



Article

Salinity-Induced Changes in the Antioxidant Status of Common Basil Plants (*Ocimum basilicum* L.) Grown under Controlled Conditions

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Abstract: Studies were performed to determine the influence of salinity on the antioxidant status of basil plants, var. Sweet Green. The experiments were conducted in a growth chamber under controlled conditions (photoperiod—14/10 h, photosynthetic photon density (PPFD)—250–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature— 24 ± 1 °C/ 16 ± 1 °C, humidity—65–70%). The plants were grown on $\frac{1}{2}$ Hoagland's nutrient solution, with the addition of elevated concentrations of sodium chloride (0, 80, 160 mM). In addition, increased content of macro- and micronutrients in the solution was applied ($\frac{4}{2}$ Hoagland's nutrient solution and 0 mM NaCl). The activity of the antioxidant enzyme guaiacol peroxidase, content of malondialdehyde and total polyphenolic compounds, concentration of free proline, and antiradical activity were characterized. The results demonstrate that the salinity of Hoagland's solution with 160 mM NaCl induced considerable changes concerning enzymatic and non-enzymatic elements of the plant defense and antioxidant systems. Enhancing the concentration of macro- and microelements in the nutrient solution ($\frac{4}{2}$ of Hoagland and 0 mM NaCl) in terms of biochemical parameters presented a similar trend to the variant with an additional lower concentration of sodium chloride (80 mM).

Keywords: antiradical activity; reducing sugars; Hoagland nutrient solution; sodium chloride



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1. Introduction

Basil (*Ocimum basilicum* L.) is one of the most popular spice herbs of the family of Lamiaceae [1–3]. The genus *Ocimum* contains between 30 and 160 species and numerous cultivars [4,5]. It can be grown in the greenhouse and field conditions [6]. Its leaves, flowers, and seeds can be used in the medicinal industry and for culinary purposes and can also be grown as an ornamental plant [5]. The herbal raw material of basil is an herb (*Basilici herba*), containing essential oils (0.5–2.5%), flavonoids, tannins, phenolic, saponin, anthocyanins, vitamins C and E, and minerals. This set of biological components allows basil to be used as a repellent against insects and nematodes and an antifungal, antibacterial, and antioxidant agent [7,8]. The share of these compounds is clearly influenced by environmental factors [9]. According to Nowak [10], the success of basil cultivation depends on maintaining a good water and plant nutrition regime.

Many studies have shown that more and more arable land in the world and about half of all irrigated lands are susceptible to salt-related stress, which can be harmful to plants [8,11–13]. According to estimates, the total area of saline soil in the world is over 952 million hectares [14].

These soils enclose almost 7% of the total land area or about 33% of the potential agricultural land area of the globe [11,14].

In most cases, salinity is triggered by an exuberance of sodium chloride (NaCl) or sodium sulfate (Na_2SO_4) in the water used for plant irrigation [11,15,16]. This problem

is particularly important for most species responsive to salinity or cultivated in arid and semi-arid regions [8,16–18].

With the upsurge of salt such as NaCl in the water, the vapor pressure of the water decreases. It indicates that fewer water molecules can remove the solution from its surface [19]. Therefore, the more elevated the concentration of salt present, the smaller the evaporation. Saltwater is a solution in a liquid condition and with its vapor tension and temperature, the saltwater loses its molecules in the form of vapors [20].

Increased salt levels in the soil reduce plant water availability, as water is retained in the soil solution by enhanced osmotic interactions. Short-term salt stress inhibits plant growth due to osmotic effects and reduces cell expansion. Long-term effects of this stress involve excessive salt absorption by plants (especially Na^+ and Cl^-), which can disturb the ionic balance and lead to premature leaf aging [21].

Recently, herb cultivation in containers under cover, which ensures continuous supply of fresh plants throughout the year, irrespective of weather conditions, has gained popularity [22–24]. An increasing number of studies have also focused on the volume of the substrate on the yield quantity and quality of pot plants. Another important aspect is urban gardening, where basil is produced in very small pots and containers, concomitantly being exposed to fertilizers abundant in salts [23].

A study by Tarchoune et al. [8] showed a link between plant antioxidant activity and tolerance to salt stress. This stress can cause secondary oxidative stress, which ensues when there is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses [8]. This leads to oxidative impairment of macromolecules and disruption of cell structure [8,25]. Therefore, reactive oxygen species have been deemed dangerous molecules whose concentrations should be kept as low as possible. Plant cells can abide ROS to diverse degrees by improving endogenous protective mechanisms implicating antioxidant molecules and enzymes [8]. Mittova et al. [26] also indicated that plant tolerance to salt stress is mainly due to the increased activity of antioxidant enzymes (glutathione reductase, peroxidase, ascorbate peroxidase), which were tested in tomatoes resistant to high sodium chloride levels.

Plants exposed to salt stress accumulate various molecules in organic matter, such as proline, glucose, betaine, etc. [27,28]. Proline content is an important indicator of plant physiological response to stress, including salt stress [15,27–29].

Lipid peroxidation, caused by free radicals, is also an important indicator of cell membrane degradation [28–31]. It occurs when unsaturated fatty acids are oxidized in the membrane, resulting in an accumulation of ROS. As they are the most common symptom denoting oxidative damage, they indicate increased damage and constitute plant response to abiotic stress [30–34]. In the research by some authors, basil was demonstrated to tolerate low concentrations of hydrochloric acid, i.e., lower than 100 mM NaCl [18,27,35].

This study aimed to analyze some biochemical markers of basil plants' enzymatic and non-enzymatic safeguard during stress caused by an increased concentration of sodium chloride (80, 160 mM) and macro- and microelements in the substrate [11]. This will provide information about the extent of this stress, which is particularly important when growing basil plants in containers, where maintaining optimal conditions in the substrate determines the success of the cultivation [11].

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out with basil plants cv. "Sweet Green", in a growth chamber under the following controlled conditions: photoperiod—14/10 h (light/dark), photosynthetic photon density (PPFD)—250–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature— $24 \pm 1^\circ\text{C}$ / $16 \pm 1^\circ\text{C}$ (day/night), relative air humidity—65–70%. In the phase of two pairs of true leaves, the seedlings were transplanted into pots (0.72 L) filled with substrate perlite and $\frac{1}{2}$ Hoagland's solution [11,36] (Figure 1).

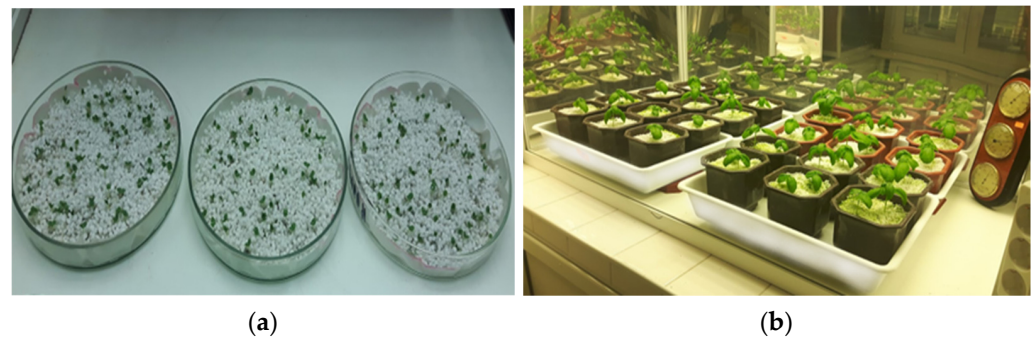


Figure 1. Setting up the experiment. (a) Eight days seedlings filled with substrate perlite; (b) Seedlings transplanted into the pots filled with substrate perlite.

In the second week of cultivation, they were split into the experimental variants: $\frac{1}{2}$ Hoagland's solution with added sodium chloride (0, 80, and 160 mM) and $\frac{4}{2}$ Hoagland's solution with 0 mM NaCl (Figure 2). Each variant was set in three replications [11].

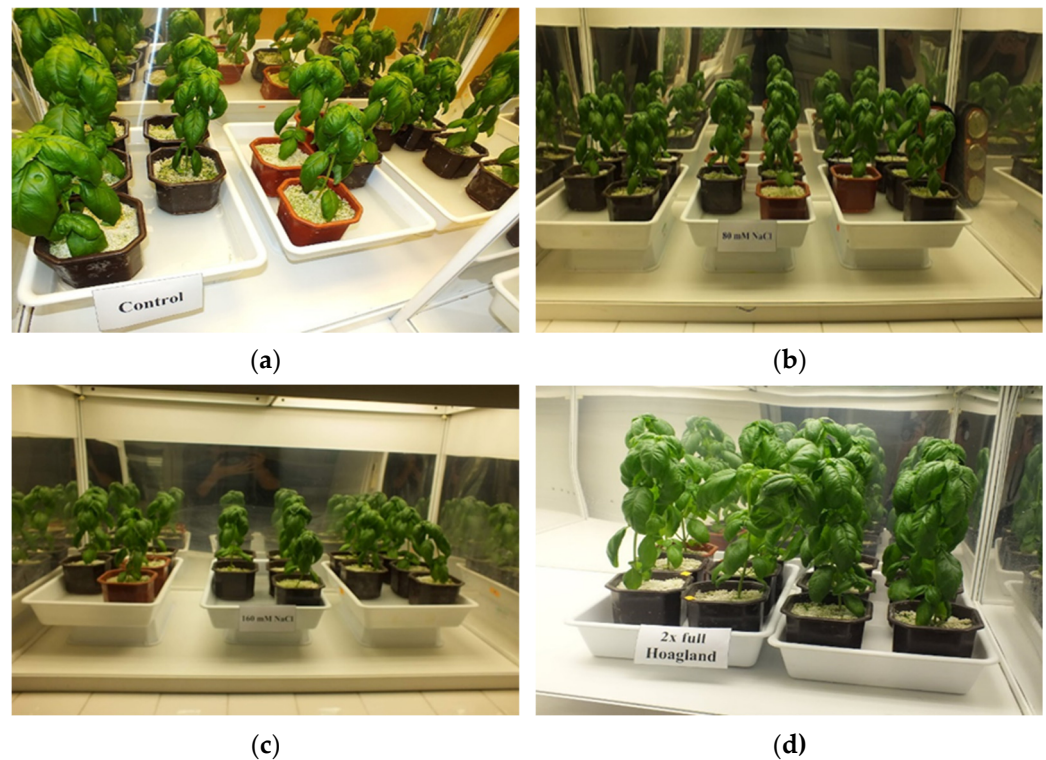


Figure 2. Design of the experiment: (a) Control ($\frac{1}{2}$ Hoagland's solution); (b) $\frac{1}{2}$ Hoagland's solution with added NaCl (80 mM); (c) $\frac{1}{2}$ Hoagland's solution with added NaCl (160 mM); (d) $\frac{4}{2}$ Hoagland's solution.

2.2. Analyses

2.2.1. Lipid Peroxidation

TBA test for lipid peroxidation indicated malondialdehyde (MDA) as the final product of the process (MDA-TBA complex) [37]. Specific absorbance at 532 nm and non-specific at 600 nm were recorded at molar extinction coefficient $E = 155 \text{ mM}^{-1} \text{ cm}^{-1}$. The results were presented as malondialdehyde (MDA) content in a unit of fresh weight (nmol MDA g^{-1} FW).

2.2.2. Enzyme Extraction

The fresh plant sample (1.0 g) was ground with 5 mL ice-cold 0.1 M Tris-HCl (pH 7.8) extraction buffer containing 1 mM DTT and 1 mM EDTA. Samples were centrifuged at $13,500 \times g$ rpm at 4 °C for 10 min. The resulting clear supernatant was used for determining the enzymatic activity spectrophotometrically (UV/Vis spectrophotometer Pharo 300), according to the methodology of Mocquot et al. [38].

2.2.3. Guaiacol Peroxidase (GPOD)

Total peroxidase activity (EC 1.11.1.7) was determined with guaiacol substrate (GPOD), and for GPOD activity, the Bergmeyer method was used [39]. The mixture of 2.3 mL of 0.1 M phosphate buffer (pH 7.0), 300 μ L of 5 mM H₂O₂ solution, 300 μ L of 8 mM guaiacol solution, and 100 μ L of enzyme extract was placed in the cuvette. The absorbance was measured at 436 nm against a blank containing the same components without enzyme extract ($E = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The values obtained were expressed as U mg g⁻¹ FW.

2.2.4. Free Proline Concentration

Proline content was indicated spectrophotometrically using a method described by Bates et al. [40] after extraction from 0.5 g of each leaf sample by grinding in 10 mL 3% sulfosalicylic acid. The mixture was then centrifuged at $10,000 \times g$ rpm for 10 min. To 2 mL of the supernatant was added 2 mL of acid solution and ninhydrin. The tubes were incubated in a water bath at 90 °C for 30 min and the reaction terminated in an ice bath. Each reaction mixture was extracted with 5 mL of toluene and vortex-mixed for 15 s. The tubes were allowed to stand for 20 min in the dark at room temperature to allow separation of the toluene and aqueous phases. Each toluene phase was then carefully collected in a test tube and its absorbance was read at 520 nm. The free proline content of the sample was determined from a standard curve prepared using an analytical proline.

2.2.5. Total Polyphenols Content

The total polyphenols content in the plant extracts was determined using the Folin–Ciocalteu reagent [41] according to the methodology of Singleton et al. [42]. The samplings (1 g of fresh leaf material) were crushed with quartz sand and 10 mL 60% acidic methanol and immersed in an ultrasound bath for 15 min. The homogenized material was repositioned to proper tubes, carefully filled, and left for 15 h in the dark at room temperature for extraction. During the incubation period, the tubes were periodically mixed. After, the tubes were centrifuged and the supernatant, which was used for gauging total phenolic content and antiradical activity, was carefully collected in new clean tubes. For the determination of total polyphenols content, 40 μ L of extract, 3160 μ L of double-distilled water, 200 μ L Folin–Ciocalteu reagent, and 600 μ L 20% NaCO₃ was mixed. The test tubes were left for 2 h at room temperature for the reaction to occur. After that, the extinction at 765 nm wavelength. Total polyphenols content was calculated as gallic acid equivalents (GAE) using a standard curve and is presented as mg g⁻¹ fresh weight. The standard curve was devised with gallic acid (Sigma-Aldrich, St. Louis, MO, USA) in the range of 0–500 mg L⁻¹.

2.2.6. Antiradical Activity

Antiradical activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), was measured in the extracts obtained for total phenolic content [43]. The incubation mixture contained 100 μ L extract and 3.9 mL $6 \times 10^{-5} \text{ mol L}^{-1}$ DPPH ($0.06 \mu\text{mol L}^{-1}$). The extract absorption was determined at 515 nm at min 0 and 30 from the initial mixture of the components. A parallel blank sample was tested, which contained double-distilled water instead of extract. The antiradical activity was expressed as % decoloration and was equal to [44]:

$$(1 - (A_{30\text{min}}/A_{0\text{min}}) \times 100).$$

2.2.7. Reducing Sugars

Reducing sugars were determined by the Hagedorn–Jensen method, modified by Popov and Koleva [45]. The method involves reducing potassium ferrocyanide ($K_3Fe(CN)_6$) in an alkaline medium using sugars. To 10 mL 0.05 n of potassium ferrocyanide was added 5 mL of the sugar solution to be tested. The tubes were placed in a boiling water bath for 20 min and then cooled in cold running water. The contents of the tubes were transferred to 100–150 mL flasks. Then, 25 mL of acetic reagent was measured and the tubes were rinsed several times; the rinsing solution was poured into the flask containing the sugar solution and 0.5 g of KJ and 1 mL of fresh starch solution were added. After 1 to 2 min, it was titrated with 0.05 n sodium thiosulphate solution until it turned from blue to white. In parallel, a blank was performed (5 mL of distilled water was placed in the tube instead of the sugar solution). The difference between the mL of sodium thiosulphate used for the titration of the blank and the sample proper corresponds to the mL of reduced potassium ferrocyanide from the sugars. The final value was read off the glucose table. The percentage of reducing sugars in the plant material, expressed as glucose, was calculated according to the formula:

$$\% CR = \frac{A \cdot V \cdot 100}{b \cdot g \cdot 1000},$$

where:

A—reducing sugars in the glucose solution taken for the test, taken as glucose, in mg;

V—volume of extract obtained, in mL (100);

b—a volume of sugar solution taken for the test in mL;

g—a mass of the sample taken in g;

100—conversion factor in %.

2.3. Analysis of Data

ANOVA was conducted to assess the significance of distinctions between means using Tukey's test for randomized blocks in a single-factor investigation. Half confidence intervals at the significance level $\alpha = 0.05$ were estimated. Statistical calculations were assembled by exploiting Statistica 13 software (StatSoft Polska Sp.o.o., Kraków, Poland).

3. Results

After 20 days of growth in increased salt concentrations, the entire plants were reaped, and biochemical analyses were conducted (Figure 3).

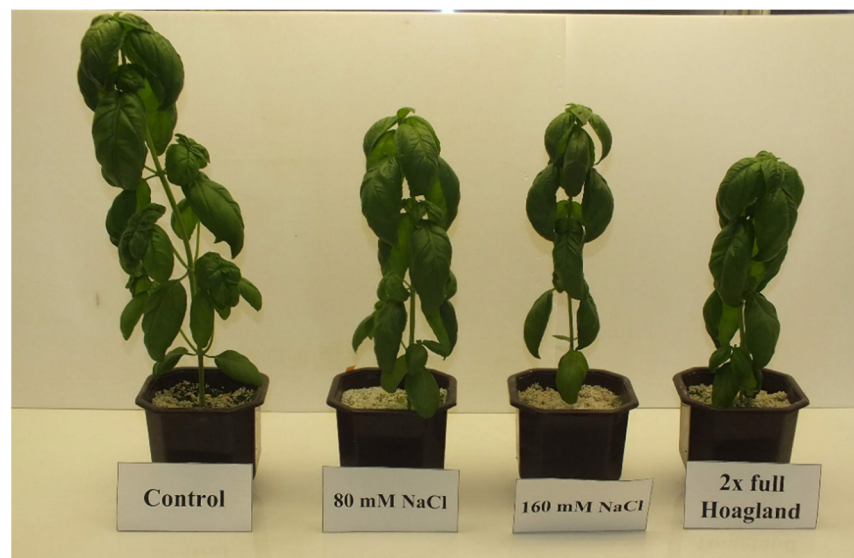


Figure 3. Basil plants at the end of the experiment.

The highest malondialdehyde content was found in the plants grown at $\frac{1}{2}$ Hoagland's solution and 160 mM NaCl ($0.32 \text{ nmol MDA} \cdot \text{g}^{-1} \text{ FW}$) (Table 1). The lack of significant differences in MDA content between control plants and other variants indicates that the integrity and stability of plasma membranes in these plants were maintained. No statistical differences were found in the malondialdehyde content between the leaves and the root system of plants.

The analysis of the results showed that the use of Hoagland's solution and 160 mM NaCl resulted in a significant increase in the guaiacol content in basil plants ($1.93 \text{ U g}^{-1} \text{ FW}$), with its significantly higher levels in the root system ($3.76 \text{ U g}^{-1} \text{ FW}$). In the other variants, roots were characterized by higher guaiacol levels than leaves. No significant differences were found in guaiacol peroxidase activity between control plants and other variants.

The highest proline level was found in basil plants grown in the presence of $\frac{1}{2}$ Hoagland's solution and 160 mM NaCl ($92.06 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$), and the lowest in the control plants ($28.74 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$). A significantly higher level of proline in the root system was found in control ($32.50 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$), a variant with Hoagland's solution and 80 mM NaCl ($68.04 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$) and a variant with $4/2$ Hoagland's solution and 0 mM NaCl ($72.68 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$). In contrast, plants grown at higher sodium chloride concentration (160 mM) contained more proline in the leaves ($110.81 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$).

Table 1. The content of malondialdehyde (MDA ($\text{nmol MDA g}^{-1} \text{ FW}$)), guaiacol (GPOD ($\text{U g}^{-1} \text{ FW}$)), and proline ($\text{mg g}^{-1} \text{ FW}$) in leaves and roots of sweet basil exposed to salinity.

Salt Concentration (A)	The Morphological Part of the Plant (B)	Malondialdehyde	GPOD	Proline
Control ($\frac{1}{2}$ Hoagland's solution with 0 mM NaCl)	Leaves	0.13 ± 0.01	0.03 ± 0.01	24.97 ± 0.10
	Roots	0.23 ± 0.01	1.32 ± 0.34	32.50 ± 5.54
Average for Control		0.18 ± 0.00	0.67 ± 0.18	28.74 ± 2.82
$\frac{1}{2}$ Hoagland's solution with NaCl (80 mM)	Leaves	0.17 ± 0.05	0.11 ± 0.01	38.38 ± 1.49
	Roots	0.25 ± 0.05	1.38 ± 0.62	68.04 ± 0.06
Average for $\frac{1}{2}$ Hoagland's solution with NaCl (80 mM)		0.21 ± 0.00	0.74 ± 0.32	53.21 ± 0.72
$\frac{1}{2}$ Hoagland's solution with NaCl (160 mM)	Leaves	0.42 ± 0.04	3.76 ± 0.76	110.81 ± 0.01
	Roots	0.22 ± 0.01	0.10 ± 0.03	73.31 ± 3.20
Average for $\frac{1}{2}$ Hoagland's solution with NaCl (160 mM)		0.32 ± 0.02	1.93 ± 0.37	92.06 ± 1.61
$4/2$ Hoagland's solution with 0 mM NaCl	Leaves	0.17 ± 0.01	0.06 ± 0.03	40.33 ± 0.47
	Roots	0.23 ± 0.13	0.88 ± 0.14	72.68 ± 4.94
Average for $4/2$ Hoagland's solution with 0 mM NaCl		0.20 ± 0.06	0.47 ± 0.08	56.51 ± 2.70
Average for:	Leaves	0.22 ± 0.04	0.99 ± 0.01	53.62 ± 0.16
	Roots	0.23 ± 0.02	0.92 ± 0.01	61.63 ± 3.05
HSD $_{\alpha=0,05}$ for:	A	0.025	0.931	6.079
	B	0.027	0.470	3.071
Interaction	B/A	0.038	0.940	6.141
	A/B	0.038	1.316	8.597

Note: Data are the means of three independent experiments ($n = 3$); \pm standard deviation, HSD—an honestly significant difference.

A correlation was found between the levels of proline and reducing sugars content in the studied plants (Table 2). In the basil plants exposed to $\frac{1}{2}$ Hoagland's solution with 160 mM NaCl, the increased free proline levels corresponded with those of reducing sugars. The highest reducing sugars level was found in basil plants grown in the presence of $4/2$ Hoagland's solution and 0 mM NaCl (0.59% FW) and $\frac{1}{2}$ Hoagland's solution and 160 mM NaCl (0.58% FW), and the lowest in the control plants (0.50% FW). In the other variants, leaves were characterized by higher reducing sugar levels (0.58% FW) than the roots system (0.53% FW). A significantly higher level of reducing sugar in the leaves was

found in a variant with 4/2 Hoagland's solution and 0 mM NaCl (0.70% FW) and lower in the control roots system (0.46% FW).

Plants cultivated at $\frac{1}{2}$ Hoagland's solution and NaCl, as well as those at 4/2 Hoagland's solution and 0 mM NaCl, had reduced amounts of polyphenolic compounds, which was more noticeable in the higher salt concentration variant (436.25 mg GAE g⁻¹ FW compared to the control—496.28 mg GAE g⁻¹ FW). These results are related to those obtained for peroxidase activity. The lowest concentration of total polyphenols was found in the plants with the highest peroxidase activity. No significant differences in total polyphenols content were found between basil leaves and the root system.

The antiradical activity was also altered in plants grown at $\frac{1}{2}$ Hoagland's solution and NaCl and those at 4/2 Hoagland's solution and 0 mM NaCl. It was significantly reduced, especially in the variant with higher NaCl concentration (21.8% compared to the control), where the lowest amounts of polyphenols were detected.

Table 2. The content of total polyphenols (mg GAE g⁻¹ FW), reducing sugars (% FW), and antiradical activity (% DPPH g⁻¹ FW) in leaves and roots of sweet basil exposed to salinity.

Salt Concentration (A)	The Morphological Part of the Plant (B)	Total Polyphenols	Reducing Sugars	Antiradical Activity
Control ($\frac{1}{2}$ Hoagland's solution with 0 mM NaCl)	Leaves	487.93 ± 44.68	0.54 ± 0.020	26.10 ± 1.72
	Roots	504.63 ± 89.21	0.46 ± 0.100	27.56 ± 5.32
Average for Control		496.28 ± 22.27	0.50 ± 0.035	26.83 ± 3.52
$\frac{1}{2}$ Hoagland's solution with NaCl (80 mM)	Leaves	424.60 ± 40.03	0.53 ± 0.010	26.34 ± 2.28
	Roots	498.69 ± 84.43	0.54 ± 0.350	24.87 ± 4.01
Average for $\frac{1}{2}$ Hoagland's solution with NaCl (80 mM)		461.64 ± 62.23	0.54 ± 0.045	25.60 ± 3.15
$\frac{1}{2}$ Hoagland's solution with NaCl (160 mM)	Leaves	402.51 ± 29.85	0.53 ± 0.010	20.81 ± 2.38
	Roots	469.99 ± 29.85	0.63 ± 0.035	21.13 ± 1.50
Average for $\frac{1}{2}$ Hoagland's solution with NaCl (160 mM)		436.25 ± 5.01	0.58 ± 0.050	20.97 ± 1.94
4/2 Hoagland's solution with 0 mM NaCl	Leaves	525.39 ± 59.44	0.70 ± 0.200	28.12 ± 2.51
	Roots	498.90 ± 12.42	0.47 ± 0.080	25.12 ± 2.23
Average for 4/2 Hoagland's solution with 0 mM NaCl		462.14 ± 23.51	0.59 ± 0.085	26.62 ± 2.37
Average for:	Leaves	435.11 ± 13.78	0.58 ± 0.050	25.34 ± 2.22
	Roots	443.05 ± 3.05	0.53 ± 0.080	24.67 ± 3.27
HSD _{α=0.05} for:	A	135.397	0.028	3.003
	B	n.s.	0.015	n.s.
Interaction	B/A	n.s.	0.029	n.s.
	A/B	n.s.	0.040	n.s.

Note: Data are the means of three independent experiments (n = 3); ± standard deviation, HSD—an honestly significant difference.

4. Discussion

The degree of lipid peroxidation in basil plants was determined by changing the malondialdehyde content. MDA is a breakdown product of polyunsaturated fatty acids in protein–lipid membranes, mainly linoleic acid. Therefore, MDA content is routinely determined and used as a good indicator for measuring oxidative cell damage [46,47].

Our study showed the highest malondialdehyde content in the plants grown at $\frac{1}{2}$ Hoagland's solution and 160 mM NaCl (0.32 nmol MDA·g⁻¹ FW). The results indicate a decrease in lipid membrane integrity and lipid peroxidation activation under the higher salt concentration. The lack of significant differences in MDA content between control plants and other variants indicates that the integrity and stability of plasma membranes in these plants were maintained. No statistical differences in malondialdehyde content were found between the leaves and root systems of the plants.

In a study, Ning et al. [48] demonstrated that those glandular trichomes have an impact on the detoxification of toxic chemicals (e.g., heavy metals and salts) and in reply to various other stress conditions [49,50]. Authors demonstrated that basil plants can exclude Na⁺ ions via leaf glands, staying intact. In addition, basil leaf glands contain various inorganic ions and organic substances which may have an impact on salt tolerance [47].

Similar results were obtained by Attia et al. [51], who showed that salinity induced an increase in malondialdehyde in the leaves of the basil genotypes they studied. Delavari et al. [27] reported a malondialdehyde (MDA) level of 0.5 $\mu\text{g g}^{-1}$ FW in the control plants, 1.2 $\mu\text{g g}^{-1}$ FW in the presence of 100 mM NaCl and 1.0 $\mu\text{g g}^{-1}$ FW following treatment with the highest salt concentration of 200 mM NaCl.

The study by Bączek-Kwinta et al. [52] showed that antioxidant activity was strongly differentiated in basil cultivars. The analysis of the results of own research showed that using Hoagland's solution and 160 mM NaCl resulted in a significant increase in the guaiacol content in basil plants (1.93 U g^{-1} FW), with significantly higher levels in leaves (3.76 U g^{-1} FW). In the other treatments, roots were characterized by higher guaiacol levels compared to leaves. The increase in the activity of the enzyme guaiacol peroxidase suggests its involvement in the defense system of the basil plants against salinity stress. No significant differences were found in guaiacol peroxidase activity between the control plants and the other variants.

The results obtained correspond with those of Tarchoune et al. [8]. According to the authors, the stability of cell membranes manifested by the unchanged level of general peroxidation is characteristic of plants coping with salinity. Tolerance to salinity is correlated with the stimulation of antioxidant enzymes and their enhanced ability to remove active oxygen compounds.

To reduce the negative effects of excess salt, plants synthesize and accumulate so-called cytoplasmic osmolytes, including endogenous amino acids. The proline accumulation in response to adverse environmental factors allows plants to acclimate to stressful conditions, including salinity [53,54].

Our study demonstrated the highest proline level in basil plants grown in the presence of $\frac{1}{2}$ Hoagland's solution and 160 mM NaCl and the lowest in the control plants. A significantly higher level of proline in the root system was found in control in Hoagland's solution and 80 mM NaCl (and a variant with $\frac{4}{2}$ Hoagland solution and 0 mM NaCl). In contrast, plants grown at higher sodium chloride concentration (160 mM) contained more proline in the leaves. This corresponds with the results obtained by Delavari et al. [27], who indicated 2 and 4 mg g^{-1} FW in roots and leaves of control basil plants (deionized water and 0 mM NaCl) and 11 and 12 mg g^{-1} FW, respectively, at 200 mM NaCl.

A correlation was observed between the levels of proline and reducing sugars content in the studied plants (Table 2). In the basil plants exposed to $\frac{1}{2}$ Hoagland's solution with 160 mM NaCl, the increased free proline levels corresponded with those of reducing sugars. According to some authors [55,56], these osmoprotectors play an important role in alleviating the effects of stress in basil plants.

Plants grown at $\frac{1}{2}$ Hoagland's solution and NaCl, as well as those at $\frac{4}{2}$ Hoagland's solution and 0 mM NaCl, had reduced amounts of polyphenolic compounds, which was more noticeable in the variant with the higher salt concentration (436.25 mg GAE g^{-1} FW compared to the control—496.28 mg GAE g^{-1} FW). These results are related to those obtained for peroxidase activity. The lowest concentration of total polyphenols was found in the plants with the highest peroxidase activity. The reduction of total polyphenols in cell walls weakens them and makes them more susceptible to the effects of stress. No significant differences in total polyphenols content were found between basil leaves and the root system.

The antiradical activity was also altered in plants grown at $\frac{1}{2}$ Hoagland's solution and NaCl and those at $\frac{4}{2}$ Hoagland's solution and 0 mM NaCl. It was significantly reduced, especially in the variant with higher NaCl concentration (21.8% compared to the control), where the lowest amounts of polyphenols were detected. The high value of DPPH-radical

scavenging activity is considered an important mechanism by antioxidants to inhibit lipid peroxidation [57,58]. Our results confirm this fact.

5. Conclusions

The present study demonstrated that the 160 mM NaCl in Hoagland's solution induced changes in the biochemical status of young basil plants. These changes included increased activity of the antioxidant enzyme guaiacol peroxidase; reduced polyphenols content; and changes in antiradical activity, MDA, and free proline concentrations. These results indicate that the plant was placed in a state of alertness by inducing its defense systems. At the same time, increasing the concentration of macro- and microelements in the nutrient solution (4/2 of Hoagland's and 0 mM NaCl) in terms of biochemical parameters showed a similar trend to the variant with an additional lower concentration of sodium chloride (80 mM).

In conclusion, it can be stated that basil plants (cv. Sweet Green) were characterized by tolerance to a lower level of salinity, as was applied in the current study.

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