Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 8 ~ Issue 11 (2021) pp: 33-53
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Alleviation of the Adverse Effects of Salinity Stress in Lettuce (*lactuca sativa* L.) by Seed Coating with *Trichoderma atroviride*

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ABSTRACT: *Environmental stresses, such as salinity, are becoming censorious constraints to plant production. This study investigated the ability of Trichoderma atroviride to alleviate salinity stress in lettuce seedlings (lactuca sativa L.) in a controlled greenhouse. The study was conducted as a randomized complete block design with three replications. Treatments included three saline solutions (0, 50 and 100 Mm NaCl), two biostimulants (Trichoderma atroviride and a commercial plant-derived product) and a non-treated control. Trichoderma strain was inoculated with lettuce seeds before sowing with respect to salt treatments. Results showed that salt stress significantly affected agronomical performance. By contrast, seed coating with Trichoderma atroviride mitigated the drastic effects of salinity by improving plant growth and biomass accumulation, leaf chlorophyll, relative water, soluble sugar, total phenolic content and the antioxidant capacity while malondialdehyde content was considerably reduced. Biostimulants also increased membrane permeability by decreasing the electrolyte leakage. Moreover, Trichoderma atroviride markedly improves the nutrient partitioning, under stress, and alleviates ion toxicity by decreasing sodium (Na^+) accumulation and maintaining a favourable K^+/Na^+ ratio. Overall, seed coating with Trichoderma strain may be considered as a sustainable tool of production to increase lettuce productivity and may represent a potential approach to counteract deleterious effects of NaCl stress.*

KEYWORDS: *salinity, Trichoderma atroviride, Lactuca sativa L., phytochemicals, mineral composition.*

Received 08 November, 2021; Revised: 22 November, 2021; Accepted 24 November, 2021 *© The author(s) 2021. Published with open access at www.questjournals.org*

I. INTRODUCTION

Plants under everyday conditions are frequently exposed to various environmental stresses that crucially affect their growth and development. Among abiotic stresses, salinity is the basic origin of crop loss, detrimental to yield quality and biomass in foremost crop plants across the world. It has been reported that high salt content affects about 20% of the total arable area [1]. Sodium accumulation induces ionic and osmotic stress that leads to retarded growth in terms of fresh and dry weight, decreased pigment content and hampers uptake of mineral elements [2]. Prolonged exposure to salinity is responsible for specific ion toxicity, hormonal and nutritional imbalance and reduced water potential [3]. This results in secondary stress, i.e.; oxidative stress caused by the overproduction of reactive oxygen species (ROS) [4].

Although plants have the capacity to generate various forms of antioxidants that can alleviate adverse effects of abiotic stress, it is necessary to further investigate other exogenous applications, including the use of plant biostimulants, which are gaining interest worldwide [5]; [6]. As defined by Du Jardin [7], biostimulants correspond to 'any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content'. The introduction of beneficial microorganisms in crop production systems is known as a good sustainable strategy to ensure competitive yields in many crops and improve the resource use efficiency [8]. Besides the classic mycorrhizal

fungi, rhizobia, and plant growth-promoting rhizobacteria, endophytic fungi are also considered promising tools to overcome the limitations of abiotic stress on crop growth and productivity [9], [10].

The fungal genus *Trichoderma* has been known as eco-friendly biocontrol agent to control plant diseases over many years. In general, *Trichoderma* spp. is found in rhizosphere, especially very close to the roots of plants. Some species may be present as parasites together with other fungi while some of those form colonies on the plant roots [11].

Trichoderma sp. are endophytic plant symbionts widely used as biofertilizers for plant growth stimulation and as biocontrol agents for plant diseases [12]; [13].

Root colonization of *Trichoderma* spp. induces numerous changes in physiological processes like photosynthesis or further transcriptomic regulation under stress [14]. Many *Trichoderma* spp. strains are able to enhance plant tolerance to biotic and abiotic stresses [15]; [16], through enhanced root growth, nutritional uptake by better solubilization of micronutrients, and also throughout the production into the rhizosphere of small peptides, volatiles and metabolites with hormone activities such as indole-3-acetic acid or auxin analogs [17]; [18]. *Trichoderma* spp. can also enhance abiotic stress tolerance by balancing ROS detoxification and modulating gene expression in related signal pathways [19]; [20].

Recent research showed that co-inoculation of *Trichoderma atroviride* combined with arbuscular mycorrhizal fungi at transplanting increased plant growth of several vegetable crops under non-stress and abiotic stress conditions [21]; [22]. The application of theses beneficial microorganisms is particularly tested, in many studies report, in transplanting and with microbial consortium based on combination of compatible strains. Nevertheless, to our knowledge, there are no reports of pre-inoculated legume seeds with *Trichoderma atroviride* alone and under conditions of salinity.

Seed coating is an interesting technology to introduce beneficial microorganisms [23]; [24] and other chemical compounds in the root zone of plants in an efficient and cost-effective way [25]. Seed coating ensures that the beneficial microorganisms are readily accessible to the root at the critical "early germination" stage, facilitating early, healthy and rapid development and improving nutrient uptake and tolerance to abiotic stresses [26]; [27]; [15].

Lettuce (*Lactuca sativa* L.) has become a much-appreciated healthy food source, from the Astercaceae family, which has garnered a crucial role in the human diet as it combines generally pleasing organoleptic properties with a rich content of bioactive compounds (minerals, vitamins, phenolic acids, and flavonoids) [28]. On the other hand, lettuce plants, like most crop plants, are considered to be moderately salt sensitive [29], with growth stunting starting at soil electrical conductivity (EC) around 2 $dS \cdot m^{-1}$ and at irrigation water electrical conductivity around to 0.9 $dS.m^{-1}$ [30]. It is one of the most important vegetable crops grown in the Mediterranean area where saline water is frequently used for irrigation [31]. Therefore, there is a need for some nature-friendly biocontrol agents that can help to alleviate adverse effects of salinity.

The present study was conducted at verifying the influence of seed coating with *Trichoderma atroviride* on lettuce (*Lactuca sativa* L.) tolerance to salinity and at understanding physiological, compositional and biochemical modifications mediated by biostimulant application under saline conditions.

II. MATERIAL AND METHODS

2.1 Plant material, growth conditions and treatments applied

The experiment was conducted in a greenhouse situated on the experimental station of the High Institute of Agronomy of Chott Mariem, Tunisia, at 30/25°C (day/night) and 14 h photoperiod, with relative humidity of 70 ± 10%. The soil used for this study was taken from the experimentation station of the higher agronomic institute of Chott Mariem (Tunisia). The soil samples air-dried, crushed to pass through a 2-mm sieve and mixed with peat (2:1 ratio). The physiochemical analysis of experimental soil was carried out before sowing and main soil properties are given in Table 1.

Table 1: Some physical and chemical properties of the experimental soil.

Soil characteristics	Value
Textural class	sandy loam
pH	7.72
EC ($dS\ m^{-1}$)	2.12
CEC ($cmol\ kg^{-1}$)	8.13
Organic matter (%)	1.25
Available nutrient ($mg\ kg^{-1}$ soil)	
N	168
P	33
K	111

The treatments studied were three levels of salinity, (0 mM NaCl, 50 mM NaCl, or 100 mM NaCl) in combination with three biostimulant applications (control, microbial inoculum, or commercial plant-derived

protein hydrolysate ("Trainer", Italpollina S.p.A., Rivoli Veronese, Italy)). The treatments were arranged into a 3 × 3 factorial in a completely randomized design with three replications, giving a total of 9 treatment combinations and 27 experimental units. Hogland nutrient solution was used across the pots as a base nutrient source. Five-week-old seedlings were transplanted in 20 cm diameter plastic pots (seven pots/treatment). The saline treatment commenced at 14 days after transplantation using different concentrations of NaCl solutions through irrigation that included 0, 50 and 100 mM of NaCl. The corresponding electrical conductivity (EC) levels for the irrigation water were 0.02, 4.25 and 10.8 Ds m^{-1}. Salt was dissolved in tap water having an initial electrical conductivity of 0.05 dS m^{-1}. The control treatment was watered by distilled water (pH 6.2; EC = 0.02 dS m^{-1}). Application of Nacl was carried out gradually at a rate of 25 mM additional Nacl per day so as to prevent salt-shock. The 50 mM Nacl and 100 mM Nacl concentrations were thus reached after 2 and 4 days respectively and were held constant until the end of the test. The volume of the saline solution was 200 mL/plant and delivered every 3 days.

Trichoderma atroviride MUCL 45632 strain was kindly provided by "Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy". Seeds of lettuce (*lactuca sativa L*) variety "*Lattuga Attrazione*" were surface sterilized with 70% ethanol for 2 min, followed by 5% sodium hypochlorite for 10 min. Thereafter, coating product was applied after dissolution of 1 g powder of *Trichoderma atroviride* in 1 ml distilled water at a rate of 1.5 g Kg^{-1} of lettuce seeds. The commercial plant-derived PH "Trainer" (Coveron Italpollina, S.p.A., Verona, Italy) was used in the current study as a positive control. The protein hydrolysate "Trainer" contained 35.5% of organic matter, 5% of total nitrogen, and 27% of amino acids and soluble peptides and obtained through an advanced enzymatic hydrolysis of proteins from legume seeds (Colla et al. 2014). This biostimulant was applied as a soil drench at weekly intervals using a volume of 100 ml/pot with a solution containing 2.5 ml l^{-1} of plant-derived protein hydrolysate. Application was initiated 5 days after transplanting until harvesting.

All combinations of treatments are listed in Table 2.

Table 2. Experimental treatments applied to lettuce seedlings.

T1	**Control: plants irrigated by the basic nutrient solution.**
T2	Plants irrigated with the basic nutrient solution with 50 mM NaCl,
T3	Plants irrigated with the basic nutrient solution with100 mM NaCl
T4	Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution.
T5	Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution with 50 mM NaCl,
T6	Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution with 100 mM NaCl.
T7	Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution.
T8	Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution with 50 mM NaCl
T9	Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution with 100 mM NaCl.

2.2 Growth measurement

Vegetative growth was assessed through the leaves number per plant, the dry matter rate and the leaf area. Lettuce plants were harvested when the first treatment reached the commercial maturity stage, after 38 days of transplantation. Number of leaves was counted, and leaf area (Cm^2) was determined by using a digital leaf meter (LI-3000 Portable Area meter). Plants were then placed in an ovenrun at 70°C up to constant weight. These dried plants were weighed to record the plant dry mass.

2.3 Physiological Measurements

All physiological measurements were conducted five weeks after transplanting.

2.3.1 Photosynthetic pigments

The pigments extracted from 100 mg of leaf fragments with 80% acetone in the darkness condition. The homogenate was centrifuged at 3000 x g for 5 min and the supernatant was collected. The absorbance of the extract was read at 665.2 and 652.4 nm for chlorophylls and 470 nm for total carotenoids. All determinations were carried out in triplicate. Pigment levels were calculated according to Lichtenthaler [32] and expressed on the basis of tissue Fresh Weight (FW).

2.3.2 Relative water content

Relative water content (RWC) was determined as described by Galmès et al. [33]. The uppermost fully expanded leaf of the main stem was weighed (fresh weight, FW) and then turgid leaf weight (TW) was obtained after submerging samples in distilled water for 24 h. The samples were then directly dried at 70 °C for 72 h and weighed (DW). All determinations were carried out in triplicate. The relative water content (RWC) was calculated from the following equations:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)]\ X\ 100$$

Where:

FW = Leaves fresh weight,

DW= Leaves dry weight,

TW = Leaves fresh weight after 24 h incubation in distilled water when their cells achieved maximum turgor.

2.3.3 Proline content

Proline content was calculated according to the method of Bates, Waldren, and Teare [34]. Dry leaf samples (0.1 g) were extracted in 3% (w/v) sulfosalicylic acid solution. After centrifugation at 12,000 rpm for 10 min, 2 ml of supernatant was homogenized with 2 ml of glacial acetic acid and ninhydrin reagent. The mixture was then incubated at 100° C in water bath for 1 h. After cooling of the tubes in ice, the homogenate was extracted with 4 ml of toluene and the upper phase was read spectrophotometrically at 520 nm. L-proline was used for standard curve construction. Analyses were carried out in triplicate.

2.3.4 Electrolyte Leakage

Electrolyte leakage was used to evaluate membrane permeability according to Kaya, Higgs, and Sakar [35]. Lettuce leaf samples of two plants per replicate were taken and cut into 1 cm segments. The samples were then rinsed three times to remove surface contamination and eventually floated in flasks containing 10 mL of distilled water and incubated at room temperature (25°C) on a shaker (100 rpm) for 24 h. The initial electrical conductivity of the solution (EC1) was recorded after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and a second reading electrical conductivity (EC2) was taken after cooling the solution at room temperature. The electrolyte leakage (percentage of membrane damage) was calculated as $EC1/EC2$ and expressed as percentage. Measurements were performed on 4 replicates per treatment.

2.4 Biochemical analyses

2.4.1 Determination of soluble sugars

The soluble sugars were estimated using the colorimetric method described by Dubois et al. [36]. For each sample, 100 mg of dried leaf material was homogenized with 3 mL of 80% ethanol (v/v) and then mixed with concentrated sulfuric acid and 5% phenol. The mixture was kept for 1 h and then the absorbance at 490 nm was determined with a spectrophotometer. Glucose was used as standard. Contents of soluble sugars were expressed as mg g^{-1} FW. Analyses were carried out in triplicate.

2.4.2 Determination of total phenolic contents

Sample extracts were obtained following Mau et al. [37] with slight modifications. To prepare the extracts, 2.5 g of dry leaf powder was extracted with 25 mL of methanol (60%) solvent. Each mixture was then magnetically stirred for 30 min and the extracts were then kept at 4°C for 24h in darkness. The mixtures were then filtered through a Whatman No. 4 filter paper and evaporated to dryness under vacuum. The extracts thus obtained were stored at 4°C for further use.

The total phenolic content of the lettuce leaf extracts was determined using Folin-Ciocalteu reagent, according to Falleh et al. [38]. Absorbance was determined against a blank at 760 nm using UV-visible spectroscopy. The content of total phenolic compound in each extract was expressed as mg of gallic acid equivalents per g fresh weight (mg GAE g^{-1} FW) from a calibration curve with gallic acid. All determinations were carried out in triplicate. Gallic acid was used as the reference standard for plotting the calibration curve.

2.4.3 Antioxidant activity estimation by DPPH assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) activity was estimated by Sánchez-Moreno, et al. [39] method. To 50 µL of shoot extract, 1.95 mL of DPPH methanolic solution (0.025 g L−1) was added, then vortexed and placed in darkness at room temperature for 30 min. The percentage of radical scavenging activity was determined as follows: ([(Abs control – Abs sample) / Abs control] ×100) at 515 nm; Abs control is the absorption of the control reaction; Abs sample is the absorption of the reaction prepared by substituting the sample under the same operating conditions.

2.4.4 Measurement of lipid peroxidation

Lipid peroxidation was estimated by determining the malondialdehyde (MDA) content in the leaves. For MDA extraction, fresh leaves samples (0.5 g) were homogenized with 0.1% trichloroacetic acid (TCA). The homogenate was then centrifuged at 15,000g for 10 min. An aliquot (1 mL) of the supernatant was mixed to 4 mL of 20% TCA prepared in 0.5% thiobarbituric acid (TBA) and incubated at 90 C for 30 min in a shaking

water bath. The reaction was stopped in ice bath. The samples were then centrifuged at 10,000 g for 5 min, and the absorbance of the supernatant was measured at 532 and 600 nm [40]. The MDA content was calculated by using an extinction coefficient of MDA (155 mM cm^{-1}) and expressed as µmol g^{-1} FW.

2.4.5 Determination of mineral elements

The concentrations of Na^+, Ca^{2+} and $^{K+}$ were determined in plant material (roots and shoots) ground finely in a mill grinder, after drying at 65 ºC for 5 days. The samples were digested in a microwave oven, reaching 200 ºC in 20 min, and held at this temperature for 2 h. Digestion of 0.1 g DW plant material was performed with 5 mL 65 % HNO3, 17 mL H_2O dist. and 3 mL 30 % (v/v) H_2O_2. For determining the sodium, potassium and calcium ion content of the xylem sap, 0.1 ml of xylem sap was diluted with distilled water to a final volume of 10 mL. The concentrations of Na^+, Ca^{2+} and K^+ were determined by inductively coupled plasma spectrometry (ICP-MS; Varian 820-MS, ICP Mass Spectrometer) [41].

2.5 Statistical analysis

Data were subjected to an analysis of variance (ANOVA), and means and standard errors were calculated. All parameters were subjected to a one-way-analysis ($P<0.001$) and compared using Tukey's test at 5% of probability. The statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS; version 20). Data presented are means ± standard errors (SEs) of three replicates. Principal component analysis (PCA) was performed using XLSTAT 2021, and the first two components (PC1 and PC2) explaining the maximum variance in the datasets were used to make biplots

III. RESULTS

3.1 Growth and Biomass Yield

The results showed that lettuce growth parameters significantly declined with the increase in NaCl concentrations, as shown in Table 3; we found a significantly negative effect of salt level on agronomic parameters in the lettuce seedlings, which started to appear significantly from 50 mM NaCl. The highest reductions were observed in shoot fresh weight (~42%) and leaf area (~ 71%) relative to the control plants. Shoot fresh weight was reduced by salinity more than shoot dry weight, indicating fresh mass was more sensitive to salinity stress than shoot dry mass. Number of leaves/plants was slightly reduced from 0 mM NaCl to 50 mM NaCl (~ 21 leaves/plant) and was significantly affected by higher NaCl concentration (17 leaves/plant).

Results showed that the application of the biostimulant "Trainer" tested in this work increased significantly the growth parameters of lettuce plants compared to control. However, seedling treated with *Trichoderma atrovidae* has shown substantial increase in plant growth. Number of leaves and leaf area increased significantly in *Trichoderma* Treated lettuce plants subjected to 0, 50 and 100 mM NaCl treatments as compared to those plants that were not treated with *Trichoderma atrovidae*.

Table 3: Effect of biostimulants application on growth parameters of lettuce plants grown under two saline levels at 38 days after transplantation.

Treatments		No. of leaves/plant	Leaf Area per plant (cm^2)	Shoot Fresh Weight (g $plant^{-1}$)	Shoot Dry Weight (g $plant^{-1}$)	Root Fresh Weight (g $plant^{-1}$)	Root Dry Weight (g $plant^{-1}$)
Biostimulant (B)	**Salinity (S)**						
Standard solution without biostimulant	**0 mMol Nacl**	22.13 ±0.30^{cd}	276,96 ±4.46^{a}	142.93 ± 5.82^{a}	12.43 ± 0.04^{a}	12.1 ± 0.43^{a}	2.13± 0.23^{a}
	50 mMol Nacl	21.53 ±0.25^{d}	214.99 ±4.69^{c}	88.53 ± 5.79^{c}	9.83 ± 0.46^{bc}	8.73 ± 0.37^{bc}	1.46 ± 0.05^{c}
	100mMol Nacl	16.76 ±1.36^{f}	80.06 ±4.60^{d}	81.66 ± 2.08^{c}	8.7 ± 0.08^{c}	7.26 ± 0.2^{c}	1.13 ± 0.11^{c}
Trainer	**0 mMol Nacl**	23.9 ±0.30^{ab}	283.41 ±3.92^{a}	128.2 ± 5.96^{b}	8.93 ± 0.29^{bc}	10.86 ± 0.25^{ab}	1.6 ± 0.1^{bc}
	50 mMol Nacl	22.18 ±0.33^{cd}	232.82 ±2.49^{b}	94.33 ± 7.58^{c}	10.6 ±1.52^{abc}	9.33 ± 0.64^{bc}	0.16 ± 0.2^{c}
	100mMol Nacl	18.64 ±0.18^{e}	82.76 ±2.47^{d}	90.6 ± 1.96^{c}	9.83 ± 0.03^{bc}	8.9 ± 0.72^{bc}	1.4 ± 0.1^{c}
Trichoderma atroviride	**0 mMol Nacl**	25.1 ±0.26^{a}	284.39 ±4.00^{a}	132.2 ± 4.3^{ab}	11.06 ± 0.24^{ab}	12.3 ± 0.2^{a}	2.03 ± 0.05^{ab}
	50 mMol Nacl	23.4 ±0.30^{bc}	236.70 ±5.23^{b}	93.36 ± 4.3^{c}	9 ± 0.69^{bc}	9.36 ± 1.17^{bc}	1.26± 0.25^{c}
	100mMol Nacl	19.63 ±0.19^{e}	91.28 ±6.85^{d}	92 ± 6^{c}	9.2 ± 0.11^{bc}	9.2 ± 1.74^{bc}	1.33 ± 0.35^{c}
				Significance			
S		**	**	**	**	**	**
B		**	**	Ns	ns	ns	ns
S X B		ns	*	**	**	ns	*

Values are mean ± standard errors of replicates. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05). Significance levels are represented by P > 0.05, ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01.

3.2 Chlorophyll and carotenoids content

Irrespective of the biostimulant treatment, the concentration of total chlorophyll, chlorophyll a and chlorophyll b in the lettuce were reduced with the rising salinity levels (Table 4). The results showed that the content of total chlorophyll and chlorophyll a at 100 mM NaCl level significantly reduced by 9% and 25% as compared to the control, respectively. Chlorophyll b exhibited similar trends to chlorophyll a with the lowest content at the highest NaCl concentration (0.12 mg g^{-1}). Results showed the same pattern for total carotenoids content which were significantly decreased with the salt stress (Table 4). Total carotenoids showed values of 0.14 mg g^{-1} FW in controls and 0.01 mg g^{-1} FW in leaves of lettuce plants at 100 mM NaCl. The highest values of total chlorophyll content were observed in "Trainer" treated plants, followed by *Trichoderma* treatment, whereas the lowest values were recorded in the untreated lettuce plants. "Trainer" induced an increment of chlorophylls, confirmed by statistical analyses in leaves treated with the biostimulant at all tested salinity levels (0, 50 and 100 Mm NaCl). In fact, *Trichoderma* treatment caused a slightly increment of the considered pigments, however the effect was not statistically relevant compared to controls. Coating seeds with *Trichoderma* gives significant results only at high salinity levels, while at medium salinity the effect is not so remarkable. Furthermore, the content of carotenoids was positively affected by the biostimulants application compared to the control treatment. "Trainer" treatment induced a significant increase in carotenoids than controls, and the increment promoted by *Trichoderma* treatment was statistically significant at 100 Mm NaCl level.

The molar ratio between a and b type chlorophylls increased gradually with salt concentration. The ratio of the two photosynthetic pigment in the leaves exposed to 50 mM and 100 mM NaCl were significantly increased with the salt stress in untreated plants. Moreover, Biostimulants tested in the study caused a pronounced increase at 50 and 100 mM NaCl conditions when compared to the non-treated control.

Table 4: Effects of biostimulants application on chlorophyll a, chlorophyll b, total chlorophyll, carotenoid (mg g^{-1} FW) and Chlorophyll a /b ratio of lettuce plants grown under two saline levels at five weeks after transplantation.

Treatments		Toatl chlorophyll content (mg g^{-1})	Chlorophyll a content (mg g^{-1})	Chlorophyll b content (mg g^{-1})	Chlorophyll a /b ratio	Carotenoids content (mg g^{-1})
Biostimulant (B)	**Salinity (S)**					
Standard solution without biostimulant	**0 mMol Nacl**	1.34±0.25^{ab}	0.91±0.01^{b}	0.37±0.09^{b}	2.45±0.12^{de}	0.14±0.01^{ab}
	50 mMol Nacl	1.28±0.14^{bc}	0.87±0.06^{bc}	0.25±0.06^{c}	3.51±0.09^{cd}	0.12±0.02^{bc}
	100mMol Nacl	1.22±0.17^{c}	0.80±0.01^{c}	0.12±0.03^{d}	6.55±0.06^{a}	0.02±0.01^{c}
Trainer	**0 mMol Nacl**	1.43±0.21^{a}	1.00±0.02^{a}	0.46±0.06^{a}	2.17±0.13^{e}	0.23±0.06^{a}
	50 mMol Nacl	1.36±0.18^{ab}	0.95±0.05^{ab}	0.33±0.02^{b}	2.86±0.1^{cde}	0.20±0.01^{ab}
	100mMol Nacl	1.35±0.21^{ab}	0.93±0.02^{ab}	0.25±0.01^{c}	3.78±0.09^{c}	0.14±0.03^{ab}
Trichoderma atroviride	**0 mMol Nacl**	1.34±0.21^{ab}	0.91±0.05^{b}	0.37±0.02^{b}	2.46±0.16^{de}	0.14±0.07^{ab}
	50 mMol Nacl	1.27±0.14^{bc}	0.88±0.01^{bc}	0.24±0.07^{c}	3.73±0.17^{c}	0.11±0.02^{bc}
	100mMol Nacl	1.33±0.02^{abc}	0.94±0.03^{ab}	0.19±0.01^{c}	5.07±0.16^{b}	0.12±0.01^{bc}
		Significance				
S		**	**	**	**	**
B		**	**	**	**	**
S X B		ns	**	ns	**	ns

Values are mean ± standard errors of replicates. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05). Significance levels are represented by P > 0.05, ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.3 Proline content

Analysis revealed that free proline level of the leaves was significantly increased by both salt regimes in all treatments (Figure 1). In untreated plants it is possible to observe that the increasing levels of salinity caused a raise in proline level of the leaves. The proline content was increased significantly by 43.4% in 100 mM NaCl-treated plants comparison to controls. The highest proline content was determined in the plant treated by *Trichoderma atrovidae* and exposed to100 mM NaCl, where free proline concentration increased significantly by 56.84% in comparison to untreated plants.

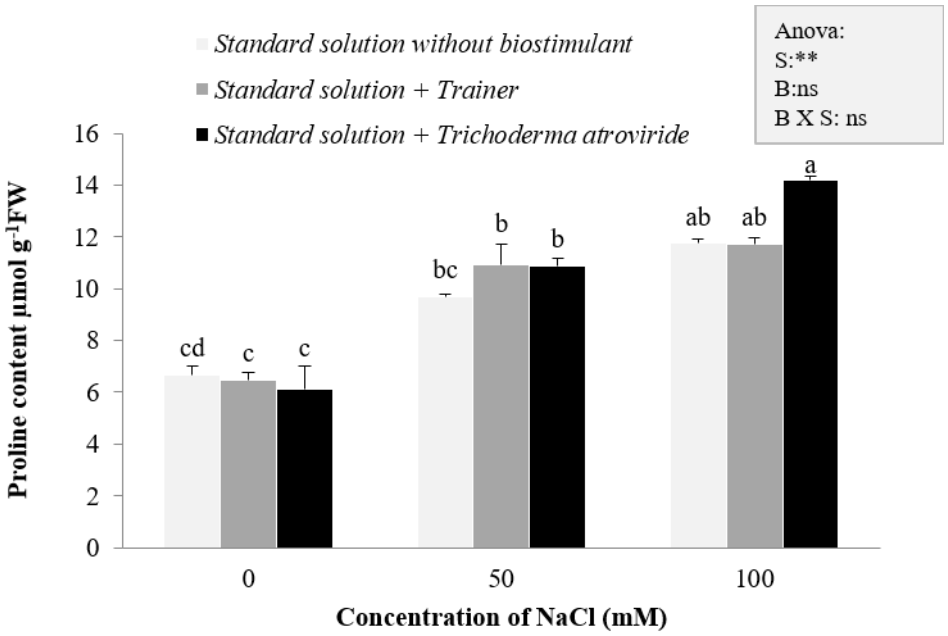

Figure 1: Effects of biostimulants application on leaf proline content of lettuce plants grown under two saline levels. Each value is the mean of the three replicates, and error bars represent ±SD. Different letters indicate significant differences according to Duncan's test (P = 0.05). ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.4 Soluble sugar content

Figure 2 show the interaction effect of salinity and Biostimulants on the soluble sugar content. There was a significant increase in the content of soluble sugars as a result of an increase in NaCl concentration. The lowest content was found in the plants subjected to 0 mM NaCl (~ 8 $mg.g^{-1}$ FW). However, the content increased significantly by 98.97% and 101.93% at 50 and 100 mM NaCl compared to the control plants respectively. Biostimulants application significantly enhanced the soluble sugar content at different salinity levels with the highest under "Trainer" application at 100 mM NaCl.

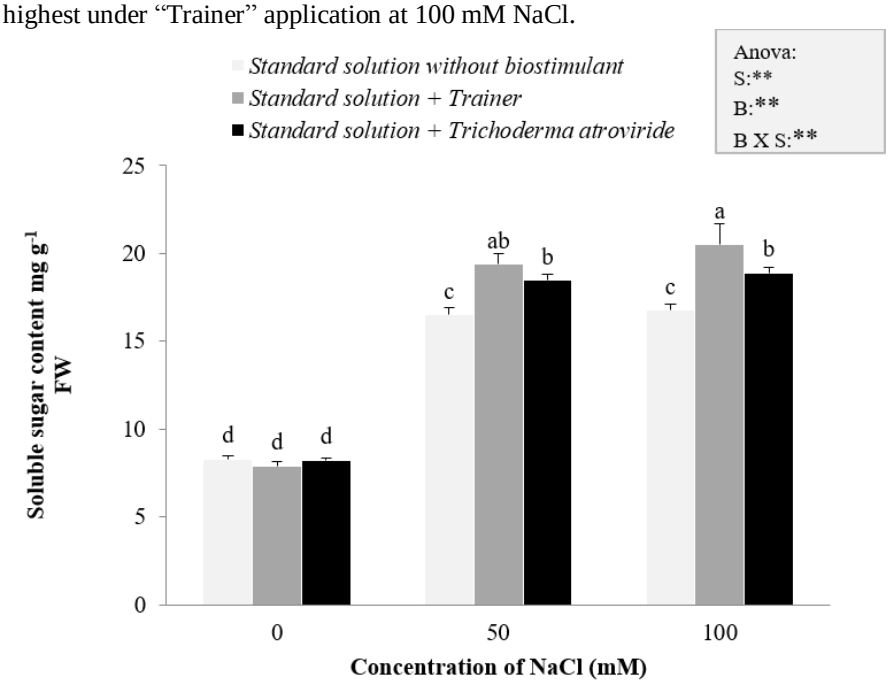

Figure 2: Effects of biostimulants application on soluble sugar content of lettuce plants grown under two saline levels. Each value is the mean of the three replicates, and error bars represent ±SD. Different letters indicate significant differences according to Duncan's test (P = 0.05). ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.5 Relative water content

Relative water content (RWC) of lettuce plants significantly decreased with increase induction of salt stress from 0-100 mM. (Figure 3). However, in treated plants, the adverse effects of salinity were alleviated, showing a substantial increase in the RWC of plants over untreated plants. **"**Trainer" and *Trichoderma* treatments maintained higher water content than uninoculated control ones regardless of salt level. The highest relative water content (84.49%) was found with non-stressed plants treated by *Trichoderma atrovidae*, and the lowest relative water content (60 %) was found in untreated plants at 100 mM salt treatment.

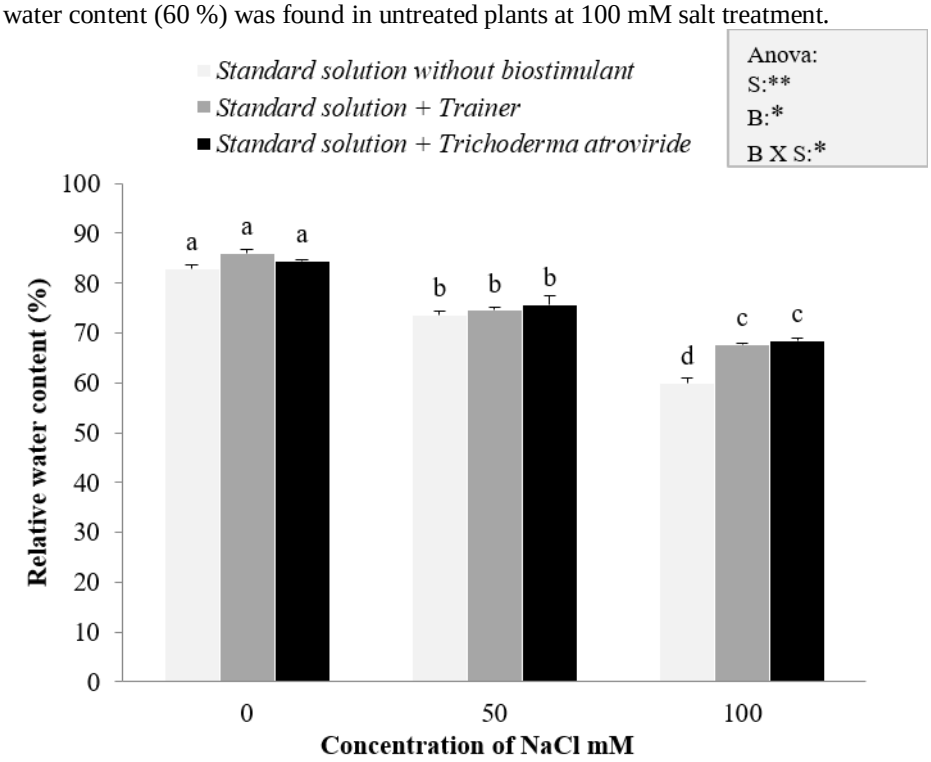

Figure 3: Effects of biostimulants application on relative water content of lettuce plants grown under two saline levels. Each value is the mean of the three replicates, and error bars represent ±SD. Different letters indicate significant differences according to Duncan's test (P = 0.05). ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.6 Electrolyte Leakage

The magnitude of membrane damage was assessed indirectly by conductometric measurements of solute leakage from cells. Our results showed that electrolyte leakage amounts were considerably enhanced by increasing salinity levels as compared to the control lettuce plants (figure 4).

In salt-stressed plants without biostimulants application, the leakage of the electrolyte was 46.3% higher than the control treatment under 100 mM NaCl, and this increase was partially reduced by the "Trainer" and *Trichoderma* treatments, with a significant interaction between the studied factors (P < 0.01).

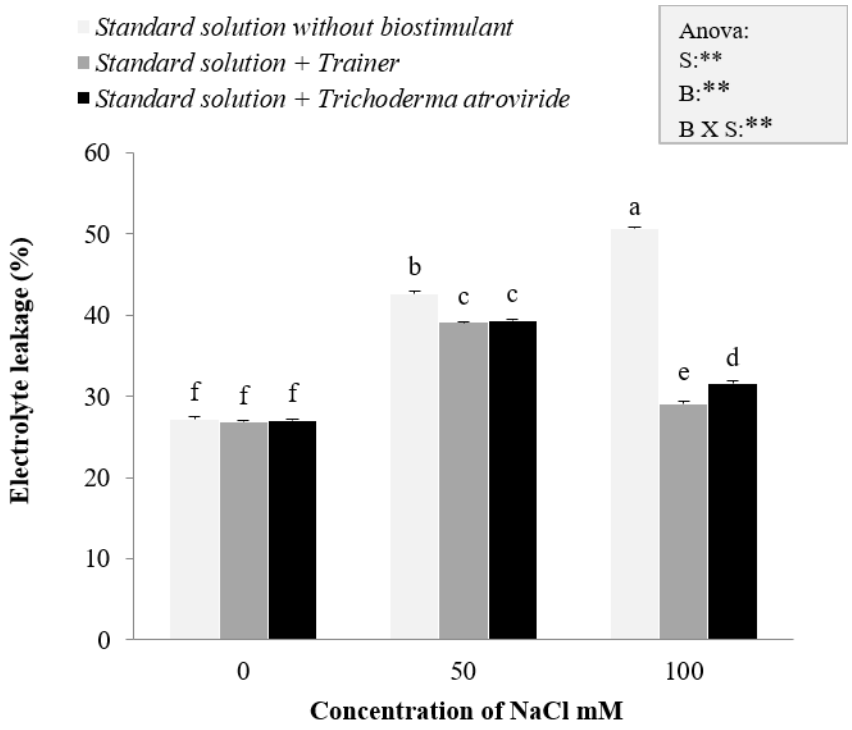

Figure 4: Effects of biostimulants application Electrolyte leakage (%) of lettuce plants grown under two saline levels. Each value is the mean of the three replicates, and error bars represent ±SD. Different letters indicate significant differences according to Duncan's test (P = 0.05). ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.7 Total phenolic content

Significant increase in total phenol content was observed towards all salt treatments as compared to control treatment (Figure 5a). Treatment with "Trainer" and *Trichoderma* at all concentration enhances significantly the total phenolic content in lettuce plants cultivated under different growth conditions (0, 50 and 100 mM of NaCl). The highest levels of total phenolic were detected in plants treated "Trainer" (35.02 mg GAE g^{-1} under 100 mM of NaCl).

3.8 Antioxidant activity (DPPH assay)

There was a significant increase in the antioxidant capacity as a result of an increase in NaCl concentration (Figure 5b). The lowest capacity was found in the plants subjected to 0 mM NaCl (67.5%). The highest antioxidant activities compared to the untreated control were recorded in lettuce treated with "Trainer" and *Trichoderma* biostimulant plants, respectively. In "Trainer" treated plants, the antioxidant capacity of lettuce plants evaluated by DPPH radical scavenging assay was increased by 3.87%, and 7.27% at 50 and 100 mM NaCl compared to the control (Figure 5b).

3.9 Lipid Peroxidation (MDA)

Salinity induced oxidative damages to the plant was determined by estimating the malondialdehyde (Figure 5c). The amount of malondialdehyde (MDA) content increased between 18.35 and 61.61 % at different concentrations of NaCl. However, "Trainer" and *Trichoderma* significantly decreased the MDA content in treated plants. The highest reduction in MDA content of 23.76 % was observed at 100 mM NaCl by "Trainer" application.

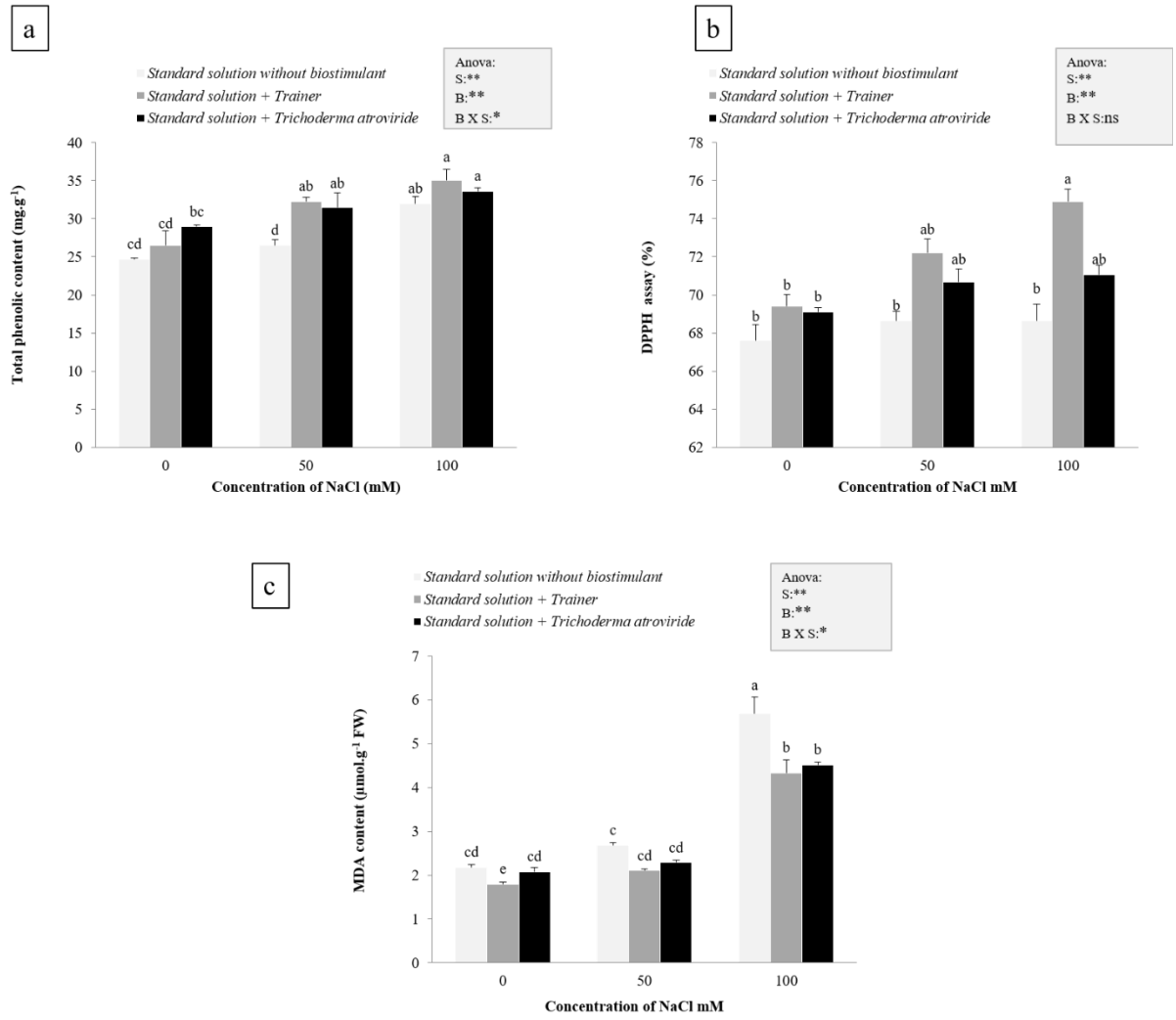

Figure 5: Effects of biostimulants application on total phenol content (a), antioxidant activity (b) and MDA content (c) of lettuce plants grown under two saline levels. Each value is the mean of the three replicates, and error bars represent ±SD. Different letters indicate significant differences according to Duncan's test (P = 0.05). ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

3.10 Mineral Composition

The mineral content was determined in leaves at harvest. Mineral concentrations are reported in the Table 5. Since the work was focused on salinity exposure, the values of sodium (Na) and calcium (Ca) have been discussed in relation to biostimulant applications. All lettuce plants growth in saline conditions showed an increase in Na^+ concentration. The highest amount of Na^+ was found in plants untreated by biostimulants at 100 mm NaCl. Across salinity, Na^+ concentration averaged 1.73 mmol/g DW in untreated lettuce plants. Calcium ion content (Ca^{2+}) was reduced as a result of salt stress. Similary, Table 5 shows also the reduction of potassium content (K^+) in shoots as a result of salt stress. The K^+/Na^+ ratio was much ($p \leq 0.05$) higher in leaves of control plants than those of salt-stressed plants.

Generally, Biostimulants significantly reduced the leaf concentration of Na^+ and increased the concentrations of Ca^{2+} and K^+ in lettuce leaves. The lowering effect of the biostimulant *Trichoderma atrovidae* in the Na^+ leaf accumulation was evident in plants grown under 100 Mm NaCl. However, there was little difference in the leave concentration of K^+ between the treated and the untreated plants.

The K^+/Na^+ ratio of the leaf was the same with or without biostimulants application in salt-stressed plants.

Table 5: Effects of biostimulants application on Na^+, Ca^{2+}, K^+, K^+/Na^+ ratio in leaves of of lettuce plants grown under two saline levels at five weeks after transplantation.

Treatments		Na^+ (mmol g^{-1} DW)	K^+(mmol g^{-1} DW)	Ca^{2+}(mmol g^{-1} DW)	K^+/Na^+
Biostimulant (B)	**Salinity (S)**				
Standard solution without biostimulant	**0 mMol Nacl**	0.05±0.01^{f}	2.91±0.02^{a}	0.22±0.02^{b}	56.82±0.02^{a}
	50 mMol Nacl	1.12±0.08^{c}	2.12±0.01^{c}	0.16±0.01^{e}	1.89±0.01^{c}
	100mMol Nacl	1.73±0.22^{a}	1.76±0.11^{d}	0.13±0.01^{f}	1.02±0.11^{c}
Trainer	**0 mMol Nacl**	0.09±0.01^{f}	2.92±0.04^{a}	0.24±0.01^{a}	31.82±0.04^{b}
	50 mMol Nacl	0.96±0.01^{de}	2.53±0.04^{b}	0.21±0.02^{bc}	2.62±0.04^{c}
	100mMol Nacl	1.52±0.02^{b}	1.96±0.02^{cd}	0.19±0.01^{cd}	1.29±0.02^{c}
Trichoderma atroviride	**0 mMol Nacl**	0.07±0.02^{f}	2.80±0.5^{ab}	0.24±0.01^{a}	38.36±0.5^{b}
	50 mMol Nacl	0.87±0.08^{e}	2.61±0.04^{ab}	0.18±0.01^{d}	3.01±0.04^{c}
	100mMol Nacl	1.02±0.01^{cd}	2.01±0.01^{cd}	0.16±0.01^{e}	1.97±0.01^{c}
		Significance			
S		**	*	**	*
B		**	**	**	**
S X B		*	ns	ns	ns

Values are mean ± standard errors of replicates. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05). Significance levels are represented by P > 0.05, ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01.

3.11 Principal component analysis

Principal component analysis was conducted on the entire experimental data set, representing average mean values of various parameters in "Trainer" and *Trichoderma atrovidae* treated plants under control and two conditions of NaCl stress, and the loading plot and scores are reported in Figure 6. PCA analysis explained, by two factors, 97.99% of the cumulative variance. The first factor explained 65.81 % and the second factor explains 14.38% (Figure 6). The abscissa axis is mainly a salinity gradient, where the variables located to the left of the axis are more affected by salinity than those located to the right (T1, T2, and T3).

PCA plots separated the treatment groups into three main groups. The first group was formed by growth attributes, contents of chlorophyll b, relative water, K^+ and Ca^{2+} level. These traits showed increasing trend in plants treated biostimulants (*Trichoderma* and "Trainer") at 0mM NaCl as compared with these of untreated plants. The second one included photosynthetic pigments, such as total chlorophyll, chlorophyll a, and carotenoid content. These parameters were mainly altered by NaCl concentrations in control plants compared with control non-stressed plants, and partial restoration was maintained in "Trainer" treated plants. The third group represents Na^+, electrolyte leakage, MDA, proline, soluble sugars and phenolic levels, which showed concentration-dependent increase in lettuce plants in response to salt stress as compared with that of control, while biostimulants treated plants showed resilience at both 50 and 100 mM NaCl when compared with untreated stressed plants.

Interestingly, the non-stressed (0 NaCl) lettuce plants treated with *Trichoderma atrovidae* produced lettuce with higher premium quality (higher growth traits and lower Sodium content).

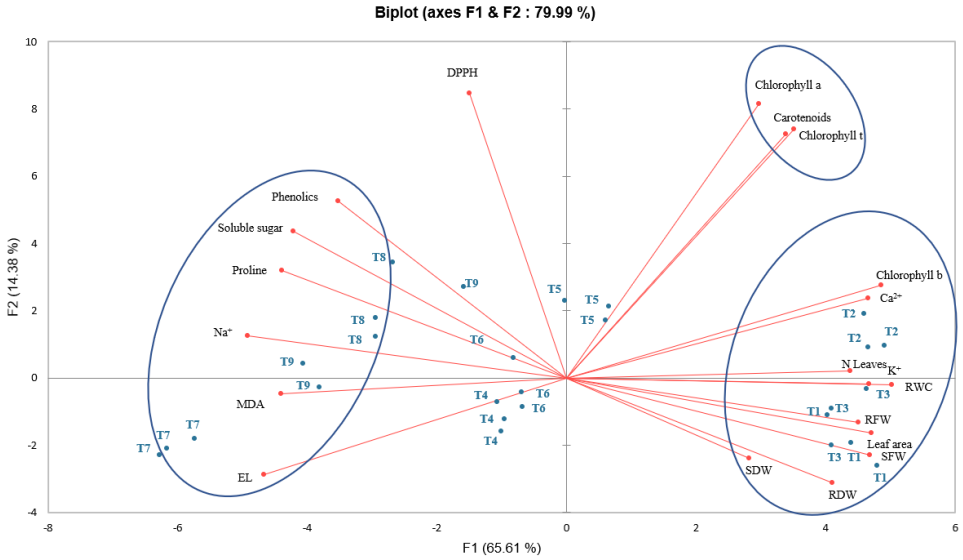

Figure 6: Principal Component Analysis (PCA 1 and PCA 2) of agronomical, physiological, biochemical traits measured in lettuce plants cultivated under three NaCl levels (0, 50 and 100 mM NaCl), and biostimulants application (control, *Trichoderma atrovidae*, Trainer).

T1: Control: plants irrigated by the basic nutrient solution, T2: Plants irrigated with the basic nutrient solution with 50 mM NaCl, T3: Plants irrigated with the basic nutrient solution with100 mM NaCl, T4: Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution, T5: Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution with 50 mM NaCl, T6: Plants from seeds coated with *Trichoderma atrovidae* and irrigated by the basic nutrient solution with 100 mM NaCl, T7: Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution, T8: Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution with 50 mM NaCl, T9: Plants treated with the protein hydrolysate "Trainer" and irrigated by the basic nutrient solution with 100 mM NaCl. RWC: Relative Water Content, RFW: Root Fresh weight, SFW: Shoot Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, N Leaves: Number of leaves per plant, EL: electrolyte leakage, MDA: malondialdehyde.

IV. DISCUSSION

Salt stress can lead to different physiological and biochemical modifications, resulting in many changes in both the structure and function of cells [42]. Plant responses to salinity differ greatly among species and to a lesser extent among varieties [43]. Their sensitivity is higher during seedling and reproductive stage [26]. Plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by many studies [43].

Most crop plants, including lettuce, are glycophytes, which cannot survive under salt concentrations greater than 100–200 mM, and lack the specialized mechanisms and structures present in euhalophytes for surviving in extreme salt concentrations. Regardless of inability or ability of glycophytes and halophytes to survive under high salt conditions, neither can tolerate high salt concentrations in their cytoplasm [44].

Based on our observations at the end of the 4-week growth period, it appears that lettuce mainly exhibits osmotic stress response, resulting in reduced growth and leaf area. The negative effect of salt stress can be commonly observed on growth reduction of several plants, including lettuce [45]; [46]; [47]; [48]. Qin et al. [49].also found a significant reduction in lettuce fresh weight, grown under higher saline concentrations than controls. The decrease in morphological traits under higher NaCl concentrations is probably due to disrupted mineral supplies, and the high presence of sodium and chloride ions [50]. The overall reduction in growth characteristics might be due to changes in photosynthesis, which can be attributed to suppression of mesophyll conductance and stomata closure [51].

Effects of salinity on plant growth and yield have been also attributed to simultaneous reduction in leaf area and root growth, affecting photosynthesis and water and mineral uptake [52]. Also, previous studies showed that lettuce leaf water content decreased in response to salinity [53]; [54]; [55]. Lower leaf water content results in stomatal closure and loss of cell turgor pressure and expansion, leading to reduced photosynthetic rate and leaf area.

In our experiment, biostimulant treatment increased significantly the growth parameters of lettuce plants compared to control. Results suggested that, the application of "Trainer" and *Trichoderma* to the lettuce plants mitigates salt-related consequences in plants, which results in considerable increases in growth and

biomass production. It was observed that salinity caused a substantial reduction in growth and biomass of those plants without 'trainer' and *Trichoderma* treatment.

Trichoderma is an endophytic symbiont, as its inoculation has antagonistic properties and therefore enhances the systemic tolerance to salt stress in plants [56]. The increase in growth and biomass production with a *Trichoderma* application may be due to its ability to produce phytohormones like cytokine and gibberellins, which could be beneficial for biological enhancements of crop fertility and may not only promote the plant growth but also increase some degree of tolerance in a saline environment [57]; [58]; [59]; [60].

Trichoderma spp. were suggested as a plant growth promoting fungi due to their ability to produce siderophores and phosphates-solubilizing enzymes [61]. This can be realized through several mechanism involved like mycoparasitism, antibiosis, degradation of toxins, inactivation of pathogenic enzymatic pathways, resistance to pathogens, enhanced nutrient uptake leading to overall development ([61]; [62]; [63].

The biostimulant "Trainer" tested in this work increased significantly the growth parameters of lettuce plants compared to control. The enhancement in the growth of lettuce plants, after treatments, could be attributed to an increased nutrient uptake, as reported by Türkmen et al. [64] who used humic acids in combination with Calcium to treat tomato seedlings.

Lucini et al. [31]observed that applications of plant derived protein hydrolysate mitigated the deleterious effects of salt stress on lettuce cv. Regina di Maggio. These results were consistent with a previous study of Ertani et al. [65], who observed that a protein hydrolysate biostimulant derived from alfalfa increased maize plant biomass, even under salinity. The effect of biostimulants can be direct on the salt sensitivity but also indirect, increasing plant biomass and fastening the growing cycle. The application of "Trainer" significantly increased the development rate, indicating a clear biostimulant effect.

Photosynthetic performance is a key aspect of how plants adapt to their growth conditions. Most stresses, including salinity stress, affect photosynthetic activity [45]. Similarly, lower chlorophyll content under higher salinities was also observed in lettuce [66]. Broadly, the negative trend of chlorophyll content under increasing NaCl concentration level was probably due to the increase in chlorophyllase activity which in turn induced the inhibition of chlorophyll biosynthesis, and other associated adverse effects on membrane stability, due to the salt in the growing media [67]; [68].

Salinity affects photosynthetic activity, both as a result of toxicity from Na^+ ion accumulation in the leaves over time, and also as a response mechanism by the plant to deal with high salt stress. The excess of energy absorbed from the leaf must be dissipated to avoid leaf photodamages. The plant needs to optimally regulate the light energy absorbed, so as to limit the photooxidative damage resulting from reduced photosynthesis, and maintain plant growth. Excess light energy that the plants absorb, if unquenched by photoprotective mechanisms or photochemistry, can lead to formation of the excited state of chlorophyll (triplet chlorophyll) in the photosystems, which can react with molecular oxygen to produce highly damaging reactive oxygen species (ROS). Thus, to maintain photosynthesis and growth and avoid cellular damage, plants use short-term and long-term acclimation to changes in their environment. This includes minimizing the light absorbed and diverting the energy from photosynthesis to photoprotective mechanisms ([44]; [69]; [70].

Carotenoids are important pigments that can also act as antioxidants protecting plasma membrane lipids from oxidative stress generated in plants exposed to salinity [71]. The data from the present study also corroborate with other data reported for other species, such as rice specimens (*Oriza sativa* L.) subjected to salinity also showing increases in carotenoid content [72].

One of the beneficial responses of plant biostimulants application is an increase in chlorophyllous pigments such as chlorophyll a, b and total, as well as carotenoids. This was the case in the current research study, since the application of "Trainer" and *Trichoderma* incurred a significant increase in carotenoids, chlorophyll a and b and consequently the total chlorophyll compared to untreated lettuce plants.

Results suggested that, in our experimental conditions, a general positive effect deriving from the application of biostimulant was observable on photosynthesis. Consistent results, regarding the effect of biostimulants on parameters of photosynthetic activity, were found, among others, in rocket treated with biostimulants of vegetable origin [73], in strawberry after seaweed extract application (Spinelli et al., 2010) and in maize treated with fulvic acid under drought [74].

Our results are in agreement with the finding of several authors [57]; [58]; [65]; [75], who demonstrated that application of *Trichoderma* was able to restore the chlorophyll content to acceptable levels under saline conditions in comparison to untreated plants.

Massa et al. [76] suggested that biostimulants could stimulate the crop performance by keeping open stomata, maintaining photosynthesis, source-sink relations and thus protecting from possible photoinhibition/photooxidation effects.

The highest pigments values observed after the application of plant biostimulants in particular "Trainer" application may be attributed to several putative mechanisms like the following: (i) better translocation of soluble sugars via the phloem, (ii) increases in the biogenesis of chloroplast and uptake of

bivalent cations principally Mg^{2+} and Fe^{2+}, that are required for chlorophyll biosynthesis as well as (iii) limited chlorophyll degradation, and thus, delayed senescence [77]; [78] ; [79].

A molecular indicator related to the photosynthetic light-harvesting function is the ratio between the two types of chlorophyll. Because the different metabolic processes implied in the synthesis and in the degradation of these pigment molecules may be specifically influenced by external stress factors and by the acclimation processes in photosynthesis, modifications of the molar ratio between chlorophyll a and b may indicate the degree of sensitivity of the light-harvesting complexes to adverse environmental conditions. The absolute amount of both chlorophyll a and chlorophyll b decreases upon salt stress, but because chlorophyll b content diminishes more than chlorophyll a content probably because of a smaller peripheral light-harvesting antenna, the chlorophyll a / chlorophyll b ratio increases moderately.

These results also corroborate previous studies indicating that NaCl stress has more effects on chlorophyll b than chlorophyll a [80]. This implies in an increase in the chlorophyll a/ chlorophyll b ratio, since the first step of chlorophyll b degradation results in its conversion into chlorophyll a [81] and therefore, a decrease in total chlorophyll content [82].

Proline is one of the most critical compounds in plants affected by salinity, and its concentration has been correlated with plant salt tolerance by significantly reducing plant Na^+ and Cl^- [83]; [84]. The presence of 100 mM NaCl in the growth medium caused a significant increase in the free proline content in all treated lettuce plants. This result was consistent with the previous results by Bartha et al. [85] and Shin et al. [86], who also found a significant increase in the proline content in all investigated NaCl concentrations in lettuce.

Increment of free proline concentration in plant cells subjected to osmotic stress is well documented in the literature, consequently different levels of proline content can indicate the degree of environmental stress that affects water balance of plants. The increase in proline content with the increase in NaCl concentration was probably due to the higher inhibitory rate of proline dehydrogenase and proline oxidase [87]. The higher proline content of leaves can be related to a higher capacity to accumulate Na^+ ions in the shoot, as osmotic adjustment is achieved by proline accumulation in the cytosol and by sodium sequestration in the vacuole [88]; [89]. Proline might detoxify plants by scavenging Reactive oxygen species or prevent them from damaging cellular structures [90]. In our study, we observed that the increasing levels of salinity caused a raise in proline concentration in control plants. Proline can play an important role in the osmotic adjustment and may participate to the scavenging of reactive oxygen species. the highest concentration was found in leaves treated with *Trichoderma*. These results support the hypothesized positive role of biostimulants in protecting plants from saline exposure. Further investigation should be performed to better understand the role of this biostimulant in the proline metabolism.

Regarding the data acquired in our experiment, it can be highlighted that the Relative water content decreased in lettuce plants under NaCl stress but increased due to the application of "Trainer" and *trichoderma*. The maintenance of a substantial amount of relative water content in leaves is a main strategy for maintaining optimal growth of plants under salinity [91]. It was reported that *Trichoderma harzianum* provides better ability to regulate additional intracellular water relations due to biomass accumulation resulting from the uptake of more water under salt stress ([92]; [8]).

Salinity stress damages the cell membrane due to increased electrolyte leakage [93]. Our data showed that electrolyte leakage content significantly increased with increasing NaCl concentrations, and these data are completely similar to the previous research on lettuce plants [35]; [94]. Many studies have shown that the increasing NaCl content results in increasing electrolyte leakage content in rice, cucumber, pepper and tomato plants [95]; [96]; [97]; [98].

The preliminary site of ion specifc salinity damage is plasma membrane [99]. Therefore, electrolyte leakage content can be considered as one of the most important indexes for plantś resistance [100]. In this study, electrolyte leakage was enhanced with increasing NaCl levels, which indicates more cell membrane damage at higher salinity levels. However, "Trainer" and *Trichoderma* treatments reduced electrolyte leakage, which likely resulted in less membrane damage.

Tuna et al.[93] who reported that silicon application decreased the electrolyte leakage in salt-stressed wheat and tomato, respectively, from that in non-Silicon treated salt stressed plants. In addition, plant-growth-promoting rhizobacteria (PGPR) inoculation reduced cell membrane damage in salt-stressed cucumber [101] (Kang et al. 2014) and maize [102], from that in the uninoculated control.

Sugars and organic acids are other organic solutes accumulated in plants under salt stress [103]: [104]. In our study, salinity induced a significant increase in sugar leaves contents. High sugar concentrations in cytoplasm could exert a feedback inhibition on CO_2 assimilation [105]. Shortage of CO_2 fixation accompanied by depressed photosystem activity and electron transport rate resulted in accelerated production of reactive oxygen species (ROS) suggesting movement of electrons to oxygen molecules rather than being used for carbon assimilation [106]. The role of amino acids and sugars in preventing photosystem inhibition had also been associated with their action in alleviation or preventing oxidative stress[107]. Soluble sugars might also

scavenge ROS in chloroplasts and vacuoles [108]. Osmolytes could either detoxify ROS indirectly. In fact, it has been demonstrated that proline and sugars mediated this role by supporting the redox cycling by the replenishment of NADPþ supply. Sugars are also essential to the synthesis of numerous non enzymatic antioxidant compounds especially ascorbate, carotenoids and glutathione [109].

Soluble sugars can directly or indirectly modulate the expression of genes involved in metabolic processes, storage functions, and defence [110]. Furthermore, according to Stoop and Pharr [111], the sugars levels rise due to the fall in carbon demand, maintaining the basal metabolism under adverse environmental conditions [112]. Soluble sugars are moreover believed to accumulate in cells and plant tissues due to salinity stress and plays a major role in maintaining a low osmotic cell sap potential [45]; [113].

Regarding the data acquired in our experiment, it can be highlighted that total phenolic content was remarkably increased as a result of increasing the level of salinity. Polyphenols represent a large family of plant secondary metabolites. The synthesis of these compounds is induced in response to biotic and abiotic stimuli and may act as antioxidants to protect the plant against oxidative stress [114]. Increase in total phenolic content by application of biostimulant in lettuce plants can be explained by enzyme activation.

Garrido et al. [66] also reported higher total phenolic content with an increase in NaCl concentration in multi-leaf lettuce, while Chisari et al. [115] and Kim et al. [116] observed a decrease in total phenolic content with an increase in NaCl concentration in romaine lettuce. These results suggest that the NaCl concentration affects phenolic content differently depending on the genotype and plant species.

Numerous studies indicate that the oxidative damage generated during the salinity stress is due to the imbalance in production of free radicals and the alteration in antioxidant activity. To avoid this damage caused by oxidative stress, plants have developed many antioxidant systems. For the most precise determination of the antioxidant capacity, different antioxidant tests were evaluated. In the present work, we used the DPPH assay.

The antioxidant activity, expressed by the ability to neutralize DPPH radical, showed significant values at 50 and 100 mM NaCl. This could be directly linked to the increase in phenolic contents, which corroborate with findings showing that accumulation of phenolic acids could be considered an adaptative strategy for overcoming oxidative damage [117]; [118].

The "Trainer" and *Trichoderma* biostimulants applied to lettuce resulted in higher antioxidant capacity and bioactive content depending on the salinity levels compared to untreated control treatment. Our findings on the effect of plant biostimulants on nutritional and functional quality of the product were in line with previous research on biostimulants (protein hydrolysate and plant extract) conducted by Caruso et al. [119], in which foliar application increases the antioxidants contents of perennial wall rocket compared to the non-treated control. Similarly, Vasantharaja et al. [120] demonstrated that the application of seaweed extract-based biostimulant boosted the antioxidant activity and the bioactive content (phenols and vitamin C) of cowpea. A mechanistic explanation of the beneficial effect of plant biostimulants, on the biosynthesis of antioxidant molecules could be due to: i) the activity stimulation of key enzymes involved in antioxidant homeostasis in cells, and ii) the higher macro- and micro-nutrient assimilation of biostimulant-treated plants which could contribute to the synthesis of amino acids, phenylalanine and tyrosine [121]; [122].

The oxidative stress induced by salinity can lead to an excess of ROS production resulting in lipid peroxidation [123], Therefore, the level of malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage [124]. The present study indicated a gradual increase of lettuce MDA content under salt stress. This increase was significant at 100 mM, suggesting that severe stress-induced lipid peroxidation resulted in deterioration of the membrane integrity.

However, no significant change of the MDA content at moderate stress (50 mM NaCl) was observed. Our results corroborate with findings showing that an unchanged lipid peroxidation level seems to be a characteristic of tolerant plants coping with moderate salt concentration [125].

In order to minimize the oxidative damage caused by ROS generated under salt stress, the plants exhibit both enzymatic and nonenzymatic antioxidant defense systems [126]. "Trainer" and *Trichoderma* applications resulted in decreased MDA content in treated plants. Thus, tested biostimulants could alleviate lipid peroxidation caused by salt stress in lettuce plants.

In this study, salt-induced toxicity (Na^+) disturbs the uptake of essential mineral nutrients such as K^+ and Ca^{2+}. Na^+ increased significantly due to salt stress. This excessive Na^+ uptake may have led to an imbalance in cellular Na^+ and K^+ contents. Under salinity, the nutritional imbalance of plants can be mainly ascribed to several mechanisms including osmotic effects of salts and also to the competition between Na^+ and K^+ uptake in roots leading to extreme ratio of Na^+/K^+ [127]; [128]; [129]. As a result, lettuce plant become more sensitive to specific ion injury as well as to mineral deficiencies resulting in stunted growth and productivity loss. Hyperaccumulation of Na^+ also reduces the influx of Ca^{2+} ions through the plasma membrane, and increases efflux of Ca^{2+} from plant cells [130]. The reduction of K^+ and Ca^{2+} content in shoots, can be explained by the pronounced decrease of the hydraulic and stomatal conductance in lettuce plants exposed to salt stress.

Reduction of potassium content in shoots as a result of salt stress is most probably due to the competition of Na^+ for the same cation transporters. K^+ is involved in different cellular functions, such as activation of enzymatic reactions, charge balancing, and osmoregulation [131]. Therefore, the control of K^+ homeostasis is fundamental in salinity tolerance.

However, the role of Ca^{2+} in antioxidant enzyme signal transduction was reported by Agarwal et al. [132] who reported that Ca^{2+} acts as a second messenger and causes a transient increase in H_2O_2, which in turn induces antioxidant enzyme activity leading to a decrease in ROS on long- time basis.

Na+/K+ ratio in tissues is additionally an important indicator of plant salinity tolerance as a lower Sodium/Potassium ratio in cytosol is indispensable for normal metabolic functions of the cell [133].

A lower K+/Na+ ratio was noted across plant partitions under salinity than in control plants. As salt stress results in ion imbalance, the need for exclusion of extra Na^+ and Cl^- is necessary for plant survival. The relative decrease in K^+/Na^+ due to salinity stress has been previously reported [134]; [135].

Therefore, two main strategies of salt stress tolerance can be considered, i.e. salt exclusion and salt sequestration, the latter one is generally used by lettuce plants. This is why the marketable biomass gets a slight salty taste. In the first phase of the salt stress, the rapidly accumulating sodium (Na^+) is an osmolite with low energy cost in the leaf vacuoles for the cell turgor adjustment, and ultimately of tissue growth under the hyperosmotic stress condition imposed by salinity [4]; [136]. Then, salt stress disturbs the uptake of essential mineral nutrients such as Ca^{2+} and $K^{+,}$ as Na^+ competitively inhibits Ca^{2+} and K^+ transport through membranes [137].

The biostimulant treatment seem to improve this adaptive mechanism with a reduced Na^+ cellular influx and consequent enhanced K^+ accumulation in leaves of lettuce treated plants under salt stress.

A presumed mechanism involved in the stimulation of crop performance after co-inoculation with beneficial microorganisms might be the improvement of mineral nutrient availability and plant uptake. Nutrient uptake is a main factor for the maintenance of homeostasis and plant development under edaphic adversities [20]. In the present study, both biostimulant treatments reduced Sodium uptake of plants and increased the Potassium uptake, compared to untreated plants under salt stress, thus increasing K^+/Na^+ ratio. The capacity to maintain a high K^+/Na^+ ratio in the shoot tissue constitutes an important salt tolerance indicator [138]. The greatest Potassium accumulation, and the reduced Sodium concentration in the microbial treated lettuce, may have helped the plants toward maintaining the osmotic potential in their cells and also preventing accumulation of cellular Na^+ to a toxic concentration.

Our results are consistent with several previous studies [57]; [139]; [140]; [58]; [75] which showed an improvement in the uptake of some macro and microelements under salinity conditions, when plants were inoculated with arbuscular mycorrhizal fungi or *Trichoderma* spp.

V. CONCLUSION

Salt stress has been found to impose deleterious effects on lettuce plants especially at higher levels of NaCl. However, coating seeds with *Trichoderma atroviride* was found to mitigate the detrimental effects of NaCl salinity on lettuce growth. Improved nutritional (higher K^+ and lower Na^+ concentration in leaf tissue) and leaf water status, may have assisted the plants to translocated minerals and alleviated the impacts of salinity on lettuce plants. *Trichoderma* inoculated plants restored the pigment and relative water contents. The increase in proline content was found to be very useful in providing tolerance to these plants under NaCl stress. *Trichoderma atroviride* not only enhanced some physiological parameters but also increased antioxidant capacity and bioactive compound. The plants inoculated with *Trichoderma atroviride* hold potential to induce relative salt tolerance and improve plant growth of lettuce plants under salt stress. Although further research is required to validate and fully understand of its mode of action, *Trichoderma atroviride* has promise as a candidate strain as a biological abiotic stress control agent. It is surely critical to generate effective bio-control strategies by enlightening the influence of such alternative strains which is perfectly safe either for rhizosphere microflora or for human being to give sustainable approaches to agriculture.

REFERENCES

[1]. Aly, A. A., F. N. Al-Barakah and M. A. El-Mahrouky. 2018. Salinity Stress Promote Drought Tolerance of Chenopodium Quinoa Willd. Communications in Soil Science and Plant Analysis 49 (11) :1331–43. doi: 10.1080/00103624.2018.1457160.

[2]. Ahmad, P., M. Ozturk, S. Sharma, and S. Gucel. 2014. Effect of sodium carbonate-induced salinity-alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (Morus alba l.) cultivars. Journal of Plant Interactions 9:460–67. doi: 10.1080/17429145.2013.855271.

[3]. Siddiqui, Z. S., and M. A. Khan. 2013. Some physiological attributes of dimorphic seeds of Halopyrum mucronatum (L.) Stapf. Pakistan Journal of Botany 45:1975-79.

[4]. Munns, R., and M. Tester. 2008. Mechanisms of salinity tolerance, Annual Review of Plant Biology 59:651–81.

[5]. Colla, G., and Y. Rouphael. 2015. Biostimulants in horticulture. Scientia Horticulturae 196:1–2. doi: 10.1016/j.scienta.2015.10.044.

[6]. Garcia-Mina, J. M., and E. Hadavi. 2016. Editorial : organic-based foliar biostimulation and nutrition in plants. Frontiers in Plant Science 6:1131. doi: 10.3389/ fpls.2015.01131.

[7]. Du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae 196:3–14. doi: 10.1016/j.scienta.2015.09.021.

[8]. Rawat, L., Y. Singh, N. Shukla, and J. Kumar. 2011. Alleviation of the adverse effects of salinity stress in wheat (Triticum aestivum L.) by seed biopriming with salinity tolerant isolates of Trichoderma harzianum. Plant and Soil 347: 387–400.

[9]. Egamberdieva, D., Z. Kucharova, K. Davranov, G. Berg, N. Makarova, T. Azarova, V. Chebotar, I. Tikhonovich, F. Kamilova, S.Z. Validov, et al. 2011. Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. Biology and Fertility of Soils 47:197–205. doi: 10.1007/s00374-010-0523-3.

[10]. Hameed, A., D. Egamberdieva, E. F. Abd-Allah, A. Hashem, A. Kumar, and P. Ahmad. 2014. Salinity stress and arbuscular mycorrhizal symbiosis in plants. In Use of Microbes for the Alleviation of Soil Stresses, ed. M. Miransari , vol. 1, 139–159. New York, NY: Springer.

[11]. Guler, N. S., N. Pehlivan, S. A. Karaoglu, S. Guzel, and A. Bozdeveci. 2016. Trichoderma atroviride ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. Acta Physiologiae Plantarum 38:1–9.

[12]. Brotman, Y., J. G. Kapuganti, and A.Viterbo. 2010. Trichoderma. Current Biology 20: 390–91. doi: 10.1016/j.cub.2010.02.042.

[13]. Harman, G. E. 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytologist 189:647–49. doi: 10.1111/j.1469-8137.2010.03614.

[14]. Mastouri, F., T. Bjorkman, and G. E. Harman. 2010. Seed treatment with Trichoderma harzianum alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100:1213–21.

[15]. Alexandru, M., D. Lazaˇr , M. Ene, and T. E. Şesan. 2013. Influence of some Trichoderma species on photosynthesis intensity and pigments in tomatoes. Romanian Biotechnological Letters 18:8499–510.

[16]. Shoresh, M., F. Mastouri, and G. Harman. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopathology 48, 21–43. doi: 10.1146/annurev-phyto-073009-114450.

[17]. López-Bucio, J., R. Pelagio-Flores, and A. Herrera-Estrella. 2015. Trichoderma as biostimulant: exploiting the multilevel properties of a beneficial fungus. Scientia Horticulturae 196:109–23. doi: 10.1016/j.scienta.2015.08.043.

[18]. Shulka, S. K., P. N. Singh, R. S. Chauhan, and R. L. Yadav. 2012. Recycling of Organic Wastes Amended with Trichoderma and Gluconacetobacter for Sustenance in Soil Health and Sugarcane Ratoon Yield in Udic Ustochrept. Communications in Soil Science and Plant Analysis 43(7):1073–1097.

[19]. Bailey, B. A., H. Bae, M. D. Strem, D. P. Roberts, S. E. Thomas, J. Crozier, G. J. Samuels, Ik-Young Choi, and K. A. Holmes. 2006. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four Trichoderma species. Planta 224:1449–64.

[20]. Mastouri, F., T. Bjorkman, and G. E. Harman. 2012. Trichoderma harzianum enhances antioxidant defense of tomato seedlings and resistance to water deficit. Molecular Plant-Microbe Interactions 25:1264–71.

[21]. Colla, G., Y. Rouphael, E. Di Mattia, C. El-Nakhel, and M. Cardarelli. 2015b. Co-inoculation of Glomus intraradices and Trichoderma atroviride acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. Journal of the Science of Food and Agriculture 95(8):1706–15. doi :10.1002/jsfa.6875.

[22]. Rouphael, Y., M. Cardarelli, P. Bonini, and G. Colla. 2017. Synergistic Action of a Microbial-based Biostimulant and a Plant Derived-Protein Hydrolysate Enhances Lettuce Tolerance to Alkalinity and Salinity. Frontiers in Plant Science 8:131. doi: 10.3389/fpls.2017.00131.

[23]. Bahrani, A., J. Pourreza, and M. H. Joo. 2010. Response of winter wheat to co-inoculation with azotobacter and arbuscular mycorrhizal fungi (AMF) under different sources of nitrogen fertilizer. American-Eurasian Journal of Agricultural & Environmental Sciences 8: 95-103.

[24]. Minaxi-Saxena, J., S. Chandra, and L. Nain. 2013. Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. Journal of Soil Science and Plant Nutrition 13:511-25.

[25]. Tavares, L. C., C. A. Rufino, A. P. Brunes, F. F. Friedrich, A. C. S. A. Barros, and F. A. Villela. 2013. Physiological performance of wheat seeds coated with micronutrients. Journal of Seed Science 35: 28-345.

[26]. Colla, G., Y. Rouphael, P. Boninic, and M. Cardarellid. 2015a. Coating seeds with endophytic fungi enhances growth, nutrient uptake, yield and grain quality of winter wheat. International Journal of Plant Production 9(2): 171-90.

[27]. Malusa, E., L. Sas-Paszt, and J. Ciesielska. 2012. Technologies for beneficial microorganism inocula used as biofertilizers. The Scientific World Journal, Article ID 491206:12. doi: 10.1100/2012/491206.

[28]. Kim, M. J., Y. Moon, J.C. Tou, B. Mou, and N.L. Waterland. 2016. Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis 49:19–34.

[29]. Shannon, M. C., and C. M. Grieve. 1998. Tolerance of vegetable crops to salinity. Scientia Horticulturae 78:5–38.

[30]. Machado, R. M. A. and R. P. Serralheiro. 2017. Soil Salinity: Effect on Vegetable Crop Growth. Management Practices to Prevent and Mitigate Soil Salinization. Horticulturae 3:30. doi:10.3390/horticulturae3020030.

[31]. Lucini, L., Y. Rouphael, M. Cardarelli, R. Canaguier, P. Kumar, and G. Colla. 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Scientia Horticulturae 182 : 124–133. doi: 10.1016/j.scienta.2014.11.022.

[32]. Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148:350–382.

[33]. Galmés, J., J. Flexas, R. Savé, and H. Medrano. 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant and Soil 290: 139–155.

[34]. Bates, L. S, R. P. Waldren, and I. D. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39:205–07.

[35]. Kaya, C., D. Higgs, and E. Sakar. 2002. Response of Two Leafy Vegetables Grown At High Salinity To Supplementary Potassium And Phosphorus During Different Growth Stages. Journal of Plant Nutrition 25(12):2663-76, doi: 10.1081/PLN-120015530.

[36]. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 3506.

[37]. Mau, J.L., G. R. Chao, and K. Wu. 2001. Antioxidant properties of methanolic extracts from several ear mushrooms. Journal of Agricultural and Food Chemistry 49: 5461–5467.

[38]. Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, and C. Abdelly.2008. Phenolic composition of Cynara cardunculus L. organs, and their biological activities. Comptes rendus Biologies 331: 372–379.

[39]. Sánchez-Moreno, C., J. A. Larrauri, and F. Saura-Calixto. 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 76 (2):270–76.

[40]. Ben Amor, B., K. Hamed, A. Debez, C. Grignon, and C. Abdelly. 2005. Physiological and antioxidant responses of the perennial halophyte Crithmum maritimum to salinity. Plant Science 168 (4) : 889–99.
[41]. Bartha, C., L. Fodorpataki, M. C. Martinez-Ballesta, O. Popescu, and M. Carvajal. 2015. Sodium accumulation contributes to salt stress tolerance in lettuce cultivars. Journal of Applied Botany and Food Quality 88: 42–48.
[42]. Takemura, T., H. Okamoto, Y. Maruyama, A. Numaguti, and A. Higurashi , and T. Nakajima. 2000. Global three-dimensional simulation of aerosol optical thickness distribution of various origins. Journal of Geophysical Research Atmospheres 105 (D14): 17853–74. doi: 10.1029/2000JD900265.
[43]. Parida, A. K., and A. B. Das. 2005. Salt tolerance and salinity effects on plants: A Review. Ecotoxicology and Environmental Safety 60: 324–49.
[44]. Adhikari, N. D., I. Simko, and B. Mou. 2019. Phenomic and Physiological Analysis of Salinity Effects on Lettuce. Sensors 19 : 4814. doi:10.3390/s19214814.
[45]. Acosta-Motos, J. R., M. F. Ortuno, A. Bernal-Vicente, P. Diaz-Vivancos, M. J. Sanchez-Blanco, and J. A. Hernandez. 2017. Plant responses to salt stress: Adaptive mechanisms. Agronomy 7: 18. doi: 10.3390/agronomy7010018.
[46]. Al-Maskri, A., L. Al-Kharusi, H. Al-Miqbali, and M. M. Khan. 2010. Effect of salinity stress on growth of lettuce (Lactuca sativa) under closed-recycle nutrient film technique. International Journal of Agriculture and Biology 12: 377–80.
[47]. Goussi, R., A. Manaa, W. Derbali, S. Cantamessa, C. Abdelly, and R. Barbato. 2018. Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in Thellungiella salsuginea. Journal of Photochemistry and Photobiology B 183: 275–87.
[48]. Shin, Y. K., S. R. Bhandari, M. C. Cho, and J. G. Lee. 2020a. Evaluation of chlorophyll fluorescence parameters and proline content in tomato seedlings grown under different salt stress conditions. Horticulture, Environment, and Biotechnology 61: 433–443.
[49]. Qin, L., S. Guo, W. Ai, Y. Tang, Q. Cheng, and G. Chen. 2013. Effect of salt stress on growth and physiology in amaranth and lettuce: Implications for bio-regenerative life support system. Advances in Space Research 51: 476–82.
[50]. Ashraf, M., P. J. C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science 166: 3–16.
[51]. Chaves, M. M., J. Flexas, and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany 103: 551–560.
[52]. Shannon, M. C., and C. M. Grieve. 1998. Tolerance of vegetable crops to salinity. Scientia Horticulturae 78:5–38.
[53]. Perez-Lopez, U., J. Miranda-Apodaca, A. Muñoz-Rueda, and A. Mena-Petite. 2013. Lettuce production and antioxidant capacity are differentially modified by salt stress and light intensity under ambient and elevated CO2. Journal of Plant Physiology170:1517–25.
[54]. Shannon, M. C., J. D. McCreight, and J. H. Draper. 1983. Screening tests for salt tolerance in lettuce. The Journal of the American Society for Horticultural Science 108:225–30.
[55]. Xu, C., and B. Mou. 2015. Evaluation of Lettuce Genotypes for Salinity Tolerance. HortScience: a Publication of the American Society for Horticultural Science 50(10):1441–46. DOI: 10.21273/HORTSCI.50.10.1441
[56]. Harman, G. E., C. R. Howell, A. Vitarbo, I. Chet, and M. Lorito. 2004. Trichoderma species – opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2: 43–56.
[57]. Ahmad, P., H. Abeer, F. A. A. Elsayed, A. A. Alqarawi, J. Riffat, E. Dilfuza, and G. Salih. 2015. Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (Brassica juncea L.) through antioxidative defense system. Frontiers in Plant Science 6: 868. doi: 10.3389/fpls.2015. 00868.
[58]. Benitez, T., A. M. Rincon, M.C. Limon, and A. C. Codon. 2004: Biocontrol mechanisms of Trichoderma strains. International Journal of Microbiology 7:249–60.
[59]. Harman, G. E. 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on Trichoderma harzianum t-22. Plant Disease 84: 377–93.
[60]. Kashyap, P. L, S. Kumar, and A.K. Srivastava. 2017. Nanodiagnostics for plant pathogens. Environmental Chemistry Letters 15:7–13.
[61]. Doni, F., A. Isahak, C. R. C. M. Zain, S. M. Ariffin, W. N. W. Mohamad, and W. M. W. Yusoff. 2014. Formulation of Trichoderma sp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. SpringerPlus 3:532. doi: 10.1186/2193-1801-3-532.
[62]. Harman, G. E. 2006. Overview of mechanisms and uses of Trichoderma spp. Phytopathology 96:190–94.
[63]. Lorito, M., S.L. Woo, G.E. Harman, and E. Monte. 2010. Translational research on Trichoderma: From 'omics to the field. Annual Review of Phytopathology 48:395–417.
[64]. Türkmen, Ö., A. Dursun, M. Turan, and Ç. Erdinç. 2004. Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (Lycopersicon esculentum L.) seedlings under saline soil conditions. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 54 : 168–74. doi: 10.1080/09064710310022014.
[65]. Ertani, A., M. Schiavon, A. Muscolo, and S. Nardi. 2013. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed Zea mays L. plants. Plant Soil 364 : 145–58. doi: 10.1007/s11104-012-1335-z.
[66]. Garrido, Y., J. A. Tudela, A. Marin, T. Mestre, V. Martinez, and M.I. Gil. 2014. Physiological, phytochemical and structural changes of multi-leaf lettuce caused by salt stress. Journal of the Science of Food and Agriculture 94, 1592–99.
[67]. Silveira, J. A. G., and F. E. L. Carvalho. 2016. Proteomics, photosynthesis and salt resistance in crops: An integrative view. Journal of Proteomics 143 : 24–35.
[68]. Taffouo, V. D., A. H. Nouck, S. D. Dibong, and A. Amougou. 2010. Effects of salinity stress on seedling growth, numeral nutrients, and total chlorophyll of some tomato (Lycopersicum esculentum L.) cultivars. The African Journal of Biotechnology 9: 5366–72.
[69]. Lee, K. P., C. Kim, F. Landgraf, and K. Appel. 2007. Executer1- and Executer2-dependent transfer of stress-related signals from the plastid to the nucleus of Arabidopsis thaliana. Proceedings of the National Academy of Sciences 104 : 10270–75.
[70]. Meskauskiene, R., M. Nater, D. Goslings, F. Kessler, R. op den Camp, and K. Appel. 2001. FLU: A negative regulator of chlorophyll biosynthesis in Arabidopsis thaliana. Proceedings of the National Academy of Sciences 98: 12826–31.
[71]. Falk, J., and S. Munné-Bosch. 2010. Tocochromanol functíons in plants: antioxidation and beyond. Journal of Experimental Botany 61(6): 1549–66. PMid:20385544. http://dx.doi.org/10.1093/ jxb/erq030.
[72]. Misra, A., A. N. Sahu, M. Misra, P. Singh, I. Merra, N. Das, M. Kar, and P. Sahu. 1997. Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biologia Plantarum 39(2) : 257–62. http:// dx.doi.org/10.1023/A:1000357323205.
[73]. Di Mola, I., L. Ottaiano, E. Cozzolino, M. Senatore, M. Giordano, C. El-Nakhel , A. Sacco, Y. Rouphael, G. Colla, and M. Mori. 2019. Plant-Based Biostimulants Influence the Agronomical, Physiological, and Qualitative Responses of Baby Rocket Leaves under Diverse Nitrogen Conditions. Plants 8: 522. doi:10.3390/plants8110522.

[74]. Anjum, S. A., L. C. Wang, M. Farooq, M. Hussain, L. L. Xue, and C. M. Zou. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. Journal of Agronomy and Crop Science 197: 177–185. doi: 10.1111/j.1439-037X.2010.00459.x.

[75]. Rouphael, Y., M. Cardarelli, E. Di Mattia, M. Tullio, E. Rea, and G. Colla. 2010. Enhancement of alkalinity tolerance in two cucumber genotypes inoculate with an arbuscular mycorrhizal biofertilizer containing Glomus intraradices. Biology and Fertility of Soils 46:409–509. doi: 10.1007/s00374-010-0457-9.

[76]. Massa, D., D. Prisa, E. Montoneri, D. Battaglini, M. Ginepro, M. Negre, and G. Burchi. 2016. Application of municipal biowaste derived products in hibiscus cultivation : effect on leaf gaseous exchange activity, and plant biomass accumulation and quality. Scientia Horticulturae 205: 59–69. doi: 10.1016/j.scienta.2016. 03.033.

[77]. Battacharyya, D., M. Z. Babgohari, P. Rathor, and B. Prithiviraj. 2015. Seaweed extracts as biostimulants in horticulture. Scientia Horticulturae196 : 39–48.

[78]. Sheng, M., M. Tang, H. Chen, B. W. Yang, F. F. Zhang, and Y. H. Huang. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 18 : 287–96. doi: 10.1007/s00572- 008-0180-7.

[79]. Tadros, M. J., H. J. Omari, and M.A. Turk. 2019. The morphological, physiological and biochemical responses of sweet corn to foliar application of amino acids biostimulants sprayed at three growth stages. Australian Journal of Crop Science 13 : 412–17.

[80]. Houimli, S. I. M., M. Denden, and B.D. Mouhandes. 2010. Effects of 24 epibrassinolide on growth, chlorophyll, electrolyte leakage and proline by pepper plants under NaCl-stress. Eurasian Journal of Biosciences 4 :96–104. http://dx.doi.org/10.5053/ejobios.2010.4.0.12.

[81]. Fang, Z., J.C. Bouwkamp, and T. Solomos. 1998. Chlorophyllase activies and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of Phaseolus vulgaris L. Journal of Experimental Botany 49: 503–10.

[82]. Pinheiro, H.A., J.V. Silva, L. Endres, V. M. Ferreira, C. A. Camara, F. F. Cabral, J. F. Oliveira, L. W. T. Carvalho, J. M. Santos, and B. J. Santos Filho. 2008. Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (Ricinus communis L) seedlings subjected to salt stress conditions. Industrial Crops and Products 27(3) : 385–92. http://dx.doi. org/10.1016/j.indcrop.2007.10.003.

[83]. Ben-Taarit, M. K., K. Msaada, K. M. Hosni, M. Hammami, E. Kchouk, and B. Marzouk. 2009. Plant growth, essential oil yield and composition of saga (Salvia officinalis L.) fruits cultivated under salt stress conditions. Industrial Crops and Products 30 (3):333–37.

[84]. Rady, M. M., V. C. Bhavya, and S. M. Howladar. 2013. Common bean (Phaseolus vulgaris) seedlings overcome NaCl stress as a result of presoaking in Moringa oleifera leaf extract. Scientia Horticulturae162 : 63–70.

[85]. Bartha, C., L. Fodorpataki, M. C. Martinez-Ballesta, O. Popescu, and M. Carvajal. 2015. Sodium accumulation contributes to salt stress tolerance in lettuce cultivars. Journal of Applied Botany and Food Quality 88: 42–48.

[86]. Shin, Y.K., S.R. Bhandari, J. S. Jo, J. W. Song, M. C. Cho, E. Y. Yang, and J. G. Lee. 2020b. Response to Salt Stress in Lettuce: Changes in Chlorophyll Fluorescence Parameters, Phytochemical Contents, and Antioxidant Activities. Agronomy 10 : 1627. doi:10.3390/agronomy10111627.

[87]. Kaur, G., and B. Asthir. 2015. Proline : A key player in plant abiotic stress tolerance. Plant Biology 59: 609–19.

[88]. Ashraf, M., and M. R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. Environmental and Experimental Botany 59 : 206–16.

[89]. Hasegawa, P. M., R. A. Bressan, J. K. Zhu, and H. J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Annual review of plant physiology and plant molecular biology 51: 463–499.

[90]. Kamiab,F. 2020. Exogenous melatonin mitigates the salinity damages and improves the growth of pistachio under salinity stress, Journal of Plant Nutrition 43 (10) :1468–84. doi: 10.1080/01904167.2020.1730898.

[91]. Siddiqui, Z. S., J. I. Cho, S. Hanpark, T. R. Kwon, B. O. Ahn, G. S. Lee, M. J. Jeong, K. Whankim, S. Konlee, and S. Chulpark. 2014: Phenotyping of rice in salt stress environment using high-throughput infrared imaging. Acta Botanica Croatica 73: 149–58.

[92]. Hashem, A., E. F. Abd-allah, A. A. Alqarawi, A. A Al-Huqail, and D. Egamberdieva. 2014. Alleviation of abiotic salt stress in Ochradenus baccatus (Del.) by Trichoderma hamatum (Bonord.) Bainier. Journal of Plant Interactions 9: 857–68.

[93]. Tuna, A. L., C. Kaya, D. Higgs, B. Murillo-Amador, S. Aydemir, and A. R. Girgin. 2008. Silicon improves salinity tolerance in wheat plants. Environmental and Experimental Botany 62: 10–16. doi: 10.1016/j.envexpbot.2007.06.006.

[94]. Mahmoudi, H., R. Kaddour, J. Huang, N. Nasri, B. Olfa, S. M'Rah, A. Hannoufa, M. Lachaâl, Z. Ouerghi. 2011. Varied tolerance to NaCl salinity is related to biochemical changes in two contrasting lettuce genotypes. Acta Physiologiae Plantarum 33(5):1613–22. https://doi. org/10.1007/s11738-010-0696-2.

[95]. Ahmad, P., M. Abass Ahanger, M. Nasser Alyemeni, L. Wijaya, P. Alam, and M. Ashraf. 2018. Mitigation of sodium chloride toxicity in Solanum lycopersicum L. by supplementation of jasmonic acid and nitric oxide. Journal of Plant Interactions 13(1):64–72. https://doi. org/10.1080/17429145.2017.1420830.

[96]. Kaya, C., H. Kirnak, and D. Higgs. 2001a. Effects of Supplementary Potassium and Phosphorus on Physiological Development and Mineral Nutrition of Cucumber and Pepper Cultivars Grown at High Salinity (NaCl). Journal of Plant Nutrition 24 (9), 1457–71.

[97]. Kaya, C., H. Kirnak, and D. Higgs. 2001b. Enhancement of Growth and Normal Growth Parameters by Foliar Application of Potassium and Phosphorus on Tomato Cultivars Grown at High (NaCl) Salinity. Journal of Plant Nutrition 24 (2):357–67.

[98]. Lutts, S., J. M. Kinet, and J. Bouharmont. 1996. NaCl-Induced Senescence in Leaves of Rice (Oryza sativa L.) Cultivars Differing in Salinity Resistance. Annals of Botany 78:389–398.

[99]. Mansour, M. M. F., K. H. A. Salama. 2004. Cellular basis of salinity tolerance in plants. Environmental and Experimental Botany 52(2):113–22.

[100]. Ashraf, M., Q. Ali. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (Brassica napus L). Environmental and Experimental Botany 63(1):266–73. https://doi.org/10.1016/j.envexpbot.2007.11.008

[101]. Kang, S. M., A. L. Khan, M. Waqas, Y. H. You, J. H. Kim, J. G. Kim, M. Hamayun, and I. J. Lee. 2014. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus. Journal of Plant Interactions 9 :673–82. doi: 10.1080/17429145.2014.894587.

[102]. Marulanda, A., R. Azcon, F. Chaumont, J. M. Ruiz-Lozano, and R. Aroca. 2010. Regulation of plasma membrane aquaporins by inoculation with a Bacillus megaterium strain in maize (Zea mays L.) plants under unstressed and salt-stressed conditions. Planta 232:533–43. doi: 10.1007/s00425-010-1196-8.

[103]. Chen, W., C. Feng, W. Guo, D. Shi, and C. Yang. 2011. Comparative effects of osmotic-, salt-and alkali stress on growth, photosynthesis, and osmotic adjustment of cotton plants. Photosynthetica 49 (3):417–25.

[104]. Baki, G. A. E., F. Siefritz, H. M. Man, H. Weiner, R. Kaldenhoff, and W. M. Kaiser. 2000. Nitrate reductase in Zea mays L. under salinity. Plant, Cell & Environment 23: 515–21. doi: 10.1046/j.1365-3040.2000.00568.

[105]. Pattanagul, W., and M. Thitisaksakul. 2008. Effect of salinity stress on growth and carbohydrate metabolism in three rice (Oryza sativa L.) cultivars differing in salinity tolerance. Indian Journal of Experimental Biology 46 (10): 736.
[106]. Zribi, L., F. Gharbi, F. Rezgui, S. Rejeb, H. Nahdi, and M. N. Rejeb. 2008. Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato ''Solanum lycopersicum (variety Rio Grande)''. Scientia Horticulturae 12.
[107]. Puniran-Hartley, N., J. Hartley, L. Shabala, and S. Shabala. 2014. Salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance: in planta evidence for cross-tolerance. Plant Physiology and Biochemistry 83:32–39.
[108]. Van den Ende, W., and R. Valluru. 2009. Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? Journal of Experimental Botany 60 (1):9–18.
[109]. Couée, I., C. Sulmon, G. Gouesbet, and A. El Amrani. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. Journal of Experimental Botany 57(3):449-59. doi: 10.1093/jxb/erj027.
[110]. Hebers, K., and V. Sonnewald. 1998. Altered gene expression: brought about by inter and pathogen interactions. Journal of Plant Research 111:323–28.
[111]. Stoop, J. M. H., and D.M. Pharr. 1994. Growth substrate and nutrient salt environment alter mannitol–hexoseportioning incelerypetioles. Journal of the American society for horticultural science 119 :237–42.
[112]. Hurry, V. M., A. Strand, M. Tobiaeson, P. Gardestrom, and G. Öquist. 1995. Cold hardening of spring and winter wheat and rape results in differential effects on growth, carbon metabolism and carbohydrate content, Plant Physiology 109: 697–706.
[113]. Yousefi, H., N. Dalir, R. Rahnemaie, and A. Babaei. 2019. The alleviation of salinity-induced stress by using boron in soilless grown rose, Journal of Plant Nutrition 43 (4) :526–37. doi: 10.1080/01904167.2019.1685103.
[114]. Sharma, P. K., and D.O. hall. 1992. Changes in carotenoid composition and photosynthesis in Sorghum under high light and salt stress. Journal of Plant Physiology 140 :661-66.
[115]. Chisari, M., A. Todaro, R.N. Barbagallo, and G. Spagna. 2010. Salinity effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (Lactuca sativa L. cv. Duende). Food Chemistry 119:1502–06.
[116]. Kim, H. J., J. M. Fonseca, J. H. Choi, C. Kubota, and D. Y. Kwon. 2008. Salt in irrigation water affects the nutritional and visual properties of Romaine lettuce (Lactuca sativa L.). Journal of Agricultural and Food Chemistry 56:3772–76.
[117]. Upadhyay, K. K, A. N. Bhatt, A. K. Mishra, B. S. Dwarakanath, S. Jain, C. Schatz, J. F. Le Meins, A. Farooque, G. Chandraiah, A. K Jain, et al. 2010. The intracellular drug delivery and antitumor activity of doxorubicin loaded poly (γ-benzyl l-glutamate)-b-hyaluronan polymersomes. Biomaterials 31 (10): 2882–92.
[118]. Vallverdú-Queralt, A., J. F. R. de Alvarenga, R. Estruch, and R. M. Lamuela-Raventos. 2013. Bioactive compounds present in the Mediterranean sofrito. Food Chemistry 141 (4): 3365–72.
[119]. Caruso, G., V. Stoleru, S. De Pascale, E. Cozzolino, A. Pannico, M. Giordano, G. Teliban, A. Cuciniello, and Y. Rouphael. 2019. Production, leaf quality and antioxidants of perennial wall rocket as affected by crop cycle and mulching type. Agronomy 9: 194.
[120]. Vasantharaja, R., L. Stanley Abraham, D. Inbakandan, R. Thirugnanasambandam, T. Senthilvelan, S. K. Ayesha Jabeen, and P. Prakash. 2019. Influence of seaweed extracts on growth, phytochemical contents and antioxidant capacity of cowpea (Vigna unguiculata L. Walp). Biocatalysis and Agricultural Biotechnology 17:589–94.
[121]. Colla, G., M. Cardarelli, P. Bonini, and Y. Rouphael. 2017. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. HortScience 52: 1214–20.
[122]. Rouphael, Y., L. Spíchal, K. Panzarová, R. Casa, and G. Colla. 2018. High-throughput plant phenotyping for developing novel biostimulants: From lab to field or from field to lab? Frontiers in Plant Science 9 :1197.
[123]. Farmer, E. E., M. J. Mueller. 2013. ROS-mediated lipid peroxidation and RES-activated signaling. Annual Review of Plant Biology 64: 429–50. doi:10.1146/annurev-arplant-050312-120132.
[124]. Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7:405–410.
[125]. Ashraf, M., P. J. C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science 166: 3–16.
[126]. Foyer, C. H., G. Noctor. 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. The Plant Cell 17 (7): 1866–75. doi:10.1105/tpc.105.033589.
[127]. Grattan, S. R., and C. M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. Scientia Horticulturae 78:127–57. doi: 10.1016/s0304-4238(98) 00192-7.
[128]. Munns, R. 2005. Genes and salt tolerance: bringing them together. New Phytologist 167: 645–63. doi: 10.1111/j.1469-8137.2005.01487.
[129]. Boshkovski, B., C. Tzerakis, G. Doupis, A. Zapolska, C. Kalaitzidis, and G. Koubouris. 2020. Relationships of Spectral Reflectance with Plant Tissue Mineral Elements of Common Bean (Phaseolus Vulgaris L.) Under Drought and Salinity Stresses. Communications in Soil Science and Plant Analysis 51 (5):675–86. doi: 10.1080/00103624.2020.172978
[130]. Cramer, G. R., E. Epstein and A. Lauchli. 1989. Na-Ca interactions in barley seedlings: relationship to ion transport and growth. Plant Cell and Environment 12: 551–558.
[131]. Wakeel, A., M. Farooq, M. Qadir, and S. Schubert. 2011. Potassium substitution by sodium in plants. Critical Reviews in Plant Sciences 30:401–13
[132]. Agarwal, S., K. R. Sairam, G. C. Srivastava, T. Aruna, and C. R. Meena. 2005. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Science 169: 559–70.
[133]. Tahir, M. A., T. Aziz, and Rahmatullah. 2011. Silicon-Induced Growth and Yield Enhancement in Two Wheat Genotypes Differing in Salinity Tolerance. Communications in Soil Science and Plant Analysis 42 (4):395–407.doi : : 10.1080/00103624.2011.542219
[134]. Ashraf, M. Y., K. Akhtar, and G. Sarwar. 2005. Role of the rooting system in salt tolerance potential of different guar accessions. Agronomy for Sustainable Development 25:243–49, doi:10.1051/agro:2005019.
[135]. Sarwar, G., M. Ashraf, and M. Naeem. 2003. Genetic variability of some primitive bread wheat varieties to salt tolerance. Pakistan Journal of Botany 35:771–77.
[136]. Shabala, S., L. Shabala, T. Cuin, J. Pang, W. Percey, Z. Chen, S. Conn, C. Eing, and L. Wegner. 2010. Xylem ionic relations and salinity tolerance in barley. Plant Journal 61: 839–53.
[137]. Zhao, Q., B. L. Ma, and C. Z. Ren. 2007. Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. Crop Science 47 :123–31.
[138]. Møller, I. S., M. Gilliham, D. Jha, G. M. Mayo, S. J. Roy, J. C. Coates, J. Haseloff, and M. Tester. 2009. Shoot Na+ exclusion and increased salinity tolerance engineered by cell typespecific alteration of Na+ transport in Arabidopsis. The Plant Cell 21:2163–78. doi: 10.1105/tpc.108.064568.

[139]. Cardarelli, M., Y. Rouphael, E. Rea, and G. Colla. 2010. Mitigation of alkaline stress by arbuscular mycorrhizal in zucchini plants grown under mineral and organic fertilization. Journal of Plant Nutrition and Soil Science 173 :778–87. doi: 10.1002/jpln. 200900378.

[140]. Cartmill, A. D., L. A. Valdez-Aguilar, D. L. Bryan, and A. Alarcón. 2008. Arbuscular mycorrhizal fungi enhance tolerance of vinca to high alkalinity in irrigation water. Scientia Horticulturae 115 :275–84. doi: 10.1016/j.scienta.2007.08.019.