, *8*, 413-419.
13. Bauder, T. A.; Davis, J. G.; Waskom, R. M. Managing saline soils. Fact sheet No. 0.503, 2014.; Colorado State University Cooperative Extension.
14. Nirit, B. Plants and salt: plant response and adaptations to salinity. *Astrobiology Exploring Life on Earth and Beyond* **2019**, 101-112.
15. Rekha, J.; Naleeni, R.; Jyoti, J.; Kumar, D.; Madanpal, S.; Akshay, T.; Tomar, S. M. S.; Dharmendra, S. Salt Stress in Pulses: A learning from global research on salinity in crop plants. *Indian Journal of Genetics and Plant Breeding* **2021**, *81*, 159-185.
16. Yildiz, H. D.; Sibel, K. Evaluation of salt tolerance in common bean genotypes by ion regulation and searching for screening parameters. *Journal of Food, Agriculture & Environment* **2009**, *7*,363-372.
17. Niknam, S. R.; Mccomb, J. A. Salt tolerance screening of selected australian woody species - A Review. *Forest Ecology and Management* **2000,** *139*,1-19.
18. Kağan, K.; Tolga, K.; Adil, B.; Mevlut, A. Determination of salinity tolerance of some lentil (*Lens culinaris* M.) varieties. *Journal of Food, Agriculture & Environment* **2009**, *8*,140-143.
19. Steven, H. G.; Janice, M. C. Tolerance of tepary and navy beans to NaCl during germination and emergence. *Hort Science* **1991**,*26*,246-249.
20. Salim, M.; Pitman, M. G. Salinity tolerance of mung bean (*Vigna radiata* L.): Seed Production. *Biologia Plantarum (PRAHA)* **2006**,*30*,53—57.
21. Bauder, T. A.; Davis, J. G.; Waskom, R.M. Salinity issues in Colorado. *Agronomy News* **1998**, *12*.

22. Rubio-Casal, A. E.; Castillo, J. M.; Luque, C. J.; Figueroa, M. E. Influence of salinity on germination and seeds viability of two primary colonizers of mediterranean salt pans. *Journal of Arid Environments* **2003**, *53*,145-154.

23. Amal, F. E.; Munira, J. E. Impact of salinity stress on germination and growth of pea (Pisum sativum L) plants. *Al-Mukhtar Journal of Sciences* **2020**,*35*,146-159

24. USDA (United State Department of Agriculture). Garrett Series. 2023. Available online: https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GARRETT.html (accessed on 7 February 2024).

25. Rhoades, J. Salinity: Electrical conductivity and total dissolved solids. Methods of soil analysis part 3—chemical methods, 1996;417-435.

26. Olsen, S. R. Estimation of available phosphorus in soils by extraction with sodium bicarbonate (No. 939), 1954; US Dept. of Agriculture. https://archive.org › details › estimationofavai939olse

27. Schwartz, H. F.; Langham, M. A. C. Growth stages of common beans (*Phaseolus vulgaris* L.), 2014; Available online: https://legume.coop/wp-content/uploads/2011/10/Card-Common-Bean-Growth-Stages.pdf (accessed on 7 February 2023).

28. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **2012**, *9*, 671–675.

29. Gutierrez, M.; Escalante-Estrada, J. A. ; Rodriguez-Gonzalez, M. T. Differences in salt tolerance between *Phaseolus vulgaris* and *Phaseolus coccineus* Cultivars. *International Journal of Agricultural Research* **2009**, *4*, 270-278.

30. Natural Resources Conservation Service Web Soil Survey. Physical Soil Properties---Weld County, Colorado, Southern Part. Version 18, 2020.

31. Corwin, D. L.; Scudiero, E. Field-scale apparent soil electrical conductivity. 2016. Methods of soil analysis 1.

32. Lesch, S.; Rhoades, J.; Corwin, D.; Robinson, D.; Suarez, D. ESAP-SaltMapper version 2.30 R. User manual and tutorial guide, Res. Report 149, December (2002). USDA-ARS. George E. Brown, Jr., Salinity Laboratory, Riverside, California.

33. Girma, W. F.; Chigigne, A. Impact of salinity on seed germination and biomass yields of field pea (*Pasum satrium* L.). *Asian Journal of Science and Technology* **2018**,*09*, 7565-7569.

34. Kaymakanova, M. Effect of salinity on germination and seed physiology in bean (*Phaseolus Vulgaris* L.), *Biotechnology & Biotechnological Equipment* **2009**, *23*, 326-329.

35. Kousik, A.; Saptarshi, M.; Shouvik, G.; Aditya, P. S.; Amrita, K.; Tuhina, G.; Arkaprava, R.; Suryakant, H.; Dinkar, J. G.; Subhasis, M.; Sudip, B.; Uday, C. J.; David, J. Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science* **2023**, *14*, DOI: 10.3389/fpls.2023.1241736

36. Munns, R.; Passioura, J. B. ; Colmer, T. D. ; Byrt, C. S. Osmotic adjustment and energy limitations to plant growth in saline soil. *New Phytol* **2020**, *225*, 1091–1096.

37. Daszkowska-Golec, A. Arabidopsis seed germination under abiotic stress as a concert of action of phytohormones. *OMICS: A Journal of Integrative Biology* **2011**, *15*, 763–774.

38. El Sabagh, A.; Islam, M. S.; Skalicky, M.; Raza, M. A.; Singh, K.; Hossain, M. A.; Hossain, A.; Mahboob, W.; Iqbal, M. A.; Ratnasekera, D.; Singhal, R. K.; Ahmed, S.; Kumari, A.; Wasaya, A.; Sytar, O.; Brestic, M.; ÇIG, F.; Erman, M.; Rahman, M. H. U.; Ullah, N.; Arshad, A. Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: adaptation and management strategies. *Frontiers in Agronomy* **2021**, *3*, DOI: 10.3389/fagro.2021.661932

39. Weiss, D.; Ori, N. Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiology* **2007**, *144*, 1240–1246.

40. Hasanuzzaman, M.; Raihan, M. R. H.; Masud, A. A.C.; Rahman, K.; Nowroz, F.; Rahman, M.; Nahar, K.; Fujita, M. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences* **2021**, *22*, 9326.

41. Peter, K. H. Calcium: A central regulator of plant growth and development. *The Plant Cell* **2005**, *17*, 2142–2155.

42. Xiao, P. Z.; Cai, X. M.; Li, R. S.; Fu, S. H. Roles and mechanisms of $Ca2^{+}$ in regulating primary root growth of plants. *Plant Signaling and Behavior* **2020**,*15*, DOI: 10.1080/15592324.2020.1748283

43. Wei, Z.; Li, J. Brassinosteroids regulate root growth, development, and symbiosis. *Molecular Plant* **2016**,*9*, 86–100.

44. Bergonci, T.; Ribeiro, B.; Ceciliato, P. H. O.; Guerrero-Abad, J. C.; Silva-Filho, M. C.; Moura, D. S. Arabidopsis thaliana RALF1 opposes brassinosteroid effects on root cell elongation and lateral root formation. *Journal of Experimental Botany* **2014**, *65*, 2219–2230.

45. Campos, W. F.; Dressano, K.; Ceciliato , P. H. O.; Guerrero-Abad, J. C.; Silva, A. L.; Fiori, C. S.; Morato Do Canto, A.; Bergonci, T.; Claus, L. A. N.; Silva-Filho, M. C.; Moura, D. S. Arabidopsis thaliana rapid alkalinization factor 1-mediated root growth inhibition is dependent on calmodulin-like protein 38. *Journal of Biological Chemistry* **2018**, *293*, 2159–2171.

46. Feng, W.; Kita, D.; Peaucelle, A.; Cartwright, H. N.; Doan, V.; Duan, Q.; Liu, M-C.; Maman, J.; Steinhorst, L.; Schmitz-Thom, I.; Yvon, R.; Kudla, J.; Wu, H.; Cheung, A. Y.; Dinneny, J. R. The feronia receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Current Biology* **2018**, *28*, 666–675.

47. Cakmak, I.; Kirkby, E. A. Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiologia Plantarum* **2008**,*133*, 692–704.

48. Mehmet, S.; Andreas, G.; Verena, W.; Heike, T. Role of magnesium fertilisers in agriculture: plant-soil continuum. *Crop and Pasture Science* **2015**,*66*,1219-1229.

49. Cakmak, I.; Yazici, A. M. Magnesium—a forgotten element in crop production. *Better Crops* **2010**,*94*, 23–25.

50. Gerendás, J.; Führs, H. The significance of magnesium for crop quality. *Plant and Soil* **2013**, *368*, 101–128.

51. Marschner, H. 'Marschner's mineral nutrition of higher plants.'(Ed. P Marschner) (Academic Press: London); 2012.

52. White, P. J.; Broadley, M. R. Biofortification of crops with seven mineral elements often lacking in human diets- iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* **2009**,*182*, 49–84.

53. Gransee, A.; Führs, H. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant and Soil* **2013**,*368*, 5–21.

54. Zheng, W.; Mahmood, U. I. H.; Faisal, N.; Liangquan, W.; Fusuo, Z.; Xuexian, L. Magnesium fertilization improves crop yield in most production systems: a meta-analysis. *Frontiers in Plant* Science **2019**, *10*.

55. Bhaskar, B., Dhananjay, D.; Sukanta, P.; Niranjan, K. Integrative effect of magnesium sulphate on the Growth of flowers and grain yield of paddy: A chemist's perspective. *RASAYAN Journal of Chemistry* **2013**,*6*,300-302

56. Narayan, O. P.; Kumar, P.; Yadav, B.; Dua, M.; Johri, A. K. Sulfur nutrition and its role in plant growth and development. *Plant Signaling & Behavior* **2023**, *18*, DOI: 10.1080/15592324.2022.2030082

57. Kopriva, S. ; Malagoli, M. ; Takahashi, H. Sulfur nutrition: impacts on plant development, metabolism, and stress responses. *Journal of Experimental Botany* **2019**, *70*,4069–4073.

58. Dietz, K. J.; Heilos, L. Carbon metabolism in spinach leaves as affected by leaf age and phosphorus and sulfur nutrition. *Plant Physiol.* **1990**, *93*, 1219-1225

59. Xu, H. L. ; Lopez, J. ; Rachidi, F. ; Tremblay, N. ; Gauthier, L. ; Desjardins, Y. ;, Gosselin, A. Effect of sulphate on photosynthesis in greenhouse-grown tomato plants. *Physiol. Plant.* **1996**, *96*, 722-726

60. Gilbert, S.M.; Clarkson, D.T.; Cambridge, M.; Lambers, H.; Hawkesford, M.J. SO/- deprivation has an early effect on the content of Ribulose-l ,5-bisphosphate carboxylase/oxygenase and photosynthesis in young leaves of wheat. *Plant Physiol.* **1997**, *115*, 1231-1239

61. Leoncini, L.; Ranieri, A.; Fergola, S.; Soldatini, G. F. Photosynthesis and metabolic changes in leaves of rapeseed grown under long-term sulfate deprivation. *Journal of Plant Nutrition* **1997**, *20*, 405-415

62. Blake-Kalff, M.M.A.; Harrison, K.R.; Hawkesford, M.J.; Zhao, F.J.; McGrath, S.P. Distribution of sulfur within oilseed rape leaves in response to sulfur deficiency during vegetative growth. *Plant Physiology* **1998**, *118*, 1337-1344

63. Resurreccion, A. P.; Makino, A.; Bennett, J.; Mae, T. Effects of sulfur nutrition on the growth and photosynthesis of rice. *Soil Science and Plant Nutrition* **2001**, *47*, 611-620

64. Place, S.; Kilcer, T.; Ketterings, Q.; Cherney, D.; Cherney, J. Sulfur for Field Crops. Fact sheet 34, 2007; Cornell University Cooperative Extension.

65. Jones, M. B. Sulfur availability indexes. M.A. Tabatabai (Ed.), Sulfur in agriculture, vol. 27, American society of agronomy, Madison, Wisconsin, USA (1986), pp. 549-566

66. Zhao, F. J. Role of sulfur for plant production in agricultural and natural ecosystems. R. Hell, C. Dahl, D. Knaff, T. Leustek (Eds.), Sulfur Metabolism in Phototrophic Organisms, Springer (2008), pp. 417-435

67. Burke, D.; Silbernagel, M.; Koehler, H. Registration of 'Othello' pinto bean. *Crop Science* **1995**, *35*,673-947.

68. Shree, P. S.; Phillip, N. M.; Mark, A. B.; Howard, F. S.; Carlos, A. U.; Henry, T.; Carlos, C.; Barry, O.; Kristen, O.; Alvaro, S. Pinto bean cultivars blackfoot, Nez Perce, and Twin Falls. *Journal of Plant Registrations* **2017**,*11*,212–217.

69. Hui, C.; Jian-Guo, J. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Canadian Science Publishing* **2010**, *18*,309-319.

70. Munns, R. Why measure osmotic adjustment? *Australian Journal of Plant Physiology* **1988**, *15*, 717 – 726.

71. Theodore, C. H.; Edmundo, A.; Fereres, E.; Henderson, D. W. Water stress, growth, and osmotic adjustment. Philosophical Transactions of the Royal Society of London. B, *Biological Sciences* **1976**, *273*,479-500

72. Stepien, P.; Johnson, G. N. Contrasting responses of photosynthesis to salt stress in the glycophyte arabidopsis and the halophyte thellungiella: Role of the Plastid Terminal Oxidase as an Alternative Electron Sink. *Plant Physiology* **2009**,*149*, 1154-1165

73. Monika, K.; Soumya, M.; Satish, C. B. Mechanisms of sodium transport in plants—progresses and challenges. *International Journal of Molecular Sciences* **2018**,*19*,647.

74. Zhu, J. K. Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology* **2003**, *6* ,441–445.