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Effects of Nutrient Solution Electrical Conductivity on the Leaf Gas Exchange, Biochemical Stress Markers, Growth, Stigma Yield, and Daughter Corm Yield of Saffron in a Plant Factory

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Abstract: Indoor saffron farming systems under controlled conditions are required to meet the high demand for this valuable crop. The aim of the present study was to determine the flowering, growth, and yield responses of saffron grown using nutrient solutions with different electrical conductivity (EC) levels (0.7, 1.4, and 2.1 dS m^{-1}). Sprouted saffron corms were cultured for 24 weeks under a volcanic rock-based aerated continuous immersion system. Vegetative growth and leaf gas exchange, but not flowering, were affected significantly by EC levels. The optimal EC in a balanced nutrient solution was 0.7 dS m^{-1}, at which level the highest plant height, leaf area, biomass, photosynthetic rate, number of daughter corms, and percentage of corms $\geq$ 25 mm were recorded. An EC level of 2.1 dS m^{-1} decreased the photosynthetic rate, stomatal conductance, and transpiration rate of saffron but increased biochemical stress marker levels and elevated various antioxidant defense enzyme levels significantly in saffron leaves, possibly reflecting a defense response to the cellular damage provoked by the higher EC level. In terms of nutrient solution EC, 0.7 dS m^{-1} was optimal in saffron, whereas 2.1 dS m^{-1} caused oxidative stress that led to reduced growth and daughter corm production.

Keywords: *Crocus sativus*; Iridaceae; controlled environment; soilless culture

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1. Introduction

Saffron (*Crocus sativus* L.; Iridaceae) is an infertile autumn-flowering perennial plant [1] known for its production of a highly priced spice that is used widely in coloring, flavoring, and therapeutics [2]. The limited farmland available in cold regions to produce saffron in fields and the labor-intensive saffron harvesting and handling processes contribute to the high cost of saffron crop production, making this spice the most expensive in the world [3–9]. Additionally, the low yield of saffron has been attributed to conventional agronomic practices, low corm progeny, and the associated microbial infections [10]. Therefore, soilless cultures and cultivation systems under controlled conditions are considered suitable replacements for conventional saffron cropping and for the production of pathogen-free corms [8,11–18]. Moreover, such systems can increase saffron yield [19].

In nature, saffron grows well in friable, loose, low-density, well-irrigated, and well-drained clay calcareous soils with electrical conductivity (EC) levels of <2 dS m^{-1} [7,20,21]. Saffron is considered sensitive to soil salinity as its estimated soluble salt threshold varies between 0.61 and 0.86 depending on irrigation water levels and planting methods [22]. Variation and imbalance in nutrient composition and concentrations as well as in pH and EC affect saffron responses markedly in soilless cultures compared with in-soil-based cultures because of the buffering capacity of soils [23]. In several previous studies on saffron growth under hydroponic conditions, the nutrient solution EC was maintained at

1.1–1.3 dS m^{-1} [17,18,24]. Conversely, Salas et al. [25] recommended using an EC level of 3 dS m^{-1} for saffron growth and corm production under hydroponic conditions.

In our previous study on saffron [26], the effects of the growing substrate, corm size, and mode of nutrient supply in the hydroponic system were investigated. A volcanic rock-based aerated continuous immersion system was found to be optimal for saffron growth and daughter corm formation with an EC level of 1.4 dS m^{-1}. The mineral nutrients in the hydroponic system affect plant growth markedly, and the optimal EC level depends on the plant species, growth conditions, and utilized hydroponic system. In the present study, we investigated the flowering, growth, photosynthetic capacity, and daughter corm production of saffron in response to various nutrient solution EC levels using a volcanic rock-based, aerated, continuous immersion, hydroponics system. The activity of antioxidant enzymes and the levels of biochemical stress markers, including free proline, malondialdehyde (MDA), electrolyte leakage (EL), and relative water content (RWC), were also determined in saffron leaves.

2. Materials and Methods

2.1. Plant Material and Corm Sprouting

Saffron corms were obtained from Bloembollenbedrijf J.C.Koot (Vennewatersweg, The Netherlands). The corms were peeled, and any injured or infected corms were discarded (Figure 1a,b). The corms were dipped in in a fungicide solution (0.5 g L^{-1} Aromil-Plus 50 WP; Mobedco-Vet, Amman, Jordan) to prevent fungal infestation and dried prior to transplanting. For corm sprouting, the corms were planted in plastic pots (6 cm in diameter) filled with a mixture of perlite and peatmoss (1:1; v/v). The pots were irrigated and incubated under dark conditions in a controlled growth chamber at 14 °C for one week and then at 12 °C for another week (Figure 1c,d).

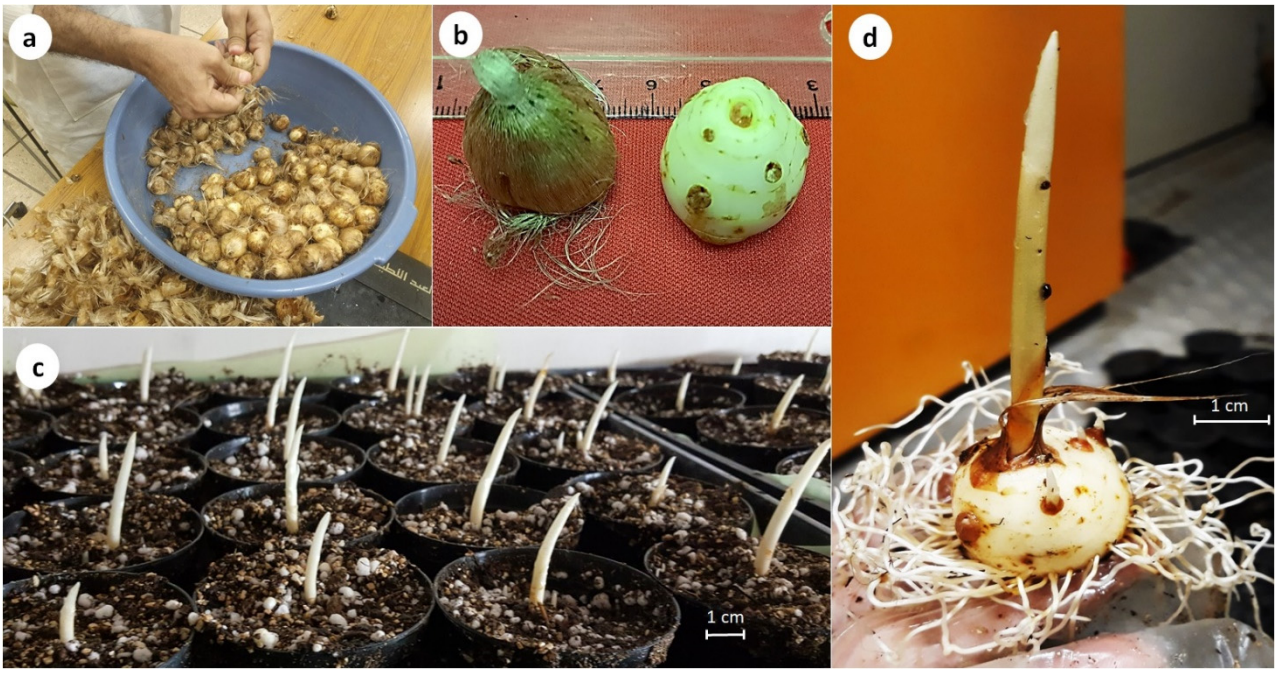

Figure 1. Dormancy breaking and corm sprouting of saffron. (**a**,**b**) Corm peeling and disinfection. (**c**,**d**) Sprouted corms and root emergence after 2 weeks of incubation at 14 °C for one week followed by 12 °C for another week in a controlled growth chamber under dark conditions.

2.2. Hydroponic System and EC Treatments

Sprouted corms (3–5 cm in length; Figure 1d) with diameters of 3.2–3.5 cm were transplanted into hydroponic plastic pots (10 × 8 cm) filled with volcanic rock and placed in floating polyurethane foam trays (15 pots per tray and 3 trays per treatment). The

trays were placed in individual tanks to supply the plants with nutrient solution (Nabtah; HydroArabia, Hasad Al-Dahab factory for liquid fertilizers, Riyadh, Saudi Arabia) via a continuous immersion system (Figure 2). The nutrient solution contained the macronutrients nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur at 163.20, 34.53, 172.56, 105.11, 33.83, and 62.70 mg·L^{-1}, respectively, and the micronutrients iron, boron, manganese, zinc, copper, and molybdenum at 1.83, 0.23, 0.27, 0.19, 0.12, and 0.07 mg·L^{-1}, respectively. Air was supplied to the nutrient solution at 0.3 vvm (air volume/nutrient solution volume/min). The plantlets were submerged continuously in the solution to maintain a constant nutrient and water status in the root zone. The EC of the nutrient solution was varied, i.e., 0.7, 1.4, or 2.1 dS m^{-1}, to determine the effects of EC on saffron flowering, growth, and corm formation. The EC of the nutrient solution was adjusted using a multiparameter bench meter (MI180-US; Milwaukee Instruments, Rocky Mount, NC, USA) and maintained by providing a fresh solution with an adjusted pH value of 5.8 ± 0.2. The environment in the growth chamber was adjusted to 8 °C ± 1 °C and 100 µmol m^{-2} s^{-1} photosynthetic photon flux density under a 16 h photoperiod using cool white fluorescent lamps.

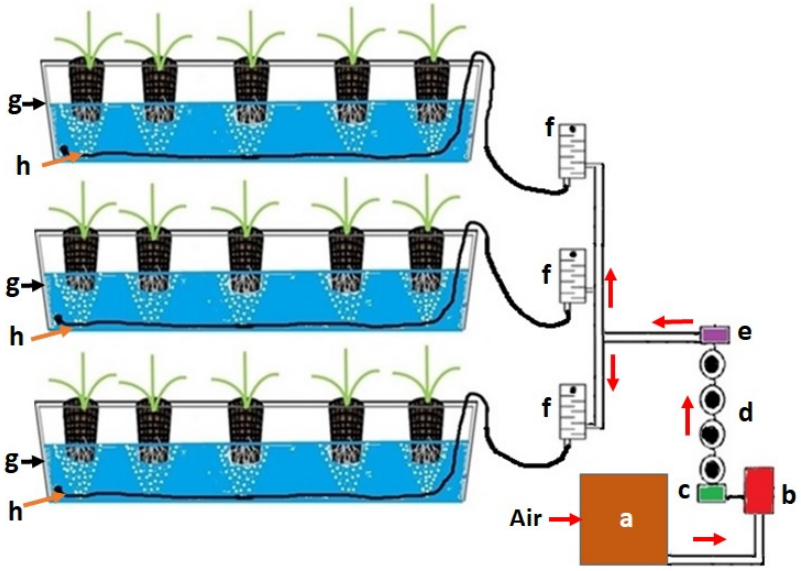

Figure 2. Schematic diagram of the continuous immersion hydroponic system used to grow saffron. (**a**) Air compressor, (**b**) air reservoir, (**c**) air cooling, (**d**) air filter system, (**e**) air dryer, (**f**) air flow meter, (**g**) growing vessels, and (**h**) airline tube.

2.3. Flowering, Vegetative Growth, and Corm Formation Parameters

Flowering parameters (number of flowers per plant, stigma length, and stigma fresh and dry weights) were recorded 29 days after corm transplanting, and the duration of the flowering period was 16 days. The stigma dry weight was determined after drying the stigmas for 1 h at 40 °C. Vegetative growth parameters (plant height, fresh and dry weight of leaves, and leaf area) were measured 22 weeks after corm sprouting. The leaf area was measured using a portable area meter (LI-3000A; LI-COR, Lincoln, NE, USA). The dry weight of leaves was determined after oven drying for 48 h at 70 °C. After leaf senescence, the plants were lifted, the mother corm parts were removed, and the replacement corms were separated. Corm formation parameters (number of corms per plant, corm diameter, and corm fresh weight) were recorded 24 weeks after corm sprouting.

2.4. Leaf Gas Exchange Parameters

Photosynthetic rate, stomatal conductance, and transpiration were monitored from the fourth week of corm sprouting until 22 weeks of plant growth. Data were recorded every 2 weeks using an LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln,

NE, USA) equipped with a standard 2 × 3 cm leaf cuvette and a Li-Cor LI-6400-02B light source. Photosynthetic parameters were measured under in-flow air with a 350-µmol mol^{-1} CO_2 concentration and a relative humidity of 60%, and leaf temperature was 8 °C. Measurements based on the saffron leaf area inside the chamber (A rectangle area of 0.3–0.5 × 3 cm; positioned at the middle of the chamber) were taken in triplicate from three plants, which were selected randomly from each treatment.

2.5. Leaf RWC

Leaf RWC was determined using leaf segments (1 cm) and calculated as follows:

$$(W_{fresh} - W_{dry})/(W_{turgid} - W_{dry}) \times 100,$$

where W_{fresh} is the weight of the freshly harvested sample, W_{turgid} is the turgid weight after saturating the sample with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry weight of the sample dried at 70 °C for 48 h [27].

2.6. Leaf EL

Leaf samples were incubated in vials containing 100 mL of distilled water for 24 h, and the primary EL (EC1) was recorded using the multiparameter bench meter. The vials were then placed in an autoclave at 121 °C and 1.2 atm for 20 min. After being allowed to cool, the secondary EL (EC2) was recorded. Leaf EL was calculated as follows:

$$(EC1 - EC0)/(EC2 - EC0) \times 100, \quad (1)$$

where EC0 is the electrical conductivity of distilled water [28].

2.7. Assay of Free Proline Content

Free proline content was measured as described by Bates et al. [29]. Leaves (0.5 g) were weighed, homogenized in 3 mL of 3% 5-sulfosalyicilic acid and liquid nitrogen using a prechilled pestle and mortar, and centrifuged for 10 min at 4000 rpm. Subsequently, 2 mL of the supernatant was collected to estimate proline content using ninhydrin reagent (0.125 g of ninhydrin, 3 mL of glacial acetic acid, and 2 mL of 6 M H_3PO_4) and incubated for 1 h at 100 °C. The reaction was stopped by placing the sample in an ice-cold bath for 15 min. Extraction was performed using 4 mL of toluene, and the absorbance at 520 nm was determined. Finally, proline concentration was estimated using a standard curve.

2.8. Assay of MDA Content

Malondialdehyde content was determined using a thiobarbituric acid (TBA) reaction [30]. Approximately 1 g of root segments was homogenized with 2 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000× *g* for 15 min. After centrifugation, 1 mL of supernatant was mixed with 2.5 mL of 0.5% TBA in 20% TCA and incubated in boiling water for 30 min. The mixture was then cooled immediately using ice to stop the reaction and centrifuged at 10,000× *g* for 5 min. Absorbances at 532 and 600 nm were determined, and the MDA concentration was estimated by subtracting the nonspecific absorption at 600 nm from the absorption at 532 nm using an absorbance coefficient of 156 mM^{-1} cm^{-1} at 532 nm.

2.9. Assay of Antioxidant Enzymes

To measure the activity of various antioxidant enzymes, 0.5 g of saffron leaves was homogenized in liquid nitrogen with 1.5 mL of the appropriate extraction buffer using a prechilled pestle and mortar. The homogenate was then filtered through four layers of cheesecloth and centrifuged at 22,000× *g* and 4 °C for 20 min. The supernatant was then recentrifuged under the same conditions for 20 min. Catalase (CAT; EC 1.11.1.6) activity was measured by following the consumption of H_2O_2 at 240 nm [31], peroxidase (POD; EC 1.11.1.7) activity was determined according to the procedure proposed by Hammer-

schmidt et al. [32], and polyphenol oxidase (PPO; EC 1.10.3.1) activity was determined according to the method described by Malik and Singh [33].

2.10. Experimental Design and Data Analysis

The experiments had a completely randomized design with three replicates per treatment. Data expressed as percentages were arcsine-transformed before analysis [34], and the treatment effects were assessed statistically using ANOVA and Tukey's range tests in SAS (Version 6.12; SAS Institute, Inc., Cary, NC, USA).

3. Results and Discussion

The EC level of the nutrient solution (0.7, 1.4, and 2.1 dS m^{-1}) did not affect the number of flowers, stigma length, and stigma fresh weight and dry weight significantly (Table 1). Conversely, plant shoot length and the fresh and dry weight of leaves were the highest when the nutrient solution EC level was low (0.7 dS m^{-1}). However, when the EC level increased, the vegetative growth and root system of saffron were reduced significantly (Figure 3). Additionally, the number, average weight, and diameter of corms were reduced when the EC level increased. The lowest EC level (0.7 dS m^{-1}) was associated with the highest percentage of saffron corms with an appropriate size for flowering in the next season (31.5% of corms were ≥25 mm in diameter) (Table 2; Figure 3). Photosynthetic rate, stomatal conductance, and transpiration rate were affected by nutrient solution EC levels (Table 1; Figure 4). Time course data on photosynthetic parameters revealed that saffron plants reached their peak photosynthetic capacity at 14–16 weeks after planting under hydroponic conditions (Figure 4). When the EC level was 0.7 dS m^{-1}, the photosynthetic rate, stomatal conductance, and transpiration rate were higher than those observed when the EC level was increased.

Table 1. Flowering and growth characteristics of *Crocus sativus* according to different electrical conductivity (EC) levels in the nutrient solution.

Flowering and Growth Parameters	EC of the Nutrient Solution (dS m^{-1})			
	0.7	**1.4**	**2.1**	**Significance**
Number of flowers/plant	1.9 a	1.9 a	1.8 a	NS
Stigma length/flower (mm)	42.46 a	42.40 a	42.41 a	NS
Stigma fresh weight/flower (mg)	43.81 a	43.90 a	43.54 a	NS
Stigma dry weight/flower (mg)	5.68 a	5.47 a	5.29 a	NS
Plant height (cm)	51.47 a	46.12 b	32.62 c	*
Leaves fresh weight/plant (g)	6.253 a	5.776 b	3.956 c	*
Leaves dry weight/plant (g)	1.683 a	1.521 b	0.986 c	*
Leaves area/plant (cm^2)	39.60 a	34.50 b	27.10 c	*
Net CO_2 assimilation (mmol CO_2 m^{-2} s^{-1})	0.00639 a	0.00548 b	0.00288 c	*
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	49.2 a	41.3 b	34.9 c	*
Transpiration rate (mmol H_2O m^{-2} s^{-1})	74.6 a	61.3 b	48.0 c	*

Values followed by the same letter in the same row are not significantly different according to Tukey's test ($p \leq 0.05$). NS = not significant, * = significant at 5% level.

Table 2. Daughter corm production of *Crocus sativus* according to the different EC levels in the nutrient solution.

EC of the Nutrient Solution (dS m^{-1})	Number of Corms/Plant	Average Weight of Corms/Plant (g)	Average Diameter of Corms/Plant (mm)	Percentage of Corms (<25 mm)	Percentage of Corms (≥25 mm)
0.7	7.21 a	7.865 a	29.37 a	68.55 c	31.45 a
1.4	5.72 b	6.245 b	24.62 b	76.22 b	23.78 b
2.1	4.36 c	3.673 c	13.92 c	89.17 a	11.83 c

Values followed by the same letter in the same column are not significantly different according to Tukey's test ($p \leq 0.05$).

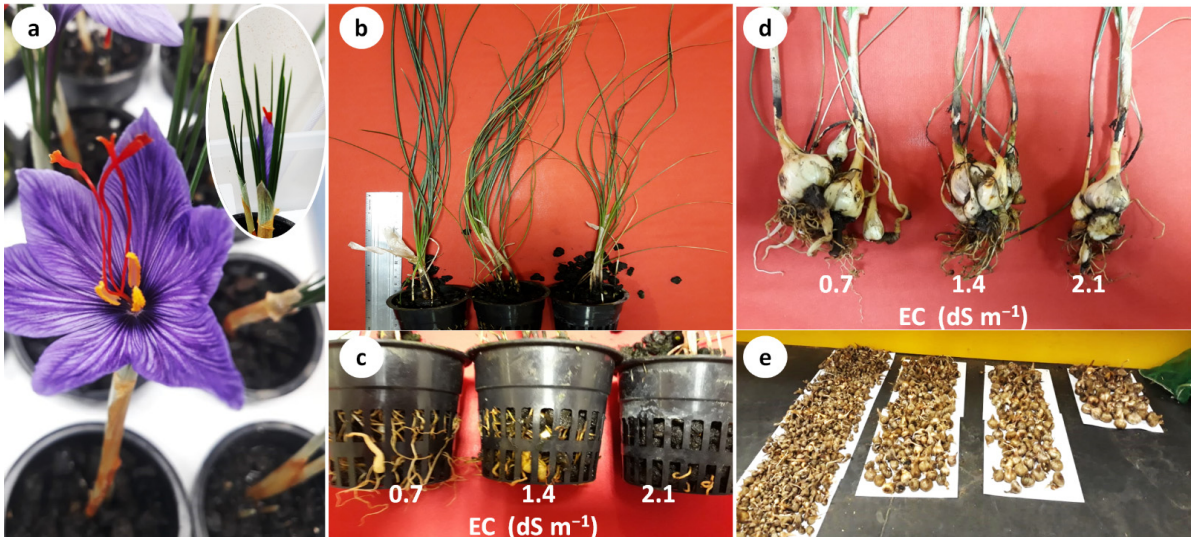

Figure 3. Flowering (**a**), vegetative growth (**b**), root system (**c**), and daughter corm production (**d**,**e**) in *Crocus sativus* according to the different EC levels (0.7, 1.4, and 2.1 dS m^{-1}) in the nutrient solution.

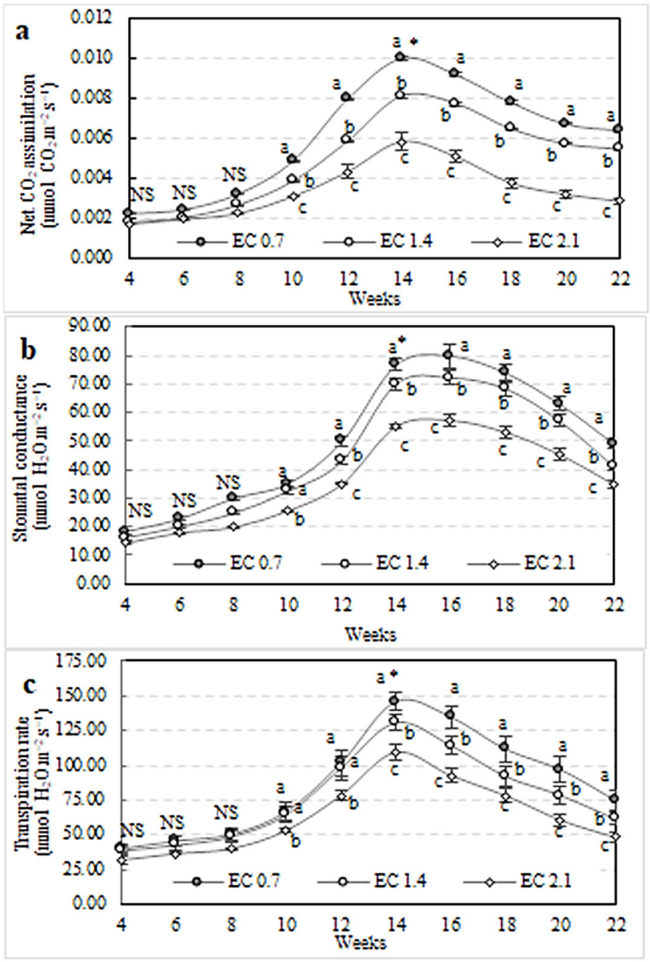

Figure 4. Temporal changes in net photosynthetic rate (**a**), stomatal conductance (**b**), and transpiration rate (**c**) in saffron plants (corm size: 10–11 cm) according to different EC levels in a nutrient solution (EC 0.7, 1.4, and 2.1 dS m^{-1}). Different letters within a set of values denote significant differences according to Tukey's test ($p \leq 0.05$). NS = not significant, * = significant at 5% level.

One of the primary skills required when running a successful hydroponic setup is managing EC levels in the fertilizer solution [35]. The elements and concentration of nutrients are important for plant growth and development [36]. Studies on nutrient requirements for saffron growth in hydroponics are limited. Several researchers have used an EC range of 1.1–1.4 dS m^{-1} for saffron growth under hydroponic conditions [17,18,24,26]. However, Salas et al. [25] investigated saffron growth and corm production under hydroponic conditions using EC levels of 2.0, 2.5, and 3.0 dS m^{-1}, finding that an EC level of 3 dS m^{-1} was optimal. The authors also found that shoot length (41.9 and 43.9 cm), the number of daughter corms (4.45 and 5.55 corms/plant), and the percentage of corms $\geq$ 25 mm in diameter (2% and 10%) differed when EC levels were 2 and 3 dS m^{-1}, respectively. In our study, when the EC was 2.1 dS m^{-1}, only the number of daughter corms (4.36) was similar to that reported by Salas et al. [25] whereas all other parameters were affected negatively compared with their values when the EC level was 0.7 dS m^{-1}. When using this lower EC level, higher values were obtained for vegetative growth parameters, the number of daughter corms, and the percentage of corms $\geq$ 25 mm in diameter compared with those recorded by Salas et al. [25] when using an EC level of 3 dS m^{-1}. Cultivation techniques and nutrient management systems can change the way in which plants absorb nutrients [37]. Therefore, different techniques may require different EC values to achieve peak growth parameter values. For example, when roots are flooded or submerged for a longer period and more frequently, lower EC levels in the cultivation of basil and lettuce can improve the growth parameters of the plants [38]. In our study, a low EC level improved saffron growth, which may have been because the saffron roots were immersed continuously in aerated nutrient solution.

The optimum EC in a balanced nutrient solution is often adjusted to 1.0–1.5 dS m^{-1}; however, the optimum EC level varies according to plant species, season, growth stage, and water quality. A high EC level can depress growth due to inhibited nutrient uptake, transport, and use in the plant's tissues. The highest growth parameters of basil and lettuce under hydroponic conditions were obtained when the EC range was 0.9–1.2 dS m^{-1} [38]. Dewir et al. [39] showed that the optimum EC in a balanced nutrient solution was 1.2 dS m^{-1} for *Spathiphyllum cannifolium*, and higher EC levels reduced plant growth and leaf pigment levels. An EC level of 2.1 dS m^{-1} reduced the leaf gas exchange of *Eruca sativa* grown hydroponically [40]. Similarly, an EC level of 2.2 dS m^{-1} reduced the photosynthetic capacity of *Solanum tuberosum* plantlets significantly [41]. The EC of a nutrient solution is reported to be related directly to photosynthetic metabolism [42], and reduced leaf photosynthesis often leads to low assimilation production [43].

Saffron plants grown in a nutrient solution with a high EC level (2.1 dS m^{-1}) exhibited increased EL, proline content, and MDA levels in the leaves as well as deceased RWC (Figure 5a–d). The EC level in the nutrient medium is known to affect a variety of plant responses, including growth and the readjustment of transport and metabolic processes. High EC levels can also induce a water deficit, even in well-watered soils, due to the reduced osmotic potential of the soil solutes, which hinders water uptake via the roots and causes symptoms similar to those observed under water deficit [44]. High EC levels also reduce turgor pressure and increase the retention of toxic ions in the root zone, resulting in limited cell expansion and ion imbalance [45]. *Wasabia japonica* plants grown for 3 weeks under hydroponic conditions with a high EC level of 5.0 dS m^{-1} exhibited a three-fold increase in EL compared with the EL recorded when the EC level was 0.5–2.0 dS m^{-1} [46].

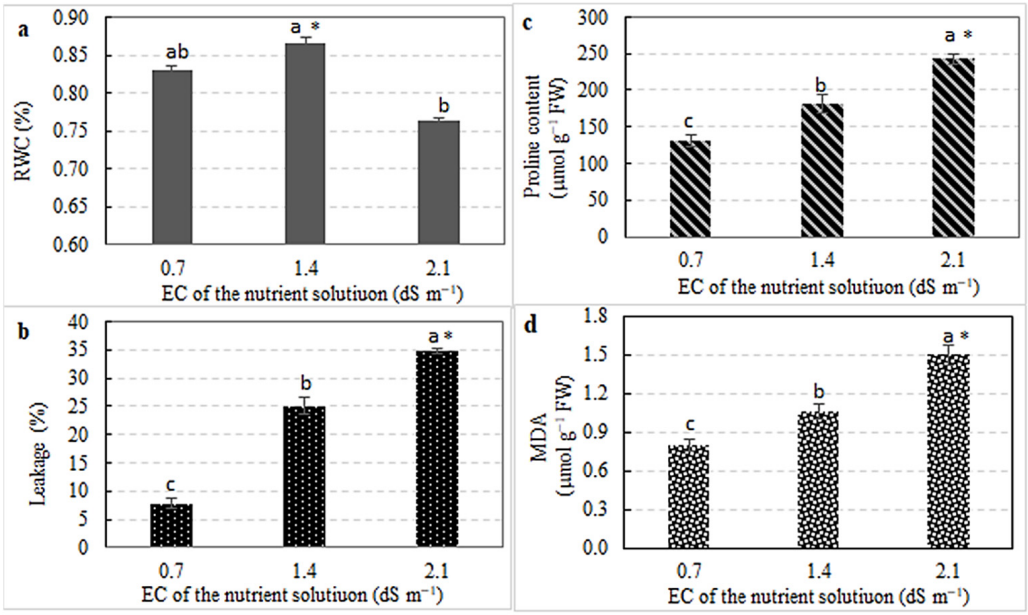

Figure 5. Relative water content (**a**), electrolyte leakage (**b**), proline content (**c**), and malondialdehyde (MDA) content (**d**) in the leaves of saffron plants grown in volcanic rock substrate according to the EC of the nutrient solution (EC 0.7, 1.4, and 2.1 dS m^{-1}). Different letters within a set of values denote significant differences according to Tukey's test ($p \leq 0.05$). * = significant at 5% level.

In plants, salt damage can be caused by a combination of several factors, including osmotic injury and specific ion toxicity primarily [47], and affects a wide variety of physiological and metabolic processes [48]. In the present study, we found a marked increase in proline content in saffron leaves (Figure 5c). The accumulation of protective solutes, including proline, is a general response to environmental stress [49,50]. Proline is an amino acid that acts as a compatible solute due to its high hydrophilic characteristics, thereby playing a prominent role in osmotic adjustment [51,52]. Therefore, proline has been considered a reliable indicator of the environmental stress imposed on plants grown hydroponically [39,53]. We also found that the water status in saffron leaves was affected by the nutrient solution EC level. Relative water content affects the physiology of cells in several ways, including changes in intercellular organelle positions, transport channels, and enzyme biochemistry as well as cell wall shrinkage [54,55]. Such changes affect the cellular metabolism of plants, including their photosynthetic capacity [55]. It has been reported that decreased leaf RWC reduces stomatal conductance progressively [55,56]. In the current study, MDA content increased markedly in saffron plants grown in a nutrient solution with an EC of 2.1 dS m^{-1} (Figure 1d). Malondialdehyde, a decomposition product of polyunsaturated fatty acid hydroperoxides, is often used as a suitable biomarker of lipid peroxidation [57], which is an effect of oxidative damage. Thus, the response of saffron plants to a high EC level could occur because they have various tolerance and avoidance mechanisms that are activated under stress conditions.

The antioxidant enzyme activities (POD, CAT, and PPO) in leaf extracts from saffron plants cultured in nutrient solutions with different EC levels are presented in Figure 6a–c. POD activity increased significantly as EC levels increased, whereas CAT and PPO activities were elevated significantly only at the highest EC level (2.1 dS m^{-1}). Salt stress can induce ionic stress and osmotic stress in plant cells, leading to increased accumulation of reactive oxygen species (ROS) that are harmful to the cells at high concentrations [58–60]. Antioxidant defense enzymes, such as CAT and POD, are deployed to minimize the concentrations of superoxide and hydrogen peroxide. H_2O_2 is eliminated by CAT and POD, including both enzymic and nonenzymic H_2O_2 degradation [61]. CAT dismutates H_2O_2 into water, whereas POD decomposes H_2O_2 through the oxidation of cosubstrates, such as phenolic compounds and/or antioxidants [62]. PPO has been reported to play

a role in oxygen scavenging through the oxidation of phenols; hence, it affects the local levels of oxygen and ROS [63]. The nutrient concentrations in hydroponic culture affect the induction of the abiotic stress response in Capsicum annuum seedlings, in which MDA content and the activities of ascorbate POD and guaiacol POD are enhanced [64]. Similarly, MDA content and the activity of antioxidant enzymes, such as ascorbate POD, CAT, and guaiacol POD, are elevated in *Spathiphyllum cannifoium* seedlings grown under hydroponic conditions [39]. Our results indicate that an EC level of 2.1 dS m^{-1} in the nutrient solution induces oxidative stress in saffron plants, which might reflect a defense response to the cellular damage elicited by the higher EC level. Although this increase in antioxidant activities was not sufficiently high to eradicate the negative effects of the salts, it apparently reduced the impact of stress, which allowed the growth and development of the saffron plants.

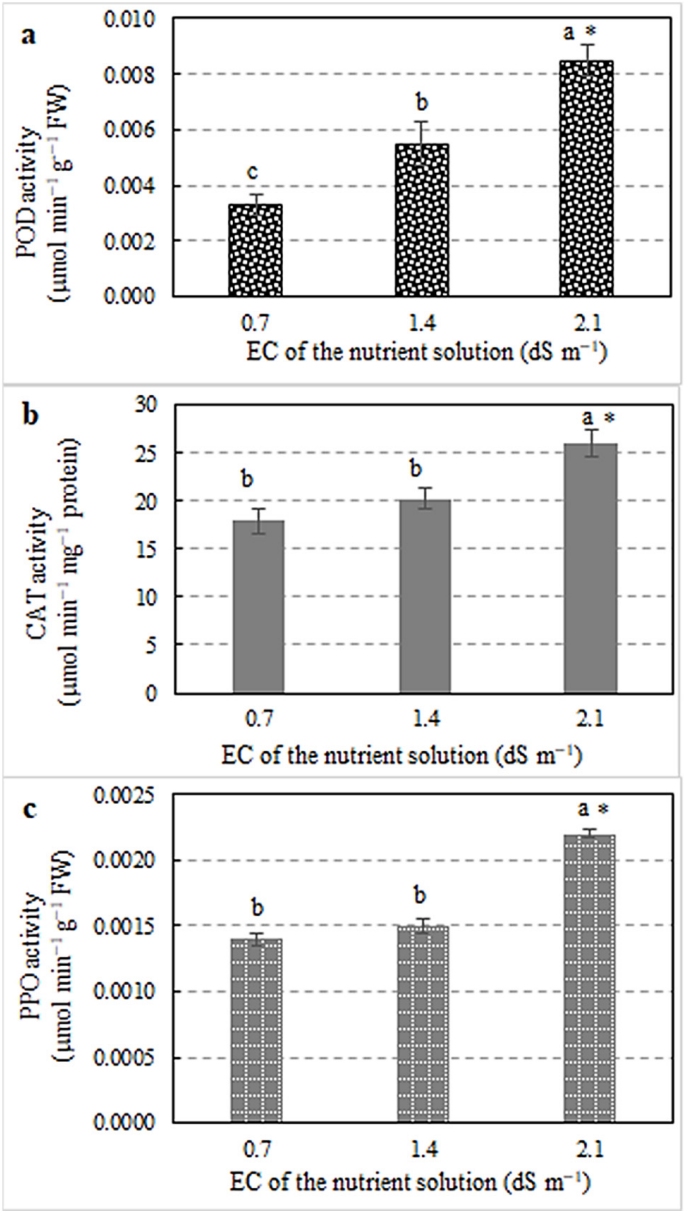

Figure 6. Antioxidant enzyme activity levels in *Crocus sativus* plants according to the different EC levels (EC 0.7, 1.4, and 2.1 dS m^{-1}) in the nutrient solution. (**a**) Peroxidase (POD), (**b**) catalase (CAT), and (**c**) polyphenol oxidase (PPO). Different letters within a set of values denote significant differences according to Tukey's test ($p \leq 0.05$). * = significant at 5% level.

4. Conclusions

In conclusion, identifying the optimal EC level is important for the vegetative growth and daughter corm formation of saffron plantlets grown under hydroponic conditions. A relatively low EC level of 0.7 dS m^{-1} was optimal for saffron growth in an aerated continuous immersion hydroponic system. In contrast, a higher EC level of 2.1 dS m^{-1} decreased leaf gas exchange, enhanced antioxidant activities, and increased stress biochemical markers, such as EL, proline, and MDA content. Thus, saffron plants suffered oxidative stress under high EC levels (i.e., 2.1 dS m^{-1}). However, individual nutrient concentrations should be further optimized to help researchers better understand the dynamics of nutrient uptake and the requirements of saffron.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nemati, Z.; Harpke, D.; Gemicioglu, A.; Kerndorff, H.; Blattner, F.R. Saffron (*Crocus sativus*) is an autotriploid that evolved in Attica (Greece) from wild Crocus cartwrightianus. *Mol. Phylogenet. Evol.* **2019**, *136*, 14–20. [CrossRef] [PubMed]
2. Abdullaev, F.I.; Frenkel, G.D. Saffron in Biological and Medical Research. In *Saffron, Crocus sativus*; Negbi, M., Ed.; Harwood Publications: Singapore, 1999; pp. 103–111.
3. Aytekin, A.; Acikgoz, A.O. Hormone and microorganism treatments in the cultivation of saffron (*Crocus sativus* L.) plants. *Molecules* **2008**, *13*, 1135–1147. [CrossRef] [PubMed]
4. Douglas, M.H.; Smallfield, B.M.; Wallace, A.R.; McGimpsey, J.A. Saffron (*Crocus sativus* L.): The effect of mother corm size on progeny multiplication, flower and stigma production. *Sci. Hortic.* **2014**, *166*, 50–58. [CrossRef]
5. Gresta, F.; Napoli, E.; Ceravolo, G.; Santonoceto, C.; Strano, T.; Ruberto, G. Stigmas yield and volatile compounds of saffron (*Crocus sativus*) in a late sowing under greenhouse with two nitrogen rates. *Acta Hort.* **2017**, *1184*, 293–300. [CrossRef]
6. Khilare, V.; Tiknaik, A.; Prakash, B.; Ughade, B.; Korhale, G.; Nalage, D.; Ahmed, N.; Khedkar, C.; Khedkar, G. Multiple tests on saffron find new adulterant materials and reveal that IST grade saffron is rare in the market. *Food Chem.* **2019**, *272*, 635–642. [CrossRef] [PubMed]
7. Kumar, R.; Singh, V.; Devi, K.; Sharma, M.; Singh, M.K.; Ahuja, P.S. State of art of saffron (*Crocus sativus* L.) agronomy: A comprehensive review. *Food Rev. Int.* **2008**, *25*, 44–85. [CrossRef]
8. Molina, R.V.; Valero, M.; Navarro, Y.; García-Luis, A.; Guardiola, J.L. The effect of time of corm lifting and duration of incubation at inductive temperature on flowering in the saffron plant (*Crocus sativus* L.). *Sci. Hortic.* **2004**, *103*, 79–91. [CrossRef]
9. Winterhalter, P.; Straubinger, M. Saffron- renewed interest in an ancient spice. *Food Rev. Int.* **2000**, *16*, 39–59. [CrossRef]
10. Devi, K.; Sharma, M.; Singh, M.; Singh Ahuja, P.S. In vitro cormlet production and growth evaluation under greenhouse condition in saffron (*Crocus sativus* L.)—A commercially important crop. *Eng. Life Sci.* **2011**, *11*, 189–194. [CrossRef]
11. García-Rodríguez, M.V.; López-Córcoles, H.; Alonso, G.L. Effect of the hydroponic growing of forced *Crocus sativus* L. on the saffron quality. *Acta Hortic.* **2017**, *1184*, 287–292. [CrossRef]
12. Helalbeigi, Y.; Khoshgoftarmanesh, A.H.; Shamsi, F.; Zamani, N. Possibility of saffron corm growth in different bed of cultivation in soillers system. In Proceedings of the 1st Congress Hydroponics in Greenhouse Production, Isfahan University Technolology, Isfahan, Iran, 22 October 2009. (In Persian with English Summary).
13. Maggio, A.; Raimondi, G.; Martino, A.; De Pascale, S. Soilless cultivation of saffron in Mediterranean environment. *Acta Hortic.* **2006**, *718*, 515–522. [CrossRef]

14. Molina, R.V.; Valero, M.; Navarro, Y.; García-Luis, A.; Guardiola, J.L. Low temperature storage of corms extends the flowering season of saffron (*Crocus sativus* L.). *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 319–326. [CrossRef]
15. Mollafilabi, A.; Koocheki, A.; Rezvani Moghaddam, P.; Nassiri Mahallati, M. Effects of bed type, corm weight and lifting time on quantitative and qualitative criteria of saffron (*Crocus sativus* L.). *J. Agroecol.* **2017**, *9*, 607–617, (In Persian with English summary). [CrossRef]
16. Poggi, L.M.; Portela, A.J.; Pontin, M.A.; Molina, R.V. Corm size and incubation effects on time to flowering and threads yield and quality in saffron production in Argentina. *Acta Hortic.* **2010**, *850*, 193–198. [CrossRef]
17. Schroeder, F.-G.; Lozoya, D.R.; Ruser, P. Hydroponic forcing of saffron (*Crocus sativus* L.). *Acta Hortic.* **2020**, *1273*, 281–288. [CrossRef]
18. Souret, F.F.; Weathers, P.J. The growth of saffron (*Crocus sativus* L.) in aeroponics and hydroponics. *J. Herbs Spices Med. Plants* **2000**, *7*, 25–35. [CrossRef]
19. Molina, R.V.; Valero, M.; Navarro, Y.; Guardiola, J.L.; García-Luis, A. Temperature effects on flower formation in saffron (*Crocus sativus* L.). *Sci. Hortic.* **2005**, *103*, 361–379. [CrossRef]
20. Koocheki, A.; Ganjeali, A.; Abbassi, F. The effect of duration of incubation and photoperiod on corm and shoot characteristics of saffron plant (*Crocus sativus* L.). *Acta Hortic.* **2006**, *739*, 61–70. [CrossRef]
21. Zarghani, F.; Karimi, A.; Khorasani, R.; Lakzian, A. To evaluate the effect of soil physical and chemical char-acteristics on the growth characteristics of saffron (*Crocus sativus* L.) corms in Tornbat-e Heydariyeh area. *J. Agroecol.* **2016**, *8*, 120–133.
22. Dastranj, M.; Sepaskhah, A. Saffron response to irrigation regime, salinity and planting method. *Sci. Hortic.* **2019**, *251*, 215–224. [CrossRef]
23. Askari-Khorasgani, O.; Pessarakli, M. Shifting saffron (*Crocus sativus* L.) culture from traditional farmland to controlled environment (greenhouse) condition to avoid the negative impact of climate changes and increase its productivity. *J. Plant Nutr.* **2019**, *42*, 2642–2665. [CrossRef]
24. Sorooshzadeh, A.; Tabibzadeh, Z. Effect of copper in hydroponic solution on leaves and roots of saffron (*Crocus sativus* L.). *Acta Hortic.* **2015**, *1062*, 83–88. [CrossRef]
25. Salas, M.d.C.; Montero, J.L.; Diaz, J.G.; Berti, F.; Quintero, M.F.; Guzmán, M.; Orsini, F. Defining optimal strength of the nutrient solution for soilless cultivation of saffron in the Mediterranean. *Agronomy* **2020**, *10*, 1311. [CrossRef]
26. Dewir, Y.H.; Alsadon, A.A.; Ibrahim, A.; El-Mahrouk, M.E. Effects of growing substrate, mode of nutrient supply, and saffron corm size on flowering, growth, photosynthetic competence, and cormlet formation in hydroponics. *HortTechnology* **2022**, *32*, 234–240. [CrossRef]
27. Weatherley, P.E. Studies in the water relations of the cotton plant. 1. the field measurement of water deficit in leaves. *New Phytol.* **1950**, *49*, 81–97. [CrossRef]
28. Sairam, R. Effect of moisture-stress on physiological activities of two contrasting wheat genotypes. *Indian J. Exp. Biol.* **1994**, *32*, 594.
29. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
30. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
31. Aebi, H. Catalases. In *Methods of Enzymatic Analysis Vol. 2*; Bergmeyer, H.U., Ed.; Academic Press: New York, NY, USA, 1984; pp. 673–684.
32. Hammerschmidt, R.; Nuckles, E.M.; Kuć, J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.* **1982**, *20*, 73–82. [CrossRef]
33. Malik, C.P.; Singh, M.B. *Plant Emymology and Histoenzymology*; Kalyani Publishers: Delhi, India, 1980.
34. Compton, M.E. Statistical methods suitable for the analysis of plant tissue culture data. *Plant Cell Tiss. Organ Cult.* **1994**, *37*, 217–242. [CrossRef]
35. Tzortzakis, N.; Nicola, S.; Savvas, D.; Voogt, W. Editorial: Soilless cultivation through an intensive crop production scheme. Management strategies, challenges and future directions. *Front. Plant Sci.* **2020**, *11*, 363. [CrossRef] [PubMed]
36. Pinter, P.J., Jr.; Hatfield, J.L.; Schepers, J.S.; Barnes, E.M.; Moran, M.S.; Daughtry, C.S.T.; Upchurch, D.R. Remote sensing for crop management. *Photogramm. Eng. Remote Sens.* **2003**, *69*, 647–664. [CrossRef]
37. Son, J.E.; Kim, H.J.; Ahn, T.I. Hydroponic systems. In *Plant Factory*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 273–283.
38. Hosseini, H.; Mozafari, V.; Roosta, H.R.; Shirani, H.; van de Vlasakker, P.C.H.; Farhangi, M. Nutrient use in vertical farming: Optimal electrical conductivity of nutrient solution for growth of lettuce and basil in hydroponic cultivation. *Horticulturae* **2021**, *7*, 283. [CrossRef]
39. Dewir, Y.H.; Chakrabarty, D.; Ali, M.B.; Hahn, E.J.; Paek, K.Y. Effects of hydroponic solution EC, substrates, PPF and nutrient scheduling on growth and photosynthetic competence during acclimatization of micropropagated *Spathiphyllum* plantlets. *Plant Growth Regul.* **2005**, *46*, 241–251. [CrossRef]
40. Yang, T.; Samarakoon, U.; Altland, J.; Ling, P. Photosynthesis, biomass production, nutritional quality, and flavor-related phytochemical properties of hydroponic-grown arugula (*Eruca sativa* Mill.) 'standard' under different electrical conductivities of nutrient solution. *Agronomy* **2021**, *11*, 1340. [CrossRef]

41. Piao, X.C.; Chakrabarty, D.; Hahn, E.J.; Paek, K.Y. Growth and photosynthetic characterstics of potato plantlets as affected by pH and EC of the nutrient solution in hydroponic system. *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 100–105. [CrossRef]
42. Lu, N.; Bernardo, E.L.; Tippayadarapanich, C.; Takagaki, M.; Kagawa, N.; Yamori, W. Growth and accumulation of secondary metabolites in perilla as affected by photosynthetic photon flux density and electrical conductivity of the nutrient solution. *Front. Plant Sci.* **2017**, *8*, 708. [CrossRef]
43. Niinemets, U. Photosynthesis and resource distribution through plant canopies. *Plant Cell Environ.* **2007**, *30*, 1071. [CrossRef]
44. Klamkowski, K.; Treder, W. Response to drought stress of three strawberry cultivars grown under greenhouse conditions. *J. Fruit Ornam. Plant Res.* **2008**, *16*, 179–188.
45. Lam, V.P.; Kim, S.J.; Park, J.S. Optimizing the electrical conductivity of a nutrient solution for plant growth and bioactive compounds of *Agastache rugosa* in a plant factory. *Agronomy* **2020**, *10*, 76. [CrossRef]
46. Choi, K.Y.; Lee, Y.B.; Cho, Y.Y. Allyl-isothiocyanate content and physiological responses of *Wasabia japonica* Matusum as affected by different EC levels in hydroponics. *Hortic. Sci. Technol.* **2011**, *29*, 311–316.
47. Nandwal, A.S.; Godara, M.; Sheokand, S.; Kamboj, D.V.; Kundu, B.S.; Kuhad, M.S.; Kumar, B.; Sharma, S.K. Salinity induced changes in plant water status, nodule functioning and ionic distribution in phenotypically differing genotypes of *Vigna radiata* L. *J. Plant Physiol.* **2000**, *156*, 350–359. [CrossRef]
48. Silveira, J.A.G.; Melo, A.R.B.; Viégas, R.A.; Oliveira, J.T.A. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. *Environ. Exp. Bot.* **2001**, *46*, 171–179. [CrossRef]
49. Hare, P.D.; Cress, W.A. Metabolic implications of stressinduced proline accumulation in plats. *Plant Growth Regul.* **1997**, *21*, 79–102. [CrossRef]
50. Sakamoto, A.; Murata, N. The role of glycine betaine in the protection of plants from stress: Clues from transgenic plants. *Plant Cell Environ.* **2002**, *25*, 163–171. [CrossRef]
51. Büssis, D.; Heineke, D. Acclimation of potato plants to polyethylene glycol-induced water deficit. II. Contents and subcellular distribution of organic solutes. *J. Exp. Bot.* **1998**, *49*, 1361–1370. [CrossRef]
52. Hayashi, F.; Ichino, T.; Osanai, M.; Wada, K. Oscillation and regulation of proline content of P5CS and ProDH gene expressions in the light/dark cycles in *Arabodopsis thaliana* L. *Plant Cell Physiol.* **2000**, *41*, 1096–1101. [CrossRef]
53. Claussen, W. Growth, water use efficiency, and proline content of hydroponically grown tomato plants as affected by nitrogen source and nutrient concentration. *Plant Soil* **2002**, *247*, 199–209. [CrossRef]
54. Lambers, H.; Chapin, F.S.; Pons, T.L. *Plant Physiological Ecology*; Springer: New York, NY, USA, 2008.
55. Lawlor, D.W.; Cornic, G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* **2002**, *25*, 275–294. [CrossRef]
56. Lawlor, D.W. Limitation to photosynthesis in water stressed leaves: Stomata vs. metabolism and the role of ATP. *Ann. Bot.* **2002**, *89*, 871–885. [CrossRef]
57. Dewir, Y.H.; Chakrabarty, D.; Ali, M.B.; Hahn, E.J.; Paek, K.Y. Lipid peroxidation and antioxidant enzyme activities of *Euphorbia millii* hyperhydric shoots. *Environ. Exp. Bot.* **2006**, *58*, 93–99. [CrossRef]
58. Chawla, S.; Jain, S.; Jain, V. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *J. Plant Biochem. Biotechnol.* **2013**, *22*, 27–34. [CrossRef]
59. Hernández, J.A.; Jiménez, A.; Mullineaux, P.; Sevilia, F. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ.* **2000**, *23*, 853–862. [CrossRef]
60. Pang, C.H.; Wang, B.S. Oxidative stress and salt tolerance in plants. In *Progress in Botany*; Springer: Berlin/Heidelberg, Germany, 2008; pp. 231–245.
61. Peltzer, D.; Dreyer, E.; Polle, A. Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fagus sylvatica* and *Coleus blumei*. *Plant Physiol. Biochem.* **2002**, *40*, 141–150. [CrossRef]
62. Blokhina, O.; Virolainen, E.; Fagerstedt, K.V. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Ann. Bot.* **2003**, *91*, 179–194. [CrossRef]
63. Boeckx, T.; Webster, R.; Winters, A.L.; Webb, K.J.; Gay, A.; Kingston-Smith, A.H. Polyphenol oxidase-mediated protection against oxidative stress is not associated with enhanced photosynthetic efficiency. *Ann. Bot.* **2015**, *116*, 529–540. [CrossRef]
64. Ding, X.; Zhang, H.; Qian, T.; He, L.; Jin, H.; Zhou, Q.; Yu, J. Nutrient concentrations induced abiotic stresses to sweet pepper seedlings in hydroponic culture. *Plants* **2022**, *11*, 1098. [CrossRef]