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The Impact of Magnesium on the Growth, Physiology and Quality of Tea Plants under Acid Stress

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Abstract: This study investigates how root environment acidification impacts tea seedlings and the role of magnesium (Mg) in mitigating these effects. We examine varying pH and Mg levels' influence on tea seedlings' resistance to abiotic stress, focusing on antioxidant capacity and nutritional content. In a hydroponic experiment, we varied root pH (3.5, 5.0, 6.5) and Mg concentrations (0.01, 0.4, 0.8 mM), assessing parameters like antioxidant capacity, peroxidative damage, and nutritional content at 1, 7, 15, and 30 days post-treatment. Root environment acidification and Mg deficiency worsened peroxidative damage in tea plant leaves and roots. Increased Mg supplementation enhanced antioxidant enzyme activity, reducing malondialdehyde and mitigating oxidative damage from root environment acid stress. Under acid stress, 0.8 mM Mg significantly increased tea leaf polyphenols, amino acids, and water-soluble extracts. Mg notably boosted chlorophyll content, surpassing lower Mg levels at pH 5. Additionally, Mg reversed root vitality inhibition induced by acid stress, leading to increased nitrogen, potassium, and Mg concentrations in leaves, promoting balanced nutrient absorption. Mg supplementation is crucial for enhancing tea plant antioxidant capacity, alleviating growth inhibition from root environment acid stress, and improving chlorophyll content and root vitality, highlighting Mg's significance in tea cultivation and broader agricultural practices.

Keywords: magnesium; tea plant; acid stress; antioxidant enzymes; lipid peroxidation; tea quality

1. Introduction

See the end of the document for further details on references. The tea plant originated from China's Guizhou Plateau [1] and holds immense cultural significance and health benefits as a traditional beverage [2]. China is the world's leading tea producer and exporter with a cultivation area of 3.165 million hectares in 2020 constituting 62.1% of global tea production [3]. However, the pursuit of higher tea yields has led to a notable increase in the use of ammonia nitrogen fertilizers in tea gardens [4,5]. This heightened usage has accelerated Al biogeochemical cycling through soils and tea plants. The result was increased soil acidification especially in regions marked by abundant rainfall and acid rain [6,7]. As a consequence, the degree of soil acidification in tea cultivation systems surpasses that observed in other economically significant crop systems. Soil pH levels < 4.0 can significantly hinder tea plant growth and this lowers both the quantity and quality of tea leaf production [8]. Additionally, soil acidification results in the leaching of crucial nutrients such as K, Na, Ca, Mg, and P that also reduce productivity and tea leaf quality [9–11].

Among the nutrients prone to leaching in acidified soils, Mg is one of the necessary elements for plant growth and development and is an essential component of chlorophyll [12]. Most soil Mg is bound within crystal mineral lattices and 90-98% is unavailable for direct plant uptake [13]. Plants can only absorb the magnesium ion (Mg^{2+}) form of this element although its large size (ionic radius) results in weak associations with root cell walls and negatively charged soil particles and thus is

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susceptible to leaching in acidic soils [13]. Plant Mg deficiency has become a significant factor inhibiting agricultural production [14].

Previous studies have also demonstrated that Mg deficiency suppresses photosynthesis, leading to carbohydrate accumulation in source leaves, inhibiting root growth and altering both leaf and root morphologies [15]. The activity of key enzymes in photosynthesis including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) also decrease under Mg deficiency resulting in reduced photosynthetic rates [16]. Mg deficiency also leads to a reduction in chlorophyll content, the occurrence of leaf chlorosis, changes in leaf ultrastructure including chloroplast swelling, deformation and disintegration as well as disruption of thylakoid continuity resulting in photooxidative damage [17–19]. These structural changes adversely affect leaf functionality and photosynthetic efficiency. Moreover, Mg deficiency interferes with carbohydrate transport, preventing the effective transport of photosynthetic products to the roots and other non-photosynthetic tissues, thus limiting plant growth and development. Replenishing Mg rapidly enhances the export of sucrose from source leaves to the phloem [20].

Both soil acidification and Mg deficiency present stress factors for plants and increase the production of reactive oxygen species (ROS) [21,22]. ROS are toxic by-products of aerobic plant processes including photosynthesis, photorespiration and respiration [23] and ROS can react with lipids, proteins and nucleic acids resulting in lipid peroxidation, protein denaturation and genomic mutations, respectively [24]. However, ROS also serve as signaling molecules in plant cells and play vital roles in programmed cell death regulation and other physiological activities. Under normal conditions, the production and removal of ROS are in dynamic equilibrium [25]. The detrimental effects of excess ROS on cellular metabolism under stress conditions are countered in plants by an antioxidant defense system that minimizes lipid peroxidation of cellular and organelle membranes [21,26,27]. Enzymatic antioxidant systems are integral to this defense system and under stress conditions, cells initially enhance their antioxidant defense system by increasing the activity of superoxide dismutase (SOD) that acts in the first line of defense from ROS. The activities of catalase (CAT) and peroxidase (POD) also increase under these conditions [15]. SOD catalyzes the breakdown of superoxide anions ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) that is then converted to water and oxygen via CAT and POD among others [28]. When ROS production exceeds the capacity of the clearance system, the latter cannot provide sufficient protection for the membrane to prevent photooxidation, This leads to oxidative stress and a significant increase in malondialdehyde (MDA) levels that are also used as an indicator of the degree of exposure of the plant to these types of processes.

Soil acidification has become an established reality. Without a focus on plant nutrition, including Mg nutrition, the combination of acid and nutrient stress could result in significant losses in productivity. Given that tea plants are predominantly grown in the rainy, acidic soils of southern China characterized by low cation exchange capacity and low Mg content, we investigated the importance of Mg nutrition on the antioxidant response and quality of one-year-old tea seedlings under acid stress as a model for remediation of these soils via Mg supplementation.

2. Materials and Methods

2.1. Plant Culture and Mg Treatments

We obtained one-year-old tea seedlings (Longjing 43) from the Tea Seedling Breeding Base located in Fuyang District, Hangzhou, Zhejiang Province, China. Longjing 43 is a commonly cultivated tea cultivar in China and is frequently used for biological research. These seedlings were propagated through asexual reproduction and were cultivated hydroponically at the Pingshan Experimental Base of Zhejiang Agriculture and Forestry University. Prior to cultivation, soil was thoroughly washed from the seedling roots using deionized water as part of a pre-cultivation process. The plants were then placed in black plastic containers (9 L) filled with deionized water, for 6 days and the water was then replaced with a 25% aerated nutrient solution. The nutrient solution concentration was gradually increased every 6 days until it reached 100%. The experiments were

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commences following a three-week acclimatization period. During the pre-cultivation phase, we adjusted the pH of the nutrient solution daily to 5.0 using 0.1 M NaOH and 0.1 M H_2SO_4. The nutrient solution was replaced every three days. Magnesium nutrition was provided using $MgSO_4$. The experiment consisted of nine different treatment levels (refer to Table 1) and for treatments lacking SO_4^{2-}, we supplemented the missing ion with a Na_2SO_4 solution.

Table 1. Root zone pH and Mg^{2+} levels (mM) for experimental tea plants used in this study.

pH	Mg^{2+} Concentrations (mM)		
	0.01	**0.4**	**0.8**
3.5	L1	M1	H1
5.0	L2	M2	H2
6.5	L3	M3	H3

2.2. Sample Preparation

Tea seedlings were separated into roots, stems and leaves at random intervals of 1, 7, 15 and 30 days and the plants were thoroughly rinsed with deionized water to remove soil. Chlorophyll concentrations were measured in fresh leaf samples. Prior to evaluation of quality parameters and nutrient element content of the tea leaves, fresh samples were blanched at 105°C for 15 minutes, then dried in an oven at 60°C to constant weight. The samples were ground to pass through a 40-mesh sieve and stored for later use. In addition, fresh tea leaf samples were promptly frozen in liquid nitrogen and preserved at -80°C.

2.3. Determination of Chlorophyll Concentration and Root Viability

The concentration of chlorophyll in fresh mature leaves was determined using a modified version previously described (Li et al., 2021). In brief, the leaves were washed, dried and primary veins were removed followed by chopping into small pieces and placement (0.20 g) in a 25 mL volumetric flask. The flask was filled with 95% ethanol and the samples were allowed to steep in the dark until the leaves turned completely white. Absorbance values were measured at 470, 649 and 665 nm and the content of chlorophyll a, chlorophyll b and carotenoids were calculated. Each treatment was replicated three times.

To determine root vitality, the *triphenyl tetrazolium chloride* (TTC) method was used. In brief, a 10 mL beaker was filled with 0.2 g of root tip sample and 5 mL of 4 $g \cdot L^{-1}$ TTC solution and 5 mL of phosphate buffer solution were added. The samples were kept in the dark at 37°C for 2 h and the reaction was terminated by adding 2 mL of 1 M sulfuric acid. Simultaneously, a control experiment was conducted, wherein sulfuric acid was initially added to the root sample, followed by the addition of other chemicals after 10 minutes. The procedural steps were consistent with those outlined in the previous experiment. Extract the roots, remove excess moisture, and grind them together with 3-4 ml of ethyl acetate and a small amount of quartz sand in a mortar. Transfer the red extract to a test tube, and wash the residue with a small amount of ethyl acetate two or three times, combining all washings in the test tube. Finally, adjust the total volume to 10 ml with ethyl acetate. After thorough mixing, the absorbance of the extraction solution was measured at 485 nm using the blank sample as a reference. TTC reduction was determined based on the absorbance values using a standard curve constructed using red formazan (1,3,5-triphenyltetrazolium formazan).

2.4. Antioxidant Enzyme and Malondialdehyde Determination

Antioxidant enzyme levels (SOD, POD and CAT) and MDA content were measured as previously described with slight modifications [29]. In brief, 0.1 g of leaf tissue (excluding the main vein) and root tissue and 3 steel balls were ground using a mill into a paste in 2 mL centrifuge tubes containing 50 mM phosphate buffer pH 7.8 containing 1.0% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,500 × g for 20 min at 4°C and antioxidant enzymes and MDA levels were measured in supernatants as follows (all assays were conducted at temperatures

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between 2-4°C): SOD activity was measured using the nitro-blue tetrazolium (NBT) photochemical reduction method; POD activity utilized the guaiacol method and CAT activity was determined via UV absorbance; MDA content was measured using the thiobarbituric acid (TBA) colorimetric method [29].

2.5. Nutrient Content Measurements

Tea samples (0.2 g) were digested in 5 mL concentrated HNO3 and 2 mL 30% H2O2 at 180°C as previously described (Batista dos Santos Espinelli Junior et al., 2020) with slight adjustments. In brief, following digestion, the mineralized samples were transferred to volumetric flasks, diluted to a final volume of 30 mL with ultrapure water and filtered through 0.22 μm filters. The filtrate was used for flame atomic absorption analysis to quantify the Mg content. Dried samples of roots, stems and leaves (0.2 g each) were boiled in concentrated H_2SO_4-H_2O_2 until the solution was clear and then diluted with ultrapure water and adjusted to 50 mL. The N content was determined using indophenol blue colorimetry, K content using flame atomic absorption and the molybdenum antimony method was used to determine P content.

2.6. Tea Quality Determination

Following the standards of GB/T 8305-2013 "Determination of Water Extracts in Tea," the differential weighing method was employed to measure water extracts. Referring to GB/T 8312-2013 "Determination of Caffeine in Tea," the caffeine content was determined using ultraviolet spectrophotometry. According to GB/T 8314-2013 "Determination of Total Free Amino Acids in Tea," the total free amino acids were quantified using the ninhydrin colorimetric method. The content of tea polyphenols was determined through the iron tartrate colorimetric method.

2.7. Statistical Analysis

Data analyses were performed using IBM SPSS statistical software for Windows, Version 26.0 (IBM , Armonk, NY, USA). Significant differences between the data were processed on the basis of one-way analysis of variance and Duncan's multiple range test ($P < 0.05$). Data are presented as mean ± SD.

3. Results

3.1. Effects of Mg Nutrition and pH on Overall Plant Health

We initially examined the effects of Mg supplementation and acidic conditions measured on the growth of our experimental tea seedlings. We tested pH 3.5, 5 and 6.5 with Mg additions of 0.01, 0.4 and 0.8 mM. We found that by 30 days, seedlings exposed to pH 5.0 exhibited the highest fresh weights. These mass increases were also positively correlated with Mg dose under similar pH conditions. We did not observe any significant distinctions between treatment groups (Table 2). Similarly, seedling dry weights did not significantly differ between the treatment groups indicating a linkage between pH and Mg (Table 3).

Table 2. Effects of Magnesium Nutrition on Fresh Weight of Tea Seedlings Under Acid Stress for the indicated times.

Treatment	Fresh weight (g)*			
	1 d	7 d	15 d	30 d
L1	4.533±0.571a	4.500±0.573a	4.477±0.655a	4.453±0.118a
L2	4.530±0.656a	4.527±0.792a	4.497±0.865a	4.500±0.866a
L3	4.533±0.853a	4.523±0.591a	4.493±0.868a	4.513±0.927a
M1	4.513±0.637a	4.527±0.554a	4.517±0.863a	4.510±0.857a
M2	4.510±0.997a	4.537±0.486a	4.550±0.631a	4.570±1.003a
M3	4.520±0.612a	4.533±0.693a	4.523±0.761a	4.553±0.901a

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H1	4.530±0.530a	4.534±0.510a	4.543±0.184a	4.610±0.799a
H2	4.533±0.156a	4.573±0.162a	4.613±1.073a	4.660±0.802a
H3	4.527±0.667a	4.540±0.932a	4.580±0.501a	4.617±0.489a

*Mean ± SD. Different lowercase letters indicate significant differences between amendments ($p < 0.05$). See Table 1 for abbreviations.

Table 3. Effects of Magnesium Nutrition on Dry Weight of Tea Seedlings Under Acid Stress for the indicated times.

Treatment	Dry Weight (g)*			
	1 d	7 d	15 d	30 d
L1	1.832±0.059a	1.791±0.070a	1.789±0.147a	1.770±0.095a
L2	1.826±0.003a	1.814±0.026a	1.805±0.135a	1.788±0.089a
L3	1.831±0.054a	1.800±0.046a	1.799±0.226a	1.796±0.080a
M1	1.820±0.099a	1.813±0.176a	1.812±0.215a	1.800±0.074a
M2	1.815±0.106a	1.811±0.380a	1.834±0.159a	1.835±0.199a
M3	1.821±0.021a	1.809±0.171a	1.819±0.092a	1.822±0.415a
H1	1.825±0.100a	1.813±0.242a	1.828±0.155a	1.846±0.204a
H2	1.827±0.138a	1.840±0.108a	1.848±0.202a	1.870±0.074a
H3	1.825±0.078a	1.818±0.143a	1.836±0.124a	1.852±0.145a

* Mean ± SD. Different lowercase letters indicate significant differences between amendments ($p < 0.05$). See Table 1 for abbreviations.

These growth effects of pH and Mg were further examined by measuring the chlorophyll content of the plant leaves. Beginning on day 7, we found a significant difference in chlorophyll a content between the pH groupings that increased with increasing Mg level. High (H) Mg treatments were similar and did not significantly differ and increased 13.9, 17.2 and 10.3% compared to the M2 treatment. Mg deficiency (L) conditions at pH 3.5 yielded the lowest chlorophyll a content; a decrease of 24.6%. By day 15, Mg treatments H1 and H3 generated chlorophyll a levels significantly lower than for the H2 treatment but did not significantly differ from the M2 treatment. On day 30, all low Mg treatments (L1, L2, and L3) and the M1 treatment yielded significantly lower chlorophyll a levels than the M2 treatment with reductions of 26.4, 13.8, 20.6 and 11.2%, respectively. The H2 treatment generated the highest chlorophyll a concentration that was significantly greater than the M2 treatment by 22.2% (Figure 1a).

The chlorophyll b content of the experimental plants displayed a pattern similar to that of chlorophyll a. On day 7, the tea seedling leaves subjected to Mg deficiency at a low pH had the lowest chlorophyll b levels that were significantly lower than for the M2 treatment with a reduction of 18%. The high Mg treatments H2 and H3 displayed significantly higher chlorophyll b levels versus M2 with increases of 24.3 and 24.2%, respectively. On day 15, H3 and M2 did not significantly differ. At the end of the experiment at day 30, low Mg and pH (except for H1) had significantly lower chlorophyll b levels than the M2 treatment (Figure 1b).

The total chlorophyll levels under Mg deficiency and acid stress (L1) were significantly less than for M2 at days 7, 15 and 30 with reductions of 22.9, 28.8 and 26.6 %, respectively. On day 7, all three high Mg treatments (H1, H2, and H3) were significantly higher than M2 with increases of 14.9, 19.1 and 16.5%, respectively. At the end of the experiment, the provision of high Mg nutrition under the same pH conditions mitigated the reduction of total chlorophyll under acid stress. H1 levels were greater that for L1 and M1 by 34.4 and 10.7%; H2 exceeded L2 and M2 by 43.8 and 23.3 % and H3 surpassed L3 and M3 by 22 and 3%, respectively. These results indicated that the combination treatment of pH 5.0 and high Mg significantly increased total chlorophyll (Figure 1c). These findings collectively suggest that acid stress is detrimental to the accumulation of total chlorophyll in tea leaf tissues.

Carotenoid content was the lowest under acid stress and Mg-deficient conditions and at days 7 and 15, the levels were significantly enhanced under high Mg and in particular, at pH 5. In contrast,

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by day 30 there were no significant differences in carotenoid content among the various treatment groups (Figure 1d).

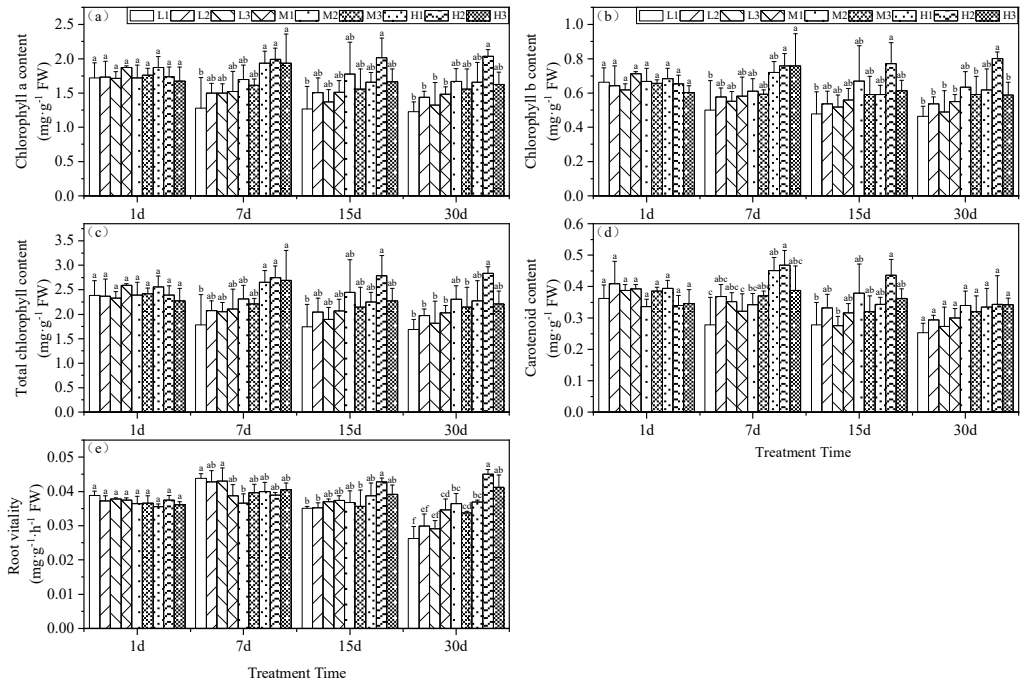

Figure 1. Effects of root zone pH and magnesium on tea plant seedlings. **(a)** Chlorophyll a, **(b)** Chlorophyll b, **(c)** Total chlorophyll, **(d)** Carotenoids, and **(e)** Root vitality. Experimental hydroponics modifications were as follows: Series 1 - pH 3.5 and Mg, 0.01 (L1), 0.4 (M1) and 0.8 (H1); Series 2 - pH 5.0 and Mg2+, 0.01 (L2), 0.4 (M2) and 0.8 mM (H2); Series 3 - pH 6.5 and Mg2+, 0.01 (L3), 0.4 (M3) and 0.8 mM (H3), respectively. FW: Fresh Weight. DW: Dry Weight. Mean ± SD. Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

Root vitality is another measure of plant health and this parameter peaked on day 7 under Mg deficiency but steadily declined thereafter in our experimental groups. By day 30, the pH 5.0 group (series 2) levels were significantly greater than for the other treatments at the same Mg level. This indicated that a continuous decrease in root environment pH reduces tea seedling root vitality. Furthermore, under the same pH conditions, increasing Mg levels enhanced tea seedling root vitality. In particular, these effects could be ranked as H1 > M1 and L1 with increases of 6.5 and 40.2%; H2 > M2 and L2 with increases of 23.6 and 50.7% and H3 > M3 and L3 with increases of 22.2 and 41.4, respectively (Figure 1e).

3.2. Mg and pH effects on Plant Oxidative Stress Indicators

MDA levels are an indicator of prior exposure to oxidative stress conditions. The MDA content in the leaves of our experimental plants significantly increased under Mg deficiency and acid stress (L1). For all treatment groups at days 7, 15 and 30, MDA levels were maximal at 87.33, 119.90 and 125.86 nmol·g^{-1}, respectively. Particularly on days 15 and 30, L1 plants increased by 144.6 and 169.6%, respectively, compared to the M2 treatment. At low pH, Mg supplementation effectively reduced MDA accumulation in the tea seedling leaves. On day 30, H1 MDA levels were reduced by 64% compared to L1 and by 13.6% compared to M1 while H3 decreased by 88% compared to L3 and by 17.2% compared to M3. Therefore, Mg deficiency led to greater levels of MDA i.e., L2 > M2 by 82.6% indicating the past exposure of greater levels of oxidative stress (Figure 2a).

The MDA content in the roots indicated that Mg deficiency generated significantly higher levels at pH 5.0 on days 15 and 30 that were increases of 131.5 and 174.4 %, respectively. Acid stress exacerbated the impact of Mg deficiency resulting in a 10.8% increase at pH 3.5 and a 6.9% increase

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at pH 6.5 compared to the pH 5.0 treatment on day 15 and by day 30, these levels were 12 and 7%, respectively. An increase in Mg in the roots significantly alleviated the increase in MDA content under acid stress. In the final phase of the experiment, 0.4 and 0.8 mM Mg under acid stress reduced MDA levels by 33.2 and 35.8%, respectively compared to the 0.01 mM Mg. Elevated pH conditions further reduced MDA levels with decreases of 51.6 and 46.2%, respectively (Figure 2b).

Mg levels also correlated with SOD activities in the tea seedling leaves. Under acid stress, high Mg generated significant increases in SOD activity versus all other treatments starting from day 7 and this high activity was maintained for the remainder of the experiments. On day 30, SOD levels were significantly increased by 60 % versus M2 treatment that surpassed L2 and H2 by 122 and 9.9%, respectively. Conversely, low Mg did not lead to elevations in SOD activity. By day 30, the low Mg group reduced SOD activity by 27.2% (Figure 2c).

The SOD activity in the roots of tea seedlings versus controls displayed significantly higher levels for M1, M3, H1, H2, and H3. These findings indicated that supplying Mg can offset the effects of acid stress. Furthermore, by day 30, Mg deficiency resulted in a significant reduction in SOD activity by 13.1, 16.4 and 9.43% compared to the M2 treatment, respectively (Figure 2d).

The POD activity in tea seedling leaves increased to a maximum at day 7 that was followed by a gradual decline. At pH 5.0, the POD activity in tea leaves consistently decreased although Mg supplementation could enhance POD activity in the leaves (Figure 2e). In contrast, POD root activity under Mg deficiency significantly decreased and by day 30, groups L1, L2 and L3 exhibited reductions of 11.6, 8.1 and 11.1%, respectively versus M2. Furthermore, POD activity for low Mg treatments continually decreased over the experimental period and conversely, Mg addition under acid stress significantly enhanced POD activity that peaked at 0.8 mM;. H1 levels increased by 58.2% increase compared to M2 and by 44.1% and 12.7% compared to L1 and M1, respectively (Figure 2f).

In tea seedling leaves, CAT activity reached its peak on day 7 day under Mg deficiency (0.01 mM) and gradually declined thereafter. On day 30, leaf CAT reached its minimum and decreased by 31.7, 30.6 and 8.6%, respectively, compared with M2. Therefore, Mg addition could increase CAT activity in tea seedling leaves and by day 30, the high-Mg, low-pH (H1) treatment possessed CAT levels significantly higher than for the other treatments and 64.1% greater versus M2 (Figure 2g). In the seedling roots, Mg deficiency initially led to an increase and subsequent decrease in CAT activity under acid stress with a decline in CAT activity over time when at pH 5.0. By day 30, CAT activity in the Mg -deficient tea seedling roots was consistently lower than for M2 . Specifically, the L1 and L2 treatments exhibited reductions of 23.9 and 27.5%, respectively. Furthermore, Mg addition had a significant enhancing effect on CAT activity in tea seedling roots and activity markedly increased as Mg was increased under similar pH conditions (Figure 2h).

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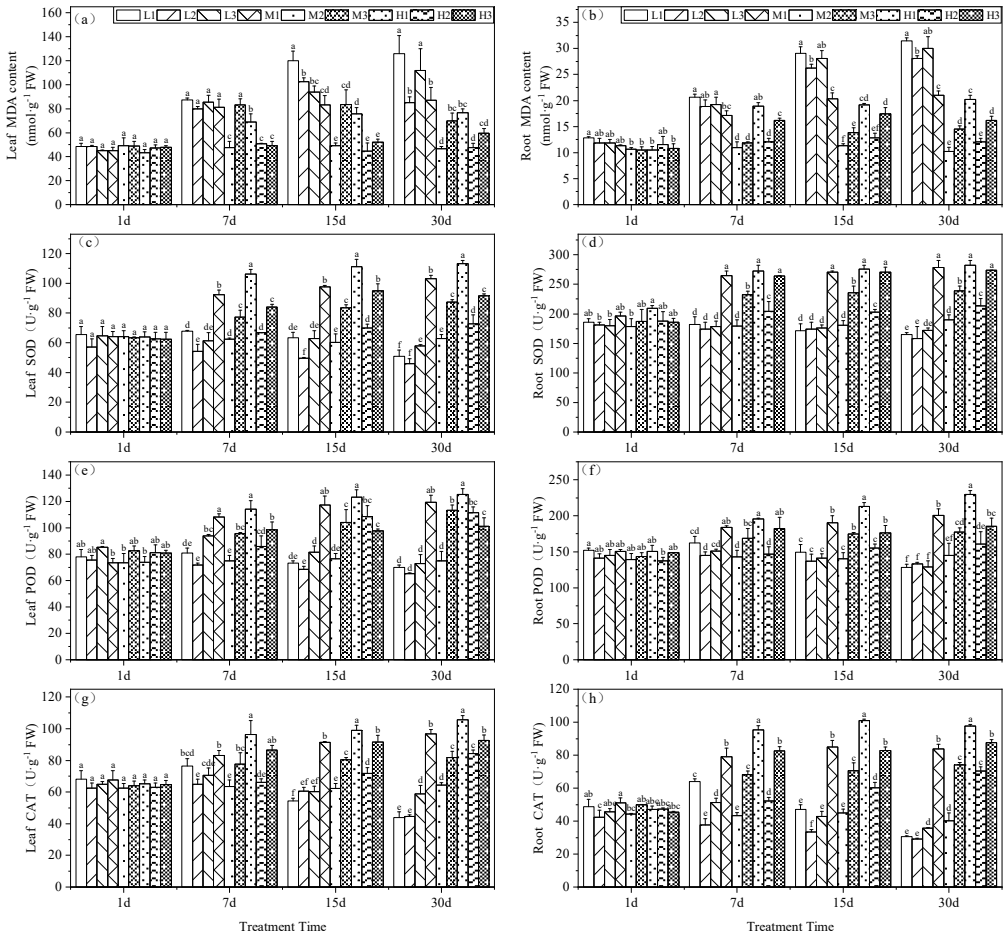

Figure 2. Effects of root zone pH and magnesium on oxidative stress markers in tea plant seedling roots and leaves. MDA content in **(a)** leaves and **(b)** roots. SOD activity in **(c)** leaves and **(d)** roots. POD activity in **(e)** leaves and **(f)** roots. CAT activity in **(g)** leaves and **(h)** roots. See Figure 1 legend for abbreviations.

3.3. Effects of Mg Nutrition on Tea Quality under Acid Stress

Tea polyphenols are complex compounds of polyhydroxy phenols found in tea leaves. Under similar Mg concentrations, the polyphenol content in tea leaves was significantly higher at pH 5.0 compared to other treatments. Interestingly, at day 15, Mg deficiency without acid stress led to maximal polyphenol values among all periods, exceeding the normal treatment by 2.07% and by day 30, the levels were stable at 2.09%, but were still higher than the other two treatment groups. By day 30, high Mg correlated with greater polyphenol content that increased by 8.1, 9.3 and 10.4 % under acid stress conditions (Figure 3a).

The total free amino acid content in the tea leaves indicated that Mg deficiency led to significantly higher levels for treatments L1, L2 and L3 that increased by 0.17, 0.62 and 0.54%, respectively. However, by day 30, all Mg deficient treatments were significantly lower than for the 0.4 mM Mg with reductions of 0.58, 0.37 and 0.09%, respectively. Additionally, at high Mg levels, acid stress did not decrease the total free amino acid content in tea leaves. Furthermore, at this point, the increase in Mg content led to 0.51 and 0.26% increases at pH 5.0 and 6.5, respectively, compared to the M2 treatment. In contrast, moderate Mg levels resulted in a reduction of total free amino acid content under acid stress with reductions of 0.18 and 0.05% in acid stress and high pH treatments, respectively (Figure 3b).

Acid stress led to a decrease in tea leaf extractable water and at days 7 and 15, L1, M1 and H1 treatments were significantly lower than M2 with reductions of 10, 7.2 and 4.7% as well as 12, 6.6 and 5%, respectively. By day 30, increased Mg could enhance the tea leaf extractable water. Specifically,

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H1 was 18.65% higher than L1 and M1, H2 were 22.1% higher than L2 and M2 and H3 was 19.4% higher than L3 and M3 (Figure 3c).

Caffeine content also varied with our experimental treatments of the seedlings. Starting from day 7, all treatments except for H2 exhibited a significant increase compared to M2. However, at days 15 and 30 the caffeine content decreased continuously under Mg deficiency and acid stress conditions. In addition, on day 30, all three Mg -deficient treatments yielded caffeine levels significantly lower than M2 with reductions of 0.35, 0.03 and 0.24% while the remaining treatments were significantly higher than M2 with increases of 0.47, 0.28, 0.45, 0.27 and 0.22%, respectively. These data indicated that Mg deficiency and acid stress promoted accumulation of caffeine in the tea plant leaves (Figure 3d).

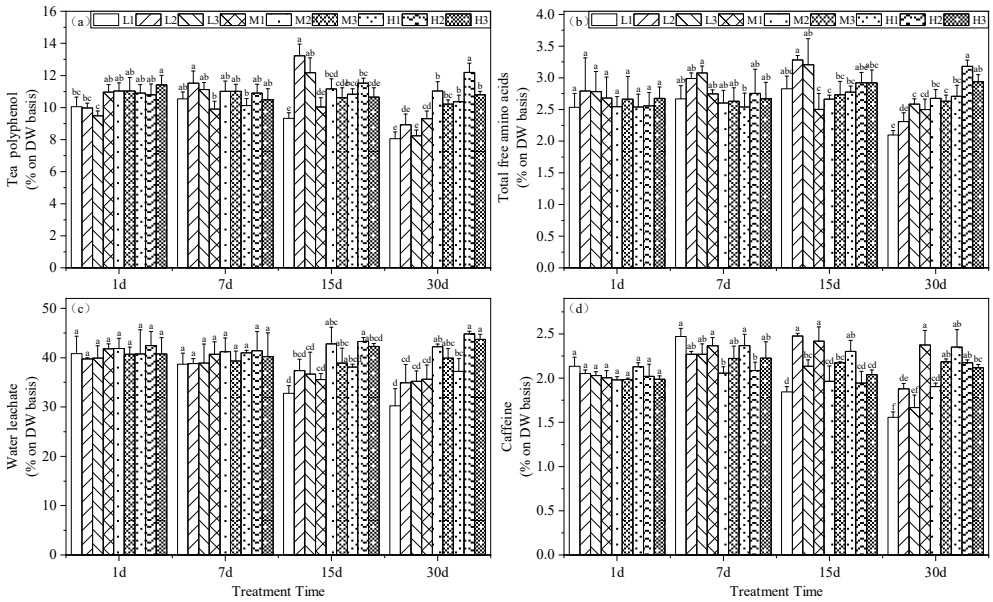

Figure 3. Effects of altered root zone pH and magnesium supplementation on tea plant physiological markers. **(a)** Polyphenols **(b)** Free amino acids **(c)** Water leachate content and **(d)** caffeine. See Figure 1 legend for abbreviations.

3.4. Nutrient Accumulation in Tea Leaves

The influence of root zone pH and Mg on plant nitrogen content indicated that by day 30 under equivalent Mg concentrations, N levels decreased with increasing pH. Under Mg deficiency, acid stress increased the N content by 9.6 and 13.4% compared to the other two groups. At Mg level of 0.4 mM, the increase was 3.3 and 5.5% and at 0.8 mM, the increases were 4.7 and 5.1%, respectively. Additionally, under Mg -deficient conditions, the N content in tea leaves was significantly lower than in the other treatments. At pH 5, the N content for M1, M2 and M3 were 3.6, 4.3 and 4.4%, respectively (Figure 4a).

The P content in leaves were also significantly increased as the Mg levels were increased although at 0.8 mM Mg, P levels decreased with time. On day 30, Mg deficiency without acid stress (L2 and L3) and high pH treatments under normal Mg conditions (M3 and H3) facilitated the accumulation of P that increased by 14.1, 14, 6.5 and 10.9%, respectively, compared to M2. Furthermore, under conditions of equivalent Mg, acid stress had a detrimental impact on the accumulation of P in the leaves (Figure 4b). In contrast, K levels did not vary for any of the experimental treatments (Figure 4c).

Mg is a necessary element for photosynthesis and is an essential component of chlorophyll. For our experimental treatments, tea leaf Mg significantly increased as Mg and pH levels in the root environment increased. At days 7, 15 and 30, leaf Mg content increased with Mg and concurrently, pH also significantly affected leaf Mg levels. On day 15 under Mg deficiency, an increase in pH led to a significant enhancement in leaf Mg level with the M3 treatment exhibiting a 9.92% increase

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compared to M1 and a 6% increase compared to M2. The H3 treatment displayed an 8.7% increase compared to H1 and a 6.2% increase compared to H2. The trends in leaf Mg content at days 7 and 30 followed a similar pattern (Figure 4d). In summary, the Mg content in tea leaves significantly increased with the rise of supplemented Mg and pH.

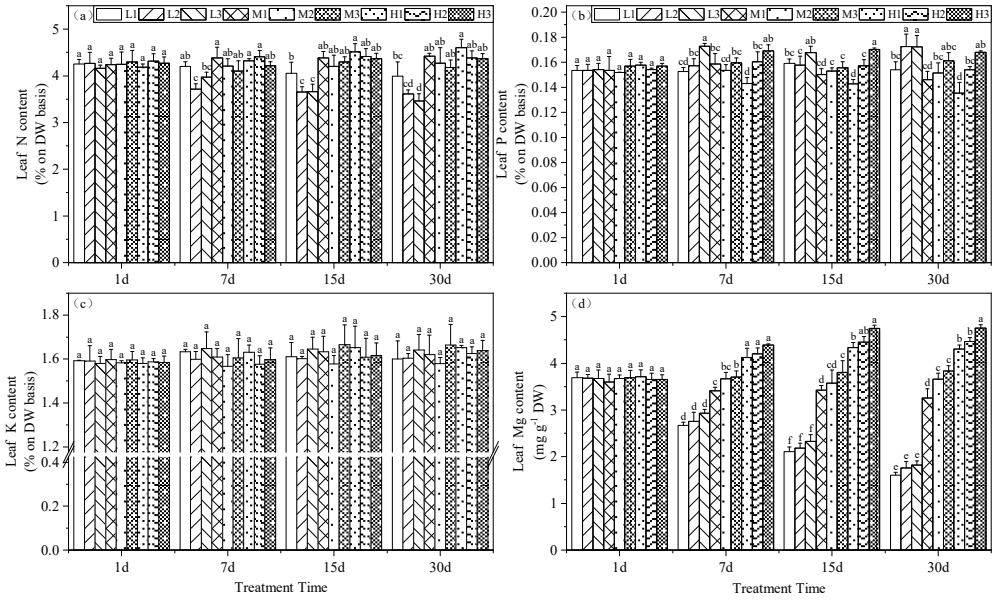

Figure 4. Effects of root zone pH and magnesium supplementation on essential elements in experimental tea seedlings. **(a)** Nitrogen **(b)** Phosphorous **(c)** Potassium and **(d)** Magnesium. See Figure 1 legend for abbreviations.

4. Discussion

4.1. Chlorophyll and Root Vitality in Tea Seedlings

Mg is an essential nutrient in plant life processes and is present in the octahedral chlorophyll structure and acts as a regulator of chlorophyll synthesis [30]. In our study, we observed an increase in chlorophyll content that positively correlated with Mg concentration while acid stress led to a decrease. Notably, Mg deficiency generated a significant reductions in chlorophyll content within 30 days indicating that the two are tightly linked as previously reported [31]. In agricultural practice, reduced chlorophyll content results in interveinal chlorosis in mature plant leaves [32] although our experimental plants displayed no significant chlorosis at any time.

Root vitality is a critical physiological indicator reflecting a plant's ability to withstand adverse environmental stress and directly affects its growth status [33–36]. Previous studies have shown that under hydroponic conditions, *Mimosa pudica* root vitality is closely linked to pH level in the root environment [37]. Consistent with this, our experiments revealed that Mg deficiency significantly inhibited root viability [38] and higher Mg (0.8 mM) enhanced the root vitality of our tea seedlings.

4.2. Oxidative Stress

Numerous environmental stressors can produce ROS in plants and this is a major contributor to significant crop productivity losses. It is estimated that approximately 1% of the O_2 consumed by plants is redirected to different subcellular sites (such as chloroplasts, mitochondria, peroxisomes) to generate ROS [39]. ROS can impact numerous cellular functions through mechanisms including nucleic acid damage, protein oxidation and membrane lipid peroxidation [40]. The effective removal of ROS generated under various environmental stress conditions relies on the action of several non-enzymatic and enzymatic antioxidants present in plant tissues [21]. Among these, the antioxidant enzyme system plays a significant role in the removal of ROS in plants and includes antioxidant enzymes and cell protective enzymes. We found that POD, SOD and CAT activities were

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induced in the leaves and roots of tea seedlings under Mg deficiency and acid stress. Prolonged Mg deficiency also resulted in reduced antioxidant enzyme activities and this has been previously reported [15,41–43]. For example, Mg deficiency leads to a widespread suppression of the antioxidant defense system accompanied by an increase in ROS. Therefore, under conditions of moderate Mg deficiency, the antioxidant defense system is generally inhibited [43]. In our research, we observed an initial increase and subsequent decrease in the activities of POD and CAT in both tea seedling leaves and roots under Mg deficiency and acid stress at 7 days. The decrease in CAT activity may be a reflection of the reduced Rubisco activity in response to Mg deficiency, leading to a reduced rate of photorespiration [44]. Additionally, our study indicated that increases in Mg supplementation also led to higher antioxidant enzyme activities. Enhancing the Mg supply to maize plants has been shown to increase their antioxidant capacity and promote expression of SOD, CAT and POD [45]. This suggests that Mg applications can be beneficial under acid stress to thereby improve the plants ability to eliminate ROS, stabilize leaf growth and enhance their adaptability to adverse rhizospheric acid environments.

When the production of ROS exceeds the clearance capacity of the antioxidant metabolic system, it fails to provide adequate protection for membrane lipids, resulting in a significant increase in the levels of MDA, a toxic metabolic byproduct of lipid peroxidation [15]. This type of elevation is thus a marker of oxidative stress exposure [46]. In our study, we found that long-term acid stress and Mg deficiency significantly increased the MDA content in tea seedling leaves and roots. This, coupled with the corresponding low antioxidant enzyme activities, indicates a notable decrease in the ability of tea seedlings to eliminate ROS under these conditions. This suggests that the rate of ROS production may exceed the cell's clearance capacity. Moreover, our findings revealed a significant reduction in MDA content with increasing Mg supplementation. This has been previously reported for Mg deficient corn [47], rice [48] and *Citrus grandis* and *Citrus sinensis* [49] leaves. Adequate Mg application can mitigate damage to cell membranes to improve to the ability to withstand environmental stressors.

4.3. Tea Quality and Nutrient Uptake in Tea Seedlings

Tea polyphenols, free amino acids and caffeine are three essential chemical components of tea leaves. Tea polyphenol content plays a significant role in determining the richness and health benefits of tea leaves and so contributes to the taste profile, offering attributes such as acidity, sweetness, and freshness. However, excessively high levels of tea polyphenols can lead to a bitter taste in the tea infusion[50–52]. Free amino acids, especially in green tea, are primary contributors to its taste profile. They provide a refreshing flavor, alleviate bitterness and enhance sweetness making them a key evaluation criterion for green tea quality [53–55]. Caffeine is the most abundant alkaloid in tea leaves, extensively studied and known for its health benefits including reducing fatigue [56].

In our study, the content of tea polyphenols, free amino acids and caffeine exhibited a brief increase within the first 15 days under Mg deficiency but subsequently decreased significantly by day 30. Furthermore, this decrease could be counteracted by increasing Mg levels and prevented the decrease in tea polyphenol and free amino acid content under acid stress. This indicates that an appropriate Mg dosage supports the maintenance of tea leaf quality under acid stress. However, it is worth noting that some studies have suggested that tea plantations with soil pH levels > 6.0 may not require Mg fertilization. Interestingly, caffeine content significantly increased under prolonged acid stress with increased Mg concentration in the nutrient solution. Field experiments have also indicated that Mg fertilization substantially increased caffeine content in black, oolong and green teas [57].

The content of tea leaf water extract is regarded as a crucial quality indicator. It reflects the abundance of soluble substances in tea leaves, influencing the tea's taste profile in terms of thickness, strength and richness and these have a decisive impact on tea quality [58,59]. Generally, higher water extract content results in a more robust tea flavor [60]. Our research found that both Mg deficiency and acid stress significantly reduced the content of tea leaf water extract. Interestingly, we observed that an increase in Mg relatively enhanced the content of tea leaf water extract. This

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implied that Mg supplementation can stabilize and even improve the water extract content of tea leaves in the root zone environment under acid stress.

Nutrient deficiency in plants can lead to an imbalance in ion metabolism and inhibit plant growth [61]. Our study indicated that N, P and Mg in tea leaves increased as Mg levels were increased. Some nutrient elements in tea leaves have been found to decrease under Mg due to reduced transport of photosynthetic products to the roots but also increases the lignification of transport tissues in roots and stems [62]. This contributes to reduced nutrient absorption capacity that can further alter nutrient uptake. Our study demonstrated that Mg supplementation can increase the accumulation of N, P and Mg in tea leaves.

Interestingly, Mg deficiency without acid stress generated P levels in leaves that were significantly higher than that for the M2 treatment. This suggests that under these conditions, tea plants may promote P uptake and transport, possibly due to Mg deficiency to alter ion balance leading to changes in ion distribution [19]. During adverse environmental stress, ion metabolism in plants is disrupted that adversely affect normal metabolism. Acid stress can markedly inhibit root growth and even lead to root tip cell death [63]. Our study results indicated that low pH environments were not conducive to the accumulation of P, N and Mg in tea leaves and increased pH facilitated Mg absorption while N absorption tends to favor a low pH root zone environment.

5 Conclusions

Soil acidification is gaining increased attention in agricultural economic crop cultivation systems. However, Mg, as a crucial nutrient element in agricultural soils appears to be relatively overlooked, especially within the context of tea plantation systems. Given that tea plantation soils are often acidic with low cation exchange capacity and Mg content, special consideration must be given to Mg supplementation. In these experiments we found that Mg deficiency negatively affected antioxidant capacity, tea quality, chlorophyll content and root vitality when tea seedlings were exposed to acid stress. The provision of Mg nutrition significantly improved the physiological status and quality of tea seedlings.

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