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Nanopotassium, Nanosilicon, and Biochar Applications Improve Potato Salt Tolerance by Modulating Photosynthesis, Water Status, and Biochemical Constituents

Abdel Wahab M. Mahmoud [1], Mahmoud M. Samy [2], Hoda Sany [1], Rasha R. Eid [2], Hassan M. Rashad [1,3] and Emad A. Abdeldaym [4,*,†]

[1] Plant Physiology Division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza 12613, Egypt; mohamed.mahmoud@agr.cu.edu.eg (A.W.M.M.); hodasany@hotmail.com (H.S.); Hali@kau.edu.sa (H.M.R.)
[2] Potato and Vegetatively Propagated Vegetables Department, Horticulture Research Institute, Agriculture Research Center, Giza 12611, Egypt; mazamahmoud@yahoo.com (M.M.S.); rashaeid46@gmail.com (R.R.E.)
[3] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[4] Department of Vegetable, Faculty of Agriculture, Cairo University, Giza 12613, Egypt
* Correspondence: emad.abdeldaym@agr.cu.edu.eg; Tel.: +20-10-1570-0774
† These authors contributed equally to this work.

Abstract: Salinity is one of the main environmental stresses, and it affects potato growth and productivity in arid and semiarid regions by disturbing physiological process, such as the photosynthesis rate, the absorption of essential nutrients and water, plant hormonal functions, and vital metabolic pathways. Few studies are available on the application of combined nanomaterials to mitigate salinity stress on potato plants (*Solanum tuberosum* L. cv. Diamont). In order to assess the effects of the sole or combined application of silicon (Si) and potassium (K) nanoparticles and biochar (Bc) on the agro-physiological properties and biochemical constituents of potato plants grown in saline soil, two open-field experiments were executed on a randomized complete block design (RCBD), with five replicates. The results show that the biochar application and nanoelements (n-K and n-Si) significantly improved the plant heights, the fresh and dry plant biomasses, the numbers of stems/plant, the leaf relative water content, the leaf chlorophyll content, the photosynthetic rate (Pn), the leaf stomatal conductance (Gc), and the tuber yields, compared to the untreated potato plants (CT). Moreover, the nanoelements and biochar improved the content of the endogenous elements of the plant tissues (N, P, K, Mg, Fe, Mn, and B), the leaf proline, and the leaf gibberellic acid (GA3), in addition to reducing the leaf abscisic acid content (ABA), the activity of catalase (CAT), and the peroxidase (POD) and polyphenol oxidase (PPO) in the leaves of salt-stressed potato plants. The combined treatment achieved maximum plant growth parameters, physiological parameters, and nutrient concentrations, and minimum transpiration rates (Tr), leaf abscisic acid content (ABA), and activities of the leaf antioxidant enzymes (CAT, POD, and PPO). Furthermore, the combined treatment also showed the highest tuber yield and tuber quality, including the contents of carbohydrates, proteins, and the endogenous nutrients of the tuber tissues (N, P, and K), and the lowest starch content. Moreover, Pearson's correlation showed that the plant growth and the tuber yields of potato plants significantly and positively correlated with the photosynthesis rate, the internal CO_2 concentration, the relative water content, the proline, the chlorophyll content, and the GA3, and that they were negatively correlated with the leaf Na content, PPO, CAT, ABA, MDA, and Tr. It might be concluded that nanoelement (n-K and n-Si) and biochar applications are a promising method to enhance the plant growth and crop productivity of potato plants grown under salinity conditions.

Keywords: *Solanum tuberosum*; plant growth; tuber quality; photosynthesis; antioxidant enzymes; plant hormones; nanoparticles; soil salinity

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1. Introduction

Potato (*Solanumtuberosum* L.) is considered to be one of the most important vegetable crops belonging to the *Solanaceae* family, and it occupies, globally, the fourth position after rice, wheat, and maize in terms of world food production [1,2]. According to a declaration by the FAO [3], the production of potatoes in Egypt was 5,078,374 tons in 2019, which was harvested from 175,161 hectares. The tubers of potato plants are used for human consumption and livestock nutrition and are considered a source of starch for alcohol fabrication [4]. Potato plants typically face an increased number of abiotic and biotic stress combinations, which seriously affect their growth and production. This might be due to global warming, which is associated with potential climate changes [4–6].

Recently, soil salinity has been one of the major abiotic stresses that causes osmotic stress and obstructs the growth and productivity of most vegetable crops in arid and semiarid regions [5,7,8]. It has been declared that around 20% of irrigated land is affected by salt, which accounts for 30% of the food-producing land [9]. In addition, approximately 50% of all the arable land might be affected by salinity throughout the 21st century [10]. The ratio of salt-affected lands is rising at a rate of 10% annually for different reasons, including the overuse the chemical fertilizers, low precipitation, high surface evaporation, poor agricultural practices, and the use of marginal water in irrigation [11]. This matter has been exacerbated by ongoing trends in global warming and climate change.

Salinity stress negatively influences the morphological, physiological, and biochemical processes of vegetable crops, and, in particular, potato plants [5]. High salt concentrations in soil or/and water not only reduce the growth, biomass, photosynthesis, and water use efficiency of potato plants, but also induce physiological drought and cause ion toxicity, thus decreasing the plant productivity [10,12]. Salinity stress also leads to ionic imbalances, the osmotic effect, and nutrient deficiency, which eventually cause oxidative stress in plants [13,14]. Reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) increase in the plant cells under abnormal environmental conditions [15,16]. Nevertheless, high ROS and RNS generation can cause oxidative damage to the metabolic compounds (e.g., proteins, lipids, nucleic acids) and the plasma membrane of the plant cell [17,18].

Numerous mitigation and amelioration techniques have been utilized to overcome the negative effects of the high salinity of water and soil [19]. Common approaches have been used for the enhancement of the plant tolerance to salinity, such as developing the rootstock of salt-tolerant plants, plant growth-promoting fungi, the foliar/land applications of elements (K, Si, Br, Zn, Mn), and the leaching of salt from the root zone [5,7,20].

However, rare information is available about the role of minerals and their dynamics in salt-tolerant plants [21]. Potassium and silicon are elements with positive effects on the plant performance under saline stress [22–26]. Several studies report that both elements are significantly able to improve plant growth and crop productivity, enhance the photosynthesis rate and water use efficiency, and increase the antioxidant enzymatic activity and plant tolerance to diseases, under adverse environmental conditions [5,27]. In recent years, particular attention has been paid to applying nanotechnologies and plant biotechnology in agriculture [28] in order to increase plant production, enhance plant tolerance to an environmental stress conditions, improve nutrient use efficiency, and mitigate the hazardous environmental effects, compared to traditional methods with bulk materials [29,30]. Therefore, this study aims to investigate the impact of biochar, nanosilicon, and nanopotassium application on the agro-physiological and biochemical traits of potato plants grown in saline soil.

2. Materials and Methods

2.1. Site Location and Experimental Design

The experiment was conducted in the sandy soil of a private farm located at Wadi El-Notron, Beheira Governorate, Egypt (longitude: 2540 E; latitude: 28,200 N; and altitude: 125 m), for two winter seasons (2019/2020 and 2020/2021), in saline soil (the soil salinity was 4.3 dS·m^{-1}). Prior to potato tuber cultivation, a soil sample was collected and analyzed at the Soil, Water and Environment Research Institute (SWERI), at the Agriculture Research Center (A.R.C), according to [31,32], in order to determine the soil physicochemical properties (Table 1). The field experiment was ploughed and subdivided into twenty-five plots (5 m × 10 m). The plots were arranged in a randomized block design with five replicates. The applied materials for different treatments were as follows: (1) Nanopotassium (n-K); (2) Nanosilicon (n-Si); (3) Biochar (Bc); (4) Combined treatment (Bc+ n-K+ n-Si); and (5) Control without addition of nanomaterials (CT). The biochar (Bc) was incorporated into the soil at a rate of 825 kg/ha before tuber cultivation, while the n-K and n-Si were applied throughout the irrigation networks, at rates of 55 and 20 ppm, respectively, at 20, 35, 45, and 60 days after planting.

Table 1. Physicochemical properties of cultivated soil before treatment application.

Particle Size of Soil (%)	Value
Sand	89.6
Silt	7.1
Clay	3.3
Textural class	Sand
Organic matter (%)	0.28
pH	7.8
EC ($dS \cdot m^{-1}$)	4.3
Calcium carbonates (%)	4.14
Soluble Anions ($Cmole \cdot Kg^{-1}$ soil)	
Cl^-	10.8
SO_4^-	12.3
HCO_3^-	18.2
Soluble Cations($Cmole \cdot Kg^{-1}$ soil)	
Mg^{2+}	11.4
Na^+	9.2
K^+	6.1
Available nutrients (ppm)	
N	14.7
P	7.01
K	46.2
Fe	10.60
Mn	1.60
B	0.22

Potato tubers (*Solanum tuberosum* L. cv. Diamont) were kept for 22 days in a ventilated storage room until sprouting. The sprouted tubers were cultivated in October (2019/2020), with 25-cm spaces between tubers, and 75 cm between the rows, and were irrigated by drip irrigation. According to the recommendation of the Ministry of Agriculture and Land Reclamation (MALR, Cairo, Egypt), the potato plants received 660, 325.5, and 352.5 $Kg \cdot ha^{-1}$ of N, P_2O_5, and K_2O, respectively. At the end of the experiment, the potato tubers were harvested, after 100 days, during January (2020/2021), and tuber samples were collected for performing the physicochemical analyses.

2.2. Biochar Preparation

The biochar was derived from rice husk, using the technique described by Hassan et al. [33]. The husk was collected after the harvesting season from El-Sharkya province, Egypt, and was then cut into small fragments (4–5 mm) and pyrolyzed in an oven at 350 °C for 24 h to produce (derive) biochar. The chemical composition of the rice-husk-derived biochar is presented in Table 2. The contents of ash, carbon (C), nitrogen (N), and hydrogen (H) were determined according to Kinney et al. [34]. The pH (in 1:1, *w*:*v*) was measured in a water suspension using a pH meter. The EC value was estimated by an EC meter. The concentrations of Si, Ca, K, Mg, and S were determined by digesting 0.5 g of biochar in concentrated H_2SO_4 (10 mL), using an atomic absorption spectrophotometer with air-acetylene fuel (Pye-Unicam, model SP-1900, Cambridge, UK), according to the method stated by Allen et al. [35]. The C:N ratio after soaking in the ammonium sulfate was calculated. The Zeta potential (ZP) was measured for the biochar by the Zeta-Meter 3.0+ system (Zeta Meter Inc., VA) at the National Research Center (Giza, Egypt).

Table 2. The chemical compositions of prepared rice-husk-derived biochar.

Properties	Rice-Husk-Derived Biochar
Moisture content (%)	3.88
Ash (%)	47.90
pH (1:1, *w*:*v*)	7.65
C (mg)	46.35
H (mg)	2.64
N (mg) (after soaking in ammonium)	3.65
Sulphate (mg)	0.22
Oxygen (mg)	2.74
C:H	0.05
C:N (after soaking in ammonium sulphate)	12.92
EC ($dS \cdot m^{-1}$)	0.14
Si ($mg \cdot kg^{-1}$)	179
Ca ($mg \cdot kg^{-1}$)	213
K ($mg \cdot kg^{-1}$)	199
Mg ($mg \cdot kg^{-1}$)	179
Zeta potential (mV)	−26.6

2.3. Synthesis of Potassium and Silicon Nanoparticles

The potassium and silicon nanoparticles were prepared and synthesized from their precursors. Silicon was prepared from silicon tetrachloride ($SiCl_4$), and potassium from potassium persulfate ($K_2S_2O_8$). All of the used precursors ($SiCl_4$ and $K_2S_2O_8$) and reagents were purchased from Sigma Chemical Co. (Saint-Louis, MO, USA).

Potassium nanoparticles (Figure 1A) were prepared according to Dong et al. [36], with modifications made by polymerizing methacrylic acid in a chitosan solution as a carrier, coated in a buffer solution for 18 h at room temperature, in two-step processes. In the first step, 0.23 g of chitosan was dissolved in a methacrylic acid aqueous solution (0.5%, v/v) for 5 h under magnetic stirring. In the second step, with continued stirring, 0.2 mmol of potassium persulfate $K_2S_2O_8$, and 0.4 mmol of potassium sulphate $K_2S_2O_4$, were added to the solution until the solution became clear. The polymerization was subsequently carried out at 75 °C under magnetic stirring for 7 h, which leads to the formation of a nanoparticle solution that was subsequently exposed to 1.5 psi of pressure for 3 days, discontinuously, for 7 h per day, and was then centrifuged at 500 rpm for 30 min, and was thereafter cooled in an ice bath for two hours.

The nanosilicon (Figure 1B) was synthesized using the method described by Zhu and Gong [37] and published elsewhere [5]. The morphologies and sizes of the nanoparticles (n-K and n-Si) and biochar were investigated using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Tokyo, Japan). One drop of the nanoparticle solution was spread onto a carbon-coated copper grid and was subsequently dried at room temperature for transmission electron microscopy (TEM) analysis. The sizes of the nanoparticles were determined according to a computer program.

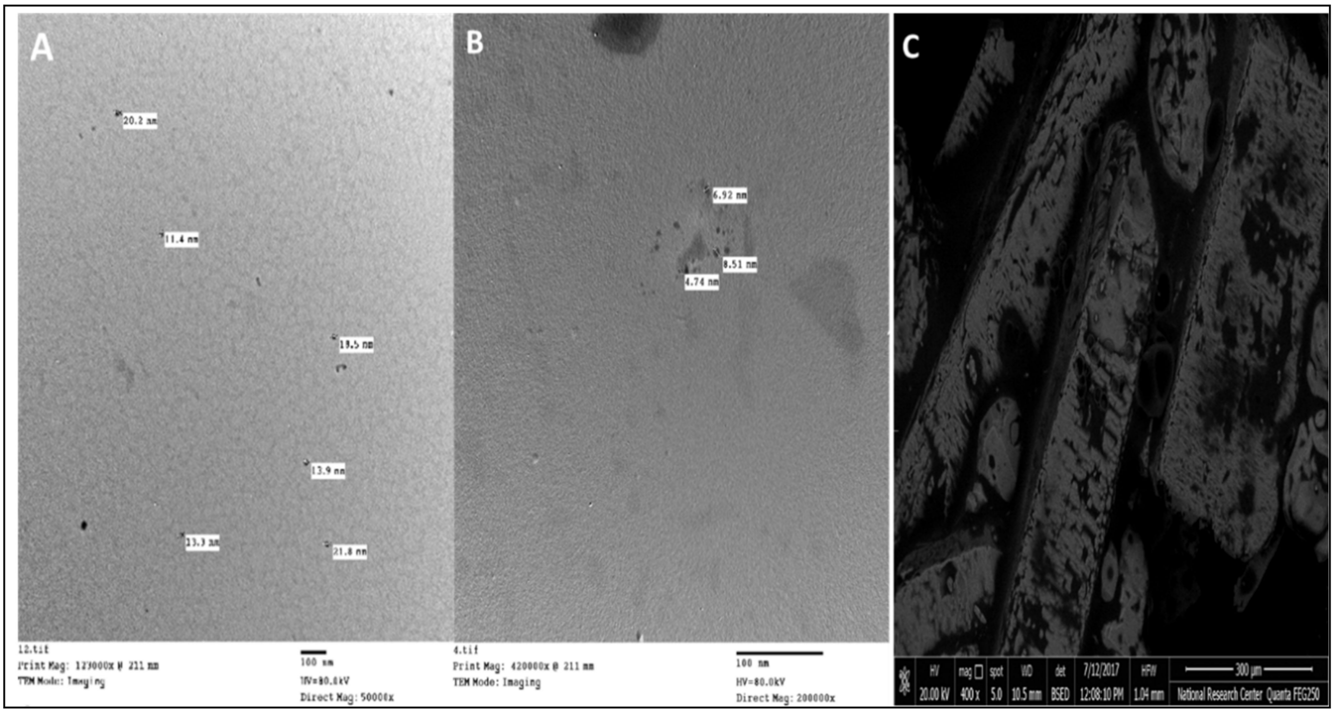

Figure 1. Scanning electron microscopy (SEM) for synthesized (**A**) potassium, and (**B**) silicon nanoparticles, and (**C**) biochar.

2.4. Data Recorded

2.4.1. Morphological Characteristics and Yield Components

Twenty plants from each treatment were selected randomly to determine the plant heights, the shoot fresh weights (FWs), the shoot dry weights (DWs), the relative water contents (RWCs), the total leaf areas, and the numbers of stems per plant. The total tuber yields of each treatment and the tuber numbers per plant, as well as the average tuber lengths, diameters, and weights, were also recorded.

2.4.2. Leaf Chlorophyll Content and Photosynthetic Parameters

The total chlorophyll content in the fresh leaves of potato plants was determined using the method stated by Lichtenthaler and Buschmann [38]. Briefly, 0.5 g of leaf samples were milled in 80% acetone (Sigma-Aldrich Co. LLC, Saint Louis, MO, USA) and were then centrifuged at 1100× *g* for 8 min at 4 °C, and the supernatant part was analyzed using a spectrophotometer (Helios UVG1702E, Cambridge, UK). The values of the total chlorophyll were expressed in mg·g^{-1} FW.

With regard to the photosynthetic parameters, the fourth leaves of fifteen plants were selected from each treatment to measure the photosynthesis (Pn), on an area basis, as well as the leaf stomatal conductance (Gc), the intercellular CO_2 concentration (int. CO_2 conc.), and the water use efficiency (WUE), using an infrared gas analyzer, the LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA). These measurements were performed on the fifth leaves of selected potato plants from 9 AM to 1:00 PM, under a light intensity of about 1300 µmol m^{-2} s^{-1}, with a relative humidity of 70%. The temperature of the leaf chamber varied from 26.2 to 28.9 °C, and the leaf chamber volume gas flow rate was 400 mL min^{-1}. The ambient CO_2 concentration was 398 µmols·mol^{-1}.

2.4.3. Activity of Antioxidant Enzymes

The leaf samples used for determining the activity of catalase (CAT) and peroxidase (POD) were prepared according to [39]. Briefly, 500 mg of the fourth fresh leaves of potato plants were ground in liquid nitrogen and homogenized in a mixture consisting of 5 mL of potassium phosphate buffer, 0.5% Triton X-100, 2% N-Vinylpyrrolidinone, 5 mM of ethylene diaminetraacetic acid disodium salt dehydrate, and 1mM of ascorbic acid. The homogenized mixture was centrifuged at 1000× g for 25 min at 4 °C, and the supernatants were used to measure the activity of the catalase by Aebi [40], and peroxidase by Nakano and Asada [41]. The values of the CAT and POD were defined as units.mg^{-1}.

The activity of the polyphenol oxidase (PPO) was assessed by the spectrophotometric method (Unico, UV2000, Rochester, NY, USA), using Catechol reagent (Qualikems, Gujarat, India) as a substrate. The absorbance was recorded at a wavelength of 495 nm, using a microplate reader (Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland). The enzyme activity was calculated and expressed as unit mg^{-1}.

2.4.4. Leaf Proline, Gibberellic Acid, and Abscisic Acid Content

The free proline content was quantified using the method defined by Bates et al. [42]. Approx. 500 mg of freeze-dried samples were homogenized in 5 mL of 3% (w/v) sulphosalicylic acid. The homogenate was filtered through filter paper (Whatman, No.1). The filtrate was mixed with a ninhydrin acid reagent (2% v/v) and acetic acid. Then the mixture was placed in a boiling water bath for 45 min at 100 °C for one hour. The mixture was then placed in a boiling water bath for 45 min at 100 °C for one hour. Then, 4 mL of toluene was added and was kept in the tubes for 20 s. The reaction was stopped by placing the tubes in crushed ice. The free proline was determined spectrophotometrically at a 520-nm wave against the reagent blank. The content of gibberellic acid (GA3) and abscisic acid (ABA) in the potato leaves was assessed using the method reported by Fales et al. [43]. Freeze-dried samples were homogenized and then mixed with 15 mL of the mixture containing methanol (80% v/v) and butylated hydroxytoluene. Additional details of the extraction and the quantification of GA_3 and ABA are stated elsewhere [5].

2.4.5. Leaf Malondialdehyde Content

A total of 50 mg of freeze-dried samples were powdered in 0.5 mL of 0.1% (w/v) trichloroacetic acid. The resulting mixture was filtered and incubated with 0.5% (v/v) thiobarbituric acid (TBA) in 20% trichloroacetic acid at 95 °C. The absorbance was measured spectrophotometrically at 440-, 532-, and 600-nm waves, using a microplate reader (Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland). The results of the malondialdehyde (MDA) content were reported as units.mg^{-1} [44].

2.4.6. Nutrient Content in Plant and Tuber Tissues

The plant and tuber samples were dried in an air-forced oven at 75 ± 2 °C for 2 days, and then the dried materials were finely powdered for the determination of endogenous nutrients. A 0.2-g dried sample (plant and tuber) was digested by adding sulfuric acid (5 mL) and perchloric acids. The resulting mixture was heated for 10 min. Then, 0.5 mL of perchloric acid was added, and heating continued till a clear solution was obtained [45,46]. The total nitrogen content (N) of the dried samples was quantified using the modified micro-Kjeldahl method, as described by AOAC [45].

The total protein content in the potato tuber was calculated by multiplying the values of the tuber nitrogen content in a conversion factor (6.25), according to [47]. The phosphorus (P) content was measured colorimetrically using the chlorostannous molybdophosphoric blue color method in sulfuric acid, according to [45]. The potassium (K), magnesium (Mg), and sodium (Na) concentrations were assessed using the flame photometer apparatus (CORNINGM410, Halstead, UK). The contents of iron (Fe), zinc (Zn), and boron (B) were measured by an atomic absorption spectrophotometer with air-acetylene fuel (PyeUnicam, model SP-1900, Cambridge, UK).

2.4.7. Tuber Starch and Carbohydrate Content

The tuber carbohydrate content was assessed using the phosphomolybdic acid method, as stated by AOAC [45]. The starch content in the potato tuber was estimated in the tuber's dry matter using the method described by AOAC [45], with few modifications.

Approximately 0.8 g of powdered samples of tubers were placed into tubes, and then 0.2 mL of 80% ethanol was added to the samples to aid dispersion, and then 3 mL of thermostable amylase was added (pH 7.0) and incubated in a boiling water bath for 6 min at 95 °C to ensure complete homogeneity. The tube was then placed in a bath at 50 °C, and 4 mL of 200 mM sodium acetate (pH 4.5) was added. The resulting mixture was centrifuged at 13,000 rpm for 4 min, and a 0.1-mL aliquot of each sample was transferred to the test tube and incubated at 50 °C for 20 min in the darkness. At the end, the concentration of starch was read spectrophotometrically, at a 630-nm wave against the reagent blank.

2.5. Statistical Analysis

The experimental design was a randomized complete block design (RCBD) for five treatments, and each treatment was replicated five times. One-way variance analysis (ANOVA) and means were compared by the Tukey test ($p < 0.05$), using the Statistica 7 program (version 2004). The principal component analysis and the Pearson's correlation between the plant growth traits and the physiochemical properties of treated potato tubers were performed using the XLSTAT program (Version 2014).

3. Results

3.1. Morphological Traits and Relative Water Content

The morphological parameters of the plants, including the plant height, the fresh and dry biomass production, and the leaf area are considered the simplest and most visible phenotypical parameters that respond clearly to nanofertilizers and environmental stresses [5,27]. The morphological characteristics of potato plants affected by application treatments (Bc, n-K, and n-Si) during both seasons are shown in Figure 2. Significant differences were observed among all of the treatments in the plant height, the shoot fresh weight (FW) and dry weight (DW), the relative water content (RWC), the total leaf area, and the number of stems per plant. The highest values of the plant growth parameters were recorded in potato plants treated with a combined treatment (Bc+n-K+n-Si), while the lowest values were noticed in untreated plants. The combination between nanoparticles significantly increased the plant height, the shoot fresh weight (FW) and dry weight, the relative water content, the total leaf area, and the number of stems per plant by 34.90, 41.48, 45.23, 28.44, 45.52, and 50%, respectively, compared to the control at the first season. The corresponding values were 28.92, 25.58, 38.44, 23.11, 33.55, and 60%, respectively, in the second year. The morphological traits of potato plants, as they were affected by the soil salinity and the application treatments (Bc, n-K, and n-Si) during both seasons, are shown in Figure 2. Significant differences were observed among all treatments in the plant height, the shoot fresh weight (FW) and dry weight (DW), the relative water content (RWC), the total leaf area, and the number of stems per plant. The highest values of the plant growth parameters were recorded in potato plants treated with a combined treatment (Bc+n-K+n-Si), while the lowest values were noticed in untreated plants. The combination between nanoparticles significantly increased the plant height, the shoot fresh weight (FW) and dry weight, the relative water content, the total leaf area, and the number of stems per plant by 34.90, 41.48, 45.23, 28.44, 45.52, and 50%, respectively, compared to the control at the first season. The corresponding values were 28.92, 25.58, 38.44, 23.11, 33.55, and 60%, respectively, in the second year.

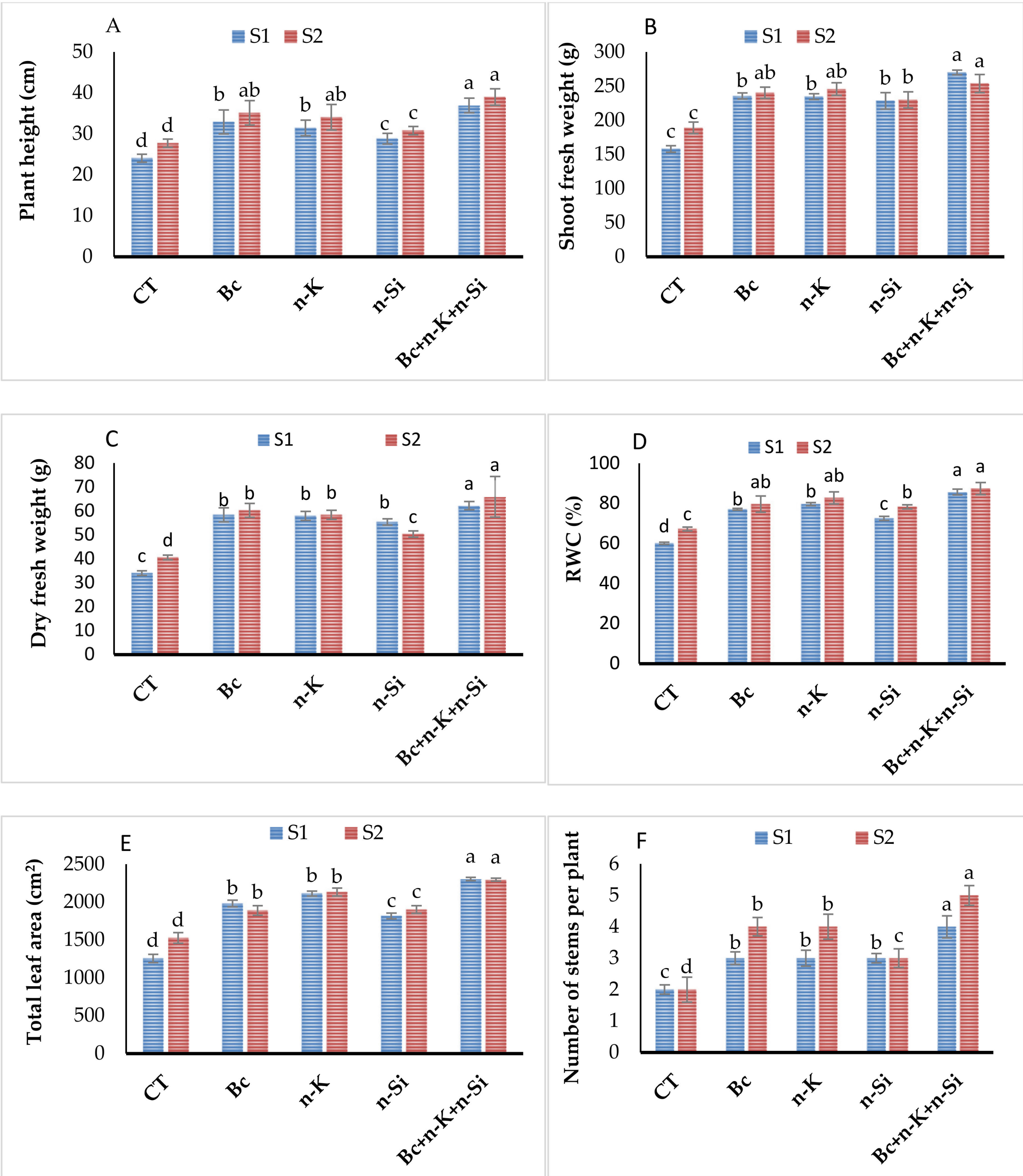

Figure 2. Effect of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on (**A**) the plant height; (**B**) the shoot fresh weight; (**C**) the shoot dry weight; (**D**) the relative water content (RWC); (**E**) the total leaf area, and (**F**) the number of stems of potato plants grown under salinity conditions. Bars followed by the same letter are not significantly different ($p < 0.05$). Error bars indicate standard error of the means (SE). S1: first season; S2: second season.

3.2. Leaf Chlorophyll and Photosynthetic Gas Exchange Parameters

The leaf chlorophyll content and the photosynthesis machinery play positive roles in plant growth and development through the increase in the carbohydrate accumulation in different plant tissues. Moreover, these parameters are more sensitive to supplementary nanofertilizers, biostimulants, and salinity stress [5,10,14,20,27]. Figure 3 shows the changes in the photosynthetic gas exchange parameters during both seasons. The results reveal that the exogenous application of the biochar (Bc) and the nanoparticle treatments (n-K and n-Si) significantly improved the leaf chlorophyll content, the photosynthesis rate (Pn), the stomatal conductance (Gc), the intercellular CO_2 concentration (Int.CO_2conc.), and the water-use efficiency (WUE) of potato plants grown in saline soils, while the transpiration rate (Tr) declined. The highest values of the photosynthetic parameters, except for the transpiration rate, were recorded in potato plants treated with a combined treatment (Bc+n-K+n-Si), followed by the single application of each material, while the minimum values were found in the untreated plants (CT), during both seasons.

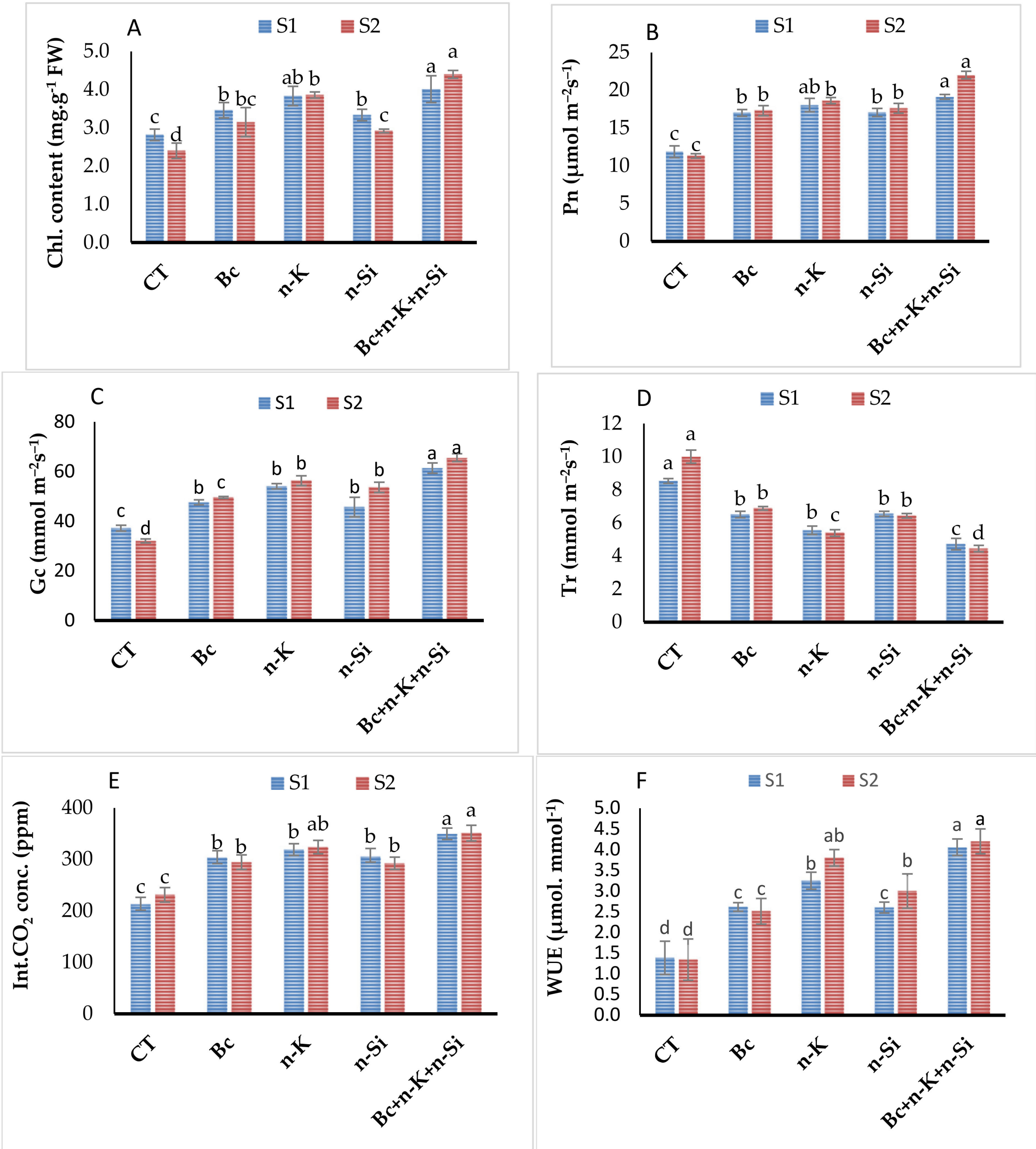

Figure 3. Effect of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on (A) the leaf chlorophyll content (Chl.); (**B**) photosynthesis rate (Pn); (**C**) stomatal conductance (Gc); (**D**) the transpiration rate (Tr); (**E**) the intercellular CO_2 concentration (Int.CO_2conc.); and (**F**) the water use efficiency (WUE) of potato plants grown under salinity conditions. Bars followed by the same letter are not significantly different ($p < 0.05$). Error bars indicate standard error of the means (SE). S1: first season; S2: second season.

3.3. Activity of Antioxidant Enzymes in Potato Leaves (CAT, POD, and PPO)

Antioxidant enzymes play an important role in scavenging reactive oxygen forms (ROS) that are generated from environmental stresses and that would lead to increased rates of cell death, thus hindering plant growth and decreasing crop productivity [8,14–18]. The data presented in Figure 4 display that the activities of antioxidant enzymes [catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO)] in potato leaves were significantly affected by the nanoparticle application and the soil salinity ($p < 0.05$). All of the nanoparticle treatments considerably reduced the activity of CAT, POD, and PPO in the leaves of salt-stressed potato plants. Compared to the control (CT), the lowest values of the antioxidant enzymes (CAT, POD, and PPO) were found in potato plants treated with a combined treatment, followed by potato plants treated with a single application of each treatment, while the highest values of the antioxidant enzymes were recorded in the leaves of untreated potato plants. This result indicates that biochar and nanoparticle (n-K and n-Si) treatments were effective in regulating the activity of the catalase, peroxidase, and polyphenol oxidase of salt-stressed potato plants.

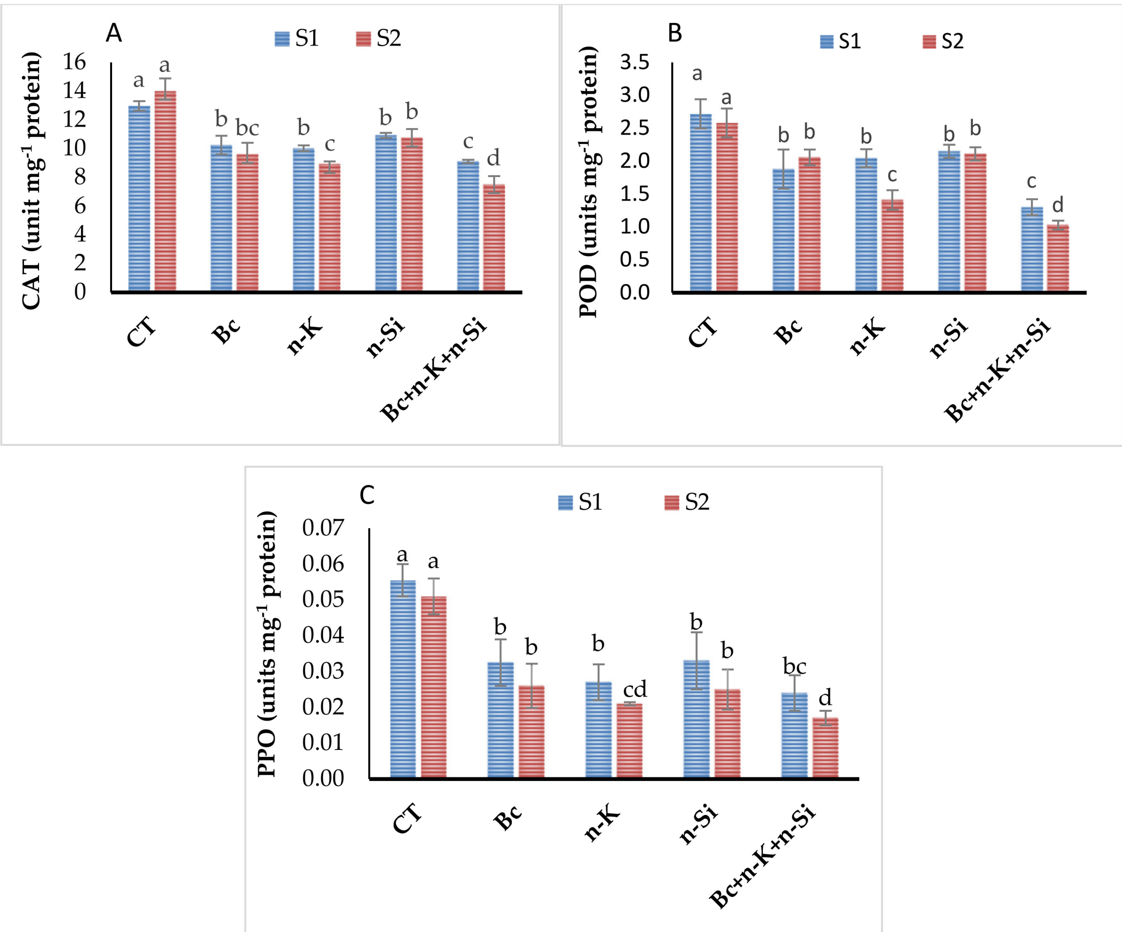

Figure 4. Effect of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on activity of (**A**) catalase (CAT); (**B**) peroxidase (POD); and (**C**) polyphenol oxidase (PPO) in leaves of potato plants grown under salinity conditions. Bars followed by the same letter are not significantly different ($p < 0.05$). Error bars indicate standard error of the means (SE). S1: first season; S2: second season.

3.4. Free Proline, Lipid Peroxidation, and Plant Hormone Contents

Environmental stresses and nanomaterial application cause significant shifts in the production of proline, lipid peroxidation (MDA), and phytohormones, which lead to large modifications in the plant performance and crop production [5,14,22,26,27,38,41]. In the current study, changes in the content of free proline, gibberellic acid (GA3), the lipid peroxidation (MDA) level, and the abscisic acid (ABA) of potato leaves as a result of the applied tested materials are shown in Figure 5. In both seasons, the single and combined application of n-Si, n-K, and biochar significantly increased the content of free proline and gibberellic acid (GA3). The maximum free proline and GA3 contents were found in the combined treatment, and the minimum values were observed in untreated salt-stressed plants.

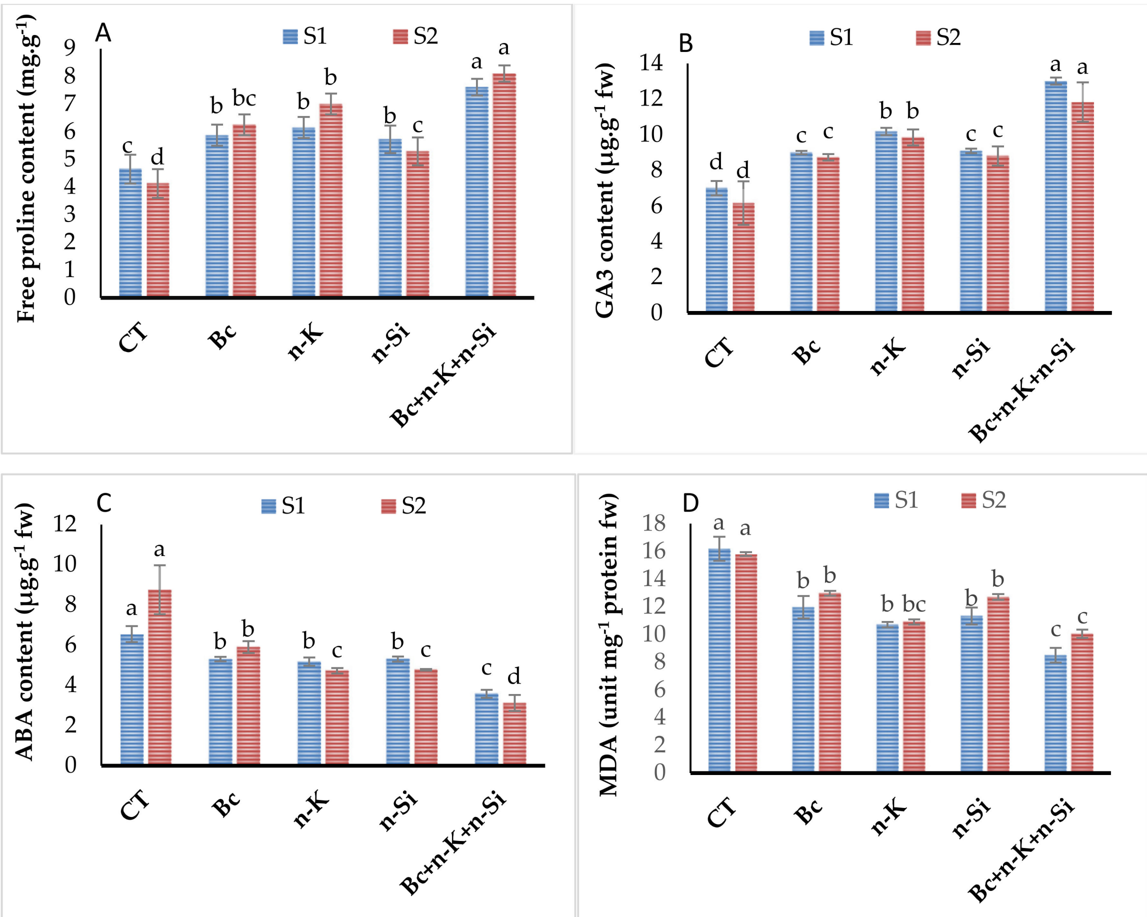

Figure 5. Effect of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on concentrations of (**A**) free proline; (**B**) gibberellic acid (GA3); (**C**) abscisic acid (ABA); and (**D**) lipid peroxidation (MDA) in the leaves of potato plants grown under salinity conditions. Bars followed by the same letter are not significantly different ($p < 0.05$). Error bars indicate standard error of the means (SE). S1: first season; S2: second season.

On the other hand, the exogenous application of biochar and nanoparticles (n-K and n-Si) greatly reduced the content of MDA and ABA in salt-stressed potato plants. The highest values of MDA and ABA were recorded in the leaves of untreated salt-stressed potato plants (CT), while the lowest values were observed in plants treated with the combined treatment (Bc+n-K+n-Si).

3.5. Endogenous Nutrient Content

One of the major outcomes of salinity is the adverse effect on the absorption, transport, and/or use of several essential nutrients [5,12,26]. The obtained results show that the application of biochar and nanotreatments (n-Si and n-K) increased the accumulation of endogenous nutrients (N, P, K, Mg, Fe, Mn, and Br) and reduced the Na accumulation in the leaf tissues of potato plants (Table 3). The combined treatment realized the best results in increasing the accumulation of N, P, K, Mg, Fe, Mn, and Br, in addition to reducing the Na accumulation in leaf tissues compared to the other treatments. By contrast, the highest concentration of Na and the lowest concentrations of N, P, K, Mg, Fe, Mn, and Br were recorded in the leaf tissue of untreated salt-stressed plants, compared to all the other treatments.

3.6. Tuber Yield and Quality

Nanoelement application induces changes in the photosynthesis machinery, the phytohormone biosynthesis, and the metabolic compound accumulation for potato plants grown under salinity conditions. These physiological changes affect the chemical compositions, diameters, sizes, weights, and numbers of potato tubers [5,17,26,28,30].

As shown in (Table 4), the application of n-K, n-Si, and biochar led to significant enhancements in the potato quantity and quality during both seasons. The single and combined application of n-Si, n-K, and biochar caused significant improvements in the numbers and weights of the tubers, as well as the total tuber yield. The highest numbers, weights, and total yields of potato tubers were achieved by the combined treatment (Bc+n-K+n-Si), compared to the other treatments. These increases in the tuber yield and its components were significant compared to the salt-stressed untreated plants, indicating the positive role of Si, K, and biochar nanoparticles. On the contrary, the minimum numbers of tubers, total tuber yields (t.h-1), and tuber weights (g) were recorded in control plants, compared to the treated plants with applied nanoparticles (n-Si and n-K) and biochar.

The physical qualities of the potato tubers, including the tuber hardnesses, diameters, and lengths showed a similar trend. In both seasons, the maximum tuber hardnesses, diameters, and lengths were recorded in plants treated with a combined treatment (Bc+n-K+n-Si), followed by plants treated with the single application of each treatment (n-Si, n-K, Bc), while the minimum values were observed in the control treatment (CT).

The chemical properties of potato tubers were significantly affected by the supplementary applications of biochar and the nanoparticles of K and Si (Table 5). The maximum contents of carbohydrates, proteins, and N, P, and k were recorded in the combined treatments, followed by Bc, n-K, and n-Si, compared to the other treatments (Table 5). On the contrary, the highest starch contents in the potato tubers were observed in the untreated plants (CT), while the lowest were found in the combined treatment (Bc+n-K+ n-Si), during both seasons.

Table 3. Impact of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on the leaf endogenous nutrient content of potato plants grown under salinity conditions.

Treatments	CT		Bc		n-K		n-Si		Bc+n-K+n-Si	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
N (%)	2.04 ± 0.14 d	1.75 ± 0.21 d	2.88 ± 0.18 b	2.73 ± 0.41 b	2.89 ± 0.23 b	3.04 ± 0.35 ab	2.50 ± 0.21 c	2.32 ± 0.17 c	3.34 ± 0.25 a	3.41 ± 0.95 a
P (%)	0.15 ± 0.02 d	0.19 ± 0.02 d	0.23 ± 0.011 c	0.26 ± 0.009 c	0.25 ± 0.01 b	0.28 ± 0.01 b	0.24 ± 0.003 cb	0.27 ± 0.001 b	0.32 ± 0.011 a	0.36 ± 0.011 a
K (%)	3.42 ± 0.27 d	3.32 ± 0.32 d	4.56 ± 0.18 c	5.10 ± 0.44 c	5.37 ± 0.18 b	5.68 ± 0.62 b	4.60 ± 0.28 c	5.16 ± 0.21 c	6.08 ± 0.21 a	6.45 ± 0.52 a
Na (%)	5.92 ± 0.9 a	6.11 ± 1.21 a	4.28 ± 0.42 c	4.58 ± 0.56 b	4.15 ± 0.9 cd	4.38 ± 0.89 c	4.40 ± 0.31 b	4.78 ± 0.27 b	3.21 ± 0.51 d	3.87 ± 0.76 d
Mg (%)	0.41 ± 0.03 d	0.40 ± 0.074 e	0.76 ± 0.86 b	0.63 ± 0.23 c	0.75 ± 0.05 b	0.80 ± 0.08 ab	0.59 ± 0.05 d	0.63 ± 0.021 c	0.88 ± 0.25 a	0.83 ± 0.13 a
Fe (ppm)	30.2 ± 1.1 d	29.50 ± 2.3d	67.80 ± 1.34 b	69.20 ± 1.91 b	66.30 ± 1.9 b	70.60 ± 2.1 b	60.70 ± 3.01 c	59.10 ± 1.55 c	88.50 ± 3.43 a	90.2 ± 4.17 a
Mn (ppm)	42.60 ± 2.30 d	50.10 ± 1.25 e	89.60 ± 3.14 b	95.50 ± 3.23 b	90.80 ± 2.9 b	86.50 ± 3.56 c	78.40 ± 1.26 c	74.60 ± 1.95 d	109.60 ± 4.32 a	100.7 ± 4.2 a
B (ppm)	17.60 ± 0.95 e	19.50 ± 1.32 e	22.50 ± 2.05 d	25.70 ± 2.45 d	31.20 ± 1.6 b	35.40 ± 1.85 b	26.96 ± 2.01 c	28.10 ± 2.05 c	37.30 ± 2.45 a	41.2 ± 2.09 a

Means within each line followed by the same letter are not significantly different ($p < 0.05$). ±values indicate standard error of the means (SE). S1: first season; S2: second season.

Table 4. Effects of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on tuber yields, their components, and the tuber physical properties of potato plants grown under salinity conditions.

Treatments	CT		Bc		n-K		n-Si		Bc+n-K+n-Si	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Total Yield ($t.ha^{-1}$)	22.3 ± 2.1c	25.75 ± 2.3 e	32.4 ± 3.21 b	34.75 ± 2.09 d	33.5 ± 1.9 b	40.75 ± 3.4 c	32.8 ± 1.44 b	39.25 ± 3.15 b	39.1 ± 0.98 a	44.5 ± 2.77 a
No. tuber	11.2 ± 0.4 c	10.1 ± 1.55 c	15.1 ± 1.61 a	16.21 ± 2.2a b	16.6 ± 1.04 a	15.5 ± 0.2 ab	15.41 ± 1.5 ab	15.52 ± 2.4 a	16.4 ± 1.24 a	17.09 ± 1.08 a
Tuber weight (g)	97.3 ± 4.6 c	101.7 ± 6.3 c	136.3 ± 6.7 b	135.68 ± 8.5b	135.92 ± 4.7 b	136.8 ± 3.8 b	131.83 ± 4.6 b	135.2 ± 4.7 b	139.6 ± 4.01 a	141.05 ± 6.1 a
Tubers Hardness ($kg.m^2$)	11 ± 1.24 b	12.1 ± 2.01c	14.9 ± 2.1ab	15.01 ± 2.8ab	14.97 ± 0.88 a	15.05 ± 1.8 a	13.67 ± 1.43 b	13.81 ± 1.7 b	16.91 ± 1.67 a	17.08 ± 1.5 a
Tuber diameter (cm)	3.5 ± 0.77 c	3.12 ± 0.32 c	5.3 ± 0.3 ab	5.54 ± 0.47 ab	5.34 ± 0.86 ab	5.51 ± 1.02 ab	4.28 ± 0.85 b	5.20 ± 0.9 ab	5.96 ± 0.74 a	6.21 ± 0.59 a
Tuber length (cm)	6.1 ± 1.62 c	6.56 ± 0.89 c	7.3 ± 1.39 b	8.07 ± 1.98 ab	7.89 ± 0.99 ab	8.01 ± 1.15 ab	7.11 ± 0.65 b	7.17 ± 1.77 b	8.65 ± 1.86 a	8.77 ± 1.32 a

Means within each line followed by the same letter are not significantly different ($p < 0.05$). ±values indicate standard error of the means (SE). S1: first season; S2: second season.

Table 5. Impact of biochar (Bc), nanopotassium (n-K), and nanosilicon (n-Si) on tuber chemical compositions of potato plants grown in saline soil.

Treatments	CT		Bc		n-K		n-Si		Bc+n-K+n-Si	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Carbohydrates (%)	66.11 ± 1.15 c	70.14 ± 2.9 c	74.13 ± 1.9 ab	75.37 ± 1.3 b	76.16 ± 1.8 ab	74.22 ± 2.1 b	73.10 ± 1.06 b	75.35 ± 3.1 ab	77.58 ± 2.02 a	79.50 ± 2.5 a
Starch content (%)	78.5 ± 2.11 a	80.4 ± 3.01 a	70.5 ± 2.3 b	69.7 ± 1.98 c	63.8 ± 0.78 c	65.9 ± 1.76 d	68.8 ± 2.85 cb	74.5 ± 2.11 b	57.4 ± 1.02 d	55.7 ± 1.23 e
Protein content (%)	6.31 ± 1.62 d	6.25 ± 1.82 c	7.50 ± 1.02 c	7.69 ± 2.91 b	7.81 ± 1.90 ab	7.87 ± 2.83 b	7.44 ± 1.85 c	7.75 ± 1.41 b	8.11 ± 1.55 a	8.35 ± 1.45 a
N (%)	1.01 ± 0.83 d	1.0 ± 0.90 c	1.2 ± 0.16 c	1.20 ± 0.18 b	1.25 ± 0.31 ab	1.26 ± 0.45 b	1.19 ± 0.30 c	1.24 ± 0.13 b	1.29 ± 0.25 a	1.4 ± 0.23 a
P (%)	0.14 ± 0.005 c	0.12 ± 0.01 c	0.17 ± 0.02 b	0.19 ± 0.006 ab	0.19 ± 0.005 ab	0.20 ± 0.01 ab	0.17 ± 0.003 b	0.18 ± 0.005 b	0.23 ± 0.007a	0.22 ± 0.01 a
K (%)	1.88 ± 0.26 d	1.95 ± 0.16 d	2.16 ± 0.10 c	2.18 ± 0.18 c	2.59 ± 0.92 b	2.71 ± 0.24 ab	2.15 ± 0.32 c	2.17 ± 0.17 c	2.8 ± 0.41 a	2.93 ± 0.38 a

Means within each line followed by the same letter are not significantly different ($p < 0.05$). ± values indicate standard error of the means (SE). S1: first season; S2: second season.

3.7. Correlation Study

Principal component analysis (PAC, Figure 6) and Pearson's correlation analysis (Table 6) were used to determine the relationships between the observed variables and a new set of nonassociated variables (parameters). Both correlations showed the changes in the agro-physiological and biochemical properties of salt-stressed potato plants. Considering the differences in the agro-physiological and biochemical properties, and the tuber quantity and quality indices of salt-stressed potato plants treated with biochar and applied nanoparticles (n-K and n-Si), 25 indexes of salt-stressed potato plants were assimilated using two-dimensional principal component analysis (PCA) (including Factor 1 (F1) and Factor 2 (F2)) with the XLSTAT software (2014). PCA was additionally used to integrate and analyze the results of the agro-physiological and biochemical properties, as well as the tuber quality indicators of salt-stressed potato plants. The principal components (F1 and F2) provided 96.85% of the total variance of the dataset. The contribution rate of F1 and F2 was 90.67% and 6.18% of the variance in the dataset, respectively. F1 had strong positive loading for the agronomical traits (plant length, dry and fresh shoots, total leaf area, stem number, tuber weight and number, and yield); physiological attributes (Chl., Pn, Gc, Inter.CO_2conc., WUE, and RWC); some biochemical components (free proline, GA3, and tuber carbohydrate contents), and a strong negative loading for the physicochemical parameters (CAT, ABA, PPO, leaf Na content, and Tr). F2 had high positive loading for the POD, MDA, and tuber starch contents.

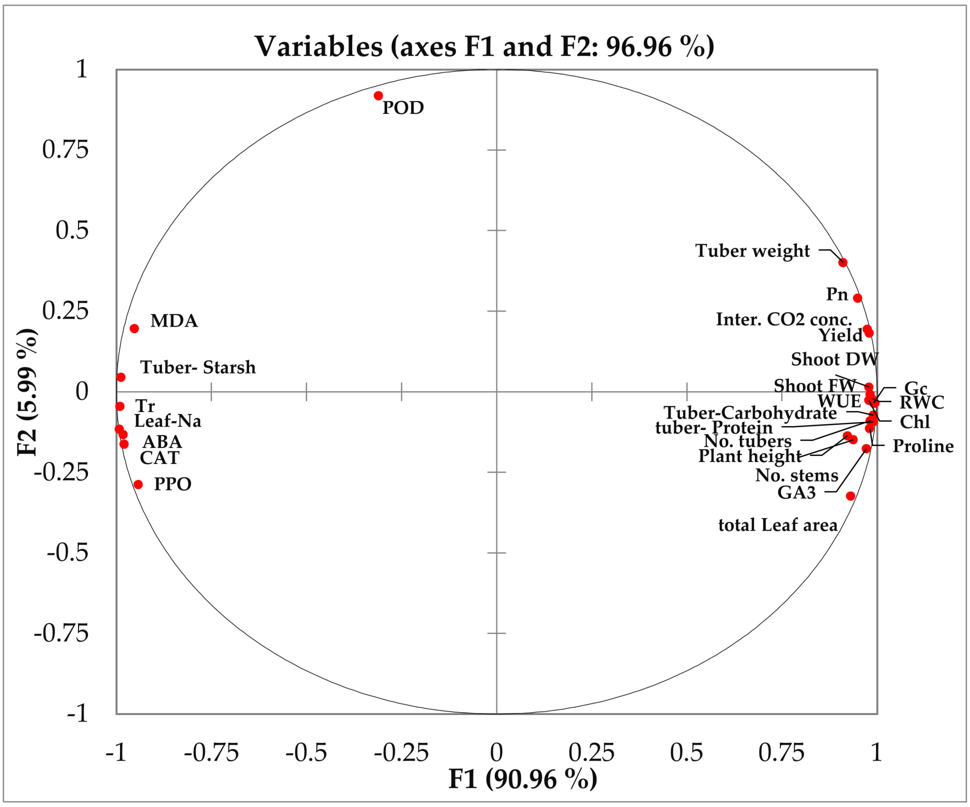

Figure 6. Principal component analysis of agro-physiological and biochemical properties of potato plants grown under salinity and treated with biochar, nanopotassium, and nanosilicon.

Table 6. Pearson's correlation analysis between the agro-physiological and biochemical properties of potato plants grown under salinity and treated with biochar, nanopotassium, and nanosilicon.

Variables	Plant Height	Shoot FW	Shoot DW	RWC	Leaf Area	No. Stems	Leaf-Na	Pn	Gc	Inter. CO_2	Tr	WUE	Chl	Proline	GA3	ABA	POD	PPO	CAT	MDA	Yield	No. Tubers	Tuber Weight	Carboh.	Starch	Protein
Plant height	**1**																									
Shoot FW	**0.93**	**1**																								
Shoot DW	**0.87**	**0.91**	**1**																							
RWC	**0.90**	**0.89**	**0.80**	**1**																						
Leaf area (tot.)	**0.93**	**0.93**	**0.90**	**0.95**	**1**																					
No. stems	0.02	**0.91**	**0.91**	**0.91**	**0.92**	**1**																				
leaf-Na	**−0.91**	**−0.85**	**−0.90**	**−0.79**	**−0.88**	**−0.92**	**1**																			
Pn	0.40	**0.90**	**0.83**	**0.94**	0.49	0.29	**−0.93**	**1**																		
Gc	0.36	**0.93**	**0.81**	**0.86**	**0.91**	**0.91**	**−0.87**	**0.94**	**1**																	
Inter. CO_2	0.11	**0.88**	**0.94**	**0.70**	0.32	0.30	−0.45	**0.86**	**0.91**	**1**																
Tr	−0.33	**−0.95**	**−0.95**	**−0.81**	**−0.86**	**−0.88**	**0.88**	**−0.96**	**−0.59**	**−0.87**	**1**															
WUE	**0.89**	**0.88**	**0.92**	**0.97**	**0.87**	**0.92**	**−0.92**	**0.73**	**0.88**	**0.90**	**−0.83**	**1**														
Chl	0.87	**0.93**	**0.85**	**0.95**	**0.73**	0.28	−0.39	**0.89**	**0.75**	**0.96**	**−0.80**	**0.98**	**1**													
Proline	**0.91**	**0.85**	**0.90**	**0.90**	**0.83**	**0.89**	**−0.97**	**0.88**	**0.64**	**0.93**	**−0.95**	**0.97**	**0.93**	**1**												
GA3	**0.91**	**0.82**	**0.94**	**0.94**	**0.93**	**0.95**	**−0.82**	0.17	**0.92**	**0.91**	**−0.94**	**0.94**	**0.91**	**0.83**	**1**											
ABA	**−0.89**	**−0.97**	**−0.96**	**−0.95**	−0.21	**−0.90**	**0.84**	**−0.96**	**−0.93**	**−0.77**	**0.97**	**−0.89**	**−0.88**	**−0.74**	**−0.90**	**1**										
POD	−0.34	-0.27	-0.26	−0.34	−0.11	−0.35	0.19	−0.06	-0.36	-0.15	0.30	−0.36	−0.37	−0.38	−0.45	0.16	**1**									
PPO	−0.10	**−0.70**	**−0.76**	**v0.87**	−0.08	−0.41	0.20	**−0.77**	**−0.93**	**−0.89**	**0.82**	**−0.80**	**−0.84**	−0.17	−0.12	**0.94**	0.07	**1**								
CAT	**−0.90**	**−0.76**	**−0.86**	**−0.81**	**−0.88**	**−0.89**	**0.87**	**−0.83**	**−0.95**	**−0.82**	**0.76**	**−0.96**	**−0.76**	**−0.89**	**−0.91**	**0.93**	0.16	**0.98**	**1**							
MDA	−0.15	**−0.86**	**−0.90**	**−0.90**	**−0.91**	**−0.66**	**0.83**	−0.24	**−0.72**	**−0.90**	**0.93**	**−0.66**	**−0.91**	**−0.93**	**−0.86**	**0.92**	0.46	0.20	0.42	**1**						
Yield	0.33	**0.95**	**0.92**	**0.85**	**0.84**	**0.88**	**−0.93**	**0.89**	**0.97**	**0.88**	**−0.84**	**0.76**	**0.70**	**0.94**	**0.93**	**−0.90**	−0.14	**−0.79**	**−0.68**	**−0.91**	**1**					
No. tubers	**0.89**	**0.94**	**0.90**	**0.87**	**0.97**	**0.95**	**−0.61**	**0.90**	**0.95**	**0.77**	**−0.90**	**0.87**	**0.89**	**0.88**	**0.95**	**−0.94**	−0.38	**−0.90**	**−0.59**	**−0.92**	**0.73**	**1**				
Tuber weight	0.32	**0.88**	**0.89**	**0.92**	0.29	0.27	−0.45	**0.88**	**0.88**	**0.68**	**−0.93**	**0.89**	**0.90**	0.01	0.05	**−0.83**	0.07	**−0.73**	**−0.66**	−0.09	**0.76**	0.06	**1**			
Carboh.	**0.89**	**0.95**	**0.91**	**0.94**	**0.75**	**0.89**	**−0.89**	**0.90**	**0.92**	**0.83**	**−0.92**	**0.96**	**0.87**	**0.89**	**0.88**	**−0.71**	−0.40	**−0.68**	**−0.96**	**−0.51**	**0.96**	**0.86**	**0.88**	**1**		
Starch	−0.27	**−0.89**	**−0.87**	**−0.85**	**−0.91**	**−0.92**	**0.77**	**−0.80**	**−0.84**	**−0.96**	**0.84**	**−0.87**	**−0.92**	**−0.79**	**−0.90**	**0.87**	0.36	**0.55**	**0.81**	**0.82**	**−0.91**	**−0.84**	−0.38	**−0.99**	**1**	
Protein	**0.90**	**0.91**	**0.85**	**0.82**	**0.94**	**0.93**	**−0.73**	**0.77**	**0.94**	**0.56**	**−0.76**	**0.78**	**0.82**	**0.95**	**0.90**	**−0.82**	−0.40	**−0.78**	**−0.86**	**−0.83**	**0.60**	**0.90**	0.04	**0.99**	**−0.77**	**1**

Values in bold are different from 0 with a significance level of alpha = 0.05.

A correlation-based method using the Pearson coefficient was used to determine the positive and negative correlations between the physicochemical and agronomical parameters of potato plants grown under salinity and treated with biochar and nanoparticles (n-K and n-Si). The significant correlations (bold numbers) and insignificant relationships (nonbolded numbers) are presented in Table 6. The Pearson's correlation analysis showed that tuber yields were significantly and positively correlated with the plant growth traits (dry and fresh shoots, total leaf area, stem number, and tuber weight and number); the physiological attributes (Chl., Pn, Gc, Inter.CO_2conc., WUE, and RWC); some biochemical components (free proline and GA3, and the tuber carbohydrate and protein contents), and that they were negatively correlated with the leaf Na content; PPO, CAT, ABA, MDA, and Tr; and the tuber starch content. These findings indicate that the single and combined applications with biochar, silicon, and potassium nanoparticles were effective at alleviating the soil salinity by reducing the sodium uptake (Table 3), and at improving the photosynthesis, water use efficiency, and essential nutrient uptake, as well as at modulating the biochemical components (antioxidant enzymes, ABA, free proline, and GA3). The results also suggest that those changes in the biochemical components that result from the biochar and nanotreatment applications might lead to improvements in the plant tolerance against salinity stress, and may increase the growth and productivity of potato plants.

4. Discussion

Soil salinity is one of the most important abiotic stresses, and it can cause a reduction in the photosynthesis parameters, biochemical constituents, nutrient and water uptake, growth characteristics, and tuber yields of potato plants. The adverse effects of salinity on the chlorophyll content could be related to the role of salinity in the inhibition of ribulose-1,5-biphosphate, accelerated chloroplast degradation, or a reduced chlorophyll concentration. Singh et al. [46] reported that the reduction in the leaf chlorophyll content could be linked to increased chlorophyllase activity and/or the inhibition in the biosynthesis of photosynthetic pigments. A reduction in the growth characteristics, including the plant height, leaf area, number of stems, and the fresh and dry biomasses of salt-stressed potato plants, has also been stated in different crops [48–50].

The unfavorable effect of the plant height, the number of stems, and the fresh and dry biomasses of increasing salinity stress might be associated with the decreased activity of the plant hormones (IAA and GA3), the cell division and expansion of the plant, the leaf area enlargement, and the minor quantity of radiation intercepted, leading to declined photosynthesis. Disruption in the photosynthesis apparatus is attributed to low electron transport through PS II and/or to the structural damages of PS II and light-harvesting complexes [51]. Bharath et al. [52] reported that the accumulation of the ABA of salt-stressed plants increased and induced stomatal closure to conserve the water; this may reduce the intercellular CO_2 concentration, and decrease the water use efficiency, the transpiration rate, and the nutrient uptake.

Excess salt concentration can cause a reduction in the osmotic potential of the growing medium and can limit the absorption of water and nutrients because of the Na accumulation in the plant root [53]. Furthermore, the leaf relative water contents of plants declined considerably as a result of the undesirable impact of the salinity on the water absorption capacity from the soil to the root system, which decreased the internal water supply and negatively affected the other plant organs [54,55]. Salt accumulation also induces the generation of reactive oxygen species (ROS), which disturbs transpiration, reduces nutrient uptake, damages vital primary metabolic compounds, increases MDA, and collapses the membrane integrity [56], which eventually lead to a decrease in the growth traits and productivity of the potato plants.

On the other hand, the application of applied biochar and nanomaterials (n-K and n-Si,) considerably reduced the harmful effects of salinity, and enhanced the vegetative growth of potato plants, such as the plant height, the fresh and dry weights, the leaf area, and the leaf chlorophyll [57]. Similar findings were observed by Kafi et al. [58] in potato plants treated with Si and K under saline conditions, where the photosynthesis machinery and the plant biomass increased in correlation to the increasing Si and K ions and the decreasing Na ions in the plant tissue. A significant improvement in the agronomical characteristics in salt-stressed potato plants treated with silicon, biochar, and potassium nanoparticles could be due to the positive role of Bc, n-K, and n-Si in decreasing the Na^+ absorption, and increasing the K^+ concentration and uptake [28,57,59], which ameliorates the activities of numerous enzymes, the chlorophyll concentration, and plant hormones (IAA and GA3), while the relative water content reduced the water loss, the intercellular CO_2 concentration, and plant photosynthesis. Moreover, the correlation study shows that the leaf area and the fresh and dry biomasses positively correlated with the photosynthesis rate, the chlorophyll content, the stomatal conductance, the Inter.CO_2conc., the WUE, the RWC, the free proline, and the GA3, and that they negatively correlated with the leaf Na content, the ABA, the MDA, and the transpiration rate (Figure 6 and Table 6.).

Furthermore, the photosynthesis apparatus, such as the net photosynthesis rate, the intercellular CO_2 concentration, the stomatal conductance, the water use efficiency, and the transpiration rate of salt-stressed potato plants were affected by n-K, n-Si, and Bc applications (Figure 3). The plant photosynthesis rate was also significantly increased by the exogenous application of nanomaterials (n-K and n-Si) and biochar (Bc), which resulted in stomatal conductance, leaf chlorophyll, leaf numbers, and leaf area increments. This may increase the intercellular CO_2 concentration, the absorption of sunlight, and the quantum yield of PS II. Moreover, the application of biochar and nanoparticles (n-Si, n-K) reduced the Na absorbance and decreased the ABA concentration in the leaves of salt-stressed plants, which resulted in an increase in the stomatal aperture and the supply of CO_2 from the stomatal cavity to the site of CO_2 fixation [5,28,58,60].

The role of applied nanomaterials and biochar (n-K, n-Si, Bc) in modulating the water status of potato plants is of interest, especially within the context of the initial reduction in the growth of salt-stressed plants being related to the osmotic effect of the salt [58,61,62]. The RWC and the water use efficiency (WUE) improved in response to Si, K, and biochar treatments under salt stress conditions, not only by reducing the Na absorbance and decreasing the transpiration rate, but also by increasing the potassium absorbance and the translocation to the stomatal guard cells, where potassium influences the stomatal conductivity [57,60,63,64]. It has been reported that the exogenous applications of Si, K, and biochar can increase a plant's water content under salinity stress because of the reduced transpiration and the increasing potassium concentration in stomatal guard cells, as well as the increased turgor pressure of the plant leaves under salt stress [26,28,60]. In the current study, the RWC of potato plants when treated with nanoelements (n-K and n-Si) and biochar was higher than that of untreated plants (Figure 3). This finding is consistent with earlier studies indicating that the RWC in wheat plants was significantly inferior under stress conditions, and that adding K and Si nutrition absolutely restored the RWC to the levels noted in the nonstressed plants [62,65–67]. The influence of Si and K can considerably increase the outcome for salt-stressed plants, and the mode of action may be avoiding the loss of water from plants by reducing the rate of transpiration [68]. The advantages of silicon and potassium nanoparticles and biochar for plants grown under salinity stress are linked to an improved photosynthetic rate, RWC, stomatal conductivity, and water use efficiency, characteristics which then enhance the tolerance to salinity of different crop plants [5,57,58,62]. Here, we recognize and examine a possible relation, at the molecular level, between n-Si, n-K, and biochar treatments and biochemical constituents, such as free proline accumulation, the activity of antioxidant enzymes, the MDA content, and the gibberellic acid level in salt-stressed potato plants (Figures 4 and 5).

The concentration of proline was also improved in potato plants under salinity stress conditions. This improvement of free proline is one of the distinctive responses to the stress aspects, especially salinity stress, which plays a vital role in reducing chlorophyll pigments from degradation [7,69]. Moreover, in its role as an osmolyte adjustment in plants, proline also plays an important role in scavenging reactive oxygen species (ROS) [70]. The application of those nanoparticles significantly increased the proline accumulation in plant tissues. Similar findings were reported by several researchers in different crops [26,69,71]. Likewise, proline increased by nanomaterial application was due to tolerance induction in plants.

The activity levels of antioxidant enzymes (CAT, POD, and PPO) and the leaf ABA content significantly increased in untreated salt-stressed potato plants; this result could be associated with the toxic effect of salinity [5,7]. Likewise, the level of malondialdehyde (MDA), which is the final product of lipid peroxidation, significantly increased as a result of salinity stress conditions. This increase in MDA could be attributed to the oxidative damage to chloroplasts and, consequently, to increasing lipid peroxidation due to increasing the activity of superoxide and hydrogen peroxide [72,73]. The results show that a positive correlation was found between the leaf Na content and ABA and MDA (Table 6 and Figure 6). Furthermore, Khoshgoftarmanesh et al. [22] reported that the MDA content positively correlated with the Na concentration in salt-stressed cucumber, but was negatively associated with Ca, K, and Si applications. Additionally, the application of biochar (Bc) and nanoparticle treatments (n-K and n-Si) reduced the harmful effects of salinity, including by reducing the concentrations of (MDA), ABA, and antioxidant enzymes (CAT, POD, and PPO) in the leaf tissues of potato plants. This may be due to the positive role of n-Si, n-K, and Bc in reducing the Na+ accumulation in plant tissues, and lipid peroxidation, regulating plasma membrane stability and improving osmolyte accumulation, which results in the scavenging of reactive oxygen species, predominantly hydrogen peroxide and superoxide [57,58,60,74].

The negative effects of salinity on the nutrient uptake might be correlated to the downregulation of some proteins, such as NRT1 and AMT1. In contrast, the significant enhancement in the endogenous essential element contents of salt-stressed potato plants (N, P, K, Mg, Fe, Mn, and Br) following n-Si, n-K, and biochar applications could be attributed to the membrane stability achieved by increasing the cell membrane H-ATPase activity, which induces nutrient uptake, but decreases the endogenous sodium (Na^+) concentration, thus ameliorating water uptake and photosynthesis. The favorable role of n-Si, n-K, and biochar treatments in nutrient uptake has been stated for different plants [5,28,57,75]. The favorable effect of the applied treatments could be due to the decreasing Na+ accumulation in salt-stressed potato plants. In fact, reductions in both the Na^+ and Cl^- levels increased K and Si [76], in addition to enhancing the water absorption, maintaining the nutrient balance, and stimulating photosynthesis. Both elements, K and Si, can preserve the antioxidative capacity, increase the osmotic adjustment, and increase the activity of the photosynthetic enzymes [5,37,66].

On the contrary, there were reductions in the tuber yields (numbers and weights of tubers) and tuber chemical compositions (including, carbohydrates, starches, proteins, and tuber N, P, and K concentrations) of the untreated salt-stressed plants (Tables 4 and 5). This could be due to the fact that salinity undesirably affects and diminishes the chlorophyll content, the water status, the nutrient uptake, the plant hormone (GA3), and the antioxidant enzymes, leading to a decreased photosynthetic rate and relative water content [5,14]. The end outcomes of these processes could be a final reduction in the tuber quantity and quality.

The obtained results reveal that n-Si, n-K, and biochar treatments confer favorable effects on the tuber yields and tuber quality of salt-stressed potato plants [5,58,62]. This may be due to the role of Si, K, and biochar in increasing the expression of RNA polymerase and ribosomal proteins, which activate the stress tolerance, increase the soil water-holding capacity, increase the Si and K uptakes and concentrations, improve the water use efficiency, enhance photosynthesis, and reduce the transpiration rate [37,66], and also reduce the oxidative stress and increase the tuber yield and quality. Several studies confirm that the improvement in the K absorbance, due to Si, K, and Bc nanoparticle application, leads to improvements in the photosynthesis rate and the CO_2 assimilation, which results in an increase in the accumulation and translocation of the carbohydrates and proteins from the leaves to the storage parts of the crops. The end findings of these processes may be a final increments in the contents of the carbohydrates and proteins of potato tubers.

On the other hand, the increased accumulation of tuber starch in the untreated salt-stressed potato plants could be clarified by a lesser accumulation of hexoses, sucrose, and the greater sucrose–phosphate synthase activity, leading the triose–phosphate pathway towards starch biosynthesis, and/or towards inhibiting the enzymes involved in starch decomposition. Similar findings were observed by several researchers [76,77]. While in salt-stressed potato plants treated with biochar, nanopotassium, and nanosilicon, the declines noted in the tuber starch content might suggest that the starch is used for various physiological processes to alleviate the salt stress [77–80].

Furthermore, improved tuber N, P, and K concentrations of salt-stressed potato plants due to the application of n-K, n-Si, and Bc could be because of the increase in the nutrient uptake.

In general, the application of n-Si, n-K, and Bc on salt-stressed potato plants induces the plant tolerance against salinity by alleviating the detrimental effects of salinity by improving the water status and the photosynthetic rate, decreasing the oxidative injuries, modulating some osmolytes and phytohormones [54,67,81,82], as well as by increasing the nutrient uptake and the activity of enzymatic and nonenzymatic antioxidants, consequently improving the plant growth and tuber quantity and quality.

5. Conclusions

It can be concluded that the applications of biochar and nanosilicon and nanopotassium have positive effects on the growth and production of potato plants grown under salinity conditions. These treatments play an important role in mitigating the harmful impacts of salinity on the potato growth and tuber yields, as well as on the physiological and biochemical traits. The single and combined applications of biochar, nanosilicon, and nanopotassium significantly improved the relative water content, the leaf area, the fresh and dry weights of the plant, the leaf chlorophyll content, the free proline content, the activity of the antioxidant enzymes, and the nutrient uptake, as well as the yields and quality of the potato tubers. To the contrary, the sodium absorption and content, lipid peroxidation (MDA), and the photosynthesis rate were decreased in salt-stressed potato plants treated with biochar and nanotreatments (n-Si and n-K). The obtained findings support enhancing potato plants and the tuber yields during salinity stress in commercial production systems by applying nanosilicon, nanopotassium, and biochar, either alone, or in combination.

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