agronomy

MDPI

Article

Influence of Silver Nanoparticles on Photosynthetic Pigment Content and Mineral Uptake in Pineapple Seedlings Grown In Vitro under Aluminum Stress

José Jesús Tejada-Alvarado [1,2], Jegnes Benjamín Meléndez-Mori [1,*], Rosmery Y. Ayala-Tocto [1], Malluri Goñas [1] and Manuel Oliva [1,*]

[1] Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM), Chachapoyas 01001, Peru; jose.tejada@untrm.edu.pe (J.J.T.-A.); rosmery.ayala@untrm.edu.pe (R.Y.A.-T.); malluri.gonas@untrm.edu.pe (M.G.)

[2] Maestría en Cambio Climático, Agricultura y Desarrollo Rural Sostenible (MACCARD), Escuela de Posgrado (EPG), Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM), Chachapoyas 01001, Peru

* Correspondence: jbenjamin@indes-ces.edu.pe (J.B.M.-M.); manuel.oliva@untrm.edu.pe (M.O.)

Abstract: The presence of toxic metals such as aluminum is described as a factor that could lead to a significant decrease in crop productivity, particularly for the cultivation of acidophilic plants. In the present study, in vitro cultivation of pineapple was used as a model to evaluate the role of silver nanoparticles (0.005, 0.01, 0.025, 0.05, and 0.1 g L^{-1} Ag NPs) against the negative effects of aluminum (0, 100, 300, and 500 µM $AlCl_3$). The results showed that the presence of 0.025 g L^{-1} Ag NPs stimulated a higher concentration of photosynthetic pigments "a", "b", "a + b" and carotenoids in treatments with high levels of $AlCl_3$. The application of Ag NPs allowed better shoot formation, improved chlorophyll a/b, and total/carotenoid ratios, as well as better levels of proline biosynthesis in response to stress. The synergistic interaction of Ag NPs and $AlCl_3$ increased the availability and assimilation of mineral elements (K, Ca, Mg, Fe, Cu, Mn, and Zn) while decreasing Al translocation. In conclusion, the implementation and validation of Ag NPs in agricultural fields would be revolutionary because they represent a novel alternative for overcoming the limitations imposed by the presence of Al.

Keywords: bioaccumulation; nutrient assimilation; phenotypic variation; phytotoxicity; toxicity of aluminum

Citation: Tejada-Alvarado, J.J.; Meléndez-Mori, J.B.; Ayala-Tocto, R.Y.; Goñas, M.; Oliva, M. Influence of Silver Nanoparticles on Photosynthetic Pigment Content and Mineral Uptake in Pineapple Seedlings Grown In Vitro under Aluminum Stress. *Agronomy* **2023**, *13*, 1186. https://doi.org/10.3390/agronomy13051186

Academic Editor: Prakash M Gopalakrishnan Nair

Received: 14 March 2023
Revised: 11 April 2023
Accepted: 12 April 2023
Published: 23 April 2023

1. Introduction

Pineapple (*Ananas comosus* L.) is the second most important tropical fruit after mango worldwide [1]. In recent years, its global production increased by 30% from 20 to 25.9 million tons between 2007 and 2017 [1]. However, it is a fruit that is usually grown on very acidic soils, making it potentially vulnerable to bio toxic forms of aluminum [2].

Aluminum (Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)^{2+}$), the third most abundant metal in the Earth's crust at 7% of its mass [3], possess a global problem for the cultivation of acidophilic plants (such as pineapple) because it can inhibit root growth and shoot formation (decreasing cell division) and limit nutrient and water uptake [4,5]. Due to Al toxicity, crops also suffer alterations at the physiological level such as the accumulation of high levels of phenolic compounds [6,7]. Physiologically, plants exposed to heavy metal stress have been found to accumulate high levels of phenolic compounds as a response to decreasing their toxicity, either by reducing oxidative stress or by capturing metal ions [6–9]. Therefore, toxic metals are a serious challenge for agriculture.

The solubility and toxicity of Al increase at low soil pH levels (<5) [2,10]. This is a serious problem as about 50% of the world's arable land and 30% of the total land area of the planet are acidic [10]. These numbers are increasing dramatically in developing countries due to the excessive use of nitrogen fertilizers, acid rain (climate change), intensive agriculture, and monoculture [11]. In pineapple cultivation, Al toxicity causes the

formation of increased callus levels as a result of the cell wall and cell membrane separation, enlargement of vacuoles, shrinkage, and reunification of mitochondria next to the inner cell membrane, which reduces the nutrient assimilation and hinders the formation of new propagules [2].

The development of nanoscience has led to a wide range of novel applications in micropropagation and other fields, promoting improved plant growth and development [12]. Recent research has shown that silver (Ag) in nanometer dimensions has a biocidal effect on a variety of explants and positively regulates ethylene production, callus formation, somatic embryo formation, organogenesis, somaclonal variation, secondary metabolite production, and genetic transformation [12]. On the one hand, the presence of metals which have an effect on crop productivity and yield is one of the main agricultural constraints that put many forms of life in the soil and water at risk [13,14]; on the other hand, the use of nanoparticles is a powerful alternative to decrease the negative effects of metal toxicity/stress [13]. For example, silver nanoparticles (Ag NPs) have significantly improved the physiological and biochemical parameters of plants grown on cadmium and lead-contaminated soils by immobilizing and modulating the toxicity of these metals [15]. The addition of Ag NPs has been demonstrated to have a high capacity to scavenge free radicals by inducing phytochemicals, leading to a significant decrease in malondialdehyde and hydrogen peroxide levels [15,16].

Ag NPs are among the current most widely used and applied nanomaterials to improve the conditions of agricultural production. For example, according to reports by Servin et al. [17] and Sillen et al. [18], Ag NPs can prevent the development of plant pathogens in the soil that damage plant roots and stems. However, altering the soil microbiome can also have an adverse effect on beneficial microorganisms that develop the symbiotic relationships necessary for nitrogen fixation and organic matter decomposition [19]. Therefore, to fully understand their precise roles in nutrient and metal (such as Al) assimilation, as well as metabolic responses and toxicological mechanisms in plants, species-specific research is needed in various species [20–22]. In this study, we aimed to evaluate the effect of silver nanoparticles on morphophysiology, biochemical behavior, and nutrient assimilation characteristics of pineapple plants grown under aluminum chloride stress conditions in vitro.

2. Materials and Methods

2.1. Plant Material

The propagation material, axillary shoots of pineapple cultivar Golden, was collected from healthy and vigorous plants grown in agricultural fields located in the district of Santa Rosa, Province of Rodriguez de Mendoza, Amazonas, Peru. The in vitro establishment phase was developed following Gómez et al. [23]. Axillary buds were grown for 60 days in PTL-100 flasks (PhytoTechnology Laboratories, Lenexa, KS, USA).

2.2. In Vitro Propagation and Growth Conditions

The culture medium with vitamins of Murashige and Skoog [24], supplemented with 30 g L^{-1} sucrose, 0.15 g L^{-1} ascorbic acid, 0.003 g L^{-1} 6-benzylaminopurine and 0.0003 g L^{-1} naphthaleneacetic acid, was used. Additionally, the medium contained different concentrations of aluminum chloride ($AlCl_3$ (0, 100, 300, and 500 µM)) and silver nanoparticles (Ag NPs (0, 0.005, 0.01, 0.025, 0.05, and 0.1 g L^{-1})). Culture media were adjusted to pH 4.5 and dispensed into 1 L disposable containers for autoclaving at 121 °C with 1.5 Kgf.cm^{-2} pressure for 20 min. The explants were grown for 45 days at 25 ± 1 °C under a photoperiod of 16 h light provided by white fluorescent rods (300 lux). Then, the number of shoots formed, and their phenotypic changes were evaluated.

In the experiment, a total of 24 treatments with 10 replicates was established and distributed according to a complete randomized design (CRD). Treatments were derived from the interaction of two factors: aluminum chloride × silver nanoparticles.

2.3. Evaluated Parameters

2.3.1. Photosynthetic Pigments

Chlorophyll a, b, a + b, and carotenoid contents were determined from 0.2 g of tender leaves and immediately isolated from the plant. The samples were crushed and then 200 mg of magnesium carbonate and 6 mL of 80% acetone were added. The procedure was performed in a Protector Basic 47 fume hood (Labconco Corp., Kansas City, MO, USA) under indirect light. The mixture obtained was transferred to amber Eppendorf tubes and centrifuged for 10 min at 2800 rpm (12 °C). Then, 1500 µL of the supernatant was transferred to quartz cuvettes for absorbance readings (663.2, 646.8, and 470 nm) on a Genesys 10S UV/Vis spectrophotometer (Thermo Scientific™, Madison, WI, USA). Photosynthetic pigment concentration was determined using the following equations [25]:

Chlorophyll a (µg/) mL: 12.25 $A_{663.2}$ − 2.79 $A_{646.8}$;
Chlorophyll b (µg/) mL: 21.50 $A_{646.8}$ − 5.10 $A_{663.2}$;
Chlorophyll a + b (µg/) mL: Chlorophyll a + Chlorophyll b;
Carotenoid (µg/) mL: (1000 A_{470} − 1.82 C_a − 85.02 C_b)/198.

2.3.2. Total Proline Content

We centrifuged a mixture of 0.5 g of lyophilized leaf tissue and 5 mL of 3% aqueous sulfosalicylic acid at 300 rpm for 60 min. A second centrifugation step was then carried out at 10,000 rpm for 30 min at 4 °C. Then, 2000 µL of the supernatant together with 0.1 M acid ninhydrin and 99.9% glacial acetic acid (hot) were added to foil-lined falcon tubes (1:1:1 ratio, *v*:*v*:*v*). The mixture was homogenized in a vortex for 1.5 min, then allowed to stand in boiling water for 60 min before cooling sharply on ice for 15 min to stabilize the reaction. Finally, 3 mL of toluene was added, stirred in a vortex for 60 s, and then allowed to stand for 10 min until phase separation occurred. The absorbance reading was taken at 520 nm on a Genesys 10S UV/Vis spectrophotometer (Thermo Scientific™, Madison, WI, USA). Readings were interpolated on a standard curve performed with L-proline [26], and expressed in µg proline/g dry plant material (µg/g).

2.3.3. Nutrient and Metal Accumulation

Leaf tissue samples were freeze-dried for 5 days with a FreeZone 4.5-L freeze dryer (Labconco Corp., Kansas City, MO, USA) for elemental analysis. The samples were then ground, and 0.2 g of crushed tissue was transferred to glass containers before digestion in a nitric–chloric acid solution (6:2, *v*:*v*). Subsequently, the solution was filtered, then ultrapure water was added up to 25 mL. The content of mineral nutrients (K, Mg, Ca, Zn, Fe, Cu, and Mn), as well as aluminum (Al) and silver (Ag), were quantified using an Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (Agilent Technologies, Santa Clara, CA, USA). Readings were expressed in ppm of dry mass after interpolating over calibration curves established with various concentrations of standard solutions for macronutrients (K, Ca, Mg (0.01, 0.5, 2, 5, and 10 ppm)), micronutrients (Fe, Cu, Mn, and Zn (0.05, 0.1, 0.4, 0.8, 2, 4 and 8 ppm)), and metals (Al and Ag (0.025, 0.5, 1, 5 and 10 ppm)).

2.4. Statistical Analysis

Statistical analysis was performed using InfoStat v.2020 software using general linear mixed model (GLMMs) techniques, which relaxes ANOVA assumptions [27,28]. Means were compared using the Di Rienzo–Guzman–Casanoves (DGC) multiple comparisons test [29] at a significance level of 5% ($p \leq 0.05$). Values were expressed as the means of 10 replicates ± standard deviation.

3. Results

3.1. Phenotypic Changes in Pineapple Seedlings Grown under Al Stress

The addition of 0.025 g L^{-1} of Ag NPs promoted the formation of the highest number of shoots in pineapples grown with 300 and 500 µM $AlCl_3$ (8.67 ± 1.21 and 7.67 ± 1.21, respectively), albeit with averages below the value recorded in the control treatment

(10.67 ± 1.63) (Table 1). Furthermore, the presence of $AlCl_3$ alone at concentrations of 100 and 300 µM promoted root formation (Figure 1); however, at higher concentrations (500 µM), there was a greater expression in the formation of undifferentiated cells (callus). The same phenomenon was observed in seedlings grown in a medium with a combination of 0.01 g L^{-1} of Ag NPs and 500 µM $AlCl_3$. On the other hand, the presence of Ag NPs alone promoted the formation of a greater number of shoots with a better visual appearance in terms of color. Particularly, the presence of 0.005 g L^{-1} of Ag NPs in the culture medium allowed the production of seedlings with a higher number of shoots and better biomass in the presence of 0 to 500 µM $AlCl_3$. Conversely, shoot malformations were more frequent at higher concentrations of Ag NPs regardless of their interaction with $AlCl_3$.

Table 1. Number of pineapples shoots in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Number of shoots	0	10.67 ± 1.63 a	7.00 ± 0.89 c	4.00 ± 0.89 d	3.17 ± 0.75 d	***	***	***
	0.005	4.50 ± 0.55 d	7.33 ± 0.82 c	6.33 ± 0.82 c	1.50 ± 0.55 e			
	0.01	1.33 ± 0.52 e	4.17 ± 0.75 d	3.50 ± 1.05 d	3.83 ± 0.41 d			
	0.025	7.50 ± 0.84 c	4.83 ± 0.75 d	8.67 ± 1.21 b	7.67 ± 1.21 c			
	0.05	3.50 ± 1.05 d	3.17 ± 0.75 d	4.50 ± 0.55 d	2.33 ± 0.82 e			
	0.1	3.67 ± 0.52 d	2.33 ± 0.52 e	3.50 ± 0.55 d	3.50 ± 0.84 d			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

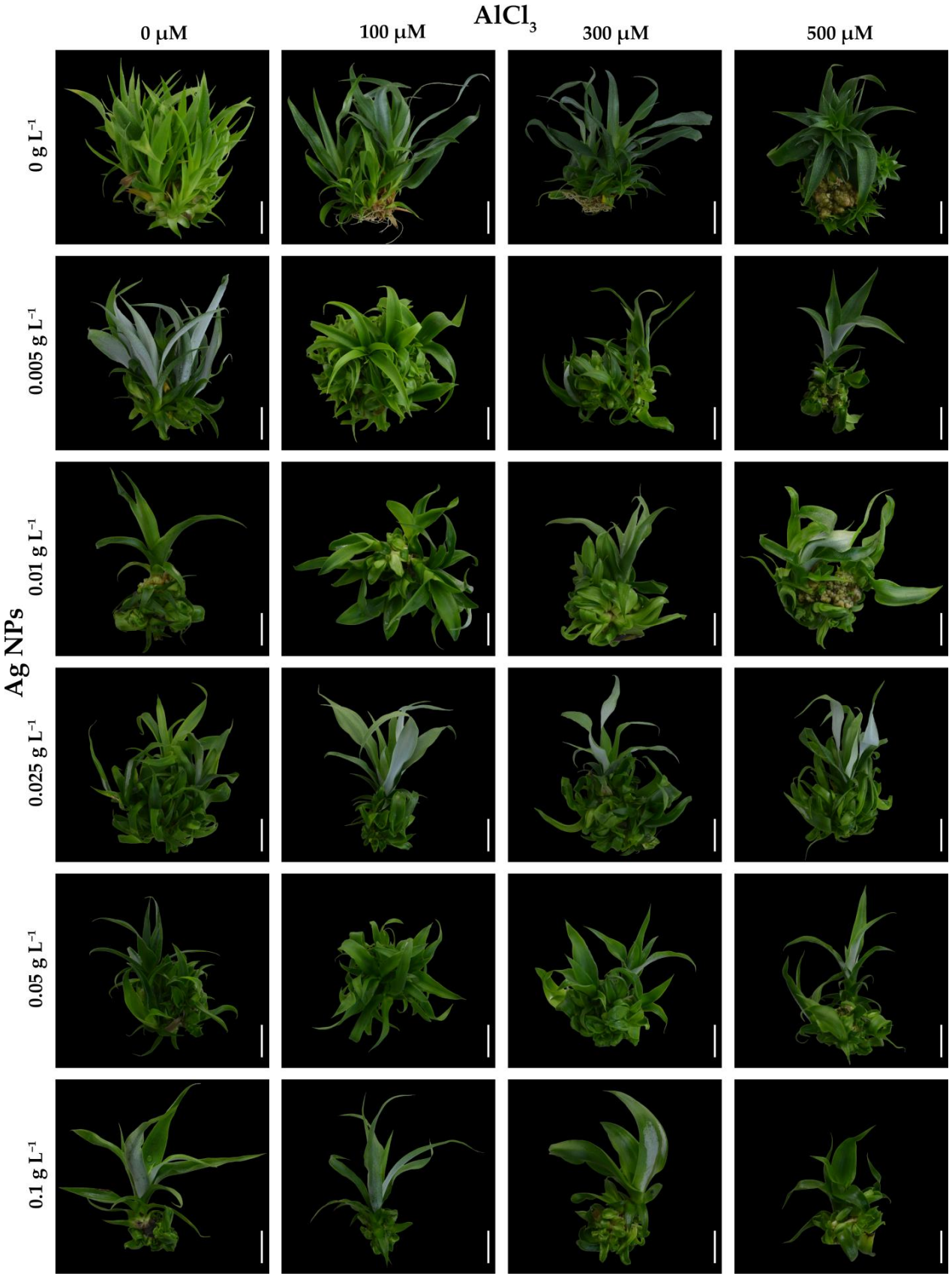

Figure 1. Phenotypic characteristics of aluminum ($AlCl_3$) adsorption mediated by silver nanoparticles (Ag NPs). Scale = 3 cm.

3.2. Photosynthetic Pigments

The factors (Ag NPs and $AlCl_3$) and their interactions significantly influenced ($p < 0.0001$) the photosynthetic pigments content (Table 2). The addition of 0.025 g L^{-1} of Ag NPs allowed pineapple seedlings grown on a medium with 100 and 500 µM $AlCl_3$ to reach higher concentrations of chlorophyll-a (30.83 ± 0.35 and 30.58 ± 0.19 µg mL^{-1}, respectively). Similar behavior was observed for chlorophyll-b content. The highest concentrations of this pigment in seedlings grown with 100, 300, and 500 µM $AlCl_3$ (23.94 ± 1.35, 34.22 ± 1.7 and 26.38 ± 1.27 µg mL^{-1}, respectively) were obtained when the medium also included the addition of 0.025 g L^{-1} of Ag NP. Importantly, the reduction in the amount of these pigments was due to an increase in the concentration of Ag NP in the culture medium, demonstrating that their useful properties are subject to specific concentration gradients.

Table 2. Photosynthetic pigment content in pineapple leaves in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Chlorophyll a (µg/)mL	0	30.04 ± 0.75 a	29.67 ± 0.62 a	30.41 ± 0.2 a	30.18 ± 0.63 a	***	***	***
	0.005	30.25 ± 0.25 a	28.76 ± 1.44 b	30.00 ± 0.42 a	30.49 ± 0.22 a			
	0.01	27.92 ± 0.88 b	29.42 ± 1.75 a	30.48 ± 0.08 a	30.33 ± 0.61 a			
	0.025	29.87 ± 1.19 a	30.83 ± 0.35 a	30.02 ± 0.24 a	30.58 ± 0.19 a			
	0.05	30.18 ± 0.83 a	30.45 ± 0.01 a	26.10 ± 0.01 c	8.07 ± 0.04 e			
	0.1	19.19 ± 1.18 d	18.12 ± 0.63 d	29.45 ± 0.75 a	29.99 ± 0.38 a			
Chlorophyll b (µg/)mL	0	26.54 ± 0.3 b	16.17 ± 0.68 d	24.68 ± 1.68 b	25.73 ± 1.06 b	***	***	***
	0.005	16.54 ± 1.77 d	20.93 ± 4.02 c	28.47 ± 3.88 b	26.05 ± 0.46 b			
	0.01	11.01 ± 0.80 f	19.90 ± 2.67 c	26.42 ± 1.2 b	22.54 ± 1.03 c			
	0.025	21.65 ± 0.11 c	23.94 ± 1.35 b	34.22 ± 1.7 a	26.38 ± 1.27 b			
	0.05	16.47 ± 0.3 d	22.25 ± 0.14 c	12.66 ± 0.03 e	10.97 ± 1.08 f			
	0.1	10.26 ± 0.96 f	6.41 ± 2.01 g	18.56 ± 0.33 c	15.84 ± 1.94 d			
Chlorophyll a + b (µg/)mL	0	56.58 ± 1.05 b	45.84 ± 0.13 d	55.09 ± 1.85 b	55.91 ± 1.22 b	***	***	***
	0.005	46.80 ± 2.02 d	49.69 ± 5.46 d	58.47 ± 3.50 b	56.54 ± 0.67 b			
	0.01	38.93 ± 1.68 e	49.32 ± 0.92 d	56.90 ± 1.27 b	52.87 ± 0.42 c			
	0.025	51.52 ± 1.10 c	54.77 ± 1.70 b	64.24 ± 1.56 a	56.96 ± 1.41 b			
	0.05	46.64 ± 0.86 d	52.70 ± 0.15 c	38.76 ± 0.04 e	19.03 ± 1.12 h			
	0.1	29.45 ± 1.06 f	24.54 ± 1.84 g	48.02 ± 1.08 d	45.83 ± 2.32 d			
Carotenoids (µg/)mL	0	5.93 ± 0.09 e	10.50 ± 0.30 b	6.56 ± 0.56 e	6.29 ± 0.80 e	***	***	***
	0.005	7.83 ± 0.73 c	6.73 ± 0.53 e	5.61 ± 0.79 e	6.42 ± 0.28 e			
	0.01	7.18 ± 0.17 d	7.74 ± 0.18 c	6.43 ± 0.20 e	7.36 ± 0.08 d			
	0.025	6.96 ± 0.09 d	7.47 ± 0.34 d	3.54 ± 0.59 g	6.44 ± 0.20 e			
	0.05	11.53 ± 0.42 a	7.28 ± 0.18 d	8.56 ± 0.01 c	4.64 ± 0.24 f			
	0.1	8.24 ± 0.81 c	10.40 ± 2.06 b	8.92 ± 0.86 c	7.59 ± 0.15 d			
Chl a/b ratio	0	1.13 ± 0.01 e	1.84 ± 0.10 c	1.24 ± 0.07 e	1.16 ± 0.28 e	***	***	***
	0.005	1.84 ± 0.15 c	1.40 ± 0.16 e	1.07 ± 0.15 e	1.17 ± 0.01 e			
	0.01	2.54 ± 0.09 b	1.50 ± 0.24 d	1.15 ± 0.04 e	1.35 ± 0.08 e			
	0.025	1.38 ± 0.05 e	1.29 ± 0.05 e	0.88 ± 0.04 f	1.17 ± 0.05 e			
	0.05	1.83 ± 0.06 c	1.37 ± 0.01 e	2.06 ± 0.00 c	0.74 ± 0.07 f			
	0.1	1.89 ± 0.23 c	3.00 ± 0.75 a	1.59 ± 0.01 d	1.91 ± 0.19 c			

Table 2. *Cont.*

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Chl a + b/Carotenoid ratio	0	9.54 ± 0.03 c	4.37 ± 0.12 f	8.44 ± 0.81 c	8.98 ± 1.08 c	***	***	***
	0.005	6.03 ± 0.78 e	7.46 ± 1.31 d	10.61 ± 1.76 b	8.82 ± 0.24 c			
	0.01	5.42 ± 0.10 e	6.38 ± 0.24 e	8.86 ± 0.40 c	7.19 ± 0.02 d			
	0.025	7.40 ± 0.21 d	7.35 ± 0.52 d	18.54 ± 3.00 a	8.85 ± 0.45 c			
	0.05	4.05 ± 0.14 f	7.24 ± 0.14 d	4.53 ± 0.01 f	4.12 ± 0.40 f			
	0.1	3.59 ± 0.27 f	2.40 ± 0.29 g	5.42 ± 0.61 e	6.04 ± 0.25 e			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

Analysis of carotenoid levels revealed that the highest levels (11.53 ± 0.42 µg mL^{-1}) were obtained from seedlings grown with 0.05 g L^{-1} of Ag NPs but free of $AlCl_3$ (Table 2). Although $AlCl_3$ was added at doses higher than 100 µM, when it interacted with 0.1 g L^{-1} of Ag NPs, higher carotenoid levels were obtained compared to the control treatment.

The chlorophyll a/b ratio (Chl a/b) shows a gradual reduction as $AlCl_3$ concentration increases (seedlings grown on medium without Ag NPs). On the other hand, the addition of 0.1 mg L^{-1} of Ag NPs improved the chlorophyll a/b ratio (3.00 ± 0.75) in seedlings grown under the influence of 100 µM $AlCl_3$. As for the total chlorophyll/carotenoid ratio, it can be observed that seedlings grown with 300 µM $AlCl_3$ and 0.025 g L^{-1} of Ag NPs reach a value (of 18.54 ± 3.00) that doubles the value observed in the control treatment (Table 2).

3.3. Total Proline Content

The factors Ag NPs and $AlCl_3$ and their interactions had a significant impact on proline content in leaves (Table 3). An increase in $AlCl_3$ dosage had an inversely proportional effect on proline concentration. However, it was observed that the incorporation of Ag NPs to the culture medium allowed the plant grown under $AlCl_3$-induced stress to increase its capacity to convert toxic amino acids into proline, reaching values up to 0.40 ± 0.01 µg g^{-1} (Table 3), suggesting that its application could improve plant tolerance to Al stress.

Table 3. Total proline content in pineapple leaves in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Proline (µg/g)	0	0.21 ± 0.01 h	0.31 ± 0.01 d	0.29 ± 0.01 d	0.16 ± 0.04 i	***	***	***
	0.005	0.37 ± 0.01 a	0.33 ± 0.02 c	0.24 ± 0.02 g	0.29 ± 0.01 d			
	0.01	0.26 ± 0.03 f	0.23 ± 0.01 g	0.20 ± 0.02 h	0.26 ± 0.01 f			
	0.025	0.23 ± 0.3 g	0.26 ± 0.01 f	0.30 ± 0.00 d	0.40 ± 0.01 a			
	0.05	0.27 ± 0.01 e	0.30 ± 0.00 d	0.39 ± 0.00 a	0.35 ± 0.00 b			
	0.1	0.26 ± 0.01 f	0.30 ± 0.00 d	0.24 ± 0.00 g	0.30 ± 0.01 d			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

3.4. Nutrient and Metal Accumulation

Ag NPs, $AlCl_3$, and their interaction had a significant effect on the accumulation of macronutrients (K, Ca, and Mg), micronutrients (Fe, Cu, Mn, and Zn), and metals (Al and Ag) in pineapple leaf tissues (Tables 4–6). It was observed that the presence of $AlCl_3$ (100 and 500 µM) reduced the K, Ca, and Mg content in seedlings grown in the absence of Ag NPs. In contrast, the results of macronutrient analysis showed that the addition of Ag NPs at doses of 0.01, 0.05, and/or 0.1 g L^{-1} stimulated nutrient accumulation in leaf tissue

under Al stress conditions. For example, compared to the control treatment, seedlings grown on a medium with 300 µM $AlCl_3$ in interaction with 0.1 g L^{-1} of Ag NPs reached the highest accumulation of K (5383.96 ± 17.60 ppm), Ca (1500.58 ± 2.51 ppm), and Mg (464.19 ± 7.39 ppm) (Table 4).

Table 4. Mineral macronutrient content in pineapple leaves in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Potassium (ppm)	0	3562.71 ± 15.50 l	3419.58 ± 9.35 m	4246.99 ± 11.99 h	3164.32 ± 12.41 n	***	***	***
	0.005	2111.66 ± 14.31 p	3997.74 ± 13.71 j	3043.93 ± 20.32 o	4791.89 ± 25.16 f			
	0.01	5365.22 ± 20.35 b	2145.60 ± 14.43 p	5005.90 ± 19.87 e	5328.77 ± 30.21 c			
	0.025	3882.33 ± 21.62 k	1635.23 ± 80.34 r	1978.81 ± 10.51 q	3432.07 ± 20.6 m			
	0.05	5190.17 ± 16.53 d	4774.33 ± 18.69 h	5334.54 ± 14.74 c	4687.19 ± 32.97 g			
	0.1	5164.60 ± 9.14 d	5710.06 ± 16.68 a	5383.96 ± 17.60 b	4096.43 ± 20.14 i			
Calcium (ppm)	0	954.01 ± 0.0 g	702.10 ± 1.63 j	717.33 ± 1.28 j	892.82 ± 3.77 h	***	***	***
	0.005	814.92 ± 2.5 i	990.88 ± 2.62 f	1091.33 ±3.6 d	942.25 ± 1.33 g			
	0.01	1086.98 ± 1.79 d	910.39 ± 1.23 h	1049.14 ± 1.65 e	1080.16 ± 1.60 d			
	0.025	1098.60 ± 8.75 d	981.27 ± 1.94 f	793.23 ± 3.05 i	938.47 ± 1.83 g			
	0.05	1195.73 ± 8.28 c	658.66 ± 1.85 k	1075.28 ± 6.38 d	947.09 ± 3.81 g			
	0.1	1035.57 ± 3.92 e	1091.85 ± 2.05 d	1500.58 ± 2.51 a	1454.25 ± 4.27 b			
Magnesium (ppm)	0	257.23 ± 2.74 k	246.36 ± 3.36 l	282.41 ± 4.18 j	252.22 ± 2.98 k	***	***	***
	0.005	312.24 ± 2.5 h	293.80 ± 3.75 i	285.42 ± 3.71 j	212.65 ± 3.59 n			
	0.01	406.07 ± 4.03 b	364.64 ± 2.42 d	366.49 ± 3.42 d	382.80 ± 3.26 c			
	0.025	332.49 ± 2.67 g	378.00 ± 2.59 c	207.83 ± 3.56 n	286.34 ± 3.68 j			
	0.05	330.46 ± 5.61 g	226.55 ± 2.81 m	351.07 ± 6.46 e	283.46 ± 5.06 j			
	0.1	343.78 ± 6.03 f	354.92 ± 6.30 e	464.19 ± 7.39 a	458.45 ± 6.72 a			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

Table 5. Mineral micronutrient content in pineapple leaves in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Iron (ppm)	0	107.78 ± 2.21 m	55.35 ± 0.93 p	93.51 ± 2.03 o	130.69 ± 2.59 k	***	***	***
	0.005	406.6 ± 4.97 b	110.41 ± 0.72 m	184.62 ± 0.84 d	123.02 ± 1.70 l			
	0.01	451.59 ± 5.64 a	124.15 ± 2.34 l	172.94 ± 2.62 f	178.89 ± 1.17 e			
	0.025	188.15 ± 2.51 b	163.23 ± 1.73 g	226.59 ± 1.86 c	140.27 ± 2.02 j			
	0.05	176.64 ± 1.47 e	97.54 ± 0.89 n	153.98 ± 0.77 h	137.92 ± 0.10 j			
	0.1	149.85 ± 0.50 i	141.63 ± 1.34 j	148.85 ± 0.10 i	179.99 ± 1.15 e			
Copper (ppm)	0	2.19 ± 0.02 a	1.85 ± 0.0 q	2.56 ± 0.02 i	1.95 ± 0.01 p	***	***	***
	0.005	2.36 ± 0.01 l	2.05 ± 0.02 n	2.38 ± 0.01 k	1.67 ± 0.01 r			
	0.01	2.35 ± 0.02 l	3.62 ± 0.03 d	2.66 ± 0.02 g	2.49 ± 0.01 j			
	0.025	2.09 ± 0.02 m	2.36 ± 0.01 l	2.00 ± 0.01 o	2.00 ± 0.01 o			
	0.05	3.71 ± 0.01 c	2.58 ± 0.02 h	2.55 ± 0.01 i	2.84 ± 0.02 f			
	0.1	3.07 ± 0.02 e	2.83 ± 0.01 f	2.66 ± 0.01 g	3.91 ± 0.01 b			

Table 5. *Cont.*

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Manganese (ppm)	0	267.76 ± 0.75 i	143.25 ± 0.56 q	163.17 ± 0.29 o	238.47 ± 1.86 k	***	***	***
	0.005	360.00 ± 2.48 b	277.65 ± 0.95 g	271.24 ± 2.56 h	292.97 ± 1.31 f			
	0.01	430.19 ± 1.16 a	250.67 ± 0.53 j	270.05 ± 0.48 h	317.32 ± 1.16 e			
	0.025	352.06 ± 3.54 c	323.97 ± 1.46 d	358.58 ± 1.25 b	237.39 ± 2.12 k			
	0.05	185.56 ± 0.83 m	91.40 ± 0.55 r	218.40 ± 0.83 l	143.49 ± 0.86 q			
	0.1	157.96 ± 1.45 p	145.05 ± 0.64 q	167.22 ± 0.43 n	219.03 ± 0.60 l			
Zinc (ppm)	0	27.41 ± 0.72 h	8.74 ± 0.14 n	14.41 ± 0.25 m	24.86 ± 0.22 j	***	***	***
	0.005	30.38 ± 0.19 e	27.32 ± 0.28 h	27.56 ± 0.32 h	26.92 ± 0.18 i			
	0.01	51.83 ± 0.24 a	20.42 ± 0.24 l	28.74 ± 0.33 f	33.54 ± 0.19 c			
	0.025	32.47 ± 0.14 d	27.35 ± 0.19 h	32.68 ± 0.11 d	20.76 ± 0.24 l			
	0.05	30.07 ± 0.48 e	21.12 ± 0.09 k	34.09 ± 0.04 b	28.31 ± 0.07 g			
	0.1	28.17 ± 0.03 g	29.29 ± 0.07 f	29.01 ± 0.08 f	33.16 ± 0.06 c			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

Table 6. Values of metal content in pineapple leaves in vitro grown under the influence of Ag NPs and $AlCl_3$.

Parameters	Silver Nanoparticles (g L^{-1})	Aluminum Chloride ($AlCl_3$)				Marginal Hypothesis Test		
		0 µM	100 µM	300 µM	500 µM	Ag NPs	$AlCl_3$	Ag NPs × $AlCl_3$
Aluminium (ppm)	0	196.89 ± 4.21 l	297.28 ± 1.63 f	322.65 ± 3.03 e	346.25 ± 2.66 d	***	***	***
	0.005	134.36 ± 26.32 m	270.63 ± 0.84 h	285.95 ± 1.27 g	248.43 ± 0.69 j			
	0.01	122.35 ± 1.93 m	327.57 ± 0.50 e	339.66 ± 0.88 d	282.93 ± 1.08 g			
	0.025	101.52 ± 0.81 n	259.15 ± 0.50 i	358.93 ± 2.16 c	318.35 ± 1.08 e			
	0.05	127.64 ± 0.59 m	110.51 ± 0.46 n	367.26 ± 2.25 b	374.18 ± 1.48 b			
	0.1	104.60 ± 0.19 n	110.16 ± 0.99 n	237.34 ± 2.27 k	426.62 ± 0.59 a			
Silver (ppm)	0	0.65 ± 0.05 s	0.59 ± 0.01 s	0.23 ± 0.01 s	0.77 ± 0.02 s	***	***	***
	0.005	54.51 ± 0.04 r	189.46 ± 0.78 h	107.44 ± 0.26 m	101.67 ± 0.29 o			
	0.01	101.00 ± 0.69 o	89.58 ± 0.10 q	99.75 ± 0.45 p	110.16 ± 0.36 l			
	0.025	223.4 ± 1.08 e	216.93 ± 1.07 f	264.20 ± 1.22 d	183.51 ± 0.16 i			
	0.05	179.69 ± 0.29 j	105.27 ± 0.40 n	207.58 ± 0.19 g	159.62 ± 0.6 k			
	0.1	217.04 ± 0.43 f	366.21 ± 0.41 a	296.90 ± 0.99 c	349.91 ± 0.83 b			

Values (±SD) not followed by same letter denote significant differences between treatments (DGC test at $p \leq 0.05$). Significance levels *** correspond to $p \leq 0.001$.

On the other hand, the incorporation of Ag NPs had a positive influence on the assimilation of micronutrients (Table 5). In other words, comparing the results obtained from plants grown in the absence of Ag NPs with those obtained from plants grown with $AlCl_3$, it was observed that $AlCl_3$-treated plants reduced their Fe, Mn, and Zn content. In the presence of 500 µM $AlCl_3$, the addition of 0.1 g L^{-1} of Ag NPs allowed the highest concentrations of Fe (179.99 ± 1.15 ppm) and Cu (3.91 ± 0.01 ppm) to be attained. The concentration of Ag NPs that allowed the highest Mn and Zn contents (317.32 ± 1.16 and 33.54 ± 0.19 ppm, respectively) to be measured in leaf tissue was 0.01 g L^{-1}.

As for Al accumulation, the presence of Ag NPs during culture had a significant impact on its uptake (Table 6). For example, it was observed that the addition of 0.1 g L^{-1} of Ag NPs to a culture medium with concentrations of 100 and 300 µM $AlCl_3$ reduced the amount of Al accumulation in the seedlings (with respect to additions of lower concentrations of Ag NPs), with levels of 110.16 ± 0.99 and 237.34 ± 2.27 ppm, respectively. On the contrary, the absence of Ag NPs led to higher Al accumulation (297.28 ± 1.63, 32.65 ± 3.03, and 346.25 ± 2.66 ppm) as $AlCl_3$ levels increased, as expected. In relation to Ag accumulation

in tissue, it was observed that higher doses of Ag NPs (0.1 g L^{-1}) were associated with higher levels of Ag accumulation in plant tissue (up to 366.21 ± 0.41 ppm). This caused the presence of hyperhydric deformities in the plants in vitro (Figure 1), drastically reducing the phenotypic characteristics of pineapple plants.

4. Discussion

Plants, as sessile organisms, are directly exposed to several stressors. For example, stress related to climate change is a latent problem in contemporary agriculture. In addition, the progressive industrialization of agricultural practices has led to soil acidification (pH < 5.0), where the toxic form of aluminum (Al) is a factor restricting crop production worldwide.

4.1. Phenotypic Changes in Pineapple Seedlings Grown under Al Stress

It was found that Ag NPs allowed pineapple plants to develop new shoots with increased biomass under Al-induced stress conditions, reducing the metal toxicity thanks to the "dilution effect" in plant tissues [30]. Likewise, it can be inferred that due to their morphology, NPs stimulate a higher formation of citric, oxalic, and malic acid, forming complexes with toxic metal ions, affecting their availability, uptake, and mobility in plants [31,32]. Strout et al. [33] found that Si NPs formed a binary film on the epidermal cell wall after absorption, improving the structural color of plants and providing resistance to diseases, drought, and high temperatures, which could also occurred in this study.

4.2. Photosynthetic Pigments

When plants are grown under $AlCl_3$-induced stress conditions, Ag NPs showed a positive impact on maintaining chloroplast function. This effect is likely related to the release of silver ions through plasmodesmata within the cell, which may inhibit ethylene signaling and perception [34,35] as chlorophyll degradation occurs via auxin translocation by ethylene [36]. Previous studies revealed that excess ethylene in leaves drastically reduces photosynthetic pigment content in *Ipomoea cairica* [37]. This illustrates the value of photosynthetic pigments as biomarkers of the physiological status of plants.

In our study, a dose less than or equal to 0.025 g L^{-1} of Ag NPs added to the culture medium allowed plants grown under $AlCl_3$ stress conditions to maintain chlorophyll-a levels comparable to the control treatment. Moreover, even with the presence of $AlCl_3$ at toxic levels, the presence of Ag NPs increased carotenoid content. This response may be due to the properties of Ag nanoparticles, which stimulate the presence of enzymatic antioxidants that scavenge free radicals, protecting plants from reactive oxygen species (ROS) which otherwise would generate disruption of cellular function [38,39]. This suggests that Ag NPs can reduce the adverse effects of Al on tissues by optimizing the production of photosynthetic pigments.

According to Afanasyeva and Ayushina [40], maintaining the proper ratio of pigment molecules is essential to ensure the proper functioning of the photosynthetic apparatus; for example, Uka et al. [41] suggest that a higher chlorophyll a/b ratio may be indicative of better tolerance to atmospheric pollution, while Öncel et al. [42] reported that the application of increasing concentrations of cadmium resulted in a decrease in the chlorophyll a/b ratio. With respect to the total chlorophyll/carotenoid ratio, low values are a sign of senescence, stress, and damage to the photosynthetic system [41]. The total chlorophyll/carotenoid ratio is an indicator of plant greenness because the intensity under stress conditions is modified by the high accumulation of pheophytin caused by chlorophyll degradation [43].

Another important aspect to consider is the formation of hyperhydric buds in vitro, which can be caused by abiotic stress such as the exposure of metals. This stress can lead to the alteration of photosynthetic pigments [44] as Al can interfere with the translocation and uptake of Mg, an essential component of chlorophyll [45]. Our results indicated that incorporating certain concentrations of Ag NPs can help overcome these limitations. This is consistent with Azeez et al. [15], who provided evidence that the addition of Ag NPs not

only eliminated Cd and Pb toxicity, but also promoted enhanced photosynthetic activity, improving the growth of *Moringa oleifera*.

4.3. Proline Content

Our research showed that Ag NPs play a protective role against $AlCl_3$ oxidative stress by promoting enhanced proline biosynthesis. This is consistent with previous studies showing that Ag NPs can interact with salt stress to increase the production of soluble sugars and proline content [46]. This suggests that proline serves as both a signaling molecule and non-enzymatic antioxidant, as its accumulation provides energy for plant survival and growth, which leads to enhanced tolerance to salt and metal stress [47,48].

The Ag NPs on pineapple growth were observed to increase the proline content in plant tissue, which in turn reduced the negative effects of $AlCl_3$ toxicity. Proline accumulation under stress acts as an osmoregulator and scavenges free radicals, stabilizing proteins and membranes [49]. Furthermore, proline is a key component of the metabolic signaling networks that regulate mitochondrial functions [50]. In addition, proline enables the efficient elimination of excess ROS through the process called "Pro–Pro cycle", and promotes the production of lignin, a plant defense compound against various stressors [50–52].

Proline is known as an inert solute that does not interfere with regular metabolic processes in plant cells [53]. However, our study found that proline biosynthesis can help mitigate the negative effects of $AlCl_3$ on pineapple seedlings, possibly because proline biosynthesis acts as a metal chelator that stimulates plant tolerance to toxic metal stress [54]. Sharma et al. [55] demonstrated that free proline can chelate Cd ions in plant tissues, transforming them into non-toxic Cd-Proline complexes. Similarly, Abdel-Haliem et al. [56] found that NPs can increase the foliar proline content and free amino acids in rice, thus enhancing resistance to NaCl penetration.

4.4. Quantification of Essential Nutrients, Al, and Ag

Plants require seventeen essential mineral elements for optimal vegetative growth and development [57]. While some metals, such as Cu, Zn, Fe, Mn, Mo, Ni, and Co, are necessary for proper plant function and lead to greater vigor and biomass, other metals, such as Cd, Ag, Al, Pb, Hg, As, and Cr, can be toxic [58]. The pH level of soil also plays an essential role, as values below 5 can increase Al toxicity and reduce the assimilation of both macro- and micronutrients. Furthermore, Mota et al. [59] showed that Al^{3+} ions are potentially toxic and highly mobile [60], leading to their accumulating in meristematic cells and vacuoles [61,62], which, in turn, can negatively affect the protein content and disrupt the regular function of the Golgi apparatus and plasmalemma [62].

In this regard, Anderson et al. [63] and Roy et al. [62] found that low pH ranges in Al-containing soils inhibit the uptake of K, Mg, Cu, Zn, Mn, P, and Fe because Al competes with Ca for space on the cell surface [62]. In response, promising results were obtained by the synergistic application of Ag NPs to reduce the deleterious effects of Al. These promising characteristics are also being reported from elements such as Ce, Co, I, Se, Si, Ti, and V, which are developing properties that are fundamental biostimulants for growth, as well as conferring plant tolerance to a stressor [57].

The balance of nutrient in plants is driven by the activity of the plasma membrane, which involves the movement of ions from the soil into the cells [64]. This activity is positively stimulated by the presence of NPs due to their mesoporous nature; they as nanocarriers of different molecules and allow for more efficient nutrient availability [65]. Moreover, NPs have a stimulating effect due to their high surface charge density, which enables them to interact non-specifically with the cell wall [66]. In other words, Ag NPs can disrupt cellular homeostasis by regulating genes that code for metal transporters [67].

Even though the advantages of NPs in helping plants overcome metal toxicity have been widely studied, our study shows that while Ag NPs can mitigate the negative effects of Al, they also result in a decrease in the levels of macro- and microelements and an increase in Ag concentrations in plant tissues. This effect has also been observed by Zuverza-Mena

et al. [68], who found that Ag NPs can reduce the uptake of macroelements such as Ca, Mg, B, Mn, and Zn in radish shoots. Similarly, Jiang et al. [69] and Zare et al. [70] found that high concentrations of Ag NPs can decrease the accumulation of Mg, Ca, Zn, Cu, B, and Mn, leading to oxidative stress and toxicity in purslane and aquatic plants. This suggests that high levels of Ag NPs can disrupt nutrient transport due to ion channel competition [71].

5. Conclusions

This study confirms that Ag NPs in specific concentrations have the potential to alleviate the negative effects of aluminum on plant growth and nutrition. This is particularly important for maintaining adequate levels of photosynthetic pigments and balanced mineral nutrition in pineapple during in vitro cultivation. These findings suggest that the incorporation of NPs into conventional agricultural practices could have a significant impact on soil preservation. Further research is needed to explore the full potential of this technology.

Author Contributions: Conceptualization, J.J.T.-A. and J.B.M.-M.; methodology, J.J.T.-A., J.B.M.-M. and R.Y.A.-T.; formal analysis, M.G.; investigation, J.J.T.-A. and R.Y.A.-T.; writing—original draft preparation, J.J.T.-A. and J.B.M.-M.; writing—review and editing, J.J.T.-A., J.B.M.-M., R.Y.A.-T., M.G. and M.O.; supervision, M.O.; funding acquisition, M.O. All authors have read and agreed to the published version of the manuscript.

Funding: The research has received technical and financial support from the SNIP project (312252)/CUI (2252878) "Creación del Servicio de un Laboratorio de Fisiología y Biotecnología Vegetal de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas" (Creation of a Plant Physiology and Biotechnology Laboratory Service of the National University Toribio Rodríguez de Mendoza de Amazonas), and the SNIP project (209950) "Creación del servicio de laboratorio de entomología y fitopatología de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas" (Creation of an Entomology and Phytopathology Laboratory Service of the National University Toribio Rodríguez de Mendoza de Amazonas), executed by the Research Institute for the Sustainable Development of Ceja de Selva. This work was also supported by Vicerrectorado de Investigación de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Jorge Díaz Valderrama and Lizzie Gill for proofreading the manuscript.

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

References

1. Mahmud, M.; Abdullah, R.; Yaacob, J.S. Effect of vermicompost on growth, plant nutrient uptake and bioactivity of ex vitro pineapple (*Ananas comosus* var. MD2). *Agronomy* **2020**, *10*, 1333. [CrossRef]
2. Lin, Y.H.; Chen, J.H. Effects of aluminum on the cell morphology in the root apices of two pineapples with different Al-resistance characteristics. *Soil Sci. Plant Nutr.* **2019**, *65*, 353–357. [CrossRef]
3. Chen, L.S.; Yang, L.T.; Guo, P.; Jiang, H.X.; Tang, N. Aluminum toxicity and fruit nutrition. In *Fruit Crops*; Srivastava, A.K., Hu, C., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 223–240.
4. Giannakoula, A.; Moustakas, M.; Syros, T.; Yupsanis, T. Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *EEB* **2010**, *67*, 487–494. [CrossRef]
5. Rahman, R.; Upadhyaya, H. Aluminium toxicity and its tolerance in plant: A review. *J. Plant Biol.* **2021**, *64*, 101–121. [CrossRef]
6. Kováčik, J.; Klejdus, B.; Hedbavny, J. Effect of aluminium uptake on physiology, phenols and amino acids in *Matricaria chamomilla* plants. *J. Hazard Mater.* **2010**, *178*, 949–955. [CrossRef] [PubMed]
7. Michalak, A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud.* **2006**, *15*, 523–530.
8. Nouman, W.; Bashir, T.; Gul, R.; Gull, T.; Olson, M.E.; Shaheen, M.; Rosa, E.; Domínguez-Perles, R.; Soliman, W.S. Metalliferous conditions induce regulation in antioxidant activities, polyphenolics and nutritional quality of *Moringa oleifera* L. *Int. J. Phytoremediat.* **2020**, *22*, 1348–1361. [CrossRef] [PubMed]

9. Tolrà, R.P.; Poschenrieder, C.; Luppi, B.; Barceló, J. Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. *EEB* **2005**, *54*, 231–238. [CrossRef]
10. Abruña-Rodríguez, F.; Vicente-Chandler, J.; Rivera, E.; Rodríguez, J. Effect of soil acidity factors on yields and foliar composition of tropical root crops. *Soil Sci. Soc. Am. J.* **1982**, *46*, 1004–1007. [CrossRef]
11. Yang, L.T.; Qi, Y.P.; Jiang, H.X.; Chen, L.S. Roles of organic acid anion secretion in aluminium tolerance of higher plants. *BioMed Res. Int.* **2012**, *2013*, e173682. [CrossRef]
12. Mahendran, D.; Geetha, N.; Venkatachalam, P. Role of silver nitrate and silver nanoparticles on tissue culture medium and enhanced the plant growth and development. In *In Vitro Plant Breeding Towards Novel Agronomic Traits: Biotic and Abiotic Stress Tolerance*; Kumar, M., Muthusamy, A., Kumar, V., Bhalla-Sarin, N., Eds.; Springer: Singapore, 2019; pp. 59–74.
13. Ahmad, B.; Zaid, A.; Zulfiqar, F.; Bovand, F.; Dar, T.A. Nanotechnology: A novel and sustainable approach towards heavy metal stress alleviation in plants. *Nanotechnol. Environ. Eng.* **2023**, *8*, 27–40. [CrossRef]
14. Verma, A.; Roy, A.; Bharadvaja, N. Remediation of heavy metals using nanophytoremediation. In *Advanced Oxidation Processes for Effluent Treatment Plants*; Shah, M.P., Ed.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 273–296.
15. Azeez, L.; Adejumo, A.L.; Lateef, A.; Adebisi, S.A.; Adetoro, R.O.; Adewuyi, S.O.; Tijani, K.O.; Olaoye, S. Zero-valent silver nanoparticles attenuate Cd and Pb toxicities on *Moringa oleifera* via immobilization and induction of phytochemicals. *Plant Physiol. Biochem.* **2019**, *139*, 283–292. [CrossRef] [PubMed]
16. Sharma, P.; Bhatt, D.; Zaidi, M.G.H.; Saradhi, P.P.; Khanna, P.K.; Arora, S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl. Biochem. Biotechnol.* **2012**, *167*, 2225–2233. [CrossRef] [PubMed]
17. Servin, A.; Elmer, W.; Mukherjee, A.; De la Torre-Roche, R.; Hamdi, H.; White, J.C.; Bindraban, P.; Dimkpa, C. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *J. Nanopart Res.* **2015**, *17*, 92. [CrossRef]
18. Sillen, W.M.A.; Thijs, S.; Abbamondi, G.R.; Janssen, J.; Weyens, N.; White, J.C.; Vangronsveld, J. Effects of silver nanoparticles on soil microorganisms and maize biomass are linked in the rhizosphere. *Soil Biol. Biochem.* **2015**, *91*, 14–22. [CrossRef]
19. Anjum, N.A.; Gill, S.S.; Duarte, A.C.; Pereira, E.; Ahmad, I. Silver nanoparticles in soil–plant systems. *J. Nanopart Res.* **2013**, *15*, 1896. [CrossRef]
20. Aqeel, M.; Khalid, N.; Nazir, A.; Irshad, M.K.; Hakami, O.; Basahi, M.A.; Alamri, S.; Hashem, M.; Noman, A. Foliar application of silver nanoparticles mitigated nutritional and biochemical perturbations in chilli pepper fertigated with domestic wastewater. *Plant Physiol. Biochem.* **2023**, *194*, 470–479. [CrossRef]
21. Bahador, E.; Einali, A.; Azizian-Shermeh, O.; Sangtarash, M.H. Metabolic responses of the green microalga *Dunaliella salina* to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment. *Aquat. Toxicol.* **2019**, *217*, 105356. [CrossRef]
22. Tripathi, A.; Liu, S.; Singh, P.K.; Kumar, N.; Pandey, A.C.; Tripathi, D.K.; Chauhan, D.K.; Sahi, S. Differential phytotoxic responses of silver nitrate ($AgNO_3$) and silver nanoparticle (AgNps) in *Cucumis sativus* L. *Plant Gene.* **2017**, *11*, 255–264. [CrossRef]
23. Gómez, D.; Hernández, L.; Valle, B.; Martínez, J.; Cid, M.; Escalona, M.; Hernández, M.; Beemster, G.T.S.; Tebbe, C.C.; Yabor, L.; et al. Temporary immersion bioreactors (TIB) provide a versatile, cost-effective and reproducible in vitro analysis of the response of pineapple shoots to salinity and drought. *Acta Physiol. Plant.* **2017**, *39*, 277. [CrossRef]
24. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
25. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F4.3.1–F4.3.8. [CrossRef]
26. 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]
27. Bolker, B.M.; Brooks, M.E.; Clark, C.J.; Geange, S.W.; Poulsen, J.R.; Stevens, M.H.H.; White, J.-S.S. Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol. Evol.* **2009**, *24*, 127–135. [CrossRef] [PubMed]
28. Dean, C.B.; Nielsen, J.D. Generalized linear mixed models: A review and some extensions. *Lifetime Data Anal.* **2007**, *13*, 497–512. [CrossRef] [PubMed]
29. Di Rienzo, J.A.; Guzman, A.W.; Casanoves, F. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *JABES* **2002**, *7*, 129–142. [CrossRef]
30. Sarwar, N.; Saifullah; Malhi, S.S.; Zia, M.H.; Naeem, A.; Bibi, S.; Farid, G. Role of mineral nutrition in minimizing cadmium accumulation by plants. *J. Sci. Food Agric.* **2010**, *90*, 925–937. [CrossRef]
31. Gong, X.; Huang, D.; Liu, Y.; Peng, Z.; Zeng, G.; Xu, P.; Cheng, M.; Wang, R.; Wan, J. Remediation of contaminated soils by biotechnology with nanomaterials: Bio-behavior, applications, and perspectives. *Crit. Rev. Biotechnol.* **2018**, *38*, 455–468. [CrossRef]
32. Xie, X.; Weiss, D.J.; Weng, B.; Liu, J.; Lu, H.; Yan, C. The short-term effect of cadmium on low molecular weight organic acid and amino acid exudation from mangrove (*Kandelia obovata* (S., L.) Yong) roots. *Environ. Sci. Pollut. Res.* **2013**, *20*, 997–1008. [CrossRef]
33. Strout, G.; Russell, S.D.; Pulsifer, D.P.; Erten, S.; Lakhtakia, A.; Lee, D.W. Silica nanoparticles aid in structural leaf coloration in the Malaysian tropical rainforest understorey herb *Mapania caudata*. *Ann. Bot.* **2013**, *112*, 1141–1148. [CrossRef]
34. Azadi, M.; Siavash Moghaddam, S.; Rahimi, A.; Pourakbar, L.; Popović-Djordjević, J. Biosynthesized silver nanoparticles ameliorate yield, leaf photosynthetic pigments, and essential oil composition of garden thyme (*Thymus vulgaris* L.) exposed to UV-B stress. *JECE* **2021**, *9*, 105919. [CrossRef]

35. Bagherzadeh Homaee, M.; Ehsanpour, A.A. Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Ind. J. Plant Physiol.* **2015**, *20*, 353–359. [CrossRef]
36. Lentini, Z.; Mussell, H.; Mutschler, M.A.; Earle, E.D. Ethylene generation and reversal of ethylene effects during development in vitro of rapid-cycling *Brassica campestris* L. *Plant Sci.* **1988**, *54*, 75–81. [CrossRef]
37. Sun, Z.-Y.; Zhang, T.-J.; Su, J.-Q.; Chow, W.S.; Liu, J.-Q.; Chen, L.-L.; Li, W.-H.; Peng, S.-L.; Peng, C.-L. A novel role of ethephon in controlling the noxious weed *Ipomoea cairica* (Linn.) Sweet. *Sci. Rep.* **2015**, *5*, 11372. [CrossRef]
38. Haghighi Pak, Z.; Karimi, N.; Abbaspour, H. Effects of silver nanoparticle exposure on growth, physiological and biochemical parameters of *Dracocephalum moldavica* L. *Iran. J. Plant Physiol.* **2017**, *7*, 2173–2183.
39. Yan, A.; Chen, Z. Impacts of silver nanoparticles on plants: A focus on the phytotoxicity and underlying mechanism. *Int. J. Mol. Sci.* **2019**, *20*, 1003. [CrossRef] [PubMed]
40. Afanasyeva, L.V.; Ayushina, T.A. Accumulation of heavy metals and biochemical responses in Siberian larch needles in urban area. *Ecotoxicology* **2019**, *28*, 578–588. [CrossRef]
41. Uka, U.N.; Belford, E.J.D.; Elebe, F.A. Effects of road traffic on photosynthetic pigments and heavy metal accumulation in tree species of Kumasi Metropolis, Ghana. *SN Appl. Sci.* **2021**, *3*, 131. [CrossRef]
42. Öncel, I.; Keleş, Y.; Üstün, A.S. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.* **2000**, *107*, 315–320. [CrossRef] [PubMed]
43. Sanmartín, P.; Villa, F.; Silva, B.; Cappitelli, F.; Prieto, B. Color measurements as a reliable method for estimating chlorophyll degradation to phaeopigments. *Biodegradation* **2011**, *22*, 763–771. [CrossRef] [PubMed]
44. Sreelekshmi, R.; Siril, E.A.; Muthukrishnan, S. Role of biogenic silver nanoparticles on hyperhydricity reversion in *Dianthus chinensis* L. an in vitro model culture. *J. Plant Growth Regul.* **2022**, *41*, 23–39. [CrossRef]
45. Ali, B.; Hasan, S.A.; Hayat, S.; Hayat, Q.; Yadav, S.; Fariduddin, Q.; Ahmad, A. A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). *EEB* **2008**, *62*, 153–159. [CrossRef]
46. Mohamed, A.K.S.H.; Qayyum, M.F.; Abdel-Hadi, A.M.; Rehman, R.A.; Ali, S.; Rizwan, M. Interactive effect of salinity and silver nanoparticles on photosynthetic and biochemical parameters of wheat. *Arch. Agron. Soil Sci.* **2017**, *63*, 1736–1747. [CrossRef]
47. Mittal, S.; Kumari, N.; Sharma, V. Differential response of salt stress on *Brassica juncea*: Photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. *Plant Physiol. Biochem.* **2012**, *54*, 17–26. [CrossRef]
48. Singh, M.; Pratap Singh, V.; Dubey, G.; Mohan Prasad, S. Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena* L. seedlings. *Ecotoxicol. Environ. Saf.* **2015**, *117*, 164–173. [CrossRef] [PubMed]
49. Aggarwal, M.; Sharma, S.; Kaur, N.; Pathania, D.; Bhandhari, K.; Kaushal, N.; Kaur, R.; Singh, K.; Srivastava, A.; Nayyar, H. Exogenous proline application reduces phytotoxic effects of selenium by minimising oxidative stress and improves growth in bean (*Phaseolus vulgaris* L.) seedlings. *Biol. Trace Elem. Res.* **2011**, *140*, 354–367. [CrossRef]
50. Szabados, L.; Savouré, A. Proline: A multifunctional amino acid. *Trends Plant Sci.* **2010**, *15*, 89–97. [CrossRef]
51. Schalk, M.; Batard, Y.; Seyer, A.; Nedelkina, S.; Durst, F.; Werck-Reichhart, D. Design of fluorescent substrates and potent inhibitors of CYP73As, P450s that catalyze 4-hydroxylation of cinnamic acid in higher plants. *Biochemistry* **1997**, *36*, 15253–15261. [CrossRef] [PubMed]
52. Signorelli, S.; Coitiño, E.L.; Borsani, O.; Monza, J. Molecular mechanisms for the reaction between •OH radicals and proline: Insights on the role as reactive oxygen species scavenger in plant stress. *J. Phys. Chem. B* **2014**, *118*, 37–47. [CrossRef]
53. Mahmoud, A.W.M.; Abdeldaym, E.A.; Abdelaziz, S.M.; El-Sawy, M.B.I.; Mottaleb, S.A. Synergetic effects of zinc, boron, silicon, and zeolite nanoparticles on confer tolerance in potato plants subjected to salinity. *Agronomy* **2020**, *10*, 19. [CrossRef]
54. Meena, M.; Divyanshu, K.; Kumar, S.; Swapnil, P.; Zehra, A.; Shukla, V.; Yadav, M.; Upadhyay, R.S. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **2019**, *5*, e02952. [CrossRef] [PubMed]
55. Sharma, S.S.; Schat, H.; Vooijs, R. In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry* **1998**, *49*, 1531–1535. [CrossRef]
56. Abdel-Haliem, M.E.F.; Hegazy, H.S.; Hassan, N.S.; Naguib, D.M. Effect of silica ions and nano silica on rice plants under salinity stress. *Ecol. Eng.* **2017**, *99*, 282–289. [CrossRef]
57. Singhal, R.K.; Fahad, S.; Kumar, P.; Choyal, P.; Javed, T.; Jinger, D.; Singh, P.; Saha, D.; Prathibha; Bose, B.; et al. Beneficial elements: New players in improving nutrient use efficiency and abiotic stress tolerance. *Plant Growth Regul.* **2022**, 1–29. [CrossRef]
58. Kalaivanan, D.; Ganeshamurthy, A.N. Mechanisms of heavy metal toxicity in plants. In *Abiotic Stress Physiology of Horticultural Crops*; Rao, N.K.S., Shivashankara, K.S., Laxman, R.H., Eds.; Springer: New Delhi, India, 2016; pp. 85–102.
59. Mota, M.F.C.; Pegoraro, R.F.; Batista, P.S.C.; Pinto, V.d.O.; Maia, V.M.; Silva, D.F.d. Macronutrients accumulation and growth of pineapple cultivars submitted to aluminum stress. *Rev. Bras. Eng. Agríc. Ambient.* **2016**, *20*, 978–983. [CrossRef]
60. Babourina, O.; Rengel, Z. Uptake of aluminium into Arabidopsis root cells measured by fluorescent lifetime imaging. *Ann. Bot.* **2009**, *104*, 189–195. [CrossRef]
61. Mustafa, G.; Komatsu, S. Toxicity of heavy metals and metal-containing nanoparticles on plants. *Biochim. Biophys. Acta.* **2016**, *1864*, 932–944. [CrossRef]
62. Roy, A.K.; Sharma, A.; Talukder, G. Some aspects of aluminum toxicity in plants. *Bot. Rev.* **1988**, *54*, 145–178. [CrossRef]
63. Anderson, J.M.; Pegg, K.G.; Scott, C.; Drenth, A. Phosphonate applied as a pre-plant dip controls *Phytophthora cinnamomi* root and heart rot in susceptible pineapple hybrids. *Australas. Plant Pathol.* **2012**, *41*, 59–68. [CrossRef]

64. El-Saadony, M.T.; Desoky, E.S.M.; Saad, A.M.; Eid, R.S.M.; Selem, E.; Elrys, A.S. Biological silicon nanoparticles improve *Phaseolus vulgaris* L. yield and minimize its contaminant contents on a heavy metals-contaminated saline soil. *JES* **2021**, *106*, 1–14. [CrossRef]
65. Rastogi, A.; Tripathi, D.K.; Yadav, S.; Chauhan, D.K.; Živčák, M.; Ghorbanpour, M.; El-Sheery, N.I.; Brestic, M. Application of silicon nanoparticles in agriculture. *3 Biotech* **2019**, *9*, 90. [CrossRef] [PubMed]
66. Juárez-Maldonado, A.; Ortega-Ortíz, H.; Morales-Díaz, A.B.; González-Morales, S.; Morelos-Moreno, Á.; Cabrera-De la Fuente, M.; Benavides-Mendoza, A. Nanoparticles and nanomaterials as plant biostimulants. *Int. J. Mol. Sci.* **2019**, *20*, 162. [CrossRef]
67. Magesky, A.; Pelletier, É. Toxicity mechanisms of ionic silver and polymer-coated silver nanoparticles with interactions of functionalized carbon nanotubes on early development stages of sea urchin. *Aquat. Toxicol.* **2015**, *167*, 106–123. [CrossRef] [PubMed]
68. Zuverza-Mena, N.; Armendariz, R.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Effects of silver nanoparticles on radish sprouts: Root growth reduction and modifications in the nutritional value. *Front. Plant Sci.* **2016**, *7*, 90. [CrossRef]
69. Jiang, H.-S.; Qiu, X.-N.; Li, G.-B.; Li, W.; Yin, L.-Y. Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. *Environ. Toxicol. Chem.* **2014**, *33*, 1398–1405. [CrossRef] [PubMed]
70. Zare, Z.; Pishkar, L.; Iranbakhsh, A.; Talei, D. Physiological and molecular effects of silver nanoparticles exposure on purslane (*Portulaca oleracea* L.). *Russ. J. Plant Physiol.* **2020**, *67*, 521–528. [CrossRef]
71. Da Costa, M.V.J.; Sharma, P.K. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in *Oryza sativa*. *Photosynthetica* **2016**, *54*, 110–119. [CrossRef]