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Effect of Gibberellic Acid on Growth, Yield, and Quality of Leaf Lettuce and Rocket Grown in a Floating System

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Abstract: Gibberellins (GAs) are growth hormones strongly involved in a wide variety of physiological activities. Currently, gibberellins are commercially used to enhance phenotypic characteristics, earliness, and productivity of many vegetable and ornamental crops. In this work, the efficacy of supplementation of low levels of gibberellic acid (0, 10^{-8}, 10^{-6}, and 10^{-4} M GA_3) through the mineral nutrient solution of a floating system on yield and quality of leaf lettuce and rocket plants was tested. The marketability of plants was lost when 10^{-4} M GA_3 was added to the mineral nutrient solution. This study demonstrated that the addition of 10^{-4} M GA_3 exceeded the acceptable threshold for use in hydroponics production systems. Below the concentration of 10^{-4} M, the presence of GA_3 in the mineral nutrient solutions (MNS), especially at 10^{-6} M GA_3, stimulated plant growth and enhanced the yield. Various morphological and physiological traits were enhanced by GA_3 treatments (biomass accumulation, leaf expansion, stomatal conductance, water use efficiency (WUE), Nitrogen use efficiency (NUE), etc.), with superimposable trends in both lettuce and rocket. The addition of 10^{-6} M GA_3 to the nutrient solution of a hydroponic floating system can promote growth and quality of lettuce and rocket plants.

Keywords: gibberellic acid; GA_3; leafy vegetables; lettuce; rocket; hydroponics; floating system

1. Introduction

Recently, consumers' consciousness of the importance of eating healthy foods [1–5] has raised notably. This is strictly related to the awareness of the consumers that an increase of vegetable intake might reduce the risk of cancers as well as many other degenerative diseases [6,7]. As a consequence of this phenomenon, the demand for vegetables increased over the past 25 years with the result that the world's value of trade in vegetables overcame that of cereals [8]. To fulfill this growing request for vegetables, it became necessary to enhance the productivity of vegetable crops with environmentally friendly, cost-effective, and easy to use techniques. These goals might be reached in many ways, such as genetic improvement, innovative cultivation systems, grafting, growth promoting microorganism, and plant growth regulators [9–13].

Since their discovery, natural and synthetic plant growth regulators have been increasingly used in agriculture and in horticulture to modify crop plants by controlling plant developmental processes (germination, vegetative growth, reproductive development, maturity, senescence, and postharvest preservation) [14]. Among these, gibberellins (GAs) are essential endogenous hormones found in plants and fungi controlling plant development by regulating several physiological mechanisms [15]. GAs can stimulate stem and root elongation, leaf expansion, flowering, fruit senescence, seed germination, or dormancy [16]. They induce transcription of genes involved in cell elongation and cell division occurring

during growth [17]; moreover, they can also stimulate the expression of hydrolytic enzymes involved in the conversion of starch to sugar [14]. By controlling starch accumulation and use, gibberellin can influence overall plant growth. Thus, the GA signal in plant tissue can be converted into alterations in gene expression, plant physiology and morphology [17]. When the gibberellin-based products became commercially available, the astonishing results obtained from their application to many crops raised great expectations of consistently increasing plant productivity [18]. Exogenous applications of gibberellins were shown to actively influence various physiological activities, such as vegetative growth, flowering and flower morphology, earliness, fruit set, ion transport and osmoregulation, leaf area expansion, internode elongation and can also increase biomass production, fruit weight and dry matter [19–28]. These effects can vary greatly depending on hormone requirement, relative concentrations, and plant responses at different growth stages [29]. Many studies have focused the attention on the use of this phytohormone for improving the productivity and quality of several crop plants [30–37]. GAs have been commercially applied to control the vegetative growth of many horticultural crops. They might increase seed yield in firm-headed lettuce, enhance growth and sugar accumulation in sugar cane, accelerate peduncle elongation and bud development in artichokes and strawberry, etc. Recently, the application of exogenous gibberellic acid (GA_3) has gained a renewed interest with the aim to promote plant growth, improve yield and increase tolerance to abiotic stresses (e.g., drought, heat, salinity) [22–24,30,32,38,39]. Foliar sprays of low concentration of GA_3 has been tested with promising results on performance, quality, and salt tolerance of various fruit and seed vegetables both in soil and in hydroponic cultivation systems [30–33,36,38,40,41]. So far, the information available on the effects of gibberellic acid application on leafy vegetables grown in hydroponic systems are quite scarce. The relations among GA_3 supply through foliar spray or through the nutrient solution and yield, quality, and post-harvest life of these vegetables still need further investigations. The quality of leafy vegetables is strictly related to leaf appearance and nutritional value (vitamin C, nitrates, antioxidants, etc.). Gibberellins may play a key role in many metabolic pathways affecting these characteristics, such as chlorophyll production and degradation, translocation of assimilates, nitrogen metabolism, and nitrogen redistribution. As stated above, these effects can vary greatly among different species, growth stages, application dose and methods, and cultivation techniques. Therefore, the present study was aimed at characterizing the effect of adding gibberellic acid (GA_3) to the mineral nutrient solution on growth, yield, and quality of leaf lettuce and rocket grown in a floating system.

2. Materials and Methods

2.1. Leafy Vegetable Cultivation

This study was carried out in greenhouse conditions at the Department of Agricultural, Food and Forest Sciences (SAAF—University of Palermo, Italy) (38°6′28″ N, 13°21′3″ E; altitude 49 m). Plants of leaf lettuce (*Lactuca sativa* L. var. *Crispa*) and rocket (*Eruca sativa* L.) were cultivated in a hydroponic floating system using nutrient solutions with four concentrations of GA_3 (Gibrelex, Biolchim, Bologna, Italy): 0, 10^{-8}, 10^{-6}, and 10^{-4} M GA_3. The mineral nutrient solutions (MNS), prepared using tap water (electrical conductivity (EC) 480 μS cm^{-1}; pH 7.6), contained 4.5 mmol L^{-1} of Ca^{2+}, 2 mmol L^{-1} of $H_2PO_4^-$, 1.25 mmol L^{-1} of NH_4^-, 1 mmol L^{-1} of Mg^{2+}, 19 mmol L^{-1} of NO_3^-, 11 mmol L^{-1} of K^+, 1.1 mmol L^{-1} of SO_4^{2-}, 40 μmol L^{-1} of Fe^{3+}, 5 μmol L^{-1} of Mn^{2+}, 4 μmol L^{-1} of Zn^{2+}, 30 μmol L^{-1} of BO_3^3, 0.75 μmol L^{-1} of Cu^{2+}, and 0.50 μmol L^{-1} of Mo [42], and differed only in GA_3 concentration. The mineral nutrient solution had an EC of 2.25 mS cm^{-1} and a pH of 5.8. Each MNS was poured into 4 different tanks (100 cm long × 50 cm wide × 15 cm deep, containing 75 L). Seedlings with 3 to 4 true leaves of leaf lettuce (cv. 'Lattuga da Taglio a Foglia Liscia', Sementi Dotto—SDD SPA, Udine, Italy) and rocket (cv. 'Coltivata da orto', Sementi Dotto—SDD SPA, Udine, Italy), grown in polystyrene trays (160 holes), were transplanted (28 February) in drilled polystyrene panels (400 plants m^{-2}) that were then placed to float in the tanks (Figure 1).

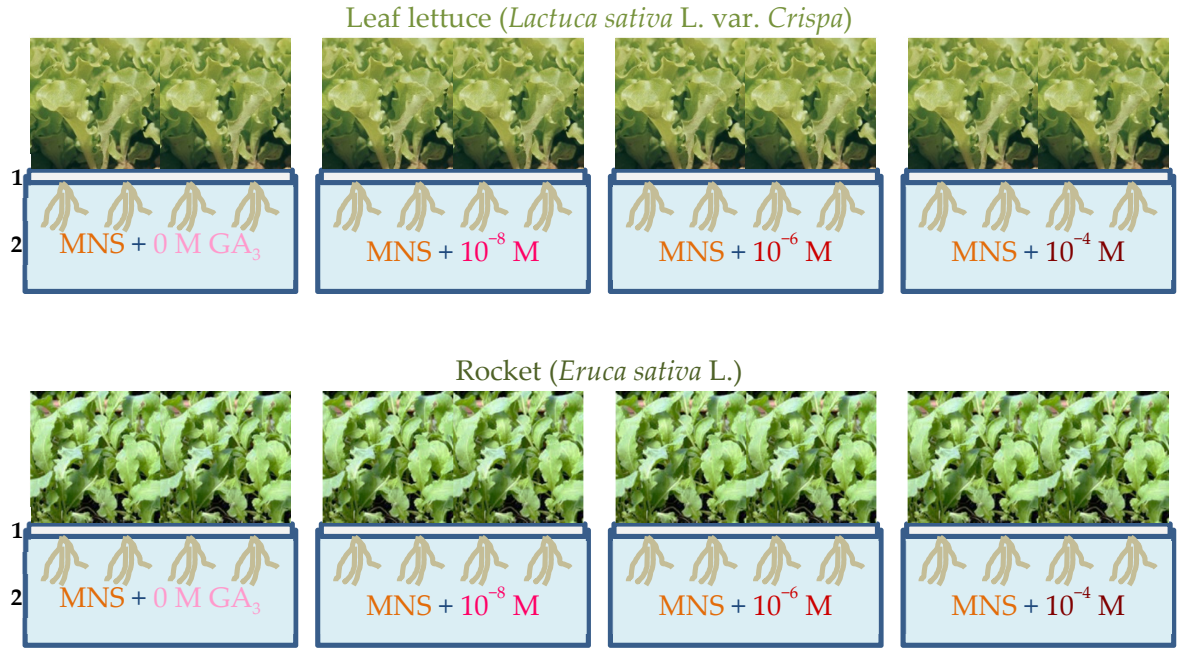

Figure 1. Graphical representation of the hydroponic floating system consisting of drilled polystyrene panels floating on mineral nutrient solutions (MNS) with different level of gibberellic acid (GA_3) ([1]Drilled polystyrene panels (400 plants m^{-2}) floating on MNS; [2]Tanks containing 75 L of MNS added with increasing concentrations of GA_3).

Each treatment was composed of four replicated tanks for each GA_3 concentration (200 plants for each tank; 16 tanks for each species). The MNSs were not aerated during crop cultivation because leafy vegetables have a fast growth and do not require high oxygen concentration in the hydroponic solution [43]. The MNS was regularly checked to assess water consumption and EC and pH modifications. The tanks were refilled with new MNS, with the same GA_3 concentration, when the volume dropped by 20%. The consumption of MNS was measured for each experimental treatment. The MNS in the tanks was completely covered by the polystyrene panels. Hence, the amount of water loss through evaporation was negligible and was not considered. This allowed calculating the water use efficiency (WUE) as WUE (g DW L^{-1} H_2O) = plant dry weight (g)/H_2O (L). At harvest, the MNS in the tanks was analyzed to calculate the residual amount of N-NH_4^+ and N-NO_3^- (determined reflectometrically by a Merck RQflex10 reflectometer according to the company protocols (Merck, Darmstadt, Germany)). This allowed the estimation of the total N uptake during the crop cycle and to calculate Nitrogen use efficiency (NUE) [44] as NUE (g DW g^{-1} N) = plant total dry weight (g)/plant N uptake (g).

Stomatal conductance was measured (15 d after transplant) with a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, England) on two recently expanded, unshaded leaves of 20 plants for each replicate.

All the plants were harvested 22 days after transplant, and marketable yield was calculated after eliminating decayed or yellowed older leaves. Then, 20 plants were randomly selected for each replicate and destructively sampled. Plant height, root length, leaf number, main leaf width (leaf lettuce), petiole length (rocket), and leaf area were determined. Leaf area was calculated for each plant by digital image analysis. Leaves were sampled, immediately weighed, then scanned with a resolution of 350 dpi (Epson Perfection 4180 Photo, Seiko Epson Corp, Suwa, Japan); the images were analyzed with the ImageJ 1.52a software (National Institutes Health, Bethesda, MD, USA). Scanned leaves were dried at 85 °C to a constant weight and re-weighed to calculate the specific leaf area (SLA cm^2 g^{-1}) as leaf area/leaf dry weight. Afterward, another 20 plants randomly selected for each replicate were

separated into epigeal (stems and leaves) and hypogeal (roots) fractions, weighed and then dried to constant weight at 85 °C for fresh and dry biomass determination.

A third sample of 20 plants for each replicate was used for leaf color measure and chemical determinations. Leaf color components (L*, a*, and b*) were recorded with a colorimeter (CR-400, Minolta Corporation, Ltd., Osaka, Japan) at two areas of photosynthetic tissue on the upper part of twenty leaves, randomly selected for each leafy vegetable and each treatment. Hue angle (h°) and Chroma (C*) were calculated as $h° = 180° + \arctan(b^*/a^*)$ [45] and $C^* = (a^{*2} + b^{*2})^{1/2}$. Twenty grams of leaves from each sample were then homogenized with H_2O (1:2 *w/v*), and the homogenates were centrifuged at 3500 rpm for 10 min. The extracts were used to determine soluble solid content (SSC), ascorbic acid and nitrate content, and titratable acidity (TA). SSC (°Brix) determination was performed with a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd., New Taipei City, Taiwan). Ascorbic acid and nitrate content (respectively, mg 100 g^{-1} and mg kg^{-1} of fresh weight) were determined reflectometrically by a Merck RQflex10 reflectometer according to the company protocols (Merck, Darmstadt, Germany). TA (expressed as mg of citric acid per 100 g of fresh weight) was determined by titrating 10 mL of extract with 0.1M NaOH up to pH 8.1.

2.2. Statistics and Principal Component Analysis

The experimental layout consisted of four replicates for each GA_3 level and each leafy vegetable randomly assigned in four blocks. To determine the effect of the GA_3 level on each leafy vegetable, a one-way ANOVA was carried out. Differences between means were determined by Tukey's multiple-range test at the 5% level.

Principal components analysis (PCA) was performed to study the correlation among the different GA_3 levels, and the agronomic and quality characteristics of leaf lettuce and rocket. The input matrix for the analysis comprised plant height, root length, whole plant fresh weight (FW), epigeal part (E) FW, roots (R) FW, E/R FW, whole plant dry weight (DW), epigeal part DW, roots DW, E/R DW, epigeal DW, root DW, yield, WUE, NUE, leaf no., plant area, leaf area, SLA, stomatal conductance, L*, a*, b*, chroma, hue, SSC, TA, ascorbic acid and N-NO_3^-. The optimum number of principal components (PCs) was assessed by retaining the factors with eigenvalues higher than 1.0. Furthermore, the plot of the PCs allowed the investigation of the correlations between the variables of the input data set. With this regard, the initial variables were projected into the subspace defined by the first and second PCs, and correlated variables were determined. Principal component analysis was performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

During plant growth, the average temperature outside the greenhouse ranged between 18.0 ± 0.3 °C (day) and 10.4 ± 0.4 °C (night) and the net solar radiation at noon was on average 467 $W \cdot m^{-2}$. The day length during the cultivation period (from sunrise to sunset) ranged between 11 and 12 hours. Inside the greenhouse, the mean air and MNS temperatures were 20.5 ± 0.6 °C and 17.3 ± 0.3 °C, respectively. Air temperature ranged between 32.0 ± 0.9 °C (day) and 13.4 ± 0.4 °C (night). During the experiment, the highest light intensity inside the greenhouse was 38,218 lux on average, ranging from 60,802 to 11,259 lux as a function of cloudiness.

The characteristics of the nutrient solutions in the tanks changed during plant growth, due to water absorption, as well as to MNS refills. During plant cultivation, the EC and pH of MNS had no significant differences due to GA_3 treatments but showed a different trend for lettuce and rocket. The EC of MNS slightly decreased only for lettuce plants and, at harvest, reached 2.00 mS cm^{-1} on average, while the MNS of rocket plants did not vary their EC. The pH increased up to 6.50 and 6.76, for lettuce and rocket, respectively.

During the first days after transplant, the plants of leaf lettuce and rocket grown in the MNS added with GA_3 showed a visibly greater growth rate than those grown without exogenous gibberellic acid. After the first (lettuce) or the second week (rocket) from transplant, the plants grown with the highest

GA_3 concentration in the MNS (10^{-4} M) started to show leaf morphology modifications (narrow and elongated leaves) and lengthened internodes so that 10 to 14 days after transplant, the plants lost their marketability (Figure 2). Hence, plants grown with 10^{-4} M GA_3 were not further examined. Lettuce and rocket plants were harvested at the same time 22 days after transplant.

Figure 2. Plants of leaf lettuce (**a**,**c**) and rocket (**b**,**d**) after two weeks of cultivation on a mineral nutrient solution with 10^{-4} M GA_3 and without GA_3, respectively.

3.1. Morpho–Physiological Parameters and Yield of Leaf Lettuce

At harvest, the height of leaf lettuce plants showed to be significantly influenced by the presence of 10^{-6} M GA_3 in the MNS. At this concentration, plants were 6.8 cm higher than control plants (25.0 cm) but had no difference in root length (Table 1). The total fresh biomass of control plants was 13.4 g plant^{-1}, whereas the fresh weight of the plants grown with 10^{-6} M GA_3 in the MNS was 41.1% higher (Table 1; Figure 2). The higher plant fresh weight derived from the increase of the epigeal part (E) weight (+44.5%), as the root weight (R), showed no significant difference due to the treatments. Therefore, the E/R ratio significantly increased from 8.3 in control plants up to 10.7 in the plants grown with 10^{-6} M GA_3 (Table 1).

Table 1. Yield and morphological parameters of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3).

	GA_3 (M)		
	0	**10^{-8}**	**10^{-6}**
Plant height (cm)	25.0 b [1]	25.2 b	31.8 a
Root length (cm)	31.1 a	30.7 a	32.0 a
Plant fresh weight			
Whole plant (g)	13.4 b	14.7 b	18. 9a
Epigeal part (g)	12.0 b	13.2 b	17.3 a
Roots (g)	1.4 a	1.5 a	1.6 a
Epigeal/Roots Ratio	8.3 b	8.8 b	10.7 a
Plant dry weight			
Whole plant (g)	0.47 b	0.57 b	0.75 a
Epigeal part (g)	0.39 b	0.49 b	0.66 a
Roots (g)	0.07 a	0.09 a	0.09 a
Epigeal/Roots Ratio	5.3 b	5.7 b	7.6 a
Epigeal dry matter (%)	3.3 b	3.7 a	3.8 a
Root dry matter (%)	5.2 b	5.4 ab	5.7 a
Yield (kg m^{-2})	4.8 b	5.3 b	6.9 a
WUE (g DW L^{-1} H_2O)	2.6 b	3.1 ab	3.5 a
NUE (g DW g^{-1} N)	11.6 b	13.6 ab	14.7 a

[1] Results indicate the mean value of four replicates. Data within a row followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test. WUE: water use efficiency. NUE: nitrogen use efficiency.

Similar to fresh biomass, the dry biomass of lettuce plants was positively affected by GA_3 treatments. Total dry weight increased significantly only in the plants grown with 10^{-6} M GA_3 in the MNS (Table 1), but to a greater extent than fresh weight as it raised by 59.9%. Root dry weight did not show changes due to the presence of GA_3 in the MNS, so, the increased total dry weight should be ascribed to the increase of the epigeal part (+67.8%) (Figure 3). Hence, the dry matter distribution between roots and shoot changed significantly as showed by the E/R ratio that increased from 5.3 of control plants up to 7.6 in the plants grown with 10^{-6} M GA_3 (Table 1). The dry matter percentage was positively influenced by GA_3 presence in the MNS with a significant difference of 0.5 between control and 10^{-6} M GA_3 in both epigeal part and roots (Table 1).

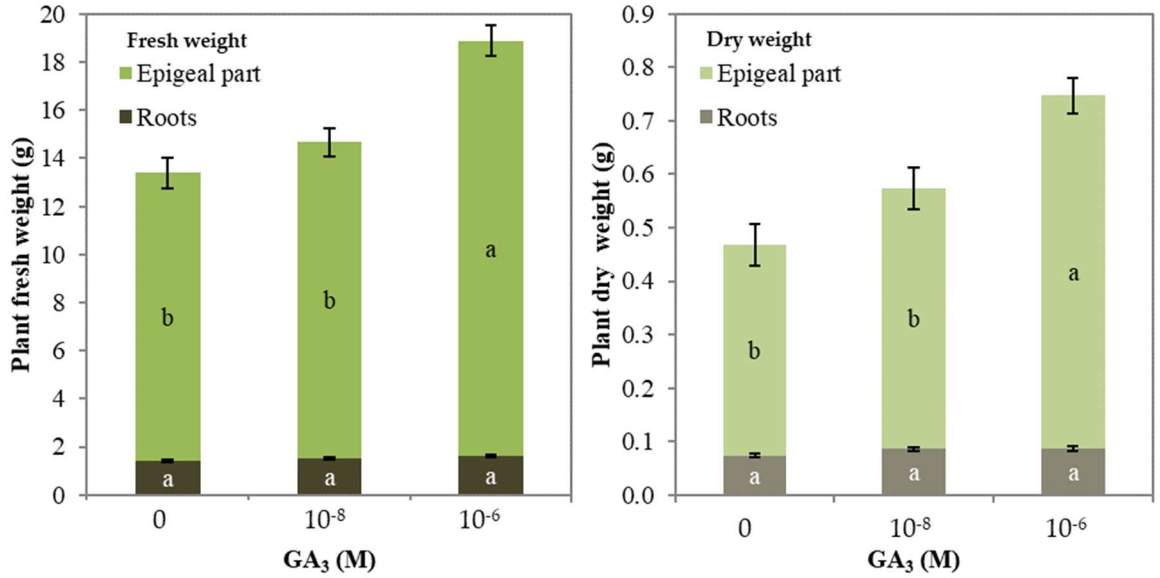

Figure 3. Fresh and dry biomass of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

The plants of lettuce grown without GA_3 yielded 4.8 kg m^{-2}; the addition of GA_3 to the MNS at the highest concentration (10^{-6} M) allowed to yield 2.1 kg m^{-2} more (+44.6%) (Table 1). During the crop cycle, the nutrient solution consumed by the plants was periodically restored, and their consumption was measured for each experimental treatment. This allowed calculating the water use efficiency (WUE) and nitrogen use efficiency which were, respectively, 2.6 g DW L^{-1} H_2O and 11.6 g DW g^{-1} N in control plants. WUE and NUE increased with increasing GA_3 in the MNS and were significantly higher at the highest concentration (10^{-6} M GA_3; + 33.4% and + 28.1%, respectively) (Table 1; Figure 4).

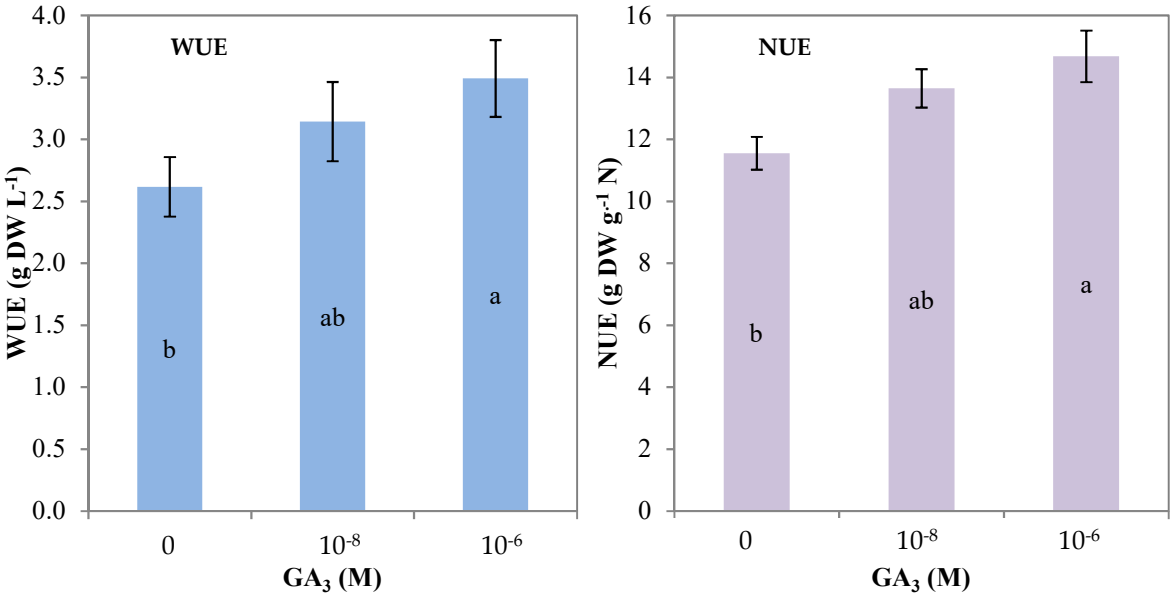

Figure 4. Water use efficiency (WUE) and nitrogen use efficiency (NUE) of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

Gibberellic acid also affected the leaf characteristics of lettuce plants, which had 8.0 leaves per plant if grown in the absence of GA_3 supplementation, whereas, plants grown with GA_3 in the MNS

were leafier, especially at 10^{-6} M GA_3 (9.1 leaves plant^{-1}) (Table 2; Figure 5). GA_3 did not change leaf width but increased leaf area when added to the MNS at 10^{-6} M GA_3 (548.3 cm^2 plant^{-1} and 60.5 cm^2 leaf^{-1}; + 25.7% and + 10.9% than control plants, respectively) (Table 2; Figure 5).

Table 2. Leaf characteristics of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3).

	GA_3 (M)		
	0	**10^{-8}**	**10^{-6}**
Number of leaves	8.0 b [1]	8.3 ab	9.1 a
Leaf width (cm)	8.8 a	9.4 a	8.7 a
Leaf area (cm^2 plant^{-1})	436.1 b	477.8 b	548.3 a
Leaf area (cm^2 leaf^{-1})	54. 5 b	57.8 b	60.5 a
Specific Leaf Area (cm^2 g DW^{-1})	704.5 b	785.2 ab	829.1 a
Stomatal conductance (mmol m^{-2} s^{-1})	610.0 b	799.0 a	882.0 a
L*	54.8 a	51.0 b	50.9 b
a*	-21.7 a	-22.0 a	-21.7 a
b*	39.0 a	38.2 ab	37.2 b
Chroma	44.7 a	44.1 ab	43.1 b
Hue°	119.1 b	120.0 ab	120.3 a
Soluble solid content (°Brix)	2.9 a	3.1 a	2.5 a
Titratable acidity (mg 100 g^{-1} FW) [2]	30.7 a	28.8 a	25.9 a
Ascorbic Acid (mg 100 g^{-1} FW)	78.5 a	53.0 a	58.0 a
N-NO3$^-$ (mg kg^{-1} FW)	2535.0 a	2305.0 a	2505.0 a

[1] Results indicate the mean value of four replicates. Data within a row followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test. [2] Titratable acidity expressed as citric acid.

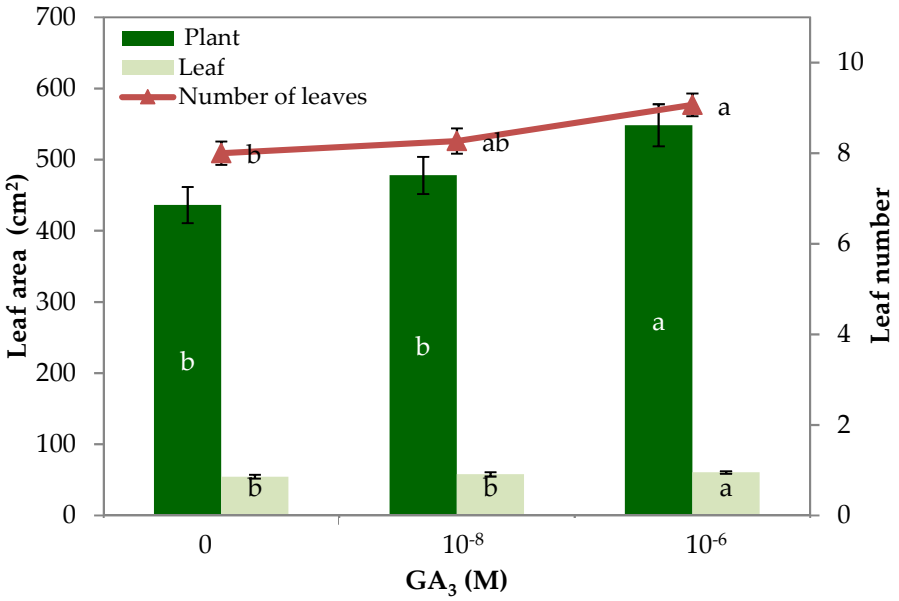

Figure 5. Leaf number and plant and leaf area of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color or points in a line with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

A significant increase in the specific leaf area (SLA) was found with increasing GA_3 concentration in the MNS, showing that GA_3 also influenced leaf thickness. SLA was 704.5 cm^2 g^{-1} DW in control plants and raised up to 829.1 cm^2 g^{-1} DW in the plants grown with 10^{-6} M GA_3 in the MNS (Table 2; Figure 6).

The measures of stomatal conductance confirmed that nutrient solution added with GA_3 affected leaf characteristics and physiology. In fact, the plants supplied with GA_3 had a significant increase

of stomatal conductance that was on average 840.5 mmol m^{-2} s^{-1}, 37.8% higher than control plants (610 mmol m^{-2} s^{-1}) (Table 2; Figure 6).

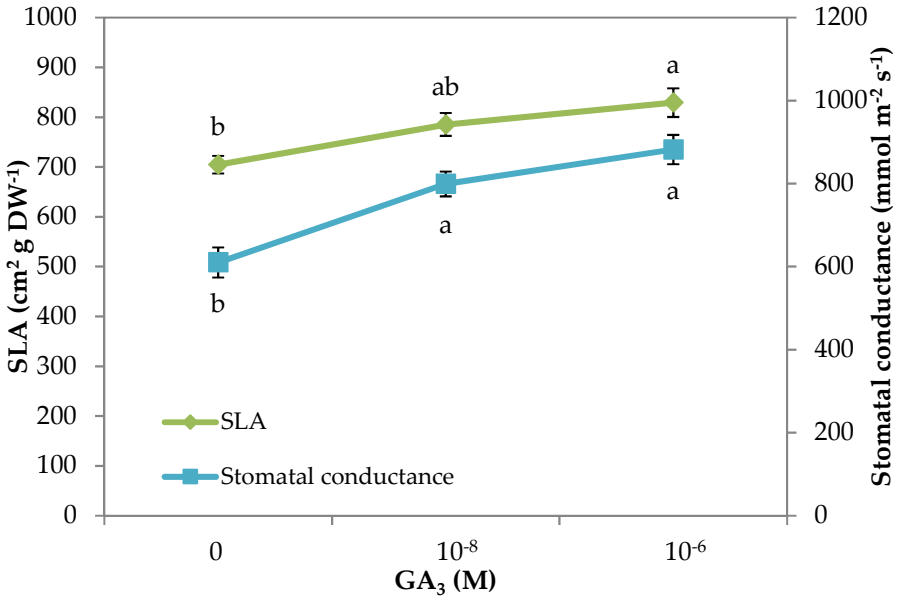

Figure 6. Specific leaf area (SLA) and stomatal conductance of leaf lettuce plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Points in a line with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

The plants grown in nutrient solutions added with GA_3 showed significant leaf color changes (Table 2). At harvest, the leaves of the plants supplied with GA_3 had lower L* value (lightness) and lower yellow component (b* value) than those of control plants, resulting in a darker and less vivid (lower chroma) greenish (higher hue angle) color (Table 2).

The quality of leaf lettuce was assessed evaluating soluble solid content (SSC), titratable acidity (TA), nitrate and ascorbic acid content of the leaves, which represent the edible part of this plant (Table 2). SSC, TA, and ascorbic acid content were not significantly influenced by GA_3 treatments even if a decreasing trend was found as increasing GA_3 dose. This trend was not found in the nitrate content of lettuce leaves, which accumulated 2448.3 mg kg^{-1} FW on average.

3.2. Morpho–Physiological Parameters and Yield of Rocket

The addition of GA_3 in the nutrient solution significantly influenced almost all the examined morpho–physiological parameters of rocket plants.

At harvest, the plants grown with 10^{-6} M GA_3 (24.0 cm) were slightly but significantly higher than control plants (21.9 cm). Root length showed a decreasing trend with increasing GA_3 concentration in the MNS but with no significant difference (20 cm on average) (Table 3).

A similar effect was also found on biomass accumulation in shoot and roots. In fact, plants treated with 10^{-6} M GA_3 had a significantly higher total fresh weight (+40.2%) than control plants (10.3 g $plant^{-1}$) (Table 3; Figure 7). This increase has to be ascribed to the epigeal part of the plant (42.9% more than control with 10^{-6} M GA_3), as the root weight did not record significant variations (0.38 g on average). The E/R ratio increased from 17.9 of control plants up to 27.9 in the plants grown with 10^{-6} M GA_3 (Table 3). The effect of GA_3 was also significant at the lower concentration with regard to the dry biomass accumulation. But, even in this case, this variation was only due to an increase of the dry weight of the epigeal part. Control plants had an epigeal dry weight of 0.36 g DW $plant^{-1}$ and an E/R ratio of 9. Epigeal dry weight increased up to 0.49 g DW $plant^{-1}$ (+36.6%) and 0.58 g DW $plant^{-1}$ (+59.0%) for 10^{-8} and 10^{-6} M GA^3, respectively. E/R reached the highest value with 10^{-6} M GA_3 (12.9)

(Table 3; Figure 7). Shoot and root dry matter percentage were influenced in different ways by the level of GA_3 in the nutrient solution. The epigeal dry matter percentage was 5.1% in control plants and increased significantly adding GA_3 in the MNS, independently of the concentration, whereas roots dry matter percentage (10.2% in control plants) increased significantly only with the highest GA_3 level (12.7%) (Table 3).

Table 3. Yield and morphological parameters of rocket plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3).

	GA_3 (M)		
	0	**10^{-8}**	**10^{-6}**
Plant height (cm)	21.9 b [1]	23.1 ab	24.0 a
Root length (cm)	21.0 a	19.9 a	19.1 a
Plant fresh weight			
Whole plant (g)	7.3 b	8.4 b	10.3 a
Epigeal part (g)	7.0 b	8.0 b	9.9 a
Roots (g)	0.39 a	0.39 a	0.36 a
Ratio Epigeal/Roots	17.9 b	20.5 b	27.9 a
Plant dry weight			
Whole plant (g)	0.39 b	0.53 a	0.62 a
Epigeal part (g)	0.36 b	0.49 a	0.58 a
Roots (g)	0.04 a	0.05 a	0.05 a
Ratio Epigeal/Roots	9.0 b	10.8 ab	12.9 a
Epigeal dry matter (%)	5.1 b	6.1 a	5.8 a
Root dry matter (%)	10.2 b	11.6 ab	12.7 a
Yield (kg m^{-2})	2.8 b	3.2 ab	4.0 a
WUE (g DW L^{-1} H_2O)	2.1 b	2.8 a	2.9 a
NUE (g DW g^{-1} N)	8.8 b	11.3 a	11.5 a

[1] Results indicate the mean value of four replicates. Data within a row followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test. WUE: water use efficiency. NUE: nitrogen use efficiency.

The yield of rocket plants grown without GA_3 in the MNS was 2.8 kg m^{-2}. The yield of rocket plants increased with the addition of GA_3 to the MNS and reached the highest value with the highest GA_3 rate (4.0 kg m^{-2}; + 42.9%) (Table 3).

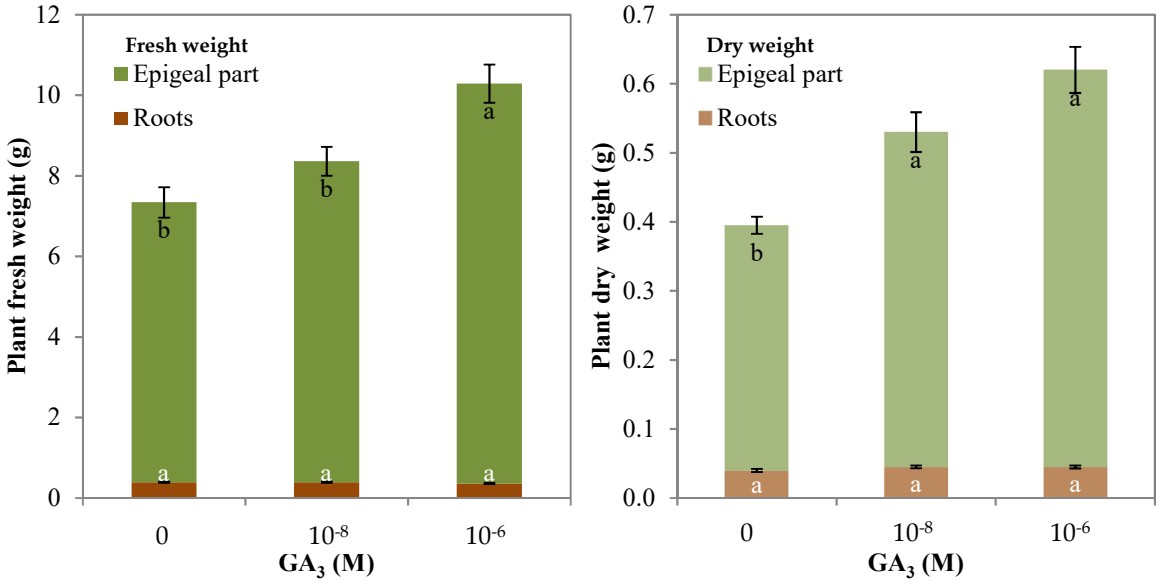

Figure 7. Fresh and dry biomass of rocket plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

A WUE of 2.1 DW L^{-1} H_2O was calculated for control rocket plants. This parameter increased significantly up to 2.8 (on average) when GA_3 was added to the MNS. GA_3 was also effective in enhancing NUE of rocket plants that increased from 8.8 g DW g^{-1} N in non-treated plants to 11.5 g DW g^{-1} N, on average, in GA_3-treated plants (Table 3; Figure 8)

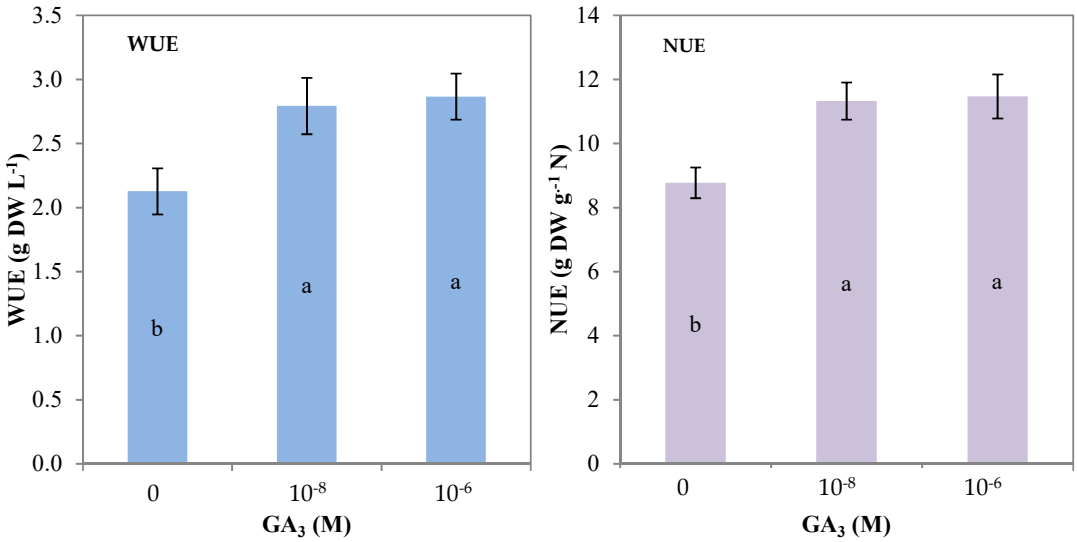

Figure 8. Water use efficiency (WUE) and nitrogen use efficiency (NUE) of rocket plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

The morphological traits of rocket leaves were significantly affected by the presence of gibberellic acid in the nutrient solution (Table 4). Plants grown with 10^{-6} M GA_3 had a higher number of leaves per plant (10.7) than control plants (9.5). The leaves of the treatment with 10^{-6} M GA_3 also differed from those of control plants in the length of their petiole (1.6 cm longer), but no difference was found in leaf blade length (11.4 cm on average) and in leaf area (10.7 cm^2 $leaf^{-1}$ on average). Thus, the higher total leaf area of the plants grown with GA_3 in the MNS (112.8 cm^2 $plant^{-1}$, + 13.6% on average) was mostly due to a higher number of leaves per plant (Figure 9).

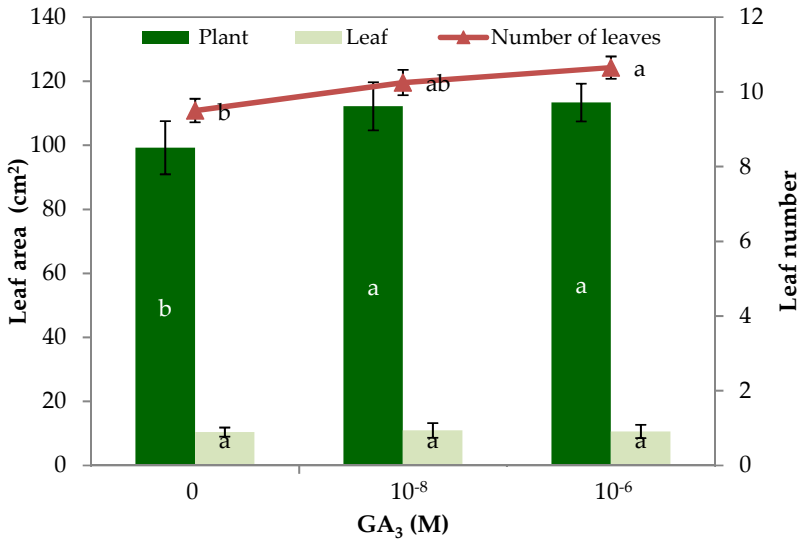

Figure 9. Leaf number and plant and leaf area of rocket plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Bars of the same color or points of a line with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

Specific leaf area was positively affected by the presence of GA_3 in the MNS, and the highest value (200.3 cm^2 g^{-1} DW) was recorded with 10^{-6} M GA_3 (Table 4; Figure 10).

The stomatal conductance of rocket leaves grown without GA_3 in the MNS was 322.9 mmol m^{-2} s^{-1} (Table 4; Figure 10); MNS supplemented with GA_3 induced a significant increase of the stomatal conductance (533.8 mmol m^{-2} s^{-1} on average), that had a positive linear correlation with SLA (R^2 = 0.901).

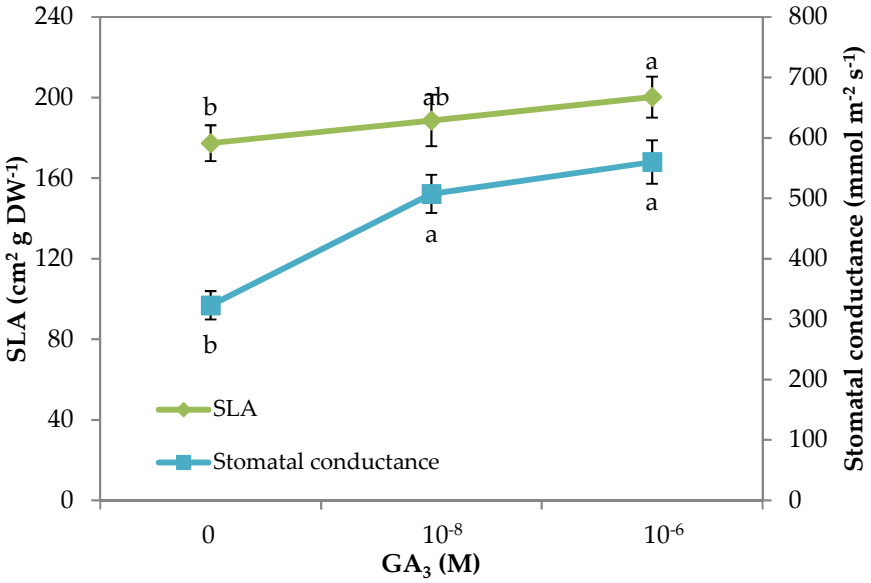

Figure 10. Specific leaf area (SLA) and stomatal conductance of rocket plants grown in nutrient solutions containing different levels of gibberellic acid (GA_3) (Points of a line with different letters are significantly different at $p \leq 0.05$ according to Tukey's test).

The color of rocket leaves at harvest changed significantly with the highest GA_3 concentration that affected L* and a* parameters; L* increased, whereas a* decreased with increasing GA_3 concentration in the MNS, corresponding to a darker greenish color (Table 4).

Table 4. Leaf characteristics of rocket plants grown in nutrient solutions containing different levels of GA_3.

	GA_3 (M)		
	0	**10^{-8}**	**10^{-6}**
Number of leaves	9.5 b [1]	10.3 ab	10.7 a
Leaf blade length (cm)	11.1 a	11.6 a	11.6 a
Petiole length (cm)	8.7 a	8.8 b	10.2 a
Leaf area (cm^2 $plant^{-1}$)	99.2 b	112.2 a	113.3 a
Leaf area (cm^2 $leaf^{-1}$)	10.4 a	10.9 a	10.6 a
Specific Leaf Area (cm^2 g^{-1} DW)	177.4 b	188.6 ab	200.3 a
Stomatal conductance (mmol m^{-2} s^{-1})	322.9 b	507.4 a	560.1 a
L*	44.7 a	43.5 ab	43.1 b
a*	-19.4 b	-19.1 ab	-18.6 a
b*	26.2 a	25.5 a	24.3 a
Chroma	32.6 a	31.8 a	30.6 a
Hue°	126.8 a	127.2 a	127.5 a
Soluble solid content (°Brix)	5.8 a	5.2 a	5.4 a
Titratable acidity (mg 100 g^{-1} FW) [2]	46.1 a	44.8 a	44.8 a
Ascorbic Acid (mg 100 g^{-1} FW)	173.0 b	221.0 a	218.0 a
N-$NO3^-$ (mg kg^{-1} FW)	2714.9 a	2364.9 ab	2069.9 b

[1] Results indicate the mean value of four replicates. Data within a row followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test. [2] Titratable acidity expressed as citric acid.

Rocket leaf quality was influenced by GA_3 treatments only regarding ascorbic acid and nitrate content. Control plants showed an ascorbic acid content of 173.0 mg 100 g^{-1} FW, whereas GA_3 treated leaves had 219.5 mg 100 g^{-1} FW on average (Table 4). On the contrary, nitrate content decreased with increasing GA_3 level in the MNS and ranged from 2714.9 mg kg^{-1} FW (control) to 2069.9 mg kg^{-1} FW (10^{-6} M GA_3).

3.3. Principal Components Analysis

The results of PCA showed two principal components (PCs) with eigenvalues higher than 1 (Table 5), accounting for 77.90% and 17.86% of the total variance, respectively. This indicated that the initial 29 variables could be expressed as a linear combination of two PCs, explaining 95.76% of the total variance. PC1 was mainly related to plant height, root length, whole plant FW, epigeal part FW, root FW, E/R FW, root DW, E/R DW, epigeal and root dry matter percentage, yield, NUE, leaf number, plant and leaf area, SLA, stomatal conductance, leaf color components, SSC, TA and ascorbic acid content; PC2 was related to whole plant and epigeal part DW, WUE and nitrate (Table 5).

Table 5. Correlation of variables to the factors of the principal components analysis (PCA) based on factor loadings.

Variables	PC1	PC2
Plant height	**0.766**	0.412
Root length	**0.990**	−0.137
Whole plant fresh weight	**0.932**	0.339
Epigeal part fresh weight	**0.905**	0.398
Root fresh weight	**0.999**	−0.049
E/R fresh weight	**−0.874**	0.439
Whole plant dry weight	0.465	**0.881**
Epigeal part dry weight	0.311	**0.943**
Roots dry weight	**−0.982**	0.179
E/R dry weight	**−0.823**	0.554
Epigeal dry weight	**−0.922**	0.340
Root dry weight	**−0.954**	0.296
Yield	**0.905**	0.398
Water Use Efficiency	0.655	**0.729**
Nitrogen Use Efficiency	**0.797**	0.566
Leaf No.	**−0.818**	0.564
Plant area	**0.999**	0.032
Leaf area	**0.998**	−0.050
Specific Leaf Area	**0.999**	−0.002
Stomatal conductance	**0.863**	0.480
L*	**0.911**	−0.342
a*	**−0.982**	0.035
b*	**0.973**	−0.213
Chroma	**0.973**	−0.216
Hue	**−0.975**	0.160
Soluble solid content	**−0.989**	−0.035
Titratable acidity	**−0.999**	−0.045
Ascorbic acid	**−0.968**	0.184
N-NO_3^-	0.151	**−0.693**

Values in bold within the same factor indicate the variable with the largest correlation.

The projection of the original variables on the plane of the two PCs could clearly demonstrate such a relationship, as reported in the plot of loadings (Figure 11a). The discrimination of the various GA_3 concentrations supplied to leaf lettuce and rocket plants can be seen in the plot of scores (Figure 11b), where two clusters could be visibly distinguished. Lettuce scores were located in the positive part of the PC1 axis and clearly separated from those of rocket, located in the negative part of the PC1 axis. The scores of 0 GA_3 had the lowest PC2 values for both leafy vegetables. The response of lettuce

and rocket differed when treated with GA_3, as the scores of treated rocket plants had both positive values of PC2, whereas only lettuce supplied with 10^{-6} M GA_3 was in the positive part of PC2 axis (first quadrant). Combining the data from the plot of loadings and scores, it can be concluded that GA_3 doses influenced the tested species in different ways (Figure 11a,b). Lettuce was related to all parameters positively related to PC1, whereas rocket was related to all parameters negatively related to PC1. The increase of GA_3 in the nutrient solution was positively related to plant dry weight, WUE, leaf number, E/R ratios, NUE, stomatal conductance, plant height, plant fresh weight and yield, and negatively related to nitrate content, L* and chrome.

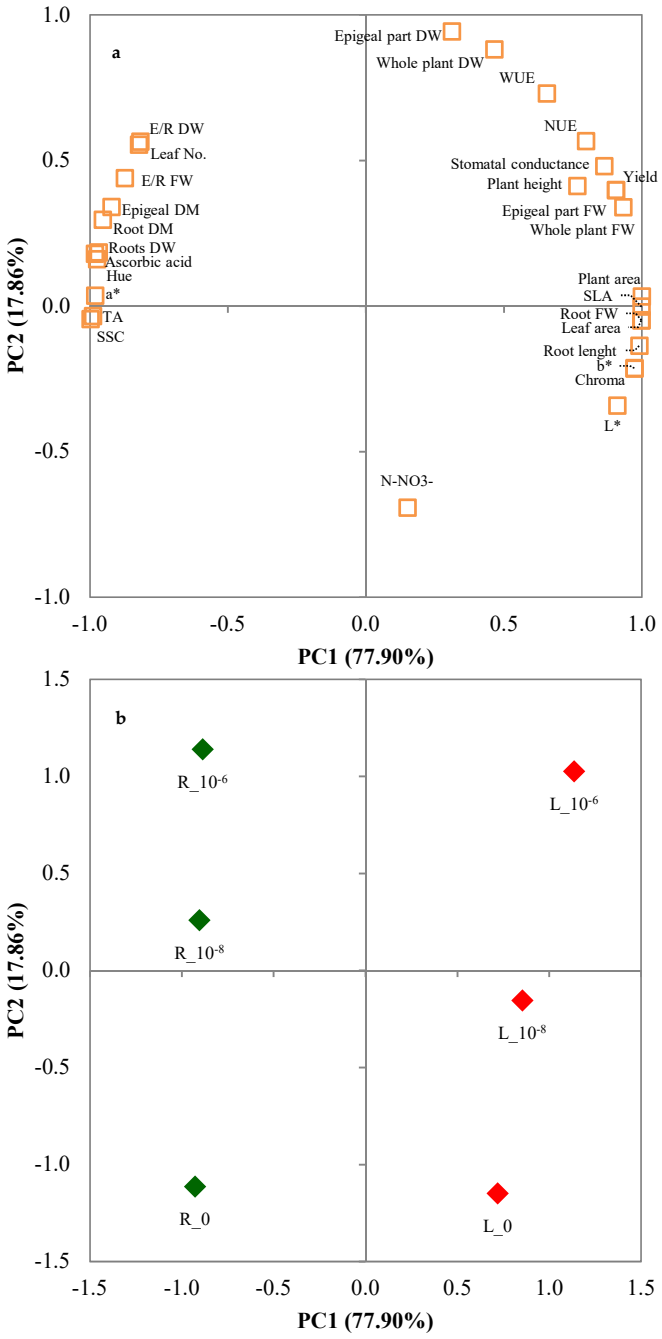

Figure 11. Plot of (**a**) loadings (morpho–physiological and quality characteristics of lettuce and rocket plants) and (**b**) scores (trials) formed by the two principal components from the PCA analysis. L_0, L_10^{-8} and L_10^{-6}: lettuce cultivated in nutrient solutions with 0, 10^{-8} and 10^{-6} M GA_3, respectively; R_0, R_10^{-8} and R_10^{-6}: rocket cultivated in nutrient solutions with 0, 10^{-8} and 10^{-6} M GA_3, respectively.

4. Discussion

Currently, GAs are used commercially to enhance morpho–physiological and yield characteristics of many vegetable and ornamental crops [9,46]. Many experiments have been carried out to study the effect of spraying exogenous gibberellic acid (GA_3) at very low concentrations on various crops [24,30,32,33,36,38,40,41], showing that hormone requirement, relative concentrations, and responses may vary for different species and different growth stages [29]. Foliar application of gibberellins has been generally adopted because these hormones are naturally synthetized in young leaves and from there are subsequently transported throughout the plant moving both acropetally and basipetally [47]. However, roots have been demonstrated to be a site of interconversion of gibberellin produced in shoots rather than a primary source [48]; in the roots, some deactivated gibberellins are converted to active GAs precursor and exported back to the shoots. In this experiment, we tested the feasibility of suppling low levels of GA_3 to leaf lettuce and rocket plants through the mineral nutrient solution of a floating system and evaluated their effects on growth and quality.

Both leaf lettuce and rocket were shown to be sensitive to exogenous GA_3 supplementation even if with a different response to tested levels. The marketability of plants was lost when 10^{-4} M GA_3 was added to the mineral nutrient solution. The leafy vegetables grown with this rate of GA_3 in the MNS responded with accelerated stem elongation that was accompanied by pale green and narrowed leaves in lettuce plants and by a relatively rapid flower development in rocket plants. It is well known that GA_3 treatments promote cell division and cell enlargements that may result in stem elongation [49–52] and that exogenous gibberellins can break the rosette or enhance and control the flowering of rosette plants by means of rapid enlargement of already differentiated tissues [53]. GAs promote cytogenesis and cell elongation in stems and can also stimulate flowering when they reach the shoot apex [54,55]. Below 10^{-4} M GA_3, the presence of GA_3 in the MNS was effective as a plant growth promoter and yield enhancer, especially at 10^{-6} M GA_3. This level of GA_3 increased significantly plant height in the tested leafy vegetables but to a different extent for lettuce and rocket. Plant hormones, such as gibberellins, brassinosteroids, and strigolactones play an important role in plant growth and development [56]. The cytological basis of GA-mediated regulation of plant height and organ size involves the promotion of both cell elongation and cell division [57,58].

GA_3 treatments also enhanced other morphological and physiological traits, with superimposable trends in both leafy vegetables. Lettuce and rocket treated with 10^{-6} M GA_3 showed an increase of total fresh biomass accumulation of 41.1% and 40.2%, respectively, as compared to controls. This increase was due to the increase of the epigeal part in both species (44.5% and 42.9%, respectively). This pattern was also found for dry biomass: the total dry biomass of plants increased by 59.9% and 59.0%, and the epigeal biomass by 67.8% and 62.0% for lettuce and rocket, respectively, as compared to controls. Hence, the addition of 10^{-6} M GA_3 affected resource repartition in the plants, promoting the growth of the aerial part as shown by the increase of the shoot:root ratio in both species. Gibberellic acid has been shown to exert a promoting effect of biomass production [20,23] of vegetative parts through the promotion of DNA, RNA, and protein synthesis [59–63] and ribose and polyribosome multiplication [64]. Biomass accumulation in GA_3 treated plants also follows from improved enzyme activity [65–68] and increased membrane permeability [69,70] that might facilitate uptake and use of mineral nutrients [33,68,71,72] and transport of photosynthates [73–76].

Moreover, GAs play important roles in regulating biomass allocation [77]. Genotypes of many species with high levels of endogenous GAs are characterized by higher leaf: root or shoot: root ratios compared to those with low levels of endogenous GAs [78]. Similarly, exogenous GA_3 may markedly change morphological traits of plants and promote biomass allocation to leaves. Sugiura et al. [79] found that the plants with high levels of endogenous GAs showed morphologies similar to those sprayed with GA_3 and highlighted a close relationship among morphological and physiological traits, thus confirming that this phytohormone is involved in the regulation of biomass allocation [80].

Many authors have shown that plant growth regulators can influence the phenotype, growth, and development of plants by changing hormonal content and their balance, which subsequently regulate

crop yield [81–85]. In our experiment, we found that the increase of biomass accumulation and the changes in biomass allocation caused by exogenous GA_3 supply (10^{-6} M GA_3), positively affected the yield of leaf lettuce and rocket with an increase of 44.6% and 42.9%, respectively.

GA_3 treated plants show enhanced carbonic anhydrase (CA) activity [86]. This enzyme has a role in photosynthetic CO_2 fixation [87] as it takes part in the hydration of CO_2 and is strictly associated with chloroplast [88]. This may guarantee enough supply of CO_2 at the site of its fixation, thus determining the high net photosynthetic rate and, consequently, high dry mass accumulation [86,89]. Yuan and Xu [90] reported that the increased net photosynthetic rate found after the application of GA_3 to broad bean leaves was accompanied by an increase in stomatal conductance and a decrease in intercellular CO_2 partial pressure. These effects of GA_3 supplementation could be very beneficial on leafy vegetables grown at high plant density in hydroponic floating systems, where self-shadowing and slow air movement inside the canopy could negatively affect light interception and CO_2 availability. The positive influence of GA_3 on CA activity and photosynthetic rate of lettuce and rocket plant could be indirectly confirmed by the increased stomatal conductance and the greater dry mass accumulation that we recorded in this trial.

The higher photosynthetic activity should also be ascribed to the morphological modification of lettuce and rocket plants grown with 10^{-6} M GA_3. Exogenous GA_3 supplied via the MNS increased leaf number and stimulated cell enlargement and leaf expansion, thus also affecting total leaf area and SLA. In addition, GA_3 treatments also influenced leaf color that showed a lower L*, b*, and chroma values and a higher hue angle than control plants. These parameters have a strong correlation with the chlorophyll content of leaves [91,92]. Hence, GA_3 supply could have also determined an increase of photosynthetic pigments. The response of chlorophyll content to GA_3 application is still controversial. Both decreases [93–95] and increases [94,96] in chlorophyll content were reported after the application of GA_3. Wheeler and Humphies [95] suggested that increases in leaf area caused by GA_3 may lead to chlorophyll dilution rather than to increased chlorophyll content. Probably these variations of chlorophyll content may depend on GA_3 dose and timing of application.

The combined positive effect of GA_3 on both stomatal conductance and leaf area development was revealed in terms of improved water use efficiency. Leaf stomatal conductance estimates rates of transpiration and gas exchange through leaf stomata. Maggio et al. [39] reported that the application of GA_3 decreased stomatal resistance and improved efficiency of water use in tomato plants. The mechanism that links GA_3 application with stomata opening is not well known; nevertheless, the increase of stomatal conductance due to GA_3 has been related to the higher accumulation of carbohydrates and potassium in guard cells of treated plants, that may influence speed and degree of stomata opening [97]. Even if the higher stomatal conductance of treated plants determines an increase of transpiration rates and water consumption, it also promotes gas exchange and photosynthetic CO_2 assimilation, thus increasing dry matter accumulation and WUE. In hydroponic floating systems, a high transpiration rate is not a problem as plants float on MNS. Thus, a higher stomatal conductance induced by GA_3 treatments could only be beneficial for this cultivation technique.

GA_3 affects nitrogen metabolism and nitrogen redistribution in plants and increases N-use efficiency (NUE) by improving better utilization of N [40]. In our work, we found an increase of NUE in treated plants of leaf lettuce and rocket (+ 27.1% and + 30.7%, respectively, with 10^{-6} M GA_3). The enhanced growth stimulated by exogenous GA_3 increases the nitrogen needs of the plants. GA_3 application may help to redistribute assimilates towards shoot apex and young leaves, aiding in the utilization of nitrogen and thus resulting in increased yield [40]. Moreover, the improvement in nitrogen utilization was possibly mediated by the effect of GA_3 on nitrate reductase activity [85,98], as confirmed by the reduction of nitrate accumulation in rocket treated with 10^{-6} M GA_3. This level of GA_3 might have affected nitrate reductase, but to a different extent in the tested leafy vegetables. The increased activity of this enzyme could have determined a reduction of nitrate accumulation in rocket during plant growth, while leaf lettuce plants could have not reduced the nitrate content during growth for a more efficient translocation from roots to leaves. SSC and TA were not significantly

affected by GA_3, whereas ascorbic acid content showed opposite trends in the tested leafy vegetables: It slightly decreased in lettuce and increased in rocket with increasing GA_3 levels. The concentration of vitamin C in some fruits and vegetables may be related to nitrogen availability, and this relationship may vary depending on genus, climate, and other factors [99,100]. It seems that to maintain ascorbic acid synthesis, leafy vegetables need to have sufficient N supply [101,102]. Thus, GA_3 treatments could have increased nitrogen needs of plants, which, in turn, have redistributed it to different biochemical processes.

The PCA analysis showed that lettuce and rocket were positively affected by GA_3 treatments even if with different extents for different parameters. Moreover, plants response to GA_3 treatment was shown to be species and dose-dependent, thus confirming that hormone requirement, relative concentrations, and responses may vary for different species [29].

These findings suggest the need to deepen the research on the dose and methods of application of GA_3 to vegetables according to the cultivation system. Moreover, GA_3 treatment during plant growth might also exert some effects on post-harvest characteristics of vegetables that could be worth to better investigating.

5. Conclusions

Growth and biomass accumulation of leaf lettuce and rocket plants were influenced by GA_3 level in the nutrient solution of a hydroponic floating system. The highest rate of GA_3 (10^{-4} M) had a negative impact on the crops as it strongly modified their morphology so that they lose marketability before harvest. Below this level, the presence of GA_3 in the MNS was effective as a plant growth promoter and yield enhancer, especially at 10^{-6} M GA_3. Various morphological and physiological traits were enhanced by GA_3 treatments (biomass accumulation, leaf expansion, stomatal conductance, WUE, NUE, etc.), with similar trends registered for both lettuce and rocket. Finally, this investigation suggested that the addition of 10^{-6} M GA_3 to the nutrient solution of a hydroponic floating system can promote growth and quality of lettuce and rocket plants.

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