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Sodium Azide Priming Enhances Waterlogging Stress Tolerance in Okra (*Abelmoschus esculentus* L.)

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Abstract: Waterlogging stress adversely affects crop growth and yield worldwide. Effect of sodium azide priming on waterlogging stress tolerance of okra plants was investigated. The study was conducted as a field experiment using two weeks old plants grown from 0%, 0.02%, and 0.05% sodium azide (NaN_3)-treated seeds. The waterlogging conditions applied were categorized into control, one week, and two weeks. Different growth and reproductive parameters were investigated. Activity and expression of antioxidant enzymes, root anatomy, and soil chemical analysis were also studied. Results showed that sodium azide priming inhibited germination. The germination percentages recorded were 92.50, 85.00, and 65.00 for 0%, 0.02%, and 0.05% NaN_3-treated seeds, respectively, nine days after planting. Waterlogging conditions depressed plant height ten weeks after planting. Under waterlogging conditions, NaN_3 promoted plant height and number of leaves formed. NaN_3 also supported the survival of plants and formation of adventitious roots under waterlogging conditions. Waterlogging conditions negatively affected the redox potential, organic C, N, and P concentrations in the soil but enhanced Soil pH, Fe, Mn, Zn, and SO_4. Under waterlogging conditions, NaN_3 increased the average number of flower buds, flowers, and fruits produced in comparison to control. Moreover, NaN_3 highly stimulated the development of aerenchyma which in turn enhanced the survival of okra plants under waterlogging conditions. NaN_3 priming also enhanced the activities and gene expression level of antioxidant enzymes (ascorbate peroxidase, APX; catalase, CAT) under waterlogging conditions. In conclusion, this study demonstrated that NaN_3 priming could improve waterlogging stress tolerance in okra.

Keywords: sodium azide; okra; waterlogging stress; antioxidants; gene expression

1. Introduction

Okra (*Abelmoschus esculentus* L.) is one of the economically important vegetable crops grown in tropical and sub-tropical regions of the world [1]. Okra originated in Ethiopia and was then reproduced in the Mediterranean area, North Africa, and India [1]. Environmental stresses negatively affect the growth, yield, and biological activities of plants worldwide [2–6]. In particular, waterlogging

conditions influence the growth and yield of okra plants through causing hypoxic or anoxic conditions, which in turn affect various physiological processes in roots, including carbohydrate metabolism, gas exchanges, and water relations [7–9]. The oxygen-deficient soil environments may lead to changes in the composition and decomposition activities of microbes. Waterlogging conditions also affect soil factors such as EC, pH, soil structure, hydraulic conductance, porosity, and organics [10,11]. Plants could adapt to waterlogging conditions via activating their self-defense mechanisms and developing adventitious roots and hypertrophied stem bases with lenticels and aerenchyma cells [7,12]. Such aerenchyma cells could enhance organ porosity and root aeration [13,14]. These morphological features help plants to manage the low oxygen tension within the tissues, prevent anoxia, and maintain root functions and plant survival.

Applications of chemicals to plants, either as foliar or seed treatments, may induce their physiological mechanisms, leading to plant growth stimulation and stress tolerance [7,15,16]. For instance, seed pretreatment with salicylic acid enhances plant growth, antioxidant activities, and tolerance to harsh environmental factors such as heavy metal, herbicides, low temperature and salt stress [17,18]. Ethylene is also described as a signaling molecule in plants and has been projected as capable of inducing survival traits and tolerance under waterlogging conditions via up-regulating the activity of antioxidant enzymes and genes linked to aerenchyma formation, leaf senescence, adventitious roots, and epinasty [7,19–21]. However, ethylene application as a proactive measure for ameliorating envisaged waterlogging condition on a wide scale may not be appreciated. Hence, seed priming techniques may be easier to enhance growth and yield. Sodium azide (NaN_3) has been successfully used for creating genetic variability and enhancing agronomic traits of crop plants. It affects crops based on the concentration applied. Gnanamurthy et al. [22] and Shagufta et al. [23] reported that NaN_3 priming delayed and inhibited the germination of maize and fenugreek, respectively. However, Vwioko and Onobun [24] reported that NaN_3 enhanced the germination percentage and height of okra plants. Al-Qurainy [25] and Zuzana et al. [26] also stated that NaN_3 stimulated the plant height of *Eruca sativa* and *Diospyros lotus*, respectively. On the other hand, Adamu and Aliyu [27] and Gnanamurthy et al. [22] revealed that NaN_3 priming inhibited plant height. NaN_3 priming also regulates various physiological and molecular mechanisms in plants and modulates the activities of catalase, peroxidase, and cytochrome oxidase [28]. Molecular changes induced by NaN_3 treatments produce mutations by base substitution, leading to changes in amino acid sequences. NaN_3 is reckoned to be an efficient reagent that induces a broad and high variation of morphological and yield parameters in cultivated species. However, it is not popularly used to initiate plants tolerance to environmental factors. Environmental stresses such as salinity and water stress [29,30] increase production of free radical in plants, and resistance to the unfavorable conditions often involves stimulation of the antioxidant response. Haq et al. [31], El Kaaby et al. [32], and Kuasha et al. [33] carried out in vitro studies on the ability of NaN_3 to confer salt tolerance in plants. Haq et al. [31] stated that one of the three cultivars of sugarcane studied regenerated plantlets that were salt tolerant, while El Kaaby et al. [32] and Kuasha et al. [33] stated that NaN_3 depressed the responses of the explants of tomato and sugarcane to salinity stress. Salim et al. [34] also studied the effect of NaN_3 on various plant traits, including disease resistance, yield, antioxidant activities, pigmentations, and salinity and drought stress tolerance. However, the role of NaN_3 in regulating waterlogging stress responses has not been studied yet. Therefore, the main aim of the present study was to assess the ability of NaN_3 to induce waterlogging stress tolerance in okra plants.

2. Materials and Methods

2.1. Plant Material and Application of Sodium Azide Treatments

Seeds of okra variety Clemson spineless produced by Technism (Longué-Jumelles, France) were obtained and used in this study. Okra seeds were soaked in sodium azide treatments, i.e., 0%, 0.02%, and 0.05% (*w*/*v*), at room temperature (27 °C) for 5 h with a continuous gentle stirring. After 5 h,

the seeds were removed and washed 5 times with deionized water to remove all traces of NaN_3. NaN_3 treatments were classed as mild (0.02%) and severe (0.05%).

2.2. Soil Preparation for Potted Field Experiment

Top soil (0–15 cm deep) was collected from the Demonstration Farm, Faculty of Agriculture, University of Benin, Nigeria. The soil type is categorized as ultisol. The composite soil sample was air-dried for three weeks and sieved to remove gravel and other particles. Each experimental pot was filled with 5 kg of soil. Thirty-six (36) pots were prepared to make twelve pots for each NaN_3 treatment. The undersides of the experimental pots were not perforated so that they could retain water.

2.3. Sowing of Seeds in Nursery Beds, Transplanting into Experimental Pots, and Acclimatization

Twelve soil nursery beds (measuring 2 feet by 2 feet) were prepared for the sowing seeds. The beds were allocated to the treated seeds, i.e., 0%, 0.02%, and 0.05% NaN_3. The seeds were sown at a depth of 2–3 cm. Germination records were collected every day for two weeks. After two weeks in the nursery beds, four plants were transferred into each experimental pot and taken to the open field. The plants were allowed to acclimatize for another two weeks in the field before flooding condition was introduced.

2.4. Application of Flooding or Waterlogging Conditions

When the plants were four weeks old, flooding of experimental pots with tap water was carried out. Three conditions of flooding or waterlogging were set up; no flooding (NF), one-week flooding (1 WF), and two weeks flooding (2 WF). Flooding of the pots was done up to 2 cm mark above the soil level. The water level was maintained in each pot by topping daily after inspection during the period.

2.5. Growth Parameters Measured

The field data collected were germination percentage, stem girth, plant height, number of leaves formed, survival percentage of plants, number of adventitious roots formed, number of flower buds formed, number of flowers, and number of fruits produced.

2.6. Soil Chemical Analyses

Soil chemical factors like pH, electrolyte conductivity (EC), redox potential (Eh), nitrogen, phosphorus, sulphate, organic carbon, iron, manganese, zinc, and total soluble phenolics were determined using standard methods. The soil analysis was carried out for the soil samples collected after plant harvested. pH, EC, and Eh were estimated in a soil-water slurry (ratio 1:3) [35]. Total nitrogen was estimated following Kjeldahl method [36]. Total soluble phenolic analysis was done based on the modified citrate extraction protocol followed by Folin–Ciocalteau colorimetric methodology [37]. The methodologies of Appiah and Ahenkorah [38] and Ben Mussa et al. [39] were used to determine sulphate content. Phosphorus measurement was conducted following the methodology of Bray and Kurtz [40]. Walkley–Black chromic acid wet oxidation methodology [41] was used to estimate the organic carbon. Iron content was determined following the hydroxylamine and 1,10- phenanthroline protocol [42]. Manganese was determined following the permanganate oxidation procedures [42]. The determination of zinc was carried using atomic absorption spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

2.7. Soil Microflora Counts

Presence of bacteria and fungi in the soil samples was investigated after plant harvest. Serial dilution processes were used in the analysis of soil microflora. Ten grams of the samples were dispensed into sterile beakers and mixed thoroughly with 90 mL sterile distilled water. Each sample was serially diluted from the stock sample and then transferred to the first tube 9 mL of sterilized water

to give 10^{-1}dilution, from which further dilution up to 10^{-4} was made. The pour plate method was utilized for inoculation on a sterilized nutrient agar (NA) or potato dextrose agar (PDA), impregnated with antifungal or antibacterial agents for the growth of bacterial or fungal isolates, respectively. Nutrient agar plates were kept for 24–48 hrs at 37 °C for bacterial growth. Potato dextrose agar was incubated at room temperature (30 ± 2 °C) for 3–5 days. Total viable colonies were then counted for the microbial isolates and represented in terms of colony forming units (cfu/g). Viable counts obtained were recorded with reference to the serial dilution used [43,44].

2.8. Root Anatomy

Harvested plant roots were washed and used to make microscopic slides to examine internal tissues. Root sections were immersed in paraffin wax and left to solidify. Sections were cut and dewaxed by clamping in the microtome. Aniline blue stain was applied to the sections to show a clear contrast of air spaces (aerenchyma) formed. Excess stains were removed by ethanol before oven-drying. Following oven-drying, slides were viewed and then photographed using the microscope IRMECO model IM-660 T1 (IRMECO GmbH & Co. KG, Geesthacht, Germany) with a camera connected to PC. Observations were done under X10 objective lens.

2.9. Antioxidant Enzyme Assays

Activities of catalase (CAT) and ascorbate peroxidase (APX) were determined in the leafy tissue of the NF, 1 WF, and 2 WF plants treated with 0%, 0.02%, and 0.05% NaN_3 collected at the tenth week after planting following the method of Zhang and Kirkham [45]. In brief, 0.25g of leafy tissue was homogenized in 3 mL of solution, composed of PBS (50 mM), EDTA (0.2 mM), and 1% PVP, and centrifuged. Supernatants were assayed to detect the absorbance at 290 nm (for APX) and 240 nm (for CAT).

2.10. RNA Isolation, cDNA Synthesis, and Quantitative RT-PCR

Quantitative real-time PCR (qRT-PCR) assay was conducted to evaluate the expression level of antioxidant enzyme-encoding genes (*APX*, *CAT*) in the leafy tissue of the NF, 1 WF, and 2 WF plants treated with 0%, 0.02%, and 0.05% NaN_3 collected at the tenth week after planting. Total RNA samples were isolated from the tissue following Qiagen RNeasy Plant Mini kit. DNA removal and cDNA synthesis were performed using Qiagen RNase-Free DNase Set and Qiagen Reverse Transcription kit, respectively. Quantitative RT-PCR was performed following Qiagen QuantiTect SYBR Green PCR kit protocol. PCR conditions, housekeeping gene, and gene-specific primers were used as reported by Vwioko et al. [7]. The primer pair 5′-TGCCCTTCTATTGTGGTTCC-3′ and 5′-GATGAGCACACTTTGGAGGA-3′ was used for *CAT* amplification, whereas the primer pair 5′-ACCAATTGGCTGGTGTTGTT-3′ and 5′-TCACAAACACGTCCCTCAAA-3′ was used for *APX* amplification. The primer pair 5′-TTCCTTGATGATGCTTGCTC-3′ and 5′-TTGACAGCTCTTGGGTGAAG-3′ was used for the housekeeping gene (*UBQ1*) amplification.

2.11. Statistical Analysis

Mean and standard deviation were measured for the data obtained for the different traits measured. Two-way analysis of variance was conducted using NaN_3 treatments and flooding conditions as factors. Tukey's test was conducted to determine the significance of values. Statistical analyses were performed using SPSS ver. 19 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Germination of NaN_3-Treated Seeds

The germination was first recorded for okra seeds given control (0%) treatments 2 days after planting (2 DAP). Germination was recorded for 0.02 and 0.05% NaN_3-treated seeds three days after

planting (3 DAP). Eight days after planting (8 DAP), the highest and least percentage of germination were recorded for 0% and 0.05% NaN_3-treated seeds, respectively (Figure 1). Twenty-four hours delay in germination was recorded for the NaN_3-treated seeds.

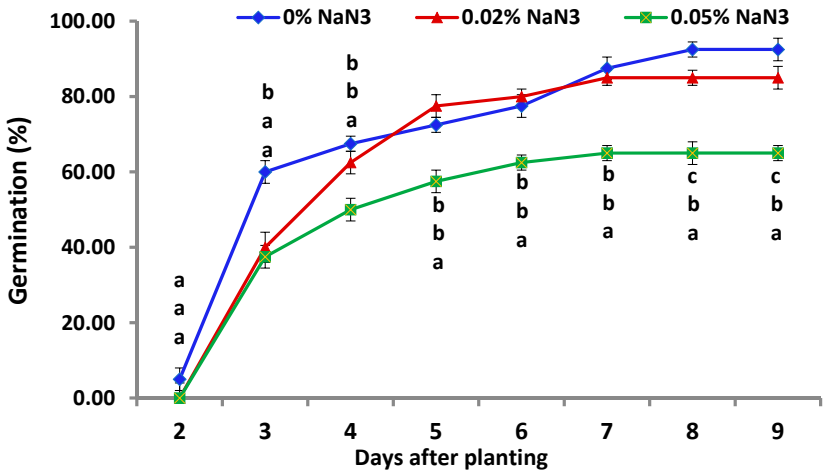


Figure 1. Percentage of germination of NaN_3-treated okra seeds sown in nursery. Values = mean ± SD, $n = 4$. Mean values with similar letters at the same day after planting (DAP) are not significantly different at $p \leq 0.05$.

3.2. Plant Height

Values obtained for plant height showed that non-waterlogged plants produced the highest values irrespective of the NaN_3 treatment given to the seeds ten weeks after planting (10 WAP). For example, mean values obtained for plant height were 31.5, 29.5, and 31.1 cm for 0%, 0.02%, and 0.05%, respectively, under non-waterlogging condition, 10 WAP (Table 1). Under one-week waterlogging condition, the values recorded for 0%, 0.02%, and 0.05% were 15.2, 21.8, and 19.4 cm, respectively, 10 WAP. Similarly, under two weeks waterlogging conditions, the values recorded for 0%, 0.02%, and 0.05% were 16.3, 22.4, and 19.9 cm, respectively, 10 WAP, indicating growth stimulations for plants grown from 0.02% and 0.05% NaN_3-treated seeds.

Table 1. Height (cm) of okra plants grown from NaN_3-treated seeds subjected to different waterlogging conditions four weeks after planting (WAP).

NaN_3 Treatment	Waterlogging Conditions	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
0%	Non-waterlogging	$8.6^{b} \pm 0.45$	$14.7^{e} \pm 0.78$	$18.8^{a} \pm 0.28$	$23.7^{a} \pm 1.19$	$31.5^{a} \pm 1.28$
	One-week waterlogging	$7.7^{c} \pm 0.47$	$17.2^{ab} \pm 0.68$	$17.9^{abc} \pm 0.43$	$18.3^{c} \pm 0.25$	$15.2^{c} \pm 0.62$
	Two weeks waterlogging	$8.3^{b} \pm 0.30$	$15.8^{cd} \pm 0.24$	$16.8^{d} \pm 0.94$	$18.9^{bc} \pm 1.05$	$19.3^{b} \pm 0.29$
0.02%	Non-waterlogging	$9.5^{a} \pm 0.62$	$12.0^{f} \pm 0.30$	$18.0^{abc} \pm 0.60$	$24.5^{a} \pm 1.31$	$29.5^{ab} \pm 0.68$
	One-week waterlogging	$8.3^{bc} \pm 0.45$	$17.9^{ab} \pm 0.66$	$18.4^{ab} \pm 0.42$	$18.8^{bc} \pm 0.47$	$21.8^{d} \pm 0.35$
	Two weeks waterlogging	$7.9^{bc} \pm 0.09$	$17.7^{ab} \pm 0.91$	$18.5^{ab} \pm 1.23$	$19.7^{b} \pm 1.30$	$22.4^{bc} \pm 0.45$
0.05%	Non-waterlogging	$8.1^{bc} \pm 0.78$	$11.9^{f} \pm 0.83$	$17.6^{bcd} \pm 0.49$	$23.5^{a} \pm 0.88$	$31.1^{a} \pm 0.98$
	One-week waterlogging	$7.9^{bc} \pm 0.21$	$17.2^{ab} \pm 0.48$	$17.7^{bcd} \pm 0.63$	$18.0^{c} \pm 0.72$	$19.4^{c} \pm 1.60$
	Two weeks waterlogging	$7.8^{bc} \pm 0.22$	$16.1^{bc} \pm 0.28$	$17.1^{cd} \pm 0.20$	$18.1^{c} \pm 0.71$	$19.9^{c} \pm 0.34$

Values = mean ± S.D., $n = 4$, WAP = weeks after planting. Mean values with similar letters as superscript in one column are not significantly different at $p \leq 0.05$.

3.3. Stem Girth

The highest stem girth values were obtained for okra plants grown under non-waterlogging conditions (Table 2). Ten weeks after planting, the values recorded for the stem girth of okra plants grown under two-week waterlogging conditions were statistically significant compared to those recorded for plants grown under and non-waterlogging conditions (Table 2).

Table 2. Stem girth (cm) of okra plants grown from NaN_3-treated seeds subjected to different waterlogging conditions four weeks after planting (WAP).

NaN_3 Treatment	Waterlogging Conditions	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
0%	Non-waterlogging	0.81 ^{a} ± 0.02	0.95 ^{c} ± 0.05	1.10 ^{b} ± 0.08	1.25 ^{a} ± 0.05	1.35 ^{a} ± 0.05
	One-week waterlogging	0.80 ^{a} ± 0.01	1.02 ^{b} ± 0.09	1.07 ^{b} ± 0.09	1.15 ^{b} ± 0.05	1.27 ^{b} ± 0.05
	Two weeks waterlogging	0.85 ^{a} ± 0.05	1.17 ^{a} ± 0.05	1.27 ^{a} ± 0.05	1.27 ^{a} ± 0.05	1.27 ^{b} ± 0.05
0.02%	Non-waterlogging	0.76 ^{b} ± 0.05	1.02 ^{b} ± 0.09	1.10 ^{c} ± 0.08	1.25 ^{a} ± 0.05	1.37 ^{a} ± 0.05
	One-week waterlogging	0.89 ^{a} ± 0.09	1.12 ^{a} ± 0.05	1.17 ^{b} ± 0.05	1.25 ^{a} ± 0.05	1.37 ^{a} ± 0.05
	Two weeks waterlogging	0.80 ^{b} ± 0.08	1.15 ^{a} ± 0.05	1.27 ^{a} ± 0.05	1.27 ^{a} ± 0.05	1.30 ^{b} ± 0.00
0.05%	Non-waterlogging	0.75 ^{b} ± 0.05	1.05 ^{b} ± 0.05	1.17 ^{b} ± 0.05	1.30 ^{a} ± 0.08	1.45 ^{a} ± 0.05
	One-week waterlogging	0.82 ^{a} ± 0.07	1.07 ^{b} ± 0.05	1.20 ^{b} ± 0.08	1.20 ^{b} ± 0.08	1.32 ^{b} ± 0.09
	Two weeks waterlogging	0.85 ^{a} ± 0.05	1.17 ^{a} ± 0.09	1.27 ^{a} ± 0.05	1.27 ^{a} ± 0.05	1.32 ^{b} ± 0.05

Values = mean ± S.D., n = 4, WAP = weeks after planting. Mean values with similar letters as superscript in one column are not significantly different at $p \leq 0.05$.

3.4. Number of Leaves Formed, Number of Adventitious Roots Produced, and Percentage of Survival of Plants

The total number of leaves formed per plant recorded indicated that the plants grown under non-waterlogging condition produced the highest number of leaves 10 WAP. The combination of waterlogging conditions and NaN_3 treatments gave higher values for number of leaves formed than when the waterlogging condition is applied only (Table 3). For example, total number of leaves under non-waterlogging conditions were 16, 16.5, and 16.5 for 0%, 0.02%, and 0.05%, respectively. Whereas in one-week waterlogging conditions, values were 13, 14, and 15 for plants grown from 0%, 0.02%, and 0.05% NaN_3-treated seeds.

Table 3. Number of leaves, average number of adventitious roots produced, and survival percentage of okra plants grown from NaN_3-treated seeds under waterlogging conditions 10 WAP.

NaN_3 Treatment	Waterlogging Conditions	No. Leaves per Plant	No. Adventitious Roots per Plant	Survival Percentage
0%	Non-waterlogging	16.0 ^{a} ± 2.30	0 ^{c}	100.0 ^{a} ± 0.00
	One-week waterlogging	13.0 ^{b} ± 1.10	10.7 ^{b} ± 7.18	33.3 ^{b} ± 27.22
	Two weeks waterlogging	12.0 ^{b} ± 0.00	13.0 ^{a} ± 8.67	25.0 ^{b} ± 16.67
0.02%	Non- waterlogging	16.5 ^{a} ± 1.00	0 ^{c}	100.0 ^{a} ± 0.00
	One-week waterlogging	14.0 ^{b} ± 1.60	15.5 ^{b} ± 1.29	50.0 ^{b} ± 19.25
	Two weeks waterlogging	13.0 ^{b} ± 1.15	21.0 ^{a} ± 0.81	33.3 ^{c} ± 0.00
0.05%	Non- waterlogging	16.5 ^{a} ± 1.00	0 ^{c}	100.0 ^{a} ± 0.00
	One-week waterlogging	15.0 ^{b} ± 1.15	18.0 ^{b} ± 1.63	50.0 ^{b} ± 19.25
	Two weeks waterlogging	13.0 ^{c} ± 1.15	22.2 ^{a} ± 1.25	50.0 ^{b} ± 19.25

Values = mean ± S.D., n = 4, WAP = weeks after planting. Mean values with similar letters as superscript in one column are not significant different at $p \leq 0.05$.

Plants did not form adventitious roots under non-waterlogging conditions. However, the production of adventitious roots was observed in plants subjected to waterlogging condition. Plants subjected to two weeks of waterlogging condition initiated higher numbers of adventitious roots (Table 3). Furthermore, plants grown from 0.05% NaN_3-treated seeds produced the highest number of adventitious roots recorded. The combination of NaN_3 concentration and waterlogging condition supported the greater production of adventitious roots in okra.

Ten weeks after planting, the number of plants that survived the waterlogging conditions is shown in Table 3. Higher percentage of survival was recorded with the combination of sodium azide and waterlogging condition. For example, under two weeks waterlogging condition, the percentage of survival of okra plants were 25, 33.3, and 50 for 0%, 0.02%, and 0.05% NaN_3-treated seeds, respectively. Similarly, for one-week waterlogging condition, percentage of survival of okra plants were 33.3, 50, and 50 for 0%, 0.02%, and 0.05% NaN_3-treated seeds, respectively.

3.5. Number of Flower Buds, Flowers, and Fruits Produced

The number of flower buds, flowers, and fruits are shown in Table 4. The waterlogging condition caused a decrease in all the reproductive parameters considered. For example, the average number of flower buds recorded for plants grown from control seeds (0.00% NaN_3 treatment) were 5.5, 2.75, and 1.75 for NF, 1 WF, and 2 WF conditions, respectively. Similarly, average number of flowers recorded for the same plants were 5, 2, and 1, respectively. Moreover, the average number of fruits recorded for the same plants were 4.5, 1.25, and 0.5, respectively. The average number of flower buds, flowers, and fruits recorded for plants grown from 0.05% NaN_3-treated seeds and subjected to waterlogging conditions were higher than those recorded for non-treated plants.

Table 4. Average number of flower buds, flowers, and fruits formed per plant of okra grown from NaN3 treated seeds subjected to waterlogging conditions ten weeks after planting.

NaN_3 Treatment	Waterlogging Conditions	Number of Flower Buds	Number of Flowers	Number of Fruits
0%	Non-waterlogging	5.5 ^{a} ± 0.57	5.0 ^{a} ± 0.81	4.5 ^{a} ± 0.57
	One-week waterlogging	2.7 ^{b} ± 1.25	2.0 ^{b} ± 1.41	1.2 ^{b} ± 0.95
	Two weeks waterlogging	1.7 ^{b} ± 1.25	1.0 ^{b} ± 0.81	0.5 ^{b} ± 0.57
0.02%	Non-waterlogging	5.0 ^{a} ± 0.81	5.0 ^{a} ± 0.81	3.5 ^{a} ± 1.29
	One-week waterlogging	2.5 ^{b} ± 0.57	1.7 ^{b} ± 0.50	1.5 ^{b} ± 1.00
	Two weeks waterlogging	1.7 ^{b} ± 0.5	1.2 ^{b} ± 0.95	1.2 ^{b} ± 0.95
0.05%	Non-waterlogging	5.0 ^{a} ± 0.81	4.5 ^{a} ± 1.29	3.7 ^{a} ± 1.89
	One-week waterlogging	3.2 ^{b} ± 0.95	2.5 ^{b} ± 0.57	2.2 ^{b} ± 0.91
	Two weeks waterlogging	2.2 ^{b} ± 0.95	1.5 ^{b} ± 0.57	1.5 ^{b} ± 0.57

Values = mean ± S.D., $n = 4$. Values with similar letters as superscript are not significantly different.

3.6. Soil Microflora Counts

The average values obtained for bacteria and fungi counts are shown in Table S1. The bacterial counts were higher than fungal counts in all soil samples analyzed. The bacterial count values were higher in soils collected from waterlogging condition, while the fungal count values were higher in soils collected from non-waterlogging condition. Soils collected from two-week waterlogging conditions gave the least fungal counts.

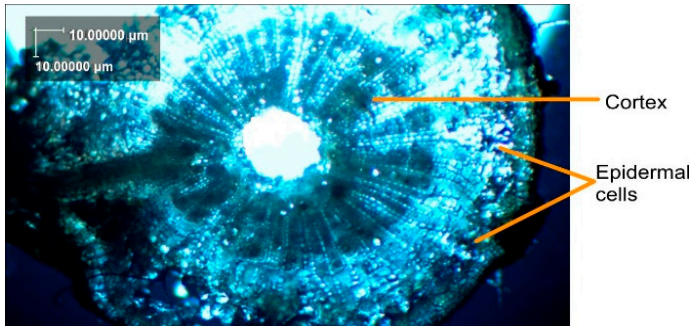
3.7. Soil Chemical Analysis

There were clear differences in many of the soil chemical parameters analyzed between soil samples collected from non-waterlogging and waterlogging experimental pots (Table S2). The differences in values obtained shows a regular pattern. For example, pH values for NF were 6.0–6.1 while higher values were recorded for 1 WF and 2 WF. Redox potential (Eh) values were consistently higher for NF than 1 WF and 2 WF. Soil Eh ranged from 23.60–24.10 for NF and 7.2–7.4 for 1 WF and 2 WF. The highest values of sulphate ion (SO_4) concentrations and electrolyte conductivity (EC) readings were observed in 1 WF soil samples. Mean values for non-treated soil EC were 228, 413, and 125 µS/cm for NF, 1 WF, and 2 WF, respectively. Similarly, mean values for SO_4 concentration in non-treated soil were 0.52, 1.13, and 0.80 mg/Kg for NF, 1 WF, and 2 WF, respectively. Organic carbon, total nitrogen and available phosphorus contents in soil followed the same reduction pattern under one- and two-week waterlogging conditions. Approximately, 10-fold reductions in organic carbon and total nitrogen contents were observed under waterlogging conditions. The records for soil metallic factors like Fe, Zn, and Mn showed the same pattern where the values were higher in soil samples collected from one- and two-week waterlogging conditions. Mean values obtained for Fe were 116.3, 242.1, and 243.3 mg/kg for NF, 1 WF, and 2 WF, respectively, for soil samples collected from pots where 0% NaN_3 plants were grown. The mean values recorded for Zn in soil samples collected from pots containing 0% NaN_3 plants were 14.2, 22.7, and 35.4 mg/kg for NF, 1 WF, and 2 WF, respectively. The mean values of Mn in

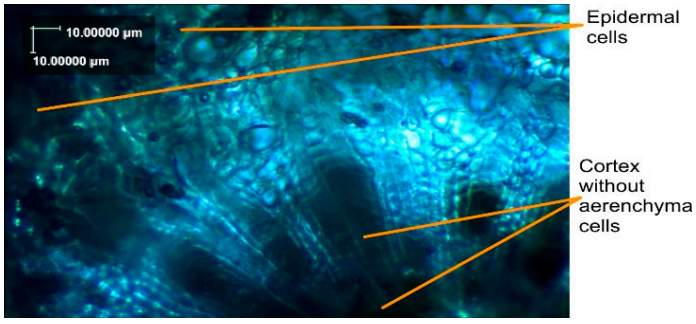
the same soil samples were 1.34, 9.68, and 12.9 mg/kg for NF, 1 WF, and 2 WF, respectively. The mean values of total phenol content show low variation.

3.8. Anatomy of Okra Roots

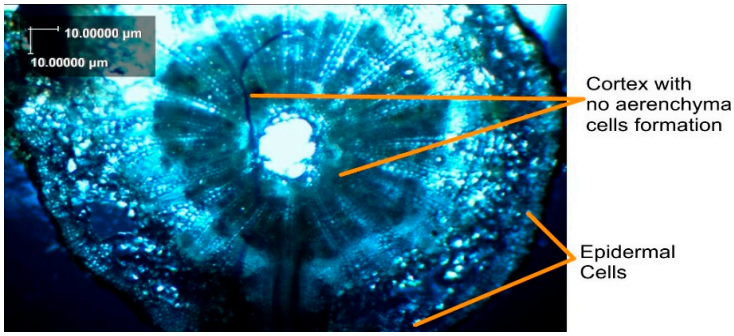
There were structural differences in the anatomy of okra root sections obtained from non-waterlogged and waterlogged plants (Figures 2–4). The presence of air channels (lacunae) was conspicuously absent in non-waterlogged root sections (Figure 2). The development of aerenchyma in the cortex and stele were very conspicuous in root sections of plants subjected to waterlogging conditions (Figures 3 and 4). Furthermore, the aerenchyma cells observed in root sections of waterlogged plants were larger in plants grown from 0.05% NaN_3-treated seeds than those from 0.02% NaN_3-treated seeds (Figures 3 and 4). This suggests an explanation for the higher percentage of survival recorded for plants grown from 0.05% NaN_3-treated seeds. The walls of the aerenchyma cells are thick to prevent their collapse.



(**A**)



(**B**)



(**C**)

Figure 2. Root sections of okra plants grown from different concentrations of NaN_3-treated seeds show no aerenchyma cells formed under non-waterlogging conditions. (**A**) 0% NaN_3, (**B**) 0.02% NaN_3, (**C**) 0.05% NaN_3.

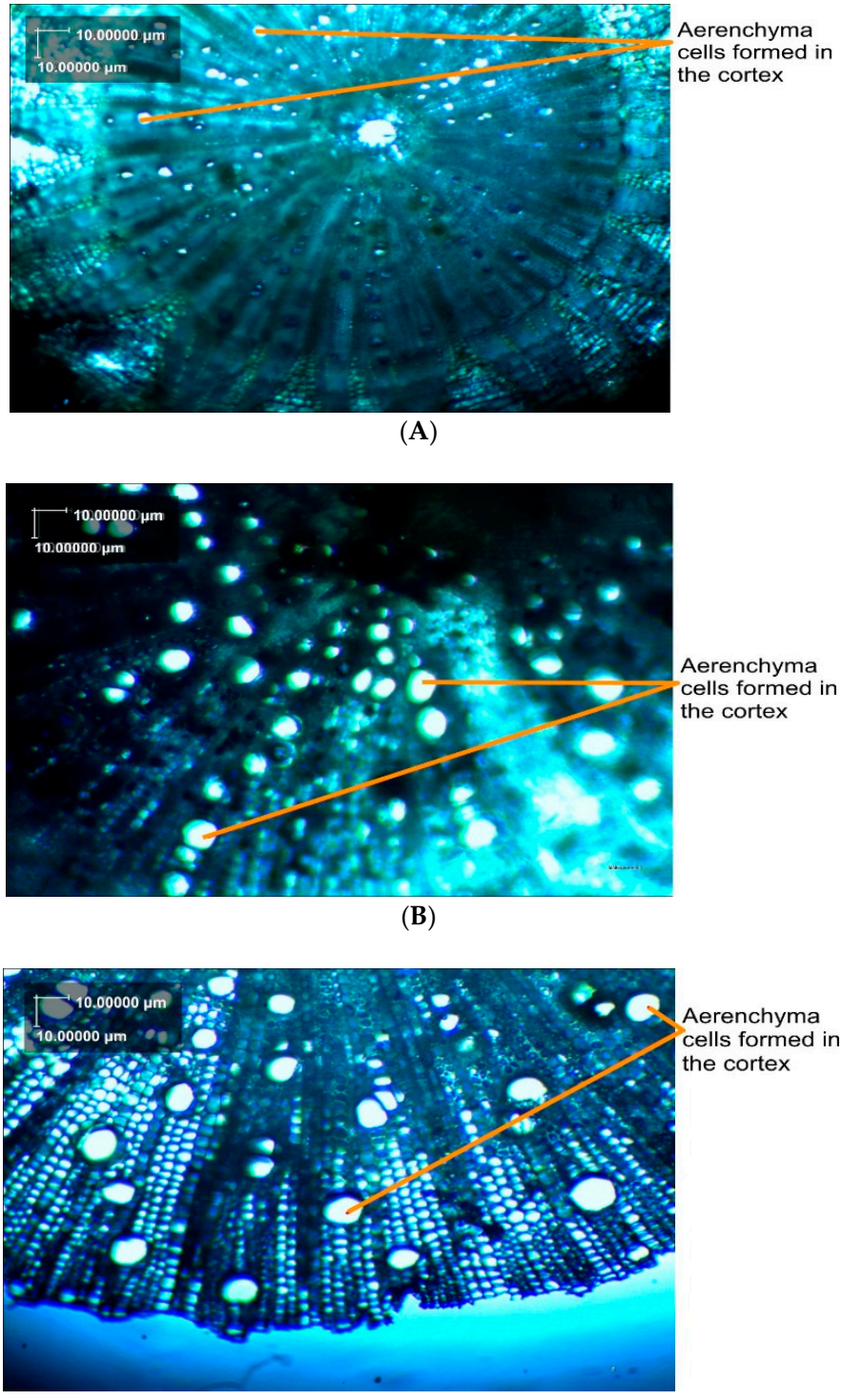
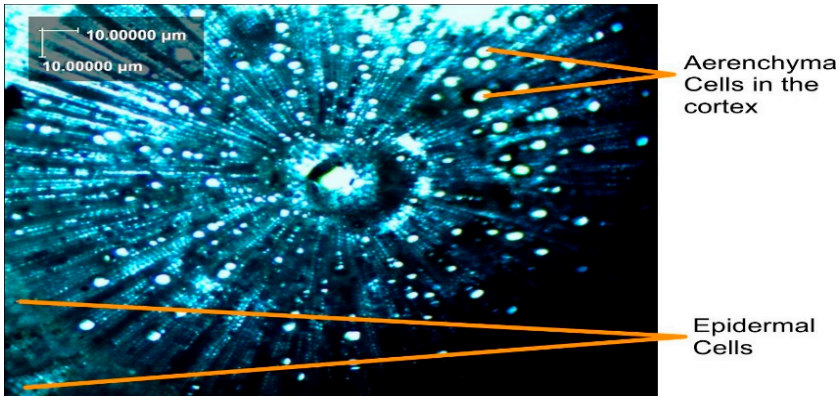
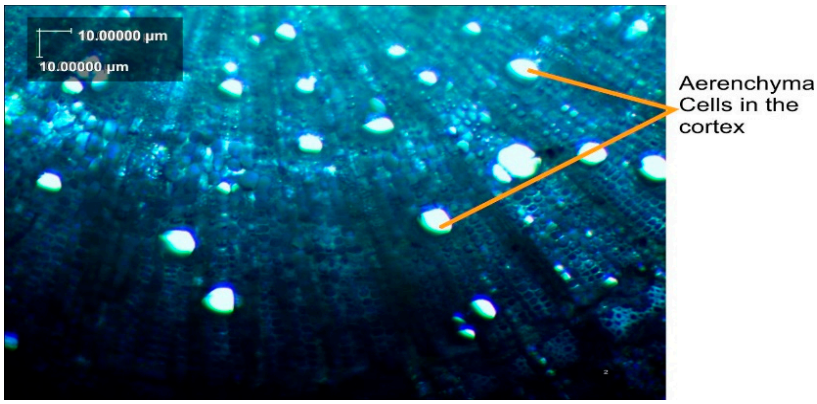


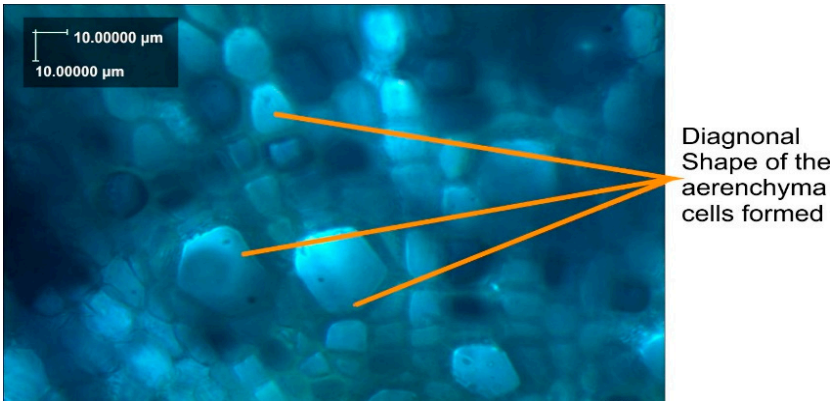
Figure 3. Root sections of okra plants grown from different concentrations of NaN_3-treated seeds show aerenchyma cells formed under one-week waterlogging conditions. (**A**) 0% NaN_3, (**B**) 0.02% NaN_3, (**C**) 0.05% NaN_3.



(**A**)



(**B**)



(**C**)

Figure 4. Root sections of okra plants grown from different concentrations of NaN_3-treated seeds show aerenchyma cells formed under two-week waterlogging conditions. (**A**) 0% NaN_3, (**B**) 0.02% NaN_3, (**C**) 0.05% NaN_3.

3.9. Antioxidant Enzymes Activity and Gene Expression Analyses

The effects of the waterlogging condition and NaN_3 treatments on the activities and expression levels of antioxidant enzymes (APX, CAT) in the leaf tissues were investigated. The activity and expression level of APX enzyme were significantly enhanced in plants exposed to waterlogging and sodium azide treatments with respect to non-treated (control) plants (Figure 5). Additionally, under waterlogging conditions, the activity and expression level of CAT enzyme were slightly enhanced in plants treated with sodium azide, as compared to non-treated plants (Figure 5).

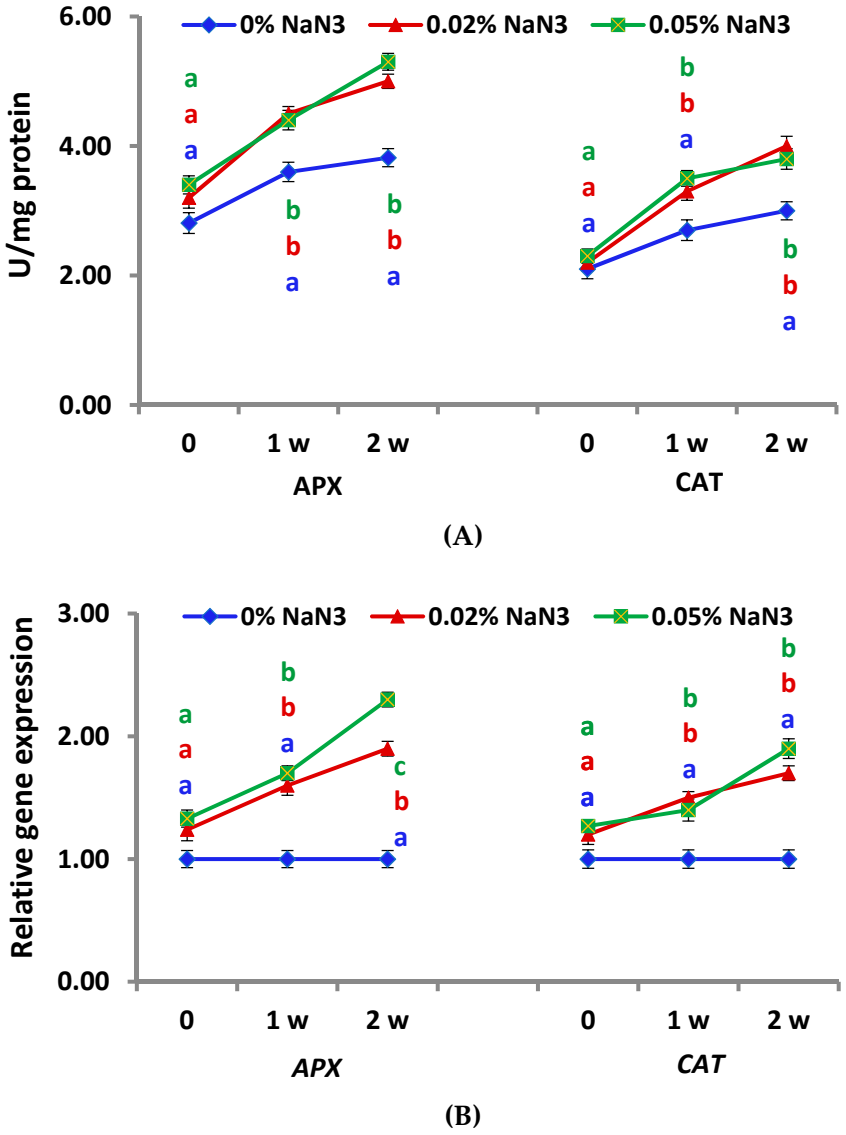


Figure 5. Activity (**A**) and gene expression levels (**B**) of APX and CAT enzymes in okra plants grown from NaN3-treated seeds under waterlogging conditions ten weeks after planting (WAP). Values = mean ± SD, $n = 4$. Mean values with similar letters at the same WAP are not significantly different at $p \leq 0.05$.

4. Discussion

Waterlogging stress has adverse impacts on crop development and productivity. Waterlogging-induced oxygen depletion results in changes in plant morphology and metabolism. Waterlogging conditions also cause inhibition of photosynthesis, leaf chlorophyll degradation, and early leaf senescence [46]. Negative impacts of flooding might be due to the reduced level of gas diffusion in water, which does not allow terrestrial plants to survive for a long period. Plants develop specific traits to improve gas exchange and cope with waterlogging conditions. These traits include formation of adventitious roots and aerenchyma cells, as well as elongation of stem root juncture above the water surface. These efficiently ameliorate the stress-induced hypoxic or anoxic conditions. The presence of aerenchyma cells facilitates exchange of gases between aerial and submerged plant parts [47]. Kawai et al. [48] proposed that the development of aerenchyma in tissues and organs decreases the number of cells requiring oxygen for respiration. However, the development of adaptive traits to waterlogging stress is species-dependent [7,49,50]. Enhanced formation of aerenchyma was observed upon treating rice plants with exogenous ethylene [14].

In the present study, NaN_3 treatments enhanced waterlogging stress tolerance and aerenchyma formation in okra. The results also showed that NaN_3 treatments affected okra germination. NaN_3-caused seed germination inhibition has also been reported in different plant species [22,23,51,52]. However, NaN_3 stimulated the germination of okra plants [24]. This germination inhibition was dependent on the concentration of NaN_3 used as seed treatment. Three days after planting (3 DAP), germination has been recorded in all NaN_3 treatments applied. Under waterlogging conditions, NaN_3 promoted okra growth 10 WAP, indicating that plants grown from 0.02% NaN_3-seed treatments exhibited better performance than those grown from 0.05% NaN_3-seed treatments. These findings were in a harmony with that reported by Al-Qurainy [25] and Zuzana et al. [26] who stated that NaN_3 could stimulate the plant growth and height of *Eruca sativa* and *Diospyros lotus*, respectively. Moreover, the difference in the number of leaves formed under waterlogging and non-waterlogging conditions was significant. Plants grown from NaN_3-treated seeds formed more leaves than those from non-treated seeds. Additionally, plants that were grown from 0.02% and 0.05% NaN_3-treated seeds produced a greater number of adventitious roots under waterlogging conditions. The emergence of adventitious roots is preceded by epidermal cell death at the nodes of submerged rice plants [47]. The activities leading to epidermal cell death for the emergence of adventitious roots occurred more in plants grown from NaN_3-treated seeds. Waterlogging conditions negatively affected the reproductive parameters recorded for okra plants in the current study. These findings are in harmony with that reported by Vwioko et al. [7]. Plants grown from 0.05% NaN_3-treated seeds formed a higher number of buds than plants produced from 0% NaN_3-treated seeds subjected to two-week waterlogging conditions. Plants grown from 0.05% NaN_3-treated seeds also produced more fruits than the control plants under two-week waterlogging conditions.

Waterlogging conditions cause depletion of soil oxygen due to microbial respiration. The reduction of soil oxygen urges anaerobic microorganisms to shift to alternative electron acceptors for their metabolic requirements [53]. Bacteria and fungi ratio in soil community are altered whenever there are soil inundations. Soil bacteria and fungi have a critical role in decomposition and nutrient cycling [54]. In the current investigation, microbial count results exhibited an increase in the bacteria populations and reduction in the fungi populations. The decrease in fungi populations has been previously reported [53,55–57]. Therefore, under waterlogging conditions, fungi presence is less prevalent than bacteria. Fungi require aerobic conditions to thrive but are inhibited by the scarcity of oxygen in the flooded soil environments. Fungi germinate from spores under flooding slowly, resulting in a decreased colonization. Unger et al. [53] suggested that some microbial groups may thrive well under flooded conditions. Gram-positive bacteria showed higher levels compared to Gram-negative bacteria under waterlogging conditions. Mentzer et al. [57] reported that flooding exhibited greater effect than nutrient loading on the microbial community and profoundly altered the composition and functional components.

Water copiously influences several physicochemical processes in soil, particularly under flooded conditions. This begins with the cutoff of oxygen supply to soil environments under waterlogging stress. The lack of oxygen promotes anaerobic metabolism by microbes through utilizing a decomposable organic matter. A reduction in soil redox potential and an increase in pH are recorded [58]. The soil Eh data recorded in a soil-water suspension rightly predicts the level of transformations present in the waterlogged soil [59]. Other important chemical changes in flooded soils indicate the prevalence of reduced forms of nitrogen, oxygen, iron, manganese, or sulphur in soil [53]. There are changes in phase or solubility because of redox reactions. For example, nitrate-nitrogen is transformed into gaseous forms (N_2, NO_2, N_2O) and lost, resulting in nitrogen depletion of soil [60]. In the present study, the soil chemical analysis showed that waterlogging conditions increased pH towards neutral, reduced soil Eh, organic carbon, total nitrogen and available phosphorus. These soil factors indicate higher reduction-oxidation reactions in soils under waterlogging conditions. These patterns of chemical environments and transformations are suspected to favor the tolerant bacteria for their higher counts recorded in waterlogged soil samples. The chemical environments attained under waterlogging

soil conditions met the metabolic needs of tolerant bacteria. The decomposition of complex organic compounds is slow under anoxic conditions and in some cases leads to detection of higher amounts of phenolics [53] in waterlogged than in non-waterlogged soils. The present study does not reveal changes in the total phenolics of soil samples, suggesting that either the soil is devoid of complex organics for microbes to degrade under waterlogging conditions, or the microbes utilized readily available forms of carbons that are root exudates. Carbon enters the soil profile via the decomposition of plant residue on the surface or via root exudates in the upper soil horizon [53].

In the current study, root anatomy showed some peculiar features with waterlogged plants. Plants did not develop air-chambers in the cortex and stele regions under non-waterlogging conditions. However, plants subjected to waterlogging conditions formed aerenchyma cells. Further examination of the micrographs showed that plants grown from NaN_3-treated seeds produce more aerenchyma cells than those grown from untreated seeds. It was evident that 0.05% NaN_3-treated seeds produce plants with the highest aerenchyma development and increased with increasing the duration of waterlogging conditions. The formation of aerenchyma in the root as an adaptive trait contributed to the survival of okra plants exposed to waterlogging conditions. Furthermore, under waterlogging conditions, the activities and expression levels of APX and CAT enzymes were enhanced in plants treated with NaN_3 compared to non-treated plants in the present study. The survival of plants in stressed environments might be attributed to the induction of expression levels of antioxidant compounds. Salim et al. [34] reported that NaN_3-treated seeds produce mutant plants that showed higher antioxidation capacities than the normal plants. Moreover, Jeng et al. [61] revealed that these mutants induced increased antioxidant capacities through the generation of scavenging metabolics (DPPH, LPI ability, FRAP, and ABTS radical scavenging activities) than the wild type. In addition, the antioxidant enhancements could be linked to the accumulation of phenolics, anthocyanin, and proanthocyanidins at higher levels in the seed coats. These results are in harmony with that reported by Elfeky et al. [62] who stated that *Helianthus annus* plants grown from NaN_3-treated seeds initiated and induced higher antioxidant capacities than those grown from untreated seeds via increasing carotenoids, peroxidase activity, and protein content. In conclusion, sodium azide priming could enhance waterlogging stress tolerance in okra plants through enhancing the growth and reproductive parameters, inducing the formation of adventitious roots and aerenchyma cells, and increasing the activities and gene expression level of antioxidant enzymes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/11/679/s1, Table S1: Total bacteria and fungi count of soil samples analyzed after plant harvest, Table S2: Values obtained for soil factors in soil samples collected from different experimental pots after plant growth under waterlogging conditions.

Author Contributions: M.A.E.-E. and E.D.V. designed and performed the experiments, analyzed the data, and wrote and revised the manuscript. M.E.I., A.A.A.-G., H.M.A., E.A.A., and M.A.A.-D. helped with analysis and revision of the manuscript. M.M.E.-S. revised the manuscript. All the authors approved the final version of the manuscript.

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References

1. Gemede, H.F.; Ratta, N.; Haki, G.D.; Woldegiorgis, A.Z.; Bey, F. Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A Review. *Int. J. Nut. Food Sci.* **2015**, *4*, 208–215. [CrossRef]
2. El-Esawi, M.A.; Al-Ghamdi, A.A.; Ali, H.M.; Alayafi, A.A.; Witczak, J.; Ahmad, M. Analysis of Genetic Variation and Enhancement of Salt Tolerance in French Pea. *Int. J. Mol. Sci.* **2018**, *19*, 2433. [CrossRef] [PubMed]
3. El-Esawi, M.A.; Al-Ghamdi, A.A.; Ali, H.M.; Ahmad, M. Overexpression of *AtWRKY30* Transcription Factor Enhances Heat and Drought Stress Tolerance in Wheat (*Triticum aestivum* L.). *Genes* **2019**, *10*, 163. [CrossRef] [PubMed]

4. El-Esawi, M.A.; Elkelish, A.; Elansary, H.O.; Ali, H.M.; Elshikh, M.; Witczak, J.; Ahmad, M. Genetic transformation and hairy root induction enhance the antioxidant potential of *Lactuca serriola* L. *Oxid. Med. Cell. Longev.* **2017**, *2017*. [CrossRef]
5. El-Esawi, M.A.; Alayafi, A.A. Overexpression of Rice *Rab7* Gene Improves Drought and Heat Tolerance and Increases Grain Yield in Rice (*Oryza sativa* L.). *Genes* **2019**, *10*, 56. [CrossRef] [PubMed]
6. El-Esawi, M.A.; Alayafi, A.A. Overexpression of *StDREB2* Transcription Factor Enhances Drought Stress Tolerance in Cotton (*Gossypium barbadense* L.). *Genes* **2019**, *10*, 142. [CrossRef]
7. Vwioko, E.; Adinkwu, O.; El-Esawi, M.A. Comparative physiological, biochemical and genetic responses to prolonged waterlogging stress in okra and maize given exogenous ethylene priming. *Front. Physiol.* **2017**, *8*, 632. [CrossRef]
8. Heschbach, C.; Mult, S.; Kreuzwieser, J.; Kopriva, S. Influence of anoxia on whole plant sulphur nutrition of flooding tolerant poplar (*Populus tremula* × *P. alba*). *Plant Cell Environ.* **2005**, *28*, 167–175. [CrossRef]
9. Herrera, A.; Tezara, W.; Marin, O.; Rengifo, E. Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. *Physiol. Plant.* **2008**, *134*, 41–48. [CrossRef]
10. Syversten, J.P.; Zablotowicz, R.M.; Smith, M.L. Soil-temperature and flooding effects on two species of citrus. 1. Plant growth and hydraulic conductivity. *Plant Soil* **1983**, *72*, 3–12.
11. Setter, T.L.; Waters, I.; Sharma, S.K.; Singh, K.N.; Kulshreshtha, N.; Yaduvanshi, N.P.S.; Ram, P.C.; Singh, B.N.; Rane, J.; McDonald, G.; et al. Review of wheat improvement for waterlogging tolerance in Australia and India: The importance of anaerobiosis and element toxicities associated with different soils. *Ann. Bot.* **2009**, *103*, 221–235. [CrossRef] [PubMed]
12. Calvo-Polanco, M.; Senorans, J.; Zwiazek, J.J. Role of adventitious roots in water relations of tamarack (*Larix* laricina) seedlings exposed to flooding. *BMC Plant Biol.* **2012**, *12*, 99–107. [CrossRef] [PubMed]
13. Sauter, M. Root responses to flooding. *Curr. Opin. Plant Biol.* **2013**, *16*, 282–286. [CrossRef] [PubMed]
14. Takahashi, H.; Yamauchi, T.; Colmer, T.; Nakazono, M. Aerenchyma Formation in Plants. In *Low-Oxygen Stress in Plants, Oxygen Sensing and Adaptive Responses to Hypoxia*, 1st ed.; Van Dongen, J.T., Licausi, F, Eds.; Plant Cell Monographs; Springer: New York, NY, USA, 2014; Volume 21, pp. 247–265.
15. Janda, T.; Szalai, G.; Tari, I.; Paldi, E. Hydroponic treatments with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. *Planta* **1999**, *208*, 175–180. [CrossRef]
16. Rajasekaran, L.R.; Blake, T.J. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. *J. Plant Growth Reg.* **1999**, *18*, 171–181. [CrossRef]
17. Gondor, O.K.; Pál, M.; Darkó, É.; Janda, T.; Szalai, G. Salicylic Acid and Sodium Salicylate Alleviate Cadmium Toxicity to Different Extents in Maize (*Zea mays* L.). *PLoS ONE* **2016**, *11*, e0160157. [CrossRef]
18. Vwioko, E.D. Performance of soybean (*Glycine max* L.) in salt-treated soil environment following salicylic acid mitigation. *NISEB J.* **2013**, *13*, 44–49.
19. Jackson, M.B. Ethylene-promoted elongation: An adaptation to submergence stress. *Ann. Bot.* **2008**, *101*, 229–248. [CrossRef]
20. Vidoz, M.L.; Loreti, E.; Mensuali, A.; Alpi, A.; Perata, P. Hormonal interplay during adventitious root formation in flooded tomato plants. *Plant J.* **2011**, *63*, 551–562. [CrossRef]
21. Sasidharan, R.; Voesenek, L.A.C.J. Ethylene-mediated acclimations to flooding stress. *Plant Physiol.* **2015**, *169*, 3–12. [CrossRef]
22. Gnanamurthy, S.; Dhanavel, D.; Girija, M.; Pavadai, P.; Bharathi, T. Effect of chemical mutagenesis on quantitative traits of maize (*Zea mays* (L.). *Int. J. Res. Bot.* **2012**, *2*, 34–36.
23. Shagufta, B.; Aijaz, A.W.; Irshad, A.N. Mutagenic sensitivity of gamma rays, EMS and sodium azide in *Trigonella foenumgraecum* L. *Sci. Res. Rep.* **2013**, *3*, 20–26.
24. Vwioko, D.E.; Onobun, E. Vegetative response of ten accessions of *Abelmoschus esculentus* (L) Moench. treated with sodium azide. *J. Life Sci. Res. Dis.* **2015**, *2*, 13–24.
25. Al-Qurainy, F. Effects of sodium azide on growth and yield traits of *Eruca sativa* (L.). *World Appl. Sci. J.* **2009**, *7*, 220–226.
26. Zuzana, K.; Katarína, R.; Elena, Z.; Maria, L.B.; Ján, B. Sodium azide induced morphological and molecular changes in persimmon (*Diospyros lotus L.*). *Agriculture* **2012**, *58*, 57–64.
27. Adamu, A.K.; Aliyu, H. Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill.). *Sci. World J.* **2007**, *2*, 9–12.

28. Gruszka, D.; Szarejko, L.; Maluszynski, M. Sodium azide as a mutagen. In *Plant Mutation Breeding and Biotechnology*; Shu, Q., Forster, B.P., Nakagawa, H., Eds.; CABI Publishing Company: Wallingford, UK, 2012; pp. 159–166.
29. Kravchik, M.; Bernstein, N. Effects of salinity on the transcriptome of growing maize leaf cells points at differential involvement of the antioxidative response in cell growth restriction. *BMC Genom.* **2013**, *16*, 14–24.
30. Mittler, R. Oxidative stress, antioxidant and stress tolerance: A review. *Trends Plant Sci.* **2002**, *7*, 405–410. [CrossRef]
31. Haq, I.U.; Memon, S.; Gill, N.P.; Rajput, M.T. Regeneration of plantlets under NaCl-stress from NaN_3 treated sugarcane explants. *Afr. J. Biotechnol.* **2011**, *10*, 16152–16156.
32. El Kaaby, E.A.J.; Al-Ajeel, S.A.; Al-Anny, J.A.; Al-Aubaidy, A.A.; Ammar, K. Effect of the chemical mutagen sodium azide on plant regeneration of two tomato cultivars under salinity stress condition in vitro. *J. Life Sci.* **2015**, *9*, 25–31. [CrossRef]
33. Kuasha, M.; Nasiruddin, K.M.; Hassan, L. Effects of sodium azide on callus in sugarcane. *Discovery* **2016**, *52*, 1683–1688.
34. Salim, K.; Fahad, A.-Q.; Firoz, A. Sodium azide: A chemical mutagen for enhancement of agronomic traits of crop plants. *Int. J. Sci. Tech.* **2009**, *4*, 1–21.
35. Ademoroti, C.A. *Standard Methods for Water and Effluent Analysis*, 1st ed.; Foludex Press Ltd.: Ibadan, Nigeria, 1996.
36. Bremner, J.M. Determination of nitrogen in soil by the Kjeldahl method. *J. Agric. Sci.* **1960**, *55*, 11–33. [CrossRef]
37. Blum, U. Benefits of citrate over EDTA for extracting phenolics from soils and plant debris. *J. Chem. Ecol.* **1997**, *23*, 347–362. [CrossRef]
38. Appiah, M.R.; Ahenkorah, Y. Determination of available sulphate in some soils of Ghana considering five extraction methods. *Biol. Fertil. Soils* **1989**, *8*, 80–86. [CrossRef]
39. Ben Mussa, S.A.; Elferjani, H.S.; Haroun, F.A.; Abdelnabi, F.F. Determination of available nitrate, phosphate and sulphate in soil samples. *Int. J. PharmTech Res.* **2009**, *1*, 598–604.
40. Bray, R.H.; Kurtz, L.T. Determination of total organic carbon and available phosphorus in soils. *Soil Sci.* **1945**, *59*, 39–48. [CrossRef]
41. Bremner, J.M.; Jenkinson, D.S. Determination of organic carbon in soil. I. oxidation by dichromate of organic matter in soil and plant materials. *J. Soil Sci.* **1960**, *11*, 394–402. [CrossRef]
42. Islam, M.S.; Halim, M.A.; Safiullah, S.; Islam, M.S.; Islam, M.M. Analysis of organic matter, ion and manganese in soil of arsenic affected Singair Area, Bangladesh. *Res. J. Environ. Toxicol.* **2009**, *3*, 31–35.
43. Harrigan, W.F.; McCance, M.E. *Laboratory Methods in Foods and Dairy Microbiology*, 8th ed.; Academic Press: London, UK, 1990.
44. Holt, J.G.; Sneath, P.H.; Krieg, N.R. *Bergey's Manual of Determinative Bacteriology*, 9th ed.; Lippincott, Williams and Wilkins Publishers: Baltimore, MD, USA, 2002; p. 787.
45. Zhang, J.; Kirkham, M.B. Enzymatic Responses of the Ascorbate-Gluta-thione Cycle to Drought in Sorghum and Sunflower Plants. *Plant Sci.* **1996**, *113*, 139–147. [CrossRef]
46. Zou, X.; Hu, C.; Zeng, L.; Xu, M.; Zhang, X. A comparison of screening methods to identify waterlogging tolerance in the field in *Brassica napus* (L.) during plant ontogeny. *PLoS ONE* **2014**, *9*, e89731. [CrossRef] [PubMed]
47. Steffens, B.; Geske, T.; Sauter, M. Aerenchyma formation in the rice stem and its promotion by H_2O_2. *New Phytol.* **2011**, *190*, 369–378. [CrossRef] [PubMed]
48. Kawai, M.; Samarajeewa, P.K.; Barrero, R.A.; Nishigushi, M.; Uchimiya, H. Cellular dissection of the degradation pattern of cortical cell death during aerenchyma formation of rice roots. *Planta* **1998**, *204*, 277–287. [CrossRef]
49. Fukao, T.; Xu, K.; Ronald, P.C.; Bailey-Serres, J. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* **2006**, *18*, 2021–2034. [CrossRef] [PubMed]
50. Hattori, Y.; Nagai, K.; Furukawa, S.; Song, X.-J.; Kawano, R.; Sakakibara, H.; Wu, J.; Matsumoto, T.; Yoshimura, A.; Kitano, H.; et al. The ethylene response factors SNORKEL 1 and SNORKEL 2 allow rice to adapt to deep water. *Nature* **2009**, *460*, 1026–1030. [CrossRef]

51. Mensah, J.K.; Obadoni, B. Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.). *Afr. J. Biotechnol.* **2007**, *6*, 668–671.
52. Nakweti, R.K.; Franche, C.; Ndiku, S.L. Effects of sodium azide (NaN_3) on seeds germination, plantlets growth and in vitro antimalarial activities of *Phyllantus odontadenius* Mull. *Arg. Amer. J. Exp. Agric.* **2015**, *5*, 226–238.
53. Unger, I.M.; Kennedy, A.C.; Muzika, R.-M. Flooding effects on soil microbial communities. *Appl. Soil Ecol.* **2009**, *42*, 1–8. [CrossRef]
54. Suzuki, C.; Kunito, T.; Aono, T.; Liu, C.-T.; Oyaizu, H. Microbial indices of soil fertility. *J. Appl. Microbiol.* **2005**, *98*, 1062–1074. [CrossRef]
55. Bossio, D.A.; Scow, K.M. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microb. Ecol.* **1998**, *35*, 265–378. [CrossRef]
56. Drenovsky, R.E.; Vo, D.; Graham, K.J.; Scow, K.M. Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microb. Ecol.* **2004**, *48*, 424–430. [CrossRef] [PubMed]
57. Mentzer, J.L.; Goodman, R.M.; Balser, T.C. Microbial responses over time to hydrologic and fertilization treatments in a simulated wet prairie. *Plant Soil* **2006**, *284*, 85–100. [CrossRef]
58. Stover, R.H. Flooding of soil for disease control. In *Soil Disinfection*; Mulder, D., Ed.; Elsevier: Amsterdam, The Netherlands, 1979.
59. Labuda, S.Z.; Vetchinnikov, A.A. Soil susceptibility on reduction as an index of soil properties applied in the investigation upon soil devastation. *Ecol. Chem. Eng.* **2011**, *18*, 333–344.
60. Vepraskas, M.J.; Faulkner, S.P. Redox chemistry of hydric soils. In *Wetlands Soils: Genesis, Hydrology, Landscapes and Classification*; Richardson, J.L., Vepraskas, M.J., Eds.; Lewis Publishers: Boca Raton, FL, USA, 2001.
61. Jeng, T.L.; Tseng, T.H.; Lai, C.C.; Wu, M.T.; Sung, J.M. Antioxidative charactrisation of NaN_3- induced common bean mutants. *Food Chemistry.* **2010**, *119*, 1006–1011. [CrossRef]
62. Elfeky, S.; Abo-Hamad, S.; Saad-Allah, K.M. Physiological impact of sodium azide on *Helianthus annus* seedlings. *Int. J. Agron. Agric. Res.* **2014**, *4*, 102–109.