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Effect of Nano-Fertilizers on Alfalfa Plants Grown under Different Salt Stresses in Hydroponic System

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Abstract: In light of climate change and the ever-increasing population, salt stress has become a critical issue for agriculture and food security. The use of nano-fertilizers in agriculture is a promising application for salt stress management. Therefore, we investigated a hydroponic experiment to evaluate the effect of different nano-fertilizers: macro-nutrient (K_2SO_4) and micro-nutrient (ZnO and SiO_2) on two alfalfa (*Medicago sativa* L.) genotypes: (Susceptible: Bulldog 505, and tolerant: Mesa-Sirsa) grown with different salt concentrations (6 and 10 dS m^{-1}) in split-split design. The results demonstrated that nano-K_2SO_4 enhanced shoot dry weight, plant height, number of flowers, number of tillers, root length, root fresh weight, and root dry weight under both salt levels. Addition of nano-K_2SO_4 enhanced plant relative water contents and electrolyte leakage with both genotypes under different salt levels. Nano-SiO_2 promoted proline and SOD production with high salinity with values of (0.78 and 1.06 µmol g^{-1} FW) and 191.15 and 143.46 U. g^{-1} FW under Bulldog and Mesa-Sirsa, respectively. The application of nano-ZnO promoted plant micro-elements under 6 dS m^{-1} with both genotypes. The incorporation of nano-fertilizers into hydroponic systems provides a promising strategy, especially in regions with low water quality.

Keywords: nano-fertilizers; hydroponic system; salt stress; alfalfa genotypes

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1. Introduction

The population will reach about 10 billion by 2050, which requires increased nutritional needs [1]. About 63 million hectares worldwide suffer from salinization due to poor irrigation practices, pollution, and irrigation with untreated water [2]. Salt stress reduces productivity by up to 50% globally [2]. Freshwater deficit is prevalent in most regions of the world, particularly in arid and semiarid regions [3]. Saline water is considered a substitutional source to freshwater [4], which is deemed as poor quality [5]. Salinity affects the biological, chemical, and physical characteristics of soils and reduces the sustainable improvement of local agriculture [6]. Plants cultivated under salt stress suffer from various impacts like drought, photosynthetic performance, and ion imbalance [7]. Therefore, to face these circumstances, plants embrace various strategies comprising physiological, biochemical, and molecular mechanisms [8].

Alfalfa (*Medicago sativa* L.) is a warm-season legume, which is spreading worldwide on 30 mega hectares. Alfalfa production is rated around 454 million tons per year globally [9]. Alfalfa could be grown in saline conditions and as a good pasturage crop resource, it could conform well with the saline-alkali environment [10]. Alfalfa is considered moderately sensitive to salts, whilst exposure to salt stress of 0.05–0.2 M L^{-1} shortens the growth and productivity level of alfalfa [11,12]. Emam et al. [13] investigated that salt stress affected two genotypes of alfalfa and found that dry matter production reduction was observed with high salinity levels as a result of salt accumulation followed by the toxic effect.

Hydroponics, the 'nutrient solution cultures' of plants, has been anticipated in both research and commercial status since the 18th century [14]. Hydroponics is used now successfully on a large scale by trade farmers of fast-growing gardener crops, as it appropriates a more use efficiency of fertilizers and water, as well as a good dominance of climate and pest factors. Moreover, hydroponic outputs in increasing of crop yield and quality, which causes higher competitiveness and economic income [15]. A nutrient solution for hydroponics is an aquatic solution composed of inorganics ions from soluble salts of essential elements for higher plants [16]. All nutrient solutions used for hydroponics culture are essentially derived from the original protocol developed by Hoagland and Arnon [17].

The development of new kinds of fertilizers using modernistic nanotechnology introduces the opportunities to potentially enhance the performance of fertilizers and to significantly improve crop production needed to face the future needs of the growing global population [18]. Nano-fertilizers are sustainable alternatives to conventional chemical fertilizers for sustainable and environmentally clean production. Liu and Lal [19] demonstrated that some engineered nanomaterials (NM) can enhance plant productivity in various concentrations values and could be used as nano-fertilizers in agriculture to boost crop yields and reduce environmental contamination or enhance the efficacy of the conventional fertilizer without providing crops with nutrients [20,21]. Moreover, nano-fertilizers could be assorted as macronutrient and micronutrient nano-fertilizers. Laware and Raskar [22] reported that the period of keeping Zn in the plant system is low and hence, the bioavailability of Zn for a long time is not confirmed with the use of conventional $ZnSO_4$ fertilizer, while nanoparticles with minimized particle size and increased surface area are expected to be the ideal material for use as Zn amendment in plants. Liu et al. [18] illustrated that the dry weight of maize plants did not change significantly from 100 to 400 mg kg^{-1} nano-ZnO, while decreased gradually from 800 to 3200 mg kg^{-1}. Lin and Xing [23] declared that nano-ZnO at a rate of 2 g L^{-1} suppressed the germination of ryegrass seeds and frustrated root protraction of all species tested. In contrast, [24] investigated that application of ZnO in nano or bulk forms at a rate of 1 g L^{-1} in a hydroponic solution caused no effects on seed germination, root prolongation, and biomass of zucchini. Silicon presents as SiO_2 has a physiological role in depositing the form of hydrated amorphous silica ($SiO_2.nH_2O$) mainly in the endoplasmic reticulum, cell wall, and intercellular spaces. Suriyaprabha et al. [25] showed that a concentration of 6 g L^{-1} of nano-SiO_2 addition in maize plants significantly increased the plant dry weight and proved to be the best in alleviating salt stress. In tomato, application of N-SiO_2 (8 g L^{-1}, size: 12 nm) increased seed germination, mean germination time, seed germination index, and seed vigor index [26]. Potassium has a critical function in plant metabolism and growth, and it participates significantly in the subsistence of plants under several abiotic stresses [27]. The amendment of K^+ mitigates the adverse effects of salinity through its roles in osmotic adjustment, stomatal regulation, and maintenance of the balance of membrane ion-charge, cellular-energy status, and protein synthesis [28]. Thus, potassium fertilizer is very fundamental for crop production and quality. As a consequence, potassium consumption has developed dramatically in most regions of the world [29]. A significant positive relevance between K fertilizer input and grain yield was manifested by Dong et al. [30]. Other studies demonstrated several impacts of using nano-fertilizers on plant growth. Li et al. [31] reported the positive effects of using nano-zero valent iron on rice growth such as increased root growth, photosynthesis, several antioxidant enzymes, and phytohormones. Under salt stress, Wang et al. [32] suggested

that using Nano-CeO_2 with rice plants grown in hydroponic conditions and exposed to either NaCl or $CdCl_2$ could mitigate their effects and increase the plant defense mechanism via chlorophyll contents and antioxidant liberation.

The objective of this work is to study the effect of using nano-fertilizers (Macro/Micronutrients) in alleviating salt stress for alfalfa under a hydroponic system.

2. Materials and Methods

Seeds of both alfalfa genotypes (*Medicago sativa* L.) germinated for three weeks in Rockwool blocks and were watered regularly, occasionally with one-half strength Hoagland's nutrient solution under different levels of salt levels. The genotype Bulldog 505 was susceptible, and *Mesa-Sirsa* was salt tolerant. Seeds from the two genotypes were planted on 1 September 2016, until the seedlings were transplanted into the hydroponic system. The Nutrient Film Technique "N.F.T" is used to establish the experiment using the "Continuous Aeration System" to pump the nutrient solutions from the reservoir usually into a manifold that connects the larger tubing to several smaller ones. Each one of these smaller tubes runs nutrient solution to one side of each one of the growing channels (PVC tubes) with the seedlings holding with the Rockwool in suitably sized holes. A thin layer (film) of the nutrient solution flows through each of the channels with the plants in it to the other side, passing by each plant and wetting the roots on the bottom of the channel as it does. The nutrient solution flows from one side to the other because the channel is sloped slightly and pumped. The excess nutrient solution flowing out of the low end of each of the channels drains into another channel or tube and is guided back to the reservoir where it is recirculated through the system again. Each tube has 16 holes and the distance between each hole is 20 cm. The experimental design comprised a split-plot replicated three times. The main plots were salt concentrations of 0, 6, and 10 dSm^{-1} (0, 0.5%, 1.0%), and the subplots were rates of different nano-fertilizers. Two alfalfa genotypes (Bulldog and Mesa-Sirsa) plants were gradually subjected to two salt levels. Calcium chloride ($CaCl_2.2H_2O$) and sodium chloride (NaCl) were mixed in a 2:1 proportion ($CaCl_2$: NaCl) and added to Hoagland solution to make two nutrient solutions of electrical conductivity 6 and 10 dSm^{-1}.

The experiment consisted of nine treatments as follows: a control with Hoagland solution (235 ppm K), a control with Hoagland solution at EC 6 dSm^{-1}, a control with Hoagland solution at EC 10 dSm^{-1}, Hoagland solution with potassium source of potassium sulfate nanoparticles (nano-K_2SO_4) at 1/4 K^+ level of the control (based on a previous experiment by [33] under salt level of 6 dS m^{-1}, Hoagland solution with potassium source of nano-K_2SO_4 at 1/4 K^+ level of the control under a salt level of 10 dS m^{-1}, Hoagland solution with Nano-ZnO at 200 $mg.L^{-1}$ (according to Liu et al., [18]) under 6 dS m^{-1}, Hoagland solution with Nano-ZnO at 200 $mg.L^{-1}$ under 10 dS m^{-1}, Hoagland solution with Nano-SiO_2 at 6 dS m^{-1} (according to Siddiqui et al. [26]) under 6 dS m^{-1}, Hoagland solution with Nano-SiO_2 at 10 dS m^{-1}. Hoagland solution with non-Nano elements with minor modification used with the following salts: 1.0 M of $NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, H_3BO_3, $MnCl_2$. $4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, EDTA-$FeSO_4.7H_2O$, and SiO_2.

The plants were harvested twice, the former was 70 days after transplanted to determine vegetative parameters, and the latter cut was harvested 40 days after the first cut. Plant biomass was determined by measuring the shoot and root dry weights using a digital scale with 0.001 g sensitivity. Shoot and root length were measured in centimeters. The number of tillers and flowers was counted at harvest time. The relative water content of shoots was measured according to Turner [34] using the equation:

$$RWC = (FW - DW)/(TW - DW) \quad (1)$$

where, FW = fresh weight, TW = turgor weight, DW = dry weight.

The relative yield was counted according to Isla and Aragüés [35] by dividing the actual yield in each saline treatment by the highest yield observed.

2.1. Salt Stress Response

2.1.1. Proline

Free proline content was determined according to the method of Bates et al. [36]. Briefly,100 mg of plant materials were homogenized in 2 mL aqueous sulfosalicylic acid (3%), centrifuged at 13,000× *g* for 10 min, then 1 mL of filtrate was placed in a test tube and reacted with 1 mL glacial acetic acid and1 mL of acid–ninhydrin. The test tubes were heated in boiling water in a water bath for 1 h and the reaction was completed by placing the test tubes in an ice bath. The reaction mixture was extracted with 2 mL of toluene and mixed vigorously by vortex. The toluene layer separated at room temperature and the absorbance of chromophore containing toluene was measured at 520 nm using a spectrophotometer (Varian Cary 50 UV-Vis spectrophotometer, Agilent Technologies, Santa Clara, CA, USA), using pure toluene as the blank. Standard curves were prepared for each trial using standard proline in 3% sulfosalicylic acid solution. The proline content was expressed as micromoles per gram of fresh weight of plant materials.

2.1.2. Electrolyte Leakage

Electrolyte leakage was determined as described by [37]. 0.2 g of alfalfa fresh leaves were placed in test tubes containing 10 mL of distilled deionized water, incubated at 25 °C on a rotary shaker for 24 h, and subsequently, the electrical conductivity of the solution (L_t) was determined. Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (L_0) was obtained after equilibration at 25 °C. Measurements of electrical conductivity were made using the H1993310 conducti-meter (HANA Instruments, DJ110E, 457260, Cluj-Napoca, Romania). The electrolyte leakage (EL) was expressed as:

$$EL\ (\%) = (L_t/L_0) \times 100 \quad (2)$$

2.2. Antioxidant Enzymes

Then, 200 mg of leaf samples were homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 2% (*w*/*v*) polyvinylpyrrolidone (PVP). The whole extraction transaction was carried out at 4 °C. The homogenate was centrifuged at 10,000× *g* for 15 min at 4 °C and the supernatant was collected and used for assaying enzyme activity.

2.2.1. Catalase (CAT, EC 1.11.1.6)

Catalase (CAT, EC 1.11.1.6) activity was measured according to [38] as the rate of H_2O_2 disappearance at 240 nm by adding 100 µL leaf crude extract to the solution mixture containing 50 mM sodium phosphate buffer (pH 7.0) and 2% H_2O_2. The activity was calculated as units (µmol H_2O_2 consumed per min) per gram of fresh weight.

2.2.2. Superoxide Dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase (SOD, EC 1.15.1.1) assay was performed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm according to the method in [39]. Three milliliters of reaction mixture consisting of 50 mM Na-phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µM NBT, 10 µM EDTA, 2.0 µM riboflavin, and 0.3 mL enzyme extract were weighed for 10 min under 4000 RPM at 35 °C. One-unit SOD activity was determined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT reduction measured at 560 nm.

2.3. Statistical Analysis

Analysis of variance (ANOVA) was performed on the data from the seed germination study and the hydroponic experiment using PROC GLM of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Each treatment was performed in triplicate and data were analyzed by comparing treatments to control at $p < 0.05$. A three-way ANOVA analysis was performed to examine the interaction between the factors: The alfalfa two genotypes (Buldog 505

and Mesa-sersa), Nano- fertilizers (K_2SO_4, ZnO and SiO_2) and salt concentrations (0, 6 and 10 dSm^{-1}). Replications were considered non-numeric, and all other variables were considered fixed effects. Means of all variables were separated using Fisher's protected LSD test. The averages were compared by the Duncan Multi-Range tests.

3. Results

3.1. Plant Biomass

A difference in shoot dry weight, plant height, number of flowers, number of tillers, roots length, root fresh, and dry weight of alfalfa plants in the treatments exposed to nano-fertilizers are statistically significant ($p < 0.01$) (Table 1). The plant shoots dry weight, plant height, and root length in both genotypes decreased significantly with the increase in the salt levels. Under both salt concentrations, the application of nano-K_2SO_4 gave the highest significant increase in the shoot dry weight, plant height, number of flowers, and root fresh weight of the alfalfa in both genotypes under 6 and 10 dSm^{-1} compared to other treatments. With the sensitive genotype (Bulldog 505), the highest value of the number of tillers was observed in the nano-K_2SO_4 treatment under the level of 10 dSm^{-1} with the value of 6.0, while in the Mesa-sirsa genotype, the highest number of tillers was observed with the nano-SiO_2 addition under 10 dSm^{-1} levels with the value of 5.7. Application of nano-SiO_2 resulted in high root length with Bulldog 505 under both salt concentrations, and under 10 dS m^{-1} with Mesa-sirsa recording 33.09, 55.42, and 58.85 cm, respectively. While nano-ZnO treatment recorded the highest root length with the Mesa-sirsa genotype under 6 dS m^{-1} with a value of 37.92 cm.

3.2. Physiological Effect

ANOVA analysis in Table 2 illustrated that there were significant effects ($p < 0.01$) with genotypes, salt levels, different treatments, and their combinations on relative water content (RWC), electrolyte leakage (EL), and proline content (PC). Proline content, RWC, and El of alfalfa genotypes were significantly affected by salinity levels and supplementary addition of Si, K, and Zn nanoparticles. Under salt stress, the addition of nano-K_2SO_4 fulfilled enhancement of RWC and EL, while proline contents were affected by different amendments under different salt levels. Relative water content, EL, and proline contents significantly increased with the addition of Si, K, and Zn nanoparticles.

3.2.1. Relative Water Content (RWC)

Relative water content in Table 2 was affected significantly ($p < 0.01$) by the application of different nano-fertilizers with both alfalfa genotypes under different salt levels. Increasing salt rate resulted in decreasing RWC in both genotypes, regardless of the application of growth-stimulant compounds. It has been noticed that the susceptible genotype (Bulldog 505) has lower RWC in comparison with the tolerant genotype (Mesa-Sirsa) with both salt levels. Application of nano-ZnO fertilizer ameliorated the RWC in both genotypes under both salt concentrations, except with Bulldog 505 with 10 dS m^{-1} recording 61.61%, 63.39, and 50.36 with Bulldog under 6 and 10 dS m^{-1} and Mesa-Sirsa with 10 dS m^{-1} respectively. Application of nano-SiO_2 recorded the lowest RWC compared to other treatments under both salt levels, but more than the control.

Table 1. Different traits of alfalfa genotypes as affected by three salt levels and different nano-fertilizers.

Genotype	Salt Conc.	Treatments	Shoot Dry Weight (gm)	Plant Height (cm)	No. of Flowers	No. of Tillers	Root Length (cm)	Root Fresh Weight (gm)	Root Dry Weight (gm)
Bulldog	2.5 dS m^{-1}	Control (Hoagland)	2.68 ± 0.31^{b}	44.69 ± 4.82^{b}	12.0 ± 0.01^{cd}	3.67 ± 0.6^{cd}	35.08 ± 14.38^{f}	16.65 ± 1.34^{f}	1.69 ± 0.11^{bc}
	6 dS m^{-1}	Control	1.20 ± 0.1^{cde}	40.23 ± 2.56^{d}	12.3 ± 4.16^{c}	4.0 ± 1.0^{bc}	33.48 ± 1.96^{g}	12.25 ± 6.97^{g}	1.31 ± 0.6^{bcdef}
		Nano-K_2SO_4	2.55 ± 0.5^{b}	22.89 ± 2.34^{i}	1.0 ± 0.0^{ij}	3.0 ± 0.0^{cdef}	26.66 ± 1.04^{k}	33.84 ± 14.6^{a}	3.73 ± 1.51^{a}
		Nano-ZnO	0.54 ± 0.13^{de}	10.97 ± 1.95^{n}	0.0 ± 0.0^{j}	2.00 ± 0.0^{f}	26.1 ± 6.26^{k}	20.2 ± 0.12^{d}	3.65 ± 0.59^{a}
		Nano-SiO_2	0.20 ± 0.08^{e}	17.31 ± 2.14^{k}	0.0 ± 0.0^{j}	3.3 ± 0.57^{cde}	33.09 ± 9.96^{gh}	10.36 ± 2.1^{h}	1.65 ± 0.3^{bcd}
	10 dS m^{-1}	Control	1.2 ± 0.1^{cde}	35.74 ± 3.89^{f}	5.3 ± 1.15^{h}	4.0 ± 0.0^{bc}	34.79 ± 8.06^{f}	16.68 ± 5.74^{f}	1.45 ± 0.43^{bcde}
		Nano-K_2SO_4	6.30 ± 0.3^{a}	48.06 ± 2.97^{a}	20.0 ± 8.89^{a}	6.0 ± 1.0^{a}	40.17 ± 12.09^{d}	28.00 ± 0.34^{b}	0.38 ± 0.04^{def}
		Nano-ZnO	0.36 ± 0.04^{de}	18.15 ± 5.17^{k}	2.0 ± 0.0^{i}	2.7 ± 0.6^{def}	30.47 ± 1.85^{i}	10.7 ± 0.04^{h}	0.12 ± 0.04^{f}
		Nano-SiO_2	2.3 ± 0.07^{bc}	43.36 ± 1.55^{c}	10.3 ± 4.5^{e}	4.0 ± 0.0^{bc}	55.42 ± 8.87^{b}	18.9 ± 0.195^{e}	0.24 ± 0.02^{ef}
Mesa-Sirsa	2.5 dS m^{-1}	Control (Hoagland)	2.16 ± 0.08^{bc}	32.13 ± 4.07^{g}	12.0 ± 0.0^{cd}	3.3 ± 0.6^{cde}	51.67 ± 5.93^{c}	16.7 ± 4.2^{f}	1.34 ± 0.01^{bcdef}
	6 dS m^{-1}	Control	0.49 ± 0.08^{de}	9.12 ± 0.55^{o}	0.0 ± 0.0^{j}	2.3 ± 0.6^{ef}	20.36 ± 8.46^{m}	10.4 ± 0.09^{h}	0.14 ± 0.01^{f}
		Nano-K_2SO_4	2.23 ± 0.12^{bc}	38.79 ± 0.78^{e}	1.0 ± 0.0^{ij}	5.0 ± 0.0^{ab}	$32.19 \pm 1.2^{9\,h}$	21.04 ± 3.97^{d}	2.26 ± 0.09^{b}
		Nano-ZnO	1.43 ± 0.13^{bcde}	28.64 ± 5.44^{h}	6.7 ± 2.5^{g}	4.0 ± 1.0^{bc}	37.92 ± 5.54^{e}	13.44 ± 1.03^{g}	1.06 ± 0.02^{bcdef}
		Nano-SiO_2	1.5 ± 0.13^{bcd}	33.07 ± 5.01^{g}	0.0 ± 0.0^{j}	5.7 ± 0.6^{a}	27.21 ± 1.94^{jk}	9.55 ± 0.15^{h}	0.28 ± 0.07^{ef}
	10 dS m^{-1}	Control	0.25 ± 0.00^{de}	12.96 ± 3.76^{m}	8.0 ± 3.0^{f}	2.0 ± 0.0^{f}	28.31 ± 5.0^{j}	6.34 ± 0.43^{i}	0.9 ± 0.2^{cdef}
		Nano-K_2SO_4	0.36 ± 0.04^{de}	29.93 ± 2.45^{j}	16.7 ± 9.8^{b}	4.0 ± 0.0^{bc}	29.71 ± 6.73^{i}	18.2 ± 0.08^{e}	0.21 ± 0.01^{ef}
		Nano-ZnO	0.87 ± 0.15^{de}	21.05 ± 3.52^{j}	0.0 ± 0.0^{j}	2.3 ± 0.6^{ef}	23.07 ± 9.64^{l}	24.0 ± 0.28^{c}	0.37 ± 0.03^{ef}
		Nano-SiO_2	0.41 ± 0.08^{de}	14.33 ± 1.8^{i}	10.7 ± 3.1^{de}	2.0 ± 0.0^{f}	58.85 ± 16.75^{a}	12.86 ± 4.79^{g}	1.71 ± 0.08^{bc}
LSD			0.098	1.86	2.02	0.30	4.56	2.66	0.82

The column values with the same letters are statistical similar according to Duncan Multiple Range Test (DMRT) at $p < 0.05$.

Table 2. Effect of different nano-fertilizers (K_2SO_4, ZnO, and SiO_2) on Relative Water Content (RWC), Electrolyte Leakage (EL), and Proline content to Alfalfa genotypes grown under three salt levels (control "Hoagland", 6 and 10 dSm^{-1}).

Genotype	Salt Conc.	Treatments	RWC (%)	EL (%)	Proline (µmol g^{-1} FW)
Bulldog	2.5 dS m^{-1}	Control (Hoagland)	72.86 ± 10.57	62.9 ± 2.8	NS
	6 dS m^{-1}	Control	44.57 ± 1.77	63.5 ± 0.01	0.19 ± 0.01
		Nano-K_2SO_4	61.61 ± 4.92	89.5 ± 2.6	0.4 ± 0.08
		Nano-ZnO	54.69 ± 0.62	ns *	ns
		Nano-SiO_2	49.17 ± 11.64	82.3 ± 7.6	0.28 ± 0.01
	10 dS m^{-1}	Control	37.97 ± 8.06	68.5 ± 1.1	0.29 ± 0.37
		Nano-K_2SO_4	42.89 ± 4.15	96.8 ± 0.5	0.31 ± 0.01
		Nano-ZnO	46.02 ± 0.39	94.7 ± 2.7	0.43 ± 0.o1
		Nano-SiO_2	39.42 ± 0.63	82.5 ± 0.95	0.78 ± 0.071
Mesa-Sirsa	2.5 dS m^{-1}	Control (Hoagland)	72.01 ± 2.5	64.7 ± 10.4	0.21 ± 0.03
	6 dS m^{-1}	Control	36.44 ± 1.78	74.1 ± 8.6	0.32 ± 0.01
		Nano-K_2SO_4	63.39 ± 2.93	88.0 ± 4.0	0.36 ± 0.02
		Nano-ZnO	60.71 ± 3.31	84.2 ± 3.3	0.53 ± 0.22
		Nano-SiO_2	50.65 ± 1.02	ns	ns
	10 dS m^{-1}	Control	37.4 ± 6.24	74.4 ± 2.4	0.94 ± 0.01
		Nano-K_2SO_4	50.36 ± 5.78	96.8 ± 2.2	1.02 ± 0.04
		Nano-ZnO	46.16 ± 0.71	77.0 ± 1.5	ns
		Nano-SiO_2	41.29 ± 1.75	84.5 ± 8.0	1.06 ± 0.06
Genotype * Salt conc.			173.17 *	4.79	0.007
Genotype * Treatments			374.33 **	943.72	0.19 *
Salt Conc. * Treatment			900.53 **	551.83 **	0.52 **
Genotype * Salt Conc. * Treatments			228.27 **	6920.94 **	0.52 **
LSD			2.03	2.50	0.005

Ns *: no samples, *: significantly at 0.05, **: significantly at 0.01.

3.2.2. Electrolyte Leakage (EL)

Salt levels and different nano-fertilizers significantly ($p < 0.01$) affected plant EL. Increasing salt concentration resulted in increasing electrolyte leakage in both genotypes and the increment with the tolerant genotype was much higher (12.7% and 13.04%) than in the susceptible genotype (0.9% and 8.1%) compared to control (Table 2). Data revealed that the addition of nano-amendments caused a better effect on plant EL. Application of nano-K_2SO_4 resulted in enhancing electrolyte leakage under both salt concentrations recording 89.5 % and 96.8% in susceptible genotype and 88.00% and 96.8% in tolerant genotype under 6 and 10 dS m^{-1} respectively. Application of nano-ZnO with Bulldog 505 with increasing salt levels encouraged the EL, recording 94.7%, while nano-SiO_2 with Mesa-Sirsa with increasing salt levels encourage the EL, recording 84.5%.

3.2.3. Proline

There was a significant effect ($p < 0.01$) between salt concentrations and plant proline content resulting in increasing plant proline with rising salt levels (Table 2). While the proline values increased in the alfalfa tolerant genotype compared to the susceptible genotype. The nano-fertilizers treatments significantly ($p < 0.01$) raised plant proline content. With a moderate increase of salinity (6 dS m^{-1}), nano-K_2SO_4 and nano-ZnO had superior effects on increasing proline content, recording 0.4 µmol g^{-1} FW and 0.53 µmol g^{-1} FW with Bulldog and Mesa-Sirsa genotypes, respectively. Application of nano-SiO_2 had a synergistic enhancement effect on proline production with high salinity levels with both genotypes recording 0.78 µmol g^{-1} FW and 1.06 µmol g^{-1} FW with Bulldog and Mesa-Sirsa genotypes, respectively.

3.3. Antioxidant Enzymes

The results concerning the effect of different treatments on catalase (CAT in mmol H_2O_2 min^{-1} g^{-1} FW) and super oxide dismutase (SOD in U. g^{-1}FW) of Alfalfa genotypes are given in Table 3. ANOVA analysis in Table 3 revealed that there were significant effects ($p < 0.01$) between genotypes, salt rates, treatments, and their combination on CAT and SOD. Catalase activity in alfalfa leaves increased with increased salt concentration by 16.8% and 31.6% and 33.3% and 50.2% at 6 and 10 dS m^{-1} with Bulldog 505 and Mesa-Sirsa genotypes, respectively. Application of nano-SiO_2 resulted in boosted alfalfa plant enzymes recording the highest values under both genotypes. nano-SiO_2 consolidated catalase production recording with Bulldog genotype 114.14 and 105.14 mmol H_2O_2 min^{-1} g^{-1} FW under 6 and 10 dS m^{-1}, respectively, and recording 108.56 mmol H_2O_2 min^{-1} g^{-1} FW under 10 dS m^{-1} with Mesa-Sirsa genotype. With regard to SOD enzyme activity, nano-SiO_2 application recorded the best effect on reproduction of SOD in both genotypes under different salt levels except for Bulldog under 6 dS m^{-1}, application of Nano-K_2SO_4 recorded the highest magnitude of SOD with values of 129.62 U. g^{-1} FW.

Table 3. Effect of different nano-fertilizers (K_2SO_4, ZnO, and SiO_2) on catalase (mmol H_2O_2 min^{-1} g^{-1} FW) and SOD (Super oxide dismutase in U. g^{-1}FW) of Alfalfa genotypes grown under three salt levels (control "Hoagland", 10 and 15 dSm^{-1} electrical conductivity).

Genotype	Salt Conc.	Treatments	CAT	SOD
Bulldog 505	2.5 dS m^{-1}	Control (Hoagland)	29.70 ± 2.39	94.19 ± 3.67
	6 dS m^{-1}	Control	35.68 ± 0.12	102.37 ± 15.54
		Nano-K_2SO_4	49.21 ± 2.75	129.62 ± 4.03
		Nano-ZnO	NS *	NS
		Nano-SiO_2	114.14 ± 12.27	122.75 ± 6.87
	10 dS m^{-1}	Control	43.4 ± 9.9	102.13 ± 11.14
		Nano-K_2SO_4	56.94 ± 4.11	186.21 ± 4.79
		Nano-ZnO	88.58 ± 11.76	144.08 ± 9.32
		Nano-SiO_2	105.14 ± 5.34	191.15 ± 13.71
Mesa-Sirsa	2.5 dS m^{-1}	Control (Hoagland)	25.49 ± 3.66	98.80 ± 6.64
	6 dS m^{-1}	Control	38.24 ± 0.04	102.20 ± 18.31
		Nano-K_2SO_4	60.66 ± 7.93	130.49 ± 6.81
		Nano-ZnO	99.76 ± 4.1	134.12 ± 15.17
		Nano-SiO_2	NS	NS
	10 dS m^{-1}	Control	51.47 ± 0.82	117.54 ± 9.48
		Nano-K_2SO_4	94.32 ± 2.46	136.18 ± 10.77
		Nano-ZnO	NS	NS
		Nano-SiO_2	108.56 ± 9.83	143.46 ± 0.71
Genotype * Salt conc.			303.81 **	657.93 **
Genotype * Treatments			2114.10 **	315.66 *
Salt Conc.* Treatment			1146.44 **	11,946.77 **
Genotype * Salt Conc.* Treatments			10,719.01 **	8393.59 **
LSD			10.46	7.73

Ns *: no samples, *: significantly at 0.05, **: significantly at 0.01.

3.4. Plant Tissue Chemical Characteristics

Data in Table 4 show that increasing salt levels resulted in increased plant chemical nutrients. Application of potassium in nano-form resulted in increased plant absorption of K and hence decreasing Na/K values under different salt concentrations with both genotypes. Also, it is noticed from Table 4 that nano-K_2SO_4 was more effective in preferable K absorption than Na absorption with susceptible germplasm (Bulldog 505) compared to tolerant one (Mesa-Sirsa) recording 7.6%, 20.8%, and 6.9% and 15.7% under 6 and 10 dS m^{-1} in both germplasms respectively. While the application of nano-ZnO caused increasing in Mg, P, and S in Bulldog 505 plants under 6 dS m^{-1} but the application of nano-K_2SO_4 gave rise to increased P and S under 10 dS m^{-1} recording 2.7% and 2.6%, respectively. With alfalfa tolerant genotype (Mesa-Sirsa), application of nano-ZnO recorded the highest Ca

and Mg values under 6 dS m^{-1} recording 3.9% and 3.4%, while nano-SiO_2 recorded the highest P and S values (0.66 and 0.83%) under 6 dS m^{-1} and highest Ca and Mg values under 10 dS m^{-1} recording 3.7% and 0.2% respectively.

Table 4. Macro-elements in plant tissue of two alfalfa genotypes as affected by three salt levels (0 and 6 dS m^{-1} Electrical Conductivity) and different nano-fertilizers (K_2SO_4, ZnO, and SiO_2).

Genotype	Salt Conc.	Treatments	Na/K	Ca	Mg	P	S
			%	mg. Kg^{-1}			
Bulldog	2.5 dS m^{-1}	Control (Hoagland)	12.0 ± 1.5 ^{i}	10,550.0 ± 2350.0 ^{q}	2700.0 ± 100.0 ^{f}	2250.0 ± 750.0 ^{k}	2133.3 ± 122.2 ^{o}
	6 dS m^{-1}	Control	13.8 ± 1.8 ^{h}	42,233.3 ± 2442.0 ^{f}	2533.3 ± 115.5 ^{i}	1600.0 ± 173.2 ^{p}	2500.0 ± 458.3 ^{h}
		Nano-K_2SO_4	7.6 ± 1.3 ^{j}	28,600.0 ± 100.0 ^{l}	2100.0 ± 100.0 ^{k}	1800.0 ± 100.0 ^{n}	2300.0 ± 100.0 ^{l}
		Nano-ZnO	18.13 ± 3.5 ^{f}	19,350.0 ± 450.0 ^{n}	7600.0 ± 10.0 ^{a}	4600 ± 0.0 ^{c}	7600.0 ± 15.0 ^{b}
		Nano-SiO_2	7.7 ± 1.6 ^{j}	75,800.0 ± 0.0 ^{a}	4200.0 ± 50.0 ^{c}	2633.3 ± 155.0 ^{h}	3233.3 ± 105.0 ^{e}
	10 dS m^{-1}	Control	36.4 ± 3.5 ^{b}	48,166.7 ± 405.1 ^{c}	2566.7 ± 57.7 ^{h}	1633.3 ± 152.8 ^{o}	2366.7 ± 57.73 ^{k}
		Nano-K_2SO_4	20.8 ± 12.4 ^{e}	36,300.0 ± 264.6 ^{i}	1666.7 ± 152.8 ^{n}	2733.3 ± 251.7 ^{f}	2566.7 ± 115.5 ^{g}
		Nano-ZnO	30.1 ± 0.0 ^{d}	43,950.0 ± 315.0 ^{e}	2733.3 ± 351.2 ^{e}	2400.0 ± 264.6 ^{j}	2100 ± 624.5 ^{p}
		Nano-SiO_2	32.8 ± 8.4 ^{c}	31,400.0 ± 1609.3 ^{k}	1850.0 ± 50.0 ^{m}	2000.0 ± 100.0 ^{l}	2266.6 ± 115.4 ^{m}
Mesa-Sirsa	2.5 dS m^{-1}	Control (Hoagland)	13.03 ± 1.1 ^{hi}	18,950.0 ± 6850.0 ^{o}	6366.7 ± 1097.0 ^{b}	4633.3 ± 251.4 ^{b}	4566.7 ± 201.1 ^{c}
	6 dS m^{-1}	Control	15.3 ± 1.1 ^{g}	48,733.3 ± 850.5 ^{b}	2266.7 ± 57.7 ^{j}	2700 ± 173.2 ^{g}	2466.7 ± 208.2 ^{i}
		Nano-K_2SO_4	6.9 ± 4.2 ^{j}	32,116.7 ± 1675.1 ^{j}	650.0 ± 35.0 ^{q}	4250.0 ± 550.0 ^{d}	3300.0 ± 900.0 ^{d}
		Nano-ZnO	7.09 ± 1.4 ^{j}	39,050.0 ± 1550.0 ^{g}	3450.0 ± 1250.0 ^{d}	1833.3 ± 107.8 ^{m}	1766.7 ± 101.1 ^{q}
		Nano-SiO_2	7.04 ± 1.6 ^{j}	16,350.0 ± 5150.0 ^{p}	2633.3 ± 251.7 ^{g}	6633.3 ± 109.0 ^{a}	8266.7 ± 134.5 ^{a}
	10 dS m^{-1}	Control	21.3 ± 3.4 ^{e}	46,891.4 ± 1370.8 ^{d}	1909.1 ± 101.2 ^{l}	2474.7 ± 198.7 ^{i}	2151.2 ± 50.04 ^{n}
		Nano-K_2SO_4	15.7 ± 3.2 ^{g}	37,066.7 ± 1285.8 ^{h}	1500 ± 0.0 ^{p}	3100 ± 100.0 ^{e}	2633.3 ± 231.0 ^{f}
		Nano-ZnO	43.8 ± 0.0 ^{a}	21,800.0 ± 0.0 ^{m}	1600.0 ± 100.0 ^{o}	900.0 ± 0.0 ^{q}	1600.0 ± 0.0 ^{r}
		Nano-SiO_2	18.4 ± 4.1 ^{f}	37,066.7 ± 1850.2 ^{h}	2100.0 ± 200.0 ^{k}	900.0 ± 100.0 ^{q}	2400 ± 435.9 ^{j}
LSD			16.8	4446.8	717.94	4335.4	5345.3

The column values with the same letters are statistical similar according to Duncan Multiple Range Test (DMRT) at $p < 0.05$.

Data in Table 5 illustrate that addition of nano-fertilizers magnificently enhanced micro-element absorbance with increasing salt levels under both genotypes. The data elucidate that the application of Nano-ZnO promoted plant micro-elements under 6 dS m^{-1} with both genotypes, while Nano-K_2SO_4 treatment elevated most plant micro-elements under 10 dS m^{-1} with both alfalfa genotypes.

Table 5. Micro-elements in plant tissue of two alfalfa genotypes as affected by three salt levels (0, 6, and10 dS m^{-1}) and different nano-fertilizers (K_2SO_4, ZnO, and SiO_2).

Genotype	Salt Conc.	Treatments	Al	B	Cd	Cr	Cu	Fe	Mn	Ni	Si	Zn
			mg. Kg^{-1}									
Bulldog	2.5 dS m^{-1}	Control (Hoagland)	12.9 ± 1.7	37.4 ± 2.8	1.6 ± 0.4	2.1 ± 0.8	8.2 ± 2.2	24.2 ± 2.4	74.2 ± 4.8	1.6 ± 0.4	31.03 ± 5.2	85.2 ± 8.7
	6 dS m^{-1}	Control	20.0 ± 0.0	53.6 ± 6.4	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	132.3 ± 7.1	140.6 ± 4.3	2.0 ± 0.0	164.9 ± 8.1	174.66 ± 24.7
		Nano-K_2SO_4	12.9 ± 0.06	26.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	10.6 ± 0.1	34.2 ± 0.2	87.0 ± 0.1	1.3 ± 0.1	131.4 ± 0.2	28.9 ± 0.1
		Nano-ZnO	40.0 ± 0.0	84.7 ± 0.0	3.1 ± 0.0	3.1 ± 0.0	15.5 ± 0.0	360.7 ± 0.0	188.2 ± 0.0	3.1 ± 0.0	160.7 ± 0.0	323.5 ± 75.1
		Nano-SiO_2	31.7 ± 1.8	47.8 ± 3.3	2.7 ± 0.2	2.7 ± 1.0	13.7 ± 6.2	137.2 ± 6.3	105.2 ± 27.2	2.7 ± 1.2	399.5 ± 2.4	113.7 ± 6.8
	10 dS m^{-1}	Control	36.7 ± 2.8	68.0 ± 6.6	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	46.5 ± 2.6	156.0 ± 24.3	2.0 ± 0.0	47.0 ± 18.3	205.9 ± 1.9
		Nano-K_2SO_4	20.0 ± 0.0	57.07 ± 8.2	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	73.7 ± 4.1	126.3 ± 6.1	2.0 ± 0.0	28.03 ± 9.8	159.2 ± 6.8
		Nano-ZnO	17.8 ± 3.9	54.6 ± 13.6	1.8 ± 0.4	1.8 ± 0.4	8.9 ± 2.0	22.3 ± 2.6	102.9 ± 33.2	1.8 ± 0.4	47.6 ± 20.6	221.4 ± 56.3
		Nano-SiO_2	20.0 ± 0.0	48.5 ± 4.8	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	35.2 ± 2.7	60.0 ± 5.3	2.0 ± 0.0	55.9 ± 16.1	87.0 ± 1.2
Mesa-Sirsa	2.5 dS m^{-1}	Control (Hoagland)	80.8 ± 13.6	92.8 ± 4.2	2.0 ± 0.05	8.2 ± 1.1	15.9 ± 1.1	511.2 ± 76.7	162.8 ± 54.7	2.0 ± 0.07	428.7 ± 4.2	213.0 ± 13.2
	6 dS m^{-1}	Control	20.2 ± 0.3	50.1 ± 5.9	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	46.0 ± 2.6	114.8 ± 8.1	2.0 ± 0.0	49.0 ± 14.1	155.7 ± 8.5
		Nano-K_2SO_4	18.7 ± 2.3	84.7 ± 4.1	1.9 ± 0.2	1.9 ± 0.2	9.7 ± 0.5	107.8 ± 6.2	127.0 ± 40.7	1.9 ± 0.2	176.0 ± 10.9	55.7 ± 1.6
		Nano-ZnO	19.7 ± 0.6	42.3 ± 2.2	2.0 ± 0.06	2.0 ± 0.06	9.8 ± 0.3	19.7 ± 0.577	85.5 ± 25.1	2.0 ± 0.06	50.7 ± 17.3	189.0 ± 9.3
		Nano-SiO_2	37.3 ± 14.8	112.2 ± 18.4	3.7 ± 1.4	3.7 ± 1.4	29.8 ± 2.6	160.4 ± 22.5	177.5 ± 25.6	3.7 ± 1.4	372.0 ± 50.7	138.8 ± 18.7
	10 dS m^{-1}	Control	20.0 ± 0.0	46.4 ± 1.3	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	35.5 ± 5.9	81.0 ± 10.6	2.0 ± 0.0	30.2 ± 11.0	163.3 ± 4.2
		Nano-K_2SO_4	20.0 ± 0.0	60.4 ± 12.1	2.0 ± 0.0	2.0 ± 0..0	10.0 ± 0.0	70.0 ± 8.4	122.7 ± 25.3	2.0 ± 0.0	29.0 ± 1.8	160.0 ± 33.8
		Nano-ZnO	17.8 ± 0.0	15.5 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	8.9 ± 0.1	39.6 ± 0.0	12.2 ± 0.0	1.7 ± 0.0	102.6 ± 0.0	13.0 ± 0.0
		Nano-SiO_2	35.0 ± 2.6	50.4 ± 3.2	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0	51.1 ± 2.3	56.6 ± 5.6	0.2 ± 0.0	65.7 ± 11.7	88.6 ± 7.7
LSD			5.33	7.68	0.8	4.3	1.14	3.06	11.1	4.0	2.74	11.42

4. Discussion

4.1. Plant Biomass

The results showed that salinity stress had a negative effect on the parameters of vegetative growth and relative water content, which is consistent with the results recorded by Rahneshan et al. [40]. Decreased growth under saline conditions has been associated with inhibition of cell division and expansion and disruption of plant physiological and biochemical processes. According to Karimi and Maleki-Kuhbanani [41], the dry and fresh weight of the roots and shoots and the height of the shoots and the leaf area have been significantly reduced. The inhibiting effect of salinity can be caused by its effects on cell division, also enlargement at the point of growth [42]. In this study, the application of Nano-K_2SO_4 improved alfalfa plant biomass under salt stress. Similar results to those in [43] found that potassium supplementation increases leaf area, improves stomata resistance under water stress conditions, and accelerates flowering and maturation. Shivay et al. [44] reported that the application of potassium in barley significantly affected the number of tillers per plant. With regard to root fresh and dry weight, application of Nano- K_2SO_4 resulted in enhancing alfalfa plant root fresh and dry weight in both genotypes under salt stress. This could be explained as an addition to its role in mitigating the adverse effects of salinity through its roles in stomatal regulation, osmotic adjustment, maintenance of the membrane ion-charge balance, cellular-energy status, and protein synthesis [28]. In hydroponic systems, the root biomass considers one of the most important parameters. Application of nano-SiO_2 increased root length with increasing salt concentrations under both genotypes recording with Bulldog and Mesa-sirsa average increments of 59.29% and 107.87%, respectively more than the control. It has been reported by [45] that application of SiO_2 to the seeds resulted in early growth and was followed by increasing the root length. Regarding zinc, the authors of [46] indicated that nano-ZnO had significantly improved the biomass and root and shoot growth of cluster bean. Also, the authors of [47] revealed that nano-ZnO had beneficial effects on stem height and root values of mung bean. Peanut yield increased with the application of nano-Zn fertilizer (30 ppm) due to the increased efficiency of nutrient use, which improves pigment formation and photosynthesis rate [48]. Under salinity pressure, treatment of pots with nano-ZnO resulted in root infiltration and nutrient uptake [49], fresh and dry weight of the rice plants [50], grain yield of the wheat plants [51], and biomass production for sunflower [52].

4.2. Physiological Effect

4.2.1. Relative Water Content

Relative water content represents a useful indicator of the state of the water balance of the plant, essentially because it expresses the absolute amount of water that the plant requires to reach artificial full saturation [53]. The reverse relation between salt concentration and RWC in plants has been reported by Saeed et al. [54]. In this study, RWC decreased under salt concentrations in both genotypes (Table 2). A similar decrease in RWC was found in the leaves of different plants that were affected by salinity [55]. This reduction can be associated with reduced Plat vigor. The addition of Nano-K_2SO_4 fostered the ability of plants to absorb water from high salt concentration media with both alfalfa genotypes (Table 2 or Figure 1). Marschner [56] reported that K plays a critical role in turgor regulation within the guard cells during stomatal movement and has a major role in osmotic adjustment [57]. The vacuole and the cytosol are the two major pools of K in plant cells. Cytosolic K^+ concentrations are essential for plant metabolism and always need to be at a constant level, while vacuolar K^+ concentrations may vary dramatically [58,59]. Under K^+ deficient conditions, a constant cytosolic K^+ concentration was attributed to the consumption of vacuolar potassium [60]. Additionally, the effect of potassium in Nano-size may be more penetrated from root cells and more accumulated in both vacuole and the cytosol cells. Kalteh et al. [61] showed that Nano-Si could reduce the negative impacts of high salinity on the development and growth of basil. In addition, silicon appears to play a role in enhancing the water state of plants under salt stress.

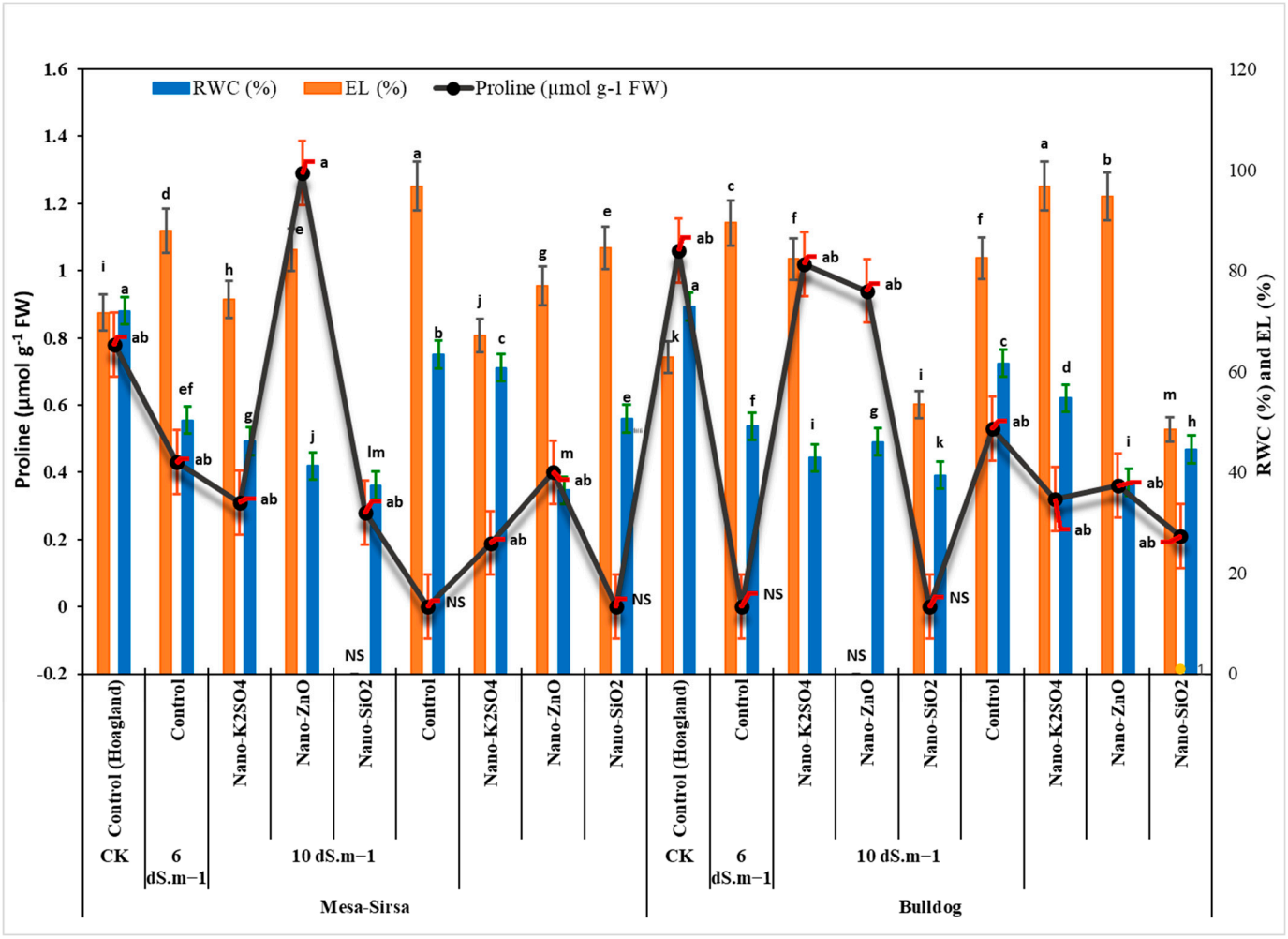

Figure 1. Effect of different Nano-fertilizers (K_2SO_4, ZnO, and SiO_2) on Relative Water Content (RWC), Electrolyte Leakage (EL), and Proline content to Alfalfa genotypes grown under three salt levels (control "Hoagland", 6 and 10 dSm^{-1}). NS: no sample. The column values with the same letters are statistical similar according to Duncan Multiple Range Test (DMRT) at $p < 0.05$.

4.2.2. Electrolyte Leakage (EL)

When RWC is considered as one aspect to examine the water balance in the plants, electrolyte leakage (EL) is another aspect of the identification of salt-tolerant plants [62,63] through its role in examining the condition of plasma membranes in cells [64]. Application of both Nano-SiO_2 and Nano-K_2SO_4 to the susceptible genotype (Bulldog 505) and tolerant genotype (Mesa-Sirsa) respectively, promoted electrolyte leakage under both salt concentrations. It has been published that Si-treated plants acquire tolerance to salt stress suggests that silicon plays a significant role in the maintenance of the integrity of cell membranes [65,66]. Furthermore, [67] showed that 2 mM Na_2SiO_3 reduced electrolyte leakage by 18.3% in water-stressed corn (50% of FC). On the other side, it has been reported that the application of silicon enhances leaf water potential [68–70]. The addition of silicon to salt-stressed plants improves the plant's water condition by resisting water loss [71]. Potassium plays an important role in plant cells as an osmo-regulator [28] and plays a crucial function in turgor regulation within the guard cells during stomatal movement [57].

4.2.3. Proline

Proline has a critical job in diminishing the deterioration effects of salt and accelerating the repair processes following stresses [72]. Proline, a common osmotic antioxidant, plays as an antioxidant and vitality source, and it regulates gene expression, leading to osmotic mod-

ification [73]. Excessive accumulation of proline increases water absorption by increasing the osmotic pressure, leading to enhanced salt stress tolerance in plants [26]. Application of nano-SiO_2 increased proline content in comparison with control. With salt susceptible genotype (Bulldog 505), nano-SiO_2 application augmented proline contents under both slat concentrations (6 and 10 dS m^{-1}), while application of nano-ZnO under 6 dS m^{-1} with Mesa-Sirsa recorded the highest proline content with a value of 0.53 µmol g^{-1} FW (Table 2 or Figure 1). Studies have shown that proline acts as an osmo-protectant and is associated with the mechanism of tolerance under salt stress [74]. Besides being an osmolyte, proline confers enzyme protection and increases membrane stability [75]. The nano-fertilizers increased the contents of total soluble sugars, total starch, and protein in the peanut plants [76]. Zinc plays an important role in protein synthesis in plants [77]. The improvement of carbohydrate and protein content in potato crops of treated plants may be due to the contribution of Zn, Si, and B nanoparticles to the activation of several enzymes related to carbohydrate metabolism and protein synthesis [78].

4.3. Antioxidant Enzymes

Catalase activity in alfalfa leaves increased with increased salt concentration by 16.8% and 31.6% and 33.3% and 50.2% at 6 and 10 dS m^{-1} with Bulldog 505 and Mesa-Sirsa genotypes, respectively. These results agree with those from the authors of [79] who clarify the activity of catalase, which increased in leaf tissues of soybean under salinity stress. Application of nano-fertilizer treatments had significant effects ($p < 0.01$) on catalase (CAT) and sodium oxide dismutase (SOD) contents in both alfalfa genotypes under different salt levels (Table 3 or Figure 2). As a result, the motivation of the active antioxidant enzymes considers an acclimation strategy that plants use to vanquish oxidative stress [80]. The response of alfalfa plants for CAT and SOD activity was assimilated with the application of nano-SiO_2 with both genotypes (Table 3 or Figure 2). These results agreed with those in [26,61,81]. Nano-SiO_2 has been shown to increase the activity of antioxidant enzymes, which has improved the plant's tolerance to salinity stress. [26,82]. Regarding zinc, when wheat plants were treated with 500 ppm non-ZnO, a marked increase in POD activity and root cell lignification was observed [83]. Similarly, an increase in activities of SOD and POD activities using non-ZnO (25–200 mg L^{-1}) in cotton plants was reported in comparison to control plants [84]. The improvement of salt tolerance resulting from the nano-SiO_2 treatment was accompanied by the improvement of membrane stability, sugar accumulation, and chloroplast formation. It was concluded that silicon Nano treatments can reduce the harmful effects of salinity on *V. faba* plants by enhancing the activity of antioxidant enzymes [85].

4.4. Plant Tissue Chemical Characteristics

Application of Nano-ZnO had superior effects on susceptible alfalfa plants' chemical components. These results are in line with the finding of Bala et al. [86] who find that the application of Nano-ZnO enhanced plant micronutrients (Fe, Mn, and Cu) and caused a significant variation in the macronutrients. Giordano et al. [87] reported that when Zn is present at 2000-fold excess in the absence of Ca^{2+}, Mn^{2+} depressed the rate of Zn absorption by about 50%. Malvi [88] reported that Zn supply led to an increase in Mg content in plants. Concerning Nano-SiO_2, it enhanced most plant micro-elements with alfalfa tolerant genotype under both salt concentrations and recorded the highest values in most plant tissue macro/micro-elements. Sabir et al. [89] demonstrated that the application of N-SiO_2 (4%), MgO (1%), and Fe_2O_3 (1%) not only improved the uptake of Ca, Mg, and Fe but also notably enhanced the intake of P with micronutrients Zn and Mn. Siddiqui [26] found that the application of SiO_2 Nano-fertilizer under saline conditions improved plant growth and productivity, improved nitrogen (N) and phosphorous (P) uptake, and reduced sodium Na accumulation in cucumber plants.

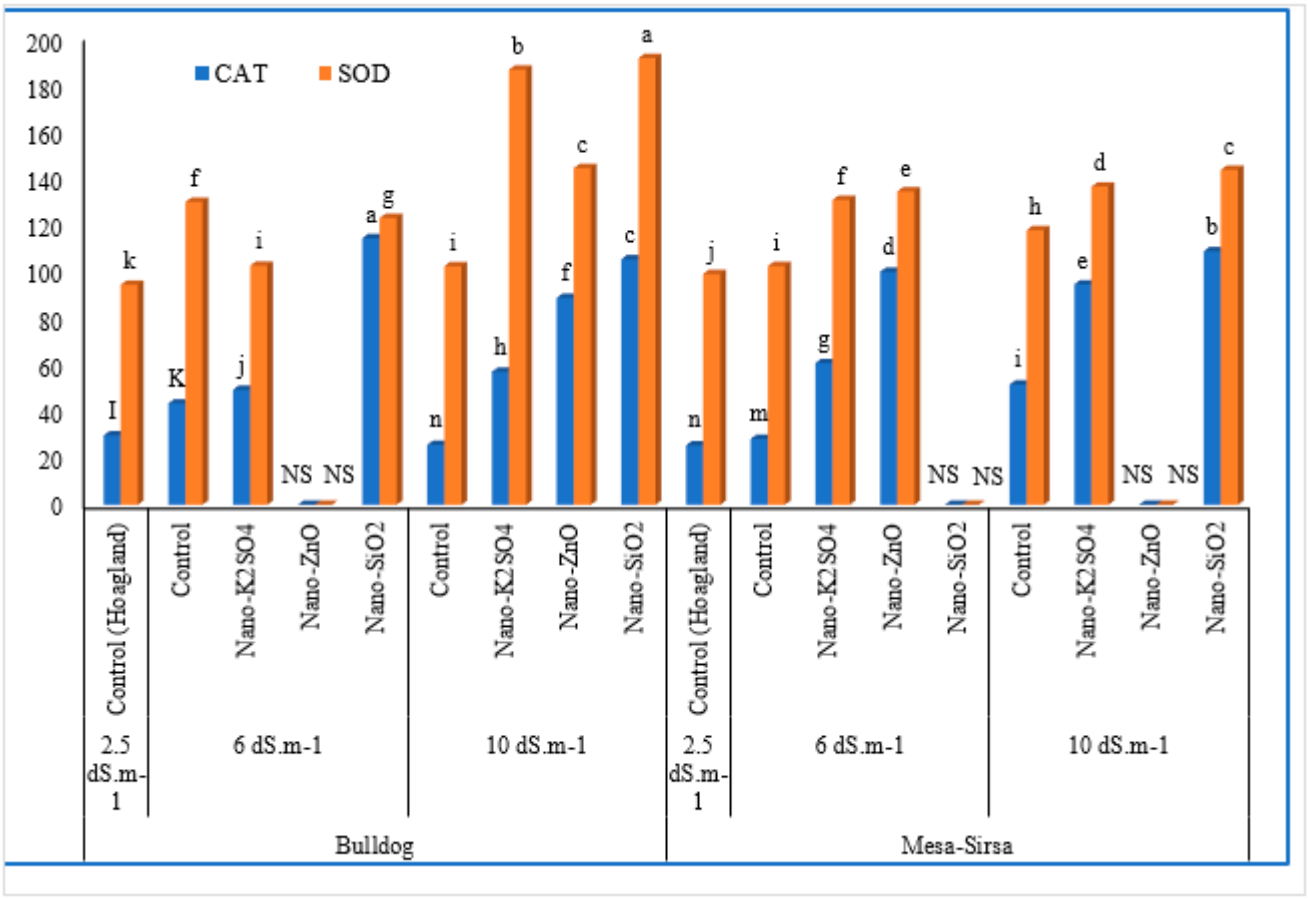

Figure 2. Effect of different Nano- fertilizers (K_2SO_4, ZnO, and SiO_2) on catalase (mmol H_2O_2 min^{-1} g^{-1} FW) and SOD (Super oxide dismutase in U. g^{-1} FW) of Alfalfa genotypes grown under three salt levels (control "Hoagland", 10 and 15 dS m^{-1} electrical conductivity). NS: no sample. The column values with the same letters are statistical similar according to Duncan Multiple Range Test (DMRT) at $p < 0.05$.

4.5. Correlation between Physiological and Phenotypic Responses

A cross genotype and treatments, there was a highly significant correlation ($p < 0.01$) between shoot dry weight and plant height (r = 0.83) (Table 6). This correlation reflected on all plant biomass traits to get also a high correlation ($p < 0.01$) with shoot dry weight such as number of tillers (r = 0.81), number of flowers (r = 0.79), electrolyte leakage (r = 0.37), root length (r = 0.47), root fresh and dry weight (r = 0.81 and r = 0.77). and proline (r = 0.52). Proline is known as an osmolyte that plays a critical role in plant response to stress conditions by maintaining cell turgor and stabilizing membranes as well as a source of energy [90,91], which is correlated with plant biomass and catalase activity (r = 0.43, $p < 0.01$). Table 6 shows a positive correlation between relative water content (RWC) and root length (r = 0.53, $p < 0.01$) as a result of salt stress as reported by Lynch and Ho [92] that under stress, plants specified more resources to root growth relative to shoot growth, which can enhance water procuration. This explains the positive correlation ($p < 0.01$) between plant root length and shoot dry weight, shoot height, number of tillers and flowers, and negative correlation between RWC and electrolyte leakage ($p < 0.01$, r = −0.07), while there was no correlation between root length and electrolyte leakage, but there was a positive correlation ($p < 0.01$) between electrolyte leakage and shoot and root biomass. Catalase (CAT) activity showed a positive correlation with root dry weight ($p < 0.01$, r = 0.4) and shoot biomass. SOD activity showed a positive correlation with plant height ($p < 0.01$, r = 0.43) and root dry weight ($p < 0.01$, r = 0.37). SOD and CAT activity had been reported that these enzymes had a greater role in scavenging H_2O_2 in both leaves and roots [93].

Table 6. Correlation among different traits of two alfalfa genotypes as affected by three salt levels (control "Hoagland", 6, and 10 dSm^{-1}) and three treatments of nano-fertilizers (K_2SO_4, ZnO, SiO_2).

	Shoot Dry Weight	Plant Height	No. of Flowers	No. of Tiller	Root Length	Root Fresh Weight	Root Dry Weight	RWC	Electrolyte Leakage	CAT	SOD	Proline
Shoot dry weight	1.00	0.83 **	0.79 **	0.81 **	0.47 **	0.81 **	0.77 **	0.36 **	0.37 **	0.17	0.18	0.52 **
Plant height	-	1.00	0.79 **	0.7 **	0.48 **	0.85 **	0.81 **	0.26	0.34 *	0.32 *	0.43 **	0.45 **
No. of Flower	-	-	1.00	0.82 **	0.39 **	0.8 **	0.85 **	0.44 **	0.55 **	0.34 *	0.34 *	0.58 **
No. of Tiller	-	-	-	1.00	0.41 **	0.7 **	0.78 **	0.35 **	0.45 **	0.36 **	0.30 *	0.58 **
Root length	-	-	-	-	1.00	0.48 **	0.53 **	0.53 **	0.2	0.32 *	0.29 *	0.34 *
Root fresh weight	-	-	-	-	-	1.00	0.86 **	0.06	0.43 **	0.16	0.35 *	0.53 **
Root dry weight	-	-	-	-	-	-	1.00	0.36 **	0.53 **	0.4 **	0.37 **	0.34 **
RWC	-	-	-	-	-	-	-	1.00	-0.07	0.32 *	0.01	0.23
Electrolyte Leakage	-	-	-	-	-	-	-	-	1.00	0.21	0.12	0.39
CAT	-	-	-	-	-	-	-	-	-	1.00	0.38	0.43 **
SOD	-	-	-	-	-	-	-	-	-	-	1.00	0.21
Proline	-	-	-	-	-	-	-	-	-	-	-	1.00

* and **: Significant at probability (0.05) and (0.01) respectively.

5. Conclusions

Two alfalfa genotypes were planted in rock wall blocks and transferred after three weeks to a hydroponic system established using the nutrient film technique using a continuous aeration system. The plants were exposed to three salt levels (0, 6, and 10 dS m^{-1}) and exposed to different Nano-fertilizers (K_2SO_4, ZnO, SiO_2). The results showed a significant difference ($p < 0.01$) in plant biomass parameters in their response to salt levels and nano-fertilizers treatments. Application of Nano-K_2SO_4 improved plant relative water, catalase activity content in both genotypes and both salt concentration, while application of Nano-SiO_2 to the susceptible genotype (Bulldog 505) resulted in enhanced electrolyte leakage and proline under both salt concentrations, however, application of Nano-K_2SO_4 with tolerant genotype (Mesa-Sirsa) under both salt concentration (6 and 10 dS m^{-1}) resulted in the lowest electrolyte leakage. Application of Nano-ZnO increased plant Ca, Mg and microelements under both salt concentrations. From the above results, it could be summarized that using nano-fertilizers with microelements or macro-elements via potassium addition, in the hydroponic system with brackish water sources could be a new technique to economically profit from both good plant production and saline water sources.

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