ENGINEERING Agriculture

Engineering Journals

RESEARCH ARTICLE

2027 / Volume 14 / Issue 1 / 27710

H_2O_2 accumulation shapes the differential recovery from nitrogen and magnesium deficiency in cucumber

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KEYWORDS

Nitrogen deficiency, magnesium deficiency, cucumber, antioxidant system, photosynthesis, H_2O_2

HIGHLIGHTS

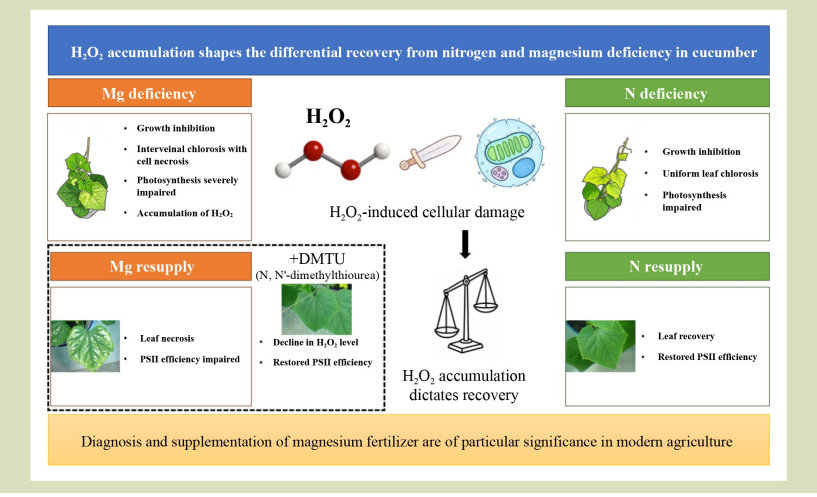
- Nitrogen and magnesium deficiencies differentially impair cucumber growth, with Mg deficiency causing more severe damage and only partial recovery upon nutrient resupply.
- It was discovered that H_2O_2 plays a crucial role in the recovery of cucumbers from nitrogen and magnesium deficiency.
- The excessive accumulation of H_2O_2 under magnesium deficiency surpasses the antioxidant capacity, resulting in irreversible cellular damage. This highlights the significance of magnesium fertilizer in modern agricultural production.
- Application of the H_2O_2 scavenger DMTU alleviates photoinhibition and partially restores photosynthetic function, providing direct evidence that H_2O_2 underlies the differential recovery from N and Mg deficiency.

Received December 12, 2025;
Accepted March 11, 2026.

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Special Issue: Magnesium Nutrition in Soil–Plant Systems

GRAPHICAL ABSTRACT



ABSTRACT

Nitrogen (N) and magnesium (Mg) deficiencies involve distinct underlying damage mechanisms and recovery responses. In this study, the physiological and biochemical responses of hydroponically grown cucumber plants to N or Mg deficiency and their recovery after nutrient resupply were evaluated. Considering that these deficiencies induce oxidative stress, we evaluated alterations in the antioxidant system and the accumulation of hydrogen peroxide (H_2O_2). The results revealed that both N deficiency and Mg deficiency inhibited cucumber growth and biomass production, with more pronounced adverse effects observed in leaves under Mg-deficient conditions. N resupply effectively restored leaf morphology, gas exchange parameters, and photosynthetic efficiency. In contrast, Mg resupply resulted in only partial recovery. This limited recovery can be attributed to irreversible damage to the photosynthetic system caused by the inability of antioxidant enzymes to effectively scavenge excessive amounts of reactive oxygen species (ROS), indicating that Mg deficiency triggers H_2O_2-induced cellular damage.

Furthermore, the application of the H_2O_2 scavenger DMTU (N,N'-dimethylthiourea) significantly reduced H_2O_2 levels under both deficiency conditions, particularly under Mg-deficient conditions. These findings demonstrate that N deficiency and Mg deficiency have distinct effects on antioxidant responses and underscore the pivotal role of H_2O_2 in plant responses to nutrient deficiency stress and subsequent recovery. These findings suggest that targeted modulation of H_2O_2 levels may serve as a promising strategy to increase crop resilience, offering a clear direction for future research.

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1 Introduction

Nitrogen (N) and magnesium (Mg) are essential macronutrients that play critical roles in the physiological and biochemical processes of plants[1,2]. Typically, N constitutes approximately 1% to 5% of the total plant dry matter, while Mg constitutes approximately 0.15% to 0.35%[3]. Most previous studies have focused on the role of N in plant physiological processes, particularly its involvement in photosynthesis through the regulation of chlorophyll content, carbon fixation enzyme activity, and the photorespiration rate[4]. Moreover, N serves as a structural component of nucleic acids, proteins, phytohormones, and secondary metabolites; therefore, N deficiency often results in an increased root-to-shoot ratio[5]. Like N, Mg plays a crucial role in photosynthetic processes and is also essential for carbon partitioning. As both a structural component of the chlorophyll molecule and an activator of various photosynthesis-related enzymes—such as carboxylases—Mg contributes significantly to plant metabolic functions[6]. In addition, sucrose phloem loading is closely associated with the proton gradient across the plasma membranes of phloem cells; consequently, any reduction in Mg-ATP levels may significantly impair the function of H^+-ATPase[7,8].

N-deficient plants typically exhibit growth inhibition and develop characteristic yellowing or reduced leaf expansion. In response to limited N availability in the soil, plants have evolved specific adaptive mechanisms to maintain growth. For example, root growth is often promoted under N deficiency to increase N uptake[9]. Plants deficient in Mg typically exhibit interveinal chlorosis in their older leaves, along with a significant reduction in the root-to-shoot ratio. The pattern of change in the root-to-shoot ratio under Mg deficiency is opposite to that observed under N deficiency, likely because of the nonsubstitutable role of Mg in assimilate transport and physiological function[10]. A reduction in the photosynthetic rate is a commonly observed response in plants under N or Mg deficiency, which can be attributed to two primary factors. First, the leaf chlorophyll content decreases because of reduced uptake of N or Mg. Both types of nutrients are highly mobile within plants and are preferentially reallocated to younger leaves to support active growth under conditions of nutrient limitation[11,12]. Second, N or Mg starvation induces a decrease in the activity of photosynthesis-related enzymes, including ribulose-1,5-bisphosphate carboxylase (Rubisco), a key rate-limiting enzyme in CO_2 assimilation[13,14]. In general, impaired CO_2 fixation leads to overreduction of the photosynthetic electron transport chain, resulting in excessive accumulation of reactive oxygen species (ROS) and subsequent damage to chloroplasts and cellular structures[15]. Although the mechanisms underlying leaf senescence under N deficiency and Mg deficiency are similar, a notable distinction in agricultural practices is that N deficiency is typically reversible upon resupply, whereas Mg deficiency often results in irreversible crop damage[10,16]. The fundamental cause of this differential recovery remains unclear.

Previous studies have demonstrated that N deficiency results in a significant reduction in the activities of superoxide dismutase (SOD), guaiacol peroxidase, and catalase (CAT) in wheat stems during the mid-grain filling stage, accompanied by a marked increase in ROS concentrations[17]. Mg deficiency results in substantial carbohydrate accumulation in leaves, a response that differs markedly from that associated with N deficiency[18]. Moreover, Mg deficiency-induced accumulation of sugars leads to feedback inhibition of photosynthesis, which may increase the production of ROS. Consequently, compared with plants

under N-deficient conditions, plants under Mg-deficient conditions may experience greater ROS-mediated damage, potentially resulting in irreversible cellular injury. Under stress conditions, plants generate various types of ROS, including hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), hydroxyl radical (·OH), and singlet oxygen ($^1O_2$)[19]. H_2O_2, the most abundant and stable ROS, plays a crucial role in various key plant resistance mechanisms[20]. H_2O_2 is proposed to function as a signaling molecule involved in the regulation of various stress responses[21]. Although elevated levels of ROS can induce oxidative damage to cellular structures, previous studies have focused primarily on plant responses to nutrient deficiency stress. Researchers have paid little attention to the specific function of reactive ROS, especially H_2O_2, in this process. It remains uncertain whether recovery from N or Mg deficiency is influenced by reactive oxygen species metabolism.

Owing to the differences in the physiological functions of N and Mg, as well as the distinct responses of plants to N deficiency and Mg deficiency, in contrast to N deficiency, Mg deficiency leads to irreversible damage once visible symptoms appear. A previous study reported that leaf senescence induced by N deficiency was reversed through N resupply, as N-sensitive regulatory pathways effectively and coordinately regulate plant growth[22,23]. In contrast, Mg resupply had limited effectiveness in reducing leaf damage, as sucrose phloem export had already been severely impaired prior to the onset of leaf senescence. Previous studies have focused primarily on plant responses to nutrient deficiency stresses, with limited attention given to the recovery mechanisms following nutrient resupply. The potential involvement of ROS, particularly H_2O_2, in N deficiency and Mg deficiency and the related differential recovery responses has not yet been fully elucidated.

To investigate the physiological responses to nutrient deficiency and subsequent resupply, cucumber plants were cultivated hydroponically under five different treatment conditions: a control group with full nutrient supply, N deficiency, Mg deficiency, N resupply following N deficiency, and Mg resupply following Mg deficiency. Parameters such as plant growth, chlorosis, biomass, photosynthesis, elemental concentration, antioxidant system activity, and H_2O_2 production were systematically evaluated. This study was designed to (1) assess the physiological effects of N deficiency or Mg deficiency on cucumber plants; (2) elucidate the distinct recovery patterns following N resupply and Mg resupply; and (3) determine the specific role of H_2O_2 in mediating the recovery processes from these nutrient deficiencies. This facilitates a more comprehensive understanding of the disparities in the physiological indicators of plants under N deficiency and Mg deficiency, as well as the disparities in the recovery processes of plants from these two types of nutrient deficiency. Comprehending these disparities offers a methodology for alleviating nutrient deficiency stress and resuming production in agricultural practices.

2 Materials and methods

2.1 Plant materials and experimental design

In this study, cucumber (*Cucumis sativus* L.) cultivar "Jinchun 4" seeds were soaked in distilled water for 1 h and germinated on moistened gauze at 25 °C in the dark for 48 h. Following germination, the seeds were sown in quartz sand and cultivated in a greenhouse under controlled conditions. The greenhouse was maintained under controlled environmental conditions, including a stable photosynthetic photon flux density (PPFD) of 500 μmol photons $m^{-2}·s^{-1}$, a 14-h photoperiod, a constant relative humidity of approximately 50%, and a day/night temperature of 28 °C/20 °C. When the seedlings reached the three-leaf stage, uniform individuals were transplanted into 1 L plastic pots filled with one-quarter strength nutrient solution. Three days after transplantation, the nutrient solution concentration was increased to half the maximum strength; another three days later, it was replaced with full-strength nutrient solution, which was maintained until the conclusion of the experiment. The nutrient solution was continuously aerated throughout the growth period. The full-strength nutrient solution contained the following essential elements: 1.25 $mmol·L^{-1}$ $(NH_4)_2SO_4$, 1.25 $mmol·L^{-1}$ $Ca(NO_3)_2$, 2.5 $mmol·L^{-1}$ K_2SO_4, 1.0 $mmol·L^{-1}$ KH_2PO_4, 2.0 $mmol·L^{-1}$ $MgSO_4$, 35.8 $μmol·L^{-1}$ Fe-EDTA, 57.8 $μmol·L^{-1}$ H_3BO_3, 11.4 $μmol·L^{-1}$ $MnCl_2$, 0.96 $μmol·L^{-1}$ $ZnSO_4$, 0.4 $μmol·L^{-1}$ $CuSO_4$, and 0.48 $μmol·L^{-1}$ H_2MoO_4. The nutrient solution was renewed every three days, and the pH was adjusted daily to 6.0 ± 0.1 using 0.1 $mol·L^{-1}$ HCl or 0.1 $mol·L^{-1}$ NaOH.

Experiment 1: Seedlings were subjected to different nutrient treatments upon full expansion of the fourth leaf (d0). Nutrient stress was imposed by removing either Mg or N from the nutrient solution. The experimental groups included a Mg-deficient group (–Mg), a Mg-deficient group for 9 days

followed by a Mg resupply for 5 days (–Mg+), a N-deficient group (–N), a N-deficient group for 9 days followed by a N resupply for 5 days (–N+), and a control group with a full supply of both Mg and N (CK). Nutrient deprivation was initiated on Day 0 (d0). On Day 9 (d9), two additional treatments were introduced: half of the Mg-deficient and N-deficient cucumber plants were selected and supplemented with Mg or N, respectively, and then cultivated for an additional 5 days, referred to as [(d9) + 5]. On Day 0 (d0), the seedlings were supplied with a standard nutrient solution, a Mg-deficient solution (0 mmol·L^{-1} $MgSO_4$), or a N-deficient solution [0 mmol·L^{-1} $(NH_4)_2SO_4$ and 0 mmol·L^{-1} $Ca(NO_3)_2$] for a duration of 9 days (d9). In the N deficiency treatment, calcium was supplied as $CaCl_2$ (1.25 mmol·L^{-1}) to replace $Ca(NO_3)_2$, ensuring equivalent Ca^{2+} availability. On Day 9 (d9), half of the Mg-deficient and N-deficient plants were resupplied with 2 mmol·L^{-1} Mg or 5 mmol·L^{-1} N, respectively.

Experiment 2: When the fourth leaves fully expanded, 2.5 μmol·L^{-1} N,N'-dimethylthiourea (DMTU) was applied via foliar spraying. After a three-day interval, normal nutrient supply, Mg-deficient, and N-deficient treatments were initiated. The seedlings were supplied with nutrient solutions according to the protocol described in Experiment 1.

2.2 Thermal imaging and microscopic observation

Nine days after the initiation of Experiment 1 (d9), thermal images of the entire plant were acquired using an infrared camera (SC620, FLIR Systems, Inc., Wilsonville, OR, USA). Imaging was conducted between 10:00 a.m. and 11:00 a.m., during which time the stomatal openings were confirmed to be fully active. Digital thermograms were analyzed using Thermal CAM Researcher Professional 2.9 software (FLIR Systems, Inc.).

The leaf abaxial epidermal microstructure was examined under a light microscope (Olympus IX71; Olympus Optical Co., Ltd., Tokyo, Japan) at a magnification of 400×.

2.3 Photosynthesis and chlorophyll fluorescence measurements

Five days after the resupply of Mg or N [(d9) + 5], gas exchange and photosynthetic efficiency parameters were measured on the upper fully expanded leaves using a portable photosynthesis system equipped with a fluorescence leaf chamber (LI-6400; LI-COR Biosciences, Lincoln, NE, USA). To measure the net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r), the leaves were maintained at a temperature of 28 °C and a relative humidity of approximately 50% under a PPFD of 1200 μmol photons m^{-2}·s^{-1}. Data were recorded after the system reached steady-state equilibrium, typically within approximately 15 min. Following the gas exchange measurements, the minimal fluorescence (F_o) and maximal fluorescence (F_m) of leaves were measured under dark-adapted conditions between 2:00 a.m. and 3:00 a.m. The variable fluorescence (F_v) is determined by calculating the difference between the maximal fluorescence (F_m) and the minimal fluorescence (F_o).

The maximum quantum efficiency of photosystem II (F_v/F_m) was determined as follows:

$$F_v/F_m = \frac{F_m - F_o}{F_m} \quad (1)$$

2.4 Determination of biomass, element concentrations and chlorophyll content

Following the measurement of photosynthesis, five plants from each treatment were randomly selected for the determination of plant height and root length. Each plant was subsequently separated into three distinct fractions: leaves, sheaths, and roots. The dry matter of each fraction was determined by oven drying at 105 °C for 30 min, followed by drying at 70 °C until a constant weight was achieved. Dried leaf samples were predigested overnight using a HNO_3-$HClO_4$ mixture (4:1, v/v) and then subjected to digestion on a heating plate with the temperature gradually increasing from 60 to 190 °C over a period of 4 h. The concentrations of various ions (P, K, Ca, Mg, Fe, Mn, Cu, Zn, B, and Mo) in the resulting digestate were analyzed using ICP–MS (NexION 300X, PerkinElmer Inc., Waltham, MA, USA). A separate portion of the dried plant material was digested with a H_2SO_4-H_2O_2 mixture at 270 °C for 1 h, and the N concentration in the digest was quantified using an AutoAnalyzer 3 Digital Colorimeter (Bran + Luebbe Inc., Norderstedt, Germany).

2.5 Quantitative determination of hydrogen peroxide and antioxidant enzyme levels

The H_2O_2 content was determined using a commercially

available assay kit (Nanjing Jiancheng Bioengineering Institute). Fresh leaf tissue (0.2 g) was rapidly frozen in liquid nitrogen and homogenized in 2 mL of ice-cold 0.1% trichloroacetic acid (TCA). The homogenate was subsequently centrifuged at 19000 × *g* for 20 min at 4 °C. Afterward, 0.5 mL of the supernatant was mixed with 0.5 mL of potassium phosphate buffer (100 mmol·L^{-1}, pH 7.8) and 2 mL of potassium iodide solution (1 mol·L^{-1}). The reaction mixture was incubated in the dark for 1 h to ensure complete development. The absorbances of both the samples and the calibration standards were measured at 390 nm using a spectrophotometer. The H_2O_2 concentration was quantified on the basis of a calibration curve generated from a series of standard solutions provided with the kit.

The activities of SOD and peroxidase (POD) were determined spectrophotometrically by measuring changes in absorbance. Fresh leaf tissue (0.2 g) was rapidly frozen in liquid nitrogen and then homogenized in 2 mL of ice-cold phosphate buffer (50 mmol·L^{-1}, pH 7.8) containing 1% insoluble polyvinylpyrrolidone (PVPP) and 0.2 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 10000 × *g* for 15 min at 4 °C, and 0.5 mL of the resulting supernatant was collected for enzymatic activity assays. SOD activity was assayed by monitoring the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to the method described by Stewart and Bewley[24]. One unit of SOD activity was defined as the 50% inhibition of NBT reduction. POD activity was determined spectrophotometrically by measuring the oxidation of guaiacol at 470 nm, following the method described by Wang et al[25].

2.6 Statistical analysis

Statistical analyses were conducted using SPSS statistical software. One-way analysis of variance (ANOVA) was employed to assess differences in the measured parameters across various treatment groups, with Duncan's multiple comparison test applied to identify significant differences among group means. A *P* value of less than 0.05 was considered to indicate statistical significance.

3 Results

3.1 Effect of Mg or N deficiency on cucumber plant growth

Following a 9-day period of nutrient deficiency cultivation (d9), cucumber seedlings grown in nutrient solutions lacking either Mg or N presented visible foliage deficiency symptoms (Fig. 1(a)). Under Mg deficiency, interveinal chlorosis developed, which is a characteristic symptom of Mg deficiency. This symptom intensified over time, particularly in older

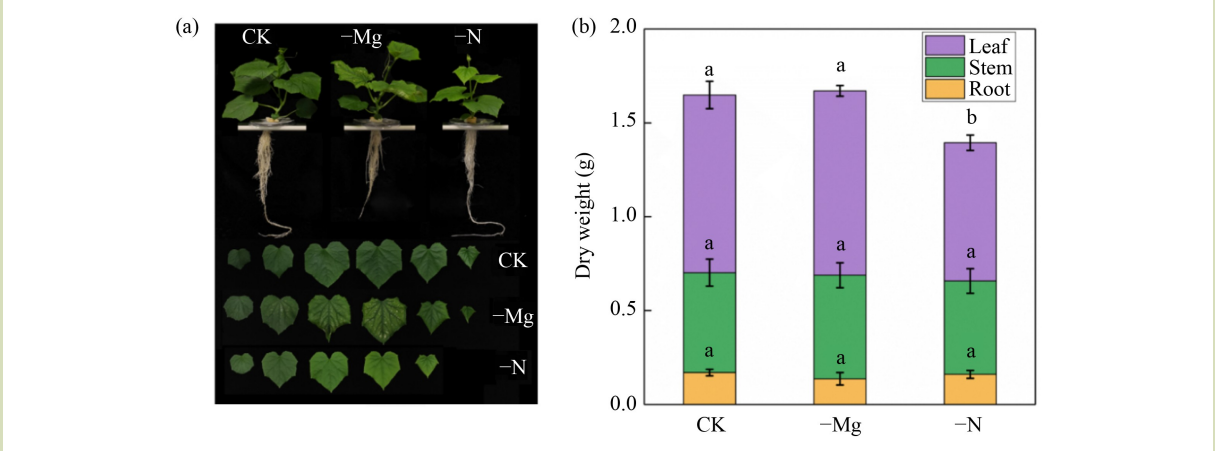


Fig. 1 Effects of Mg or N deficiency on cucumber growth (d9). (a) Growth status of cucumber plants; (b) dry weight of cucumber plants. Cucumber plants were cultivated under three treatments: normal nutrient solution (CK), Mg-deficient conditions (–Mg), and N-deficient conditions (–N) for a period of 9 days. The data are presented as the means and standard deviations from five independent replicates. Different lowercase letters indicate statistically significant differences ($P < 0.05$) according to Duncan's multiple range test, with comparisons made between treatments for the same plant organ.

leaves, as the duration of Mg deficiency increased. Similarly, N deficiency led to leaf chlorosis and inhibited overall plant growth (Fig. 1(a)). N-deficient plants displayed shorter shoots and reduced shoot growth rates, whereas root growth remained largely unaffected. In contrast, compared with that in the control plants, shoot growth in the Mg-deficient plants was comparable to that in the control, but compared with that in the control plants, root growth in the former decreased. When deficiency symptoms became apparent, total dry matter accumulation was not significantly affected by Mg deficiency; however, N deficiency resulted in an average reduction in whole-plant dry matter of approximately 16% (Fig. 1(b)).

Thermal imaging and microscopic observations revealed distinct effects of Mg deficiency and N deficiency on leaf physiology. N deficiency led to a significant increase in leaf temperature, as shown in Fig. 2. In contrast, Mg deficiency resulted in elevated temperatures primarily between the veins of middle leaves Microscopic analysis further demonstrated that visible cell necrosis occurred exclusively under Mg-deficient conditions (Fig. 2(a)).

3.2 Effects of Mg or N resupply on the performance and photosynthetic parameters of cucumber plants

Five days after nutrient supplementation [(d9) + 5], the Mg resupply increased plant height and root and stem biomass (Table 1) and partially alleviated deficiency symptoms in the

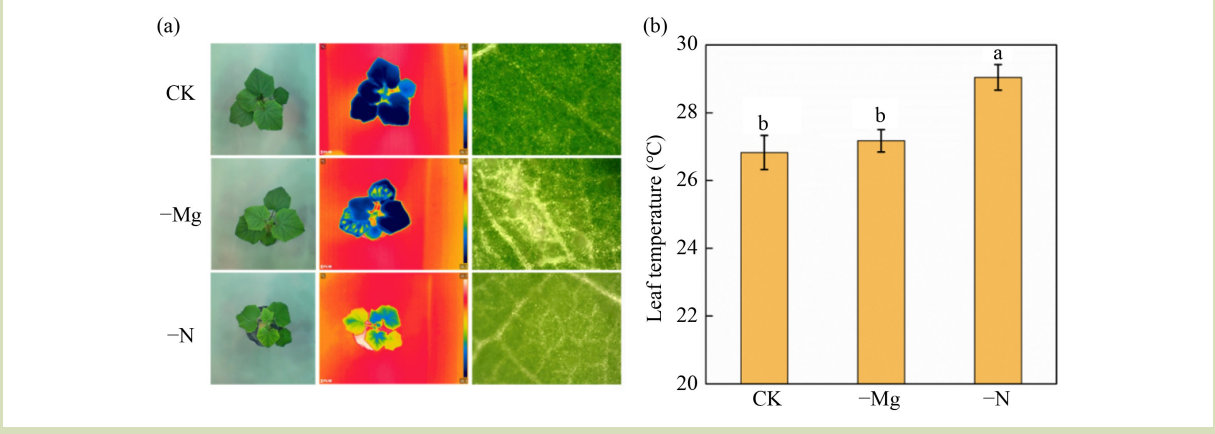


Fig. 2 Effects of Mg or N deficiency on the leaf temperature of cucumber plants (d9). (a) Digital color reflectance images, thermal images, and microscopy images of cucumber leaves following treatment with a standard nutrient solution (CK), Mg-deficient solution (–Mg), and N-deficient solution (–N) for 9 days; (b) leaf temperature measurements in cucumber plants. The data are presented as the means and standard deviations from five independent replicates. Different lowercase letters indicate statistically significant differences ($P < 0.05$) according to Duncan's multiple range test.

Table 1 Effects of Mg or N resupply on the growth of Mg or N deficient cucumber plants [(d9) + 5]

Treatment	Plant height (cm)	Root length (cm)	Dry weight (g)				Root/Shoot
			Total	Root	Stem	Leaf	
CK	65.3 ± 5.1a	40.5 ± 2.1a	3.25 ± 0.06a	0.32 ± 0.03a	1.34 ± 0.06a	1.58 ± 0.05a	0.11 ± 0.01b
–Mg	55.5 ± 1.3b	37.7 ± 2.6a	2.46 ± 0.10c	0.18 ± 0.01c	0.82 ± 0.06cd	1.46 ± 0.04b	0.08 ± 0.01c
–Mg+	63.3 ± 2.5a	39.8 ± 7.6a	2.72 ± 0.23b	0.25 ± 0.03b	1.04 ± 0.14b	1.43 ± 0.08b	0.10 ± 0.01b
–N	34.0 ± 3.0d	42.5 ± 7.3a	2.12 ± 0.08d	0.26 ± 0.03b	0.80 ± 0.06d	1.06 ± 0.04d	0.14 ± 0.01a
–N+	44.7 ± 1.5c	41.0 ± 5.1a	2.41 ± 0.14c	0.25 ± 0.03b	0.97 ± 0.06bc	1.19 ± 0.06c	0.12 ± 0.01b

Note: The treatments were as follows: CK, complete supply of Mg and N; –Mg, Mg deficiency. –N, N deficiency; the cultivation duration was 14 days. –Mg+, Mg deprivation for 9 days followed by Mg resupply for 5 days; –N+, N deprivation for 9 days followed by N resupply for 5 days. The data are presented as the means and standard deviations from five replicates. Different lowercase letters indicate statistically significant differences ($P<0.05$) according to Duncan's multiple range test.

upper leaves but not in the middle leaves (Fig. 3). In contrast, under N starvation, N resupply not only significantly affected dry weight but also increased plant height (Table 1). Following N resupply, the chlorosis observed in the cucumber plants was markedly alleviated (Fig. 3).

Both Mg deficiency and N deficiency significantly decreased the photosynthetic rate in plants; however, these inhibitory effects were partially alleviated following the resupply of Mg or N. N resupply markedly increased key gas exchange parameters, including the net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r), while it concomitantly reduced the intercellular CO_2 concentration (C_i) in cucumber (Fig. 4). Under N deficiency, all measured photosynthetic parameters were restored to control levels after N resupply. In contrast, although Mg resupply increased the photosynthetic rate, it remained below control levels. While Mg resupply had no significant effect on the maximum quantum efficiency of PSII (F_v/F_m) in the middle leaves (Fig. 4(e)), it effectively mitigated leaf necrosis and supported the maintenance of normal growth in the upper leaves (Fig. 3).

3.3 Effects of Mg or N resupply on element concentrations and antioxidant enzyme activity in cucumber plants

Compared with the control (CK), Mg deficiency significantly reduced the Mg concentration in plant leaves but markedly increased the concentrations of Mn and Mo by 73.3% and 72.8%, respectively (Fig. 5(a)). Additionally, the concentrations of Ca, Zn, and Cu increased by 53.2%, 46.9%, and 28.2%, respectively. N deficiency severely inhibited leaf growth, leading to a general decline in the concentrations of most measured elements, with the exception of P and Ca; among all the elements examined, the concentration of N decreased the most under N-deficient conditions (Fig. 5(b)). Following Mg resupply, the concentrations of most elements increased and remained at elevated levels, although the leaf Mg concentration remained substantially low. In contrast, N resupply increased the concentrations of the studied elements (excluding P and Ca), with the concentrations of all the elements eventually recovering to levels comparable to those of the control plants.

Compared with the control treatment, the Mg deficiency and N deficiency treatments significantly increased SOD activity by 185% and 91% in the lower leaves, 284% and 105% in the middle leaves, and 98% and 26% in the upper leaves, respectively (Fig. 6(a)). POD activity exhibited differential responses under the various deficiency conditions. In the Mg-deficient plants, POD activity in the lower and middle leaves was 7.47-fold and 36.85-fold greater, respectively, than that in the control plants, whereas no significant difference was detected between the N-deficient and control plants (Fig. 6(b)).



Fig. 3 Cucumber leaves from different canopy positions under Mg or N deficiency and subsequent recovery conditions. Color images of representative leaves were captured after 5 days of nutrient resupply [(d9) + 5], with leaf samples arranged from lower to upper canopy positions from left to right. CK: plants supplied with sufficient amounts of Mg and N; –Mg: Mg deficiency treatment; –N: N deficiency treatment; the duration of cultivation was 14 days. –Mg+: Mg was withheld for 9 days, followed by 5 days of Mg resupply; –N+: N was withheld for 9 days, followed by 5 days of N resupply.

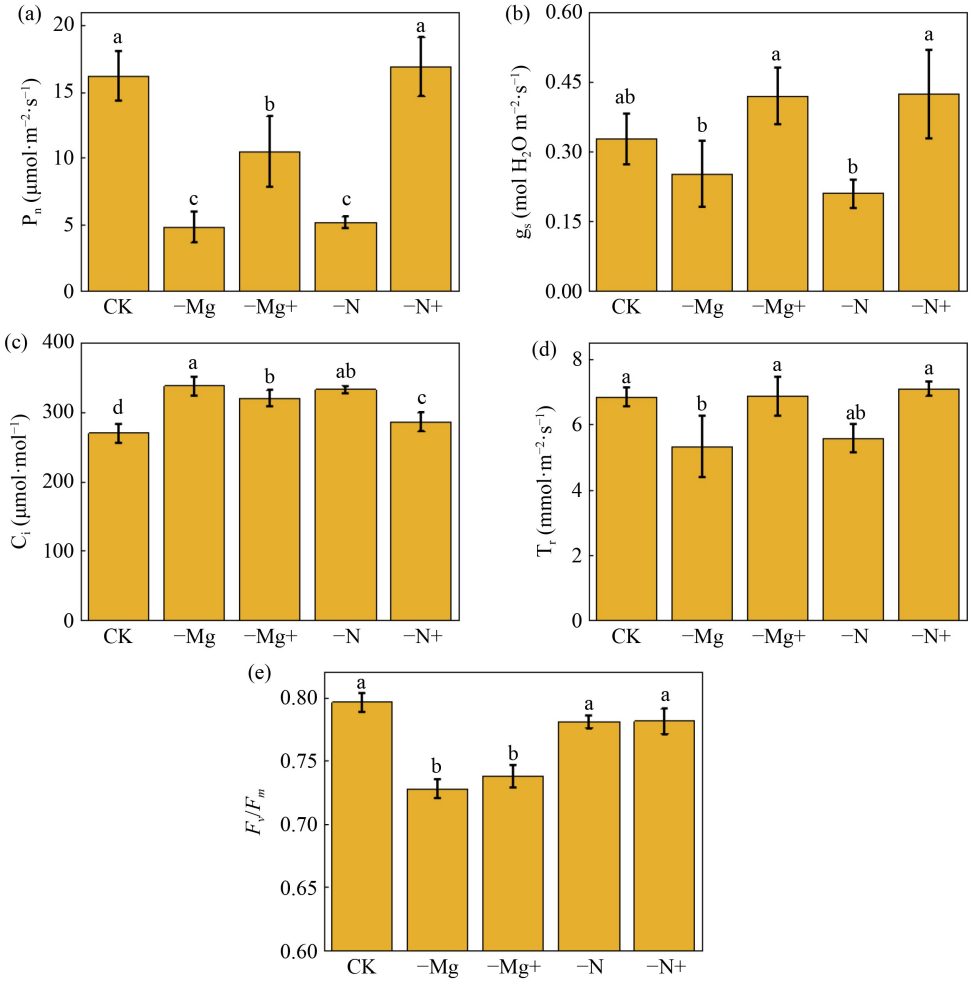


Fig. 4 Effects of Mg or N deficiency and resupply on the photosynthetic performance and chlorophyll fluorescence characteristics of plants at 5 days after treatment initiation [(d9) + 5]. (a) Net photosynthetic rate (P_n); (b) stomatal conductance (g_s); (c) intercellular CO_2 concentration (C_i); (d) transpiration rate (T_r); (e) maximum quantum efficiency of photosystem II (F_v/F_m). The data are presented as the means and standard deviations of five biological replicates. Different letters indicate statistically significant differences ($P < 0.05$) according to Duncan's multiple range test.

The resupply of Mg had a minimal effect on enzyme activity, whereas N resupply decreased SOD activity in the lower leaves but increased POD activity in the middle leaves. Compared with the control treatment, Mg deficiency significantly increased the H_2O_2 content by 130% in the middle leaves, and Mg resupply significantly reduced H_2O_2 levels (Fig. 6(c)). In the N-deficient plants, the H_2O_2 content in leaves at different positions was comparable to that in the control. Upon N resupply, the H_2O_2 content in leaves at all positions decreased significantly in the N-deficient plants.

3.4 Effects of spraying DMTU on leaf H_2O_2 production and F_v/F_m

Following the application of the H_2O_2 scavenger DMTU, the H_2O_2 content the leaves at different positions of Mg-deficient plants was comparable to that in the control (CK) and N+DMTU-treated plants (Fig. 7(a)). In the N-deficient plants, H_2O_2 levels in the middle leaves remained slightly elevated relative to those in the middle leaves of control plants after DMTU application. Although the F_v/F_m values in the Mg deficiency and Mg resupply groups were significantly lower

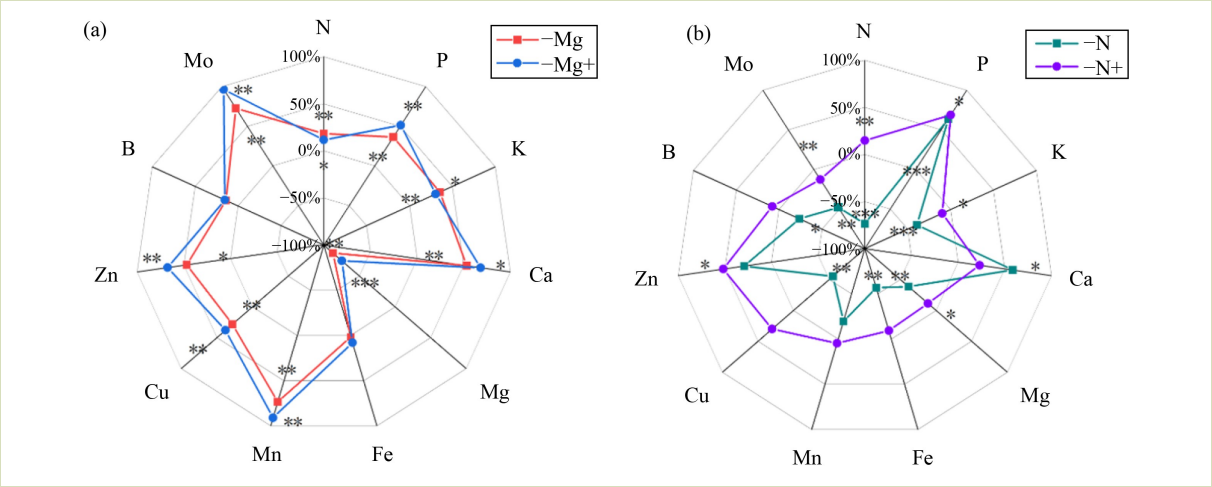


Fig. 5 Effects of Mg (a) and N (b) deficiency and subsequent resupply on the concentrations of elements in leaves at 5 days after treatment initiation [(d9) + 5]. Changes in the elemental concentrations relative to those in the control group (CK), which received the standard nutrient solution, were calculated as follows: (elemental concentration in treatment–elemental concentration in CK)/(elemental concentration in CK). Asterisks denote statistically significant differences compared with the control group ($^{*}P < 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$), as determined by two-tailed Student's *t* test.

than those in the control group (Fig. 5), DMTU application substantially alleviated the extent of photoinhibition (Fig. 7(b)), which corresponded to reduced H_2O_2 accumulation in leaves (Fig. 7(a)).

4 Discussion

4.1 Divergent stress effects: distinct physiological signatures of N deficiency versus Mg deficiency

Mg or N deficiency suppressed overall growth; however, different mechanisms were involved in their effects on biomass allocation. Deficiencies in Mg or N inhibited cucumber growth and biomass accumulation (Fig. 1). The observed symptoms were similar to those reported in previous studies[4,26]. The results revealed that the shoot/root ratios of the plants differed significantly among the –Mg, –N, and control treatment groups. Mg deficiency more strongly inhibited root growth than shoot growth; this increase in the shoot/root ratio under Mg deficiency has been previously reported in other plant species[27,28]. In contrast, N deficiency significantly reduced shoot growth, whereas root growth was less strongly affected, suggesting that the mechanisms through which Mg deficiency and N deficiency influence plant growth differ[29]. Previous studies have suggested that Mg deficiency may impair phloem loading, thereby limiting the translocation of carbohydrates from source leaves to sink tissues, such as roots[30,31]. The reduced shoot/root ratio under N deficiency can be attributed to a biphasic response: suppression of the leaf elongation rate coupled with the maintenance or stimulation of root growth through the preferential allocation of assimilated carbon to the roots.

Compared with N deficiency, Mg deficiency resulted in more severe structural and functional impairments, as evidenced by leaf chlorosis and confirmed at the cellular level. More pronounced negative effects were observed in plants under Mg deficiency, particularly in the middle and lower leaves (Fig. 1, Fig. 2(a)). Mg deficiency resulted in the development of typical interveinal chlorosis, a phenomenon consistent with the findings of Cai et al[2]. Leaves under N deficiency exhibited a pale green appearance, with no necrotic lesions observed. In contrast, light microscopic examination confirmed structural damage in leaves under Mg deficiency, whereas leaves under N deficiency maintained an intact cellular architecture. Further evidence indicating greater cellular damage in leaves under Mg deficiency was provided by thermal imaging, which, through digital infrared thermography, clearly displayed the leaf temperature and water status on the monitor, offering direct visualization of physiological stress[32,33]. Both Mg deficiency

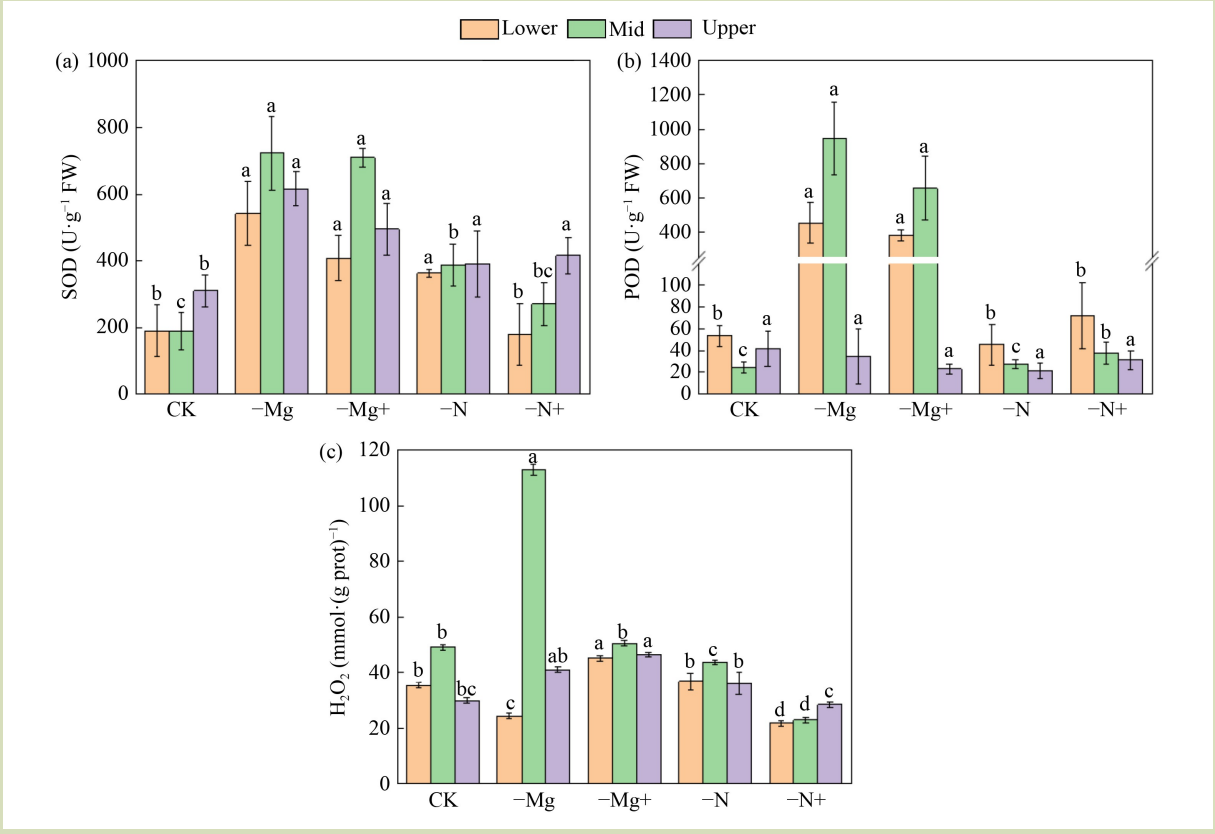


Fig. 6 Effects of Mg or N deficiency and resupply on antioxidant enzyme activity and H_2O_2 content in leaves at 5 days after treatment initiation [(d9) + 5]. (a) SOD enzyme activity; (b) POD enzyme activity; (c) H_2O_2 content. The data represent the means ± standard deviations of five biological replicates. Different lowercase letters indicate statistically significant differences ($P < 0.05$) determined by Duncan's multiple range test among treatments within the same leaf position. U·g^{-1} FW, units per gram fresh weight; mmol·(g prot)$^{-1}$, millimoles per gram of protein.

and N deficiency negatively affected the T_r, whereas the leaf temperature increased exclusively under N deficiency (Fig. 2). Leaf temperature consistently increases because of the inhibition of T_r caused by water loss from cellular damage resulting from Mg deficiency. Cakmak and Kirkby proposed that Mg deficiency-induced leaf necrosis may be attributed to impaired photosynthetic CO_2 fixation and the subsequent increase in ROS production[8]. Unlike Mg deficiency, N deficiency reduces photosynthetic activity, thereby leading to excess electron flow and promoting the overproduction of ROS[34].

N and Mg are essential elements for plant photosynthesis. Both Mg deficiency and N deficiency significantly reduced gas exchange parameters, including P_n, g_s, and T_r, and increased C_i (Fig. 4); these observations are consistent with findings reported in previous studies[4,35,36]. Rubisco is the key rate-limiting enzyme in CO_2 assimilation, and its activity has been demonstrated to be positively correlated with Mg and N concentrations, as Mg and N serve as activators or enzymatic activators or structural components[13,37]. Moreover, both Mg and N are critical components of chlorophyll; therefore, the decline in photosynthetic performance under deficient conditions can be attributed not only to reduced carbon fixation but also to impaired light-dependent reactions.

4.2 Differential recovery efficacy: the effects of N resupply versus Mg resupply in on alleviating deficiency symptoms

Nine days after the initiation of treatment, the plants were

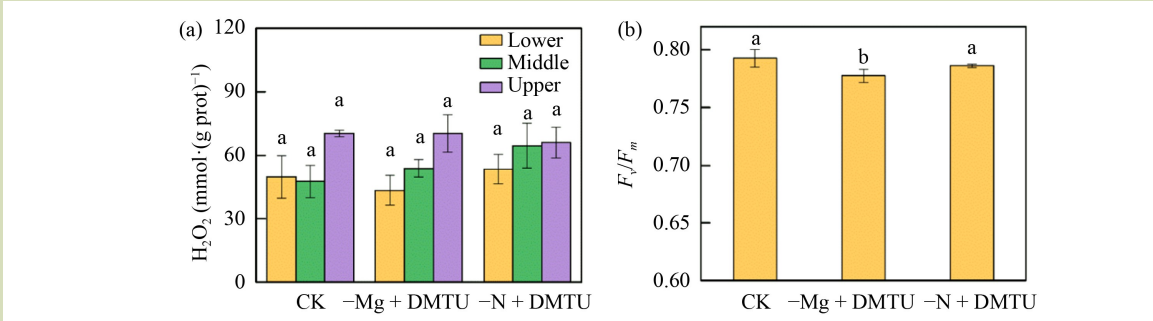


Fig. 7 Effects of the foliar application of DMTU on the hydrogen peroxide (H_2O_2) content and maximum quantum efficiency of photosystem II (F_v/F_m) in leaves. (a) H_2O_2 content across leaves in different positions; statistical analysis was conducted to compare the H_2O_2 concentrations at the same leaf positions under different treatments; (b) F_v/F_m values in the middle leaves. The data are presented as the means ± standard deviations of five biological replicates. Different lowercase letters indicate statistically significant differences ($P < 0.05$) determined by Duncan's multiple range test among treatments within the same leaf position. mmol·(g prot)$^{-1}$, millimoles per gram of protein.

resupplied with Mg or N to further investigate the distinct physiological responses under Mg deficiency and N deficiency. Five days later, leaves subjected to N deficiency exhibited signs of recovery, whereas necrotic symptoms persisted in leaves subjected to Mg deficiency (Fig. 3). These observations can be attributed to the more severe and detrimental effects of Mg deficiency on cucumber plants. Upon N resupply, the photosynthetic rate was restored to normal levels; however, although the improvement in photosynthetic capacity by Mg resupply remained significantly lower than that in the control treatment. This discrepancy may be explained by the disruption of the photosynthetic electron transport chain under Mg deficiency. Consequently, Mg deficiency likely induced irreversible damage to the leaf tissues.

Photosystem II (PSII) activity plays a crucial role in mediating the photosynthetic response to environmental stress; previous studies have indicated that the F_v/F_m ratio is a key indicator of photoinhibition in PSII[36]. Therefore, we assessed the F_v/F_m ratios under various treatment conditions to evaluate and compare the extent of leaf damage associated with Mg deficiency and N deficiency. Similarly, Mg deficiency significantly reduced the F_v/F_m ratio, whereas N deficiency did not significantly affect this ratio (Fig. 4). Previous studies have demonstrated that Mg deficiency reduces the F_m and the F_v/F_m while increasing the minimum fluorescence (F_o) in sugar beet[38]. Furthermore, additional studies have demonstrated that Mg deficiency reduces photosynthetic efficiency, F_v/F_m, and the photochemical quenching coefficient (qP) but increases nonphotochemical quenching (NPQ), with no significant effect on F_o[39]. Photoinhibition under Mg deficiency may be attributed to the absorption of photon energy by light-harvesting pigments exceeding the capacity of the photosynthetic electron transport chain to utilize it; the resulting excess excitation energy may lead to the formation of ROS[31,40]. A significant accumulation of ROS occurs in leaf cells, particularly within chloroplasts, which may lead to chlorosis and cellular death[41]. This explains the limited recovery of photosynthetic capacity following Mg resupply.

Nutrient deficiency can trigger the generation of ROS, resulting in elevated levels of H_2O_2 in plant cells[42]. A previous study demonstrated that elevated accumulation of H_2O_2 can inhibit photosynthetic efficiency. The removal of H_2O_2 may therefore be a critical factor in alleviating photoinhibition[43]. Therefore, the differences in damage caused by N deficiency and Mg deficiency may be attributed to variations in H_2O_2 accumulation levels. Furthermore, the differential recovery of photosynthetic capacity in cucumber leaves following N or Mg supplementation is closely associated with the respective efficiencies in scavenging H_2O_2.

4.3 The recovery of N-deficient and Mg-deficient plants is dependent on the H_2O_2 concentration

H_2O_2 is a component of oxidative metabolism and plays important roles in biochemical and physiological processes[44,45]. Therefore, we further investigated the H_2O_2 content in leaves under various treatment conditions. The –Mg

treatment resulted in the most severe deficiency symptoms, with H_2O_2 levels reaching twice those of the control (Fig. 6(c)). H_2O_2 is an inevitable byproduct of the photosynthetic electron transport chain, and its excessive accumulation can lead to damage to photosystems[46]. ROS are continuously generated at a basal level, and a balance is maintained between their generation and elimination. They play crucial roles in plants. Nevertheless, once the endogenous ROS balance is disrupted by biotic and abiotic environmental stresses, they can also lead to extensive cellular damage. In the event of Mg deficiency, the export of sucrose from the phloem is severely impeded, which subsequently inhibits CO_2 fixation. Excessive absorbed light energy is subsequently transferred to molecular oxygen, resulting in the generation of ROS[8,15]. In the case of N deficiency, the transfer of nitrogen nutrients to the cell wall to resist stress-induced damage weakens nitrogen allocation from the cytoplasm to photosynthetic components. The activity of photosynthesis-related enzymes in chloroplasts decreases notably, leading to the accumulation of CO_2 and a substantial increase in the concentration of ROS[17]. As previously noted, nutrient deficiency may contribute to increased concentrations of ROS, such as H_2O_2. The main site where ROS are produced is the chloroplasts. Therefore, we believe that the primary factor underlying the differential effects of N deficiency and Mg deficiency on photosynthetic damage is H_2O_2.

Plants possess diverse antioxidant enzymes, such as SOD, CAT, POD, and APX, to eliminate ROS and protect cells against oxidative stress[2]. The Mg deficiency-induced increase in antioxidant enzyme activity is a common phenomenon in the leaves of various plant species, including beans, maize, rice, and citrus[2,47,48]. In addition to Mg deficiency, N deficiency also increases ROS production, thereby inducing DNA strand breaks, lipid peroxidation, and ultimately cell death[49]. Previous studies have demonstrated that the application of ammonium nitrogen enhances antioxidant enzyme activity in blackberries[50], and under heat stress, the application of Mg significantly increased the activities of SOD, POD, and CAT in broad beans while reducing the accumulation of malondialdehyde (MDA) and H_2O_2[51]. An increase in the activities of SOD and POD, which can remove free radicals and maintain the steady-state cellular level of ROS, is likely associated with oxidative stress in plants[52].

The activities of SOD and POD exhibited distinct patterns under N or Mg deficiency and during the subsequent resupply. The variation in enzyme activities among the different treatments might be associated with the differences in leaf ion concentrations. Mg deficiency significantly altered the levels of Mn and Zn in cucumber leaves (Fig. 5). Ye et al.[36] reported antagonistic interactions between Mg and Mn, as well as Zn. These findings suggest that Mg deficiency may increase the uptake of Mn and Mo. Similar results have been reported for potato, wheat, maize, and oil rape[53–56]. Mn has been reported to act as an activator of numerous enzymes, particularly those involved in the antioxidant system. In plants, the oxygen-evolving complex (OEC) in PSII, manganese superoxide dismutase (MnSOD), and oxalate oxidase have been demonstrated to exclusively require Mn[57]. Moreover, the activities of antioxidant system enzymes, including POD, SOD, and APX, increase with increasing manganese concentration[58]. In the present study, Mg deficiency led to a substantial increase in the Mn absorption rate, exceeding 50%, which remained elevated even after Mg resupply. In contrast, compared with the control treatment, N deficiency did not significantly affect the Mn concentration. Thus, alterations in ion uptake, specifically that of Mn, may constitute a component of the adaptive response of plants to alleviate the accumulation of ROS under nutrient stress[59].

In addition to Mn, elevated Cu concentrations are also observed under Mg deficiency and during Mg resupply. Excessive Cu is toxic to plant cells and can adversely affect various biochemical and physiological processes, particularly by disrupting photosynthesis and N metabolism[60]. Compared with N deficiency and resupply treatments, minimal differences in ion concentrations were observed following Mg resupply, suggesting that the irreversible effects of Mg deficiency may be attributed to cellular damage caused by H_2O_2 rather than ion imbalance. Furthermore, ion concentrations were restored to normal levels upon N resupply. Given the low levels of H_2O_2 in leaves under N deficiency or N resupply conditions, it is reasonable to conclude that N supplementation can gradually mitigate the effects of reactive oxygen species (ROS), enabling leaves to recover to a normal physiological state.

To further validate our hypothesis, we attempted to protect the leaves by scavenging H_2O_2. Dimethylthiourea (DMTU), a widely used scavenger of H_2O_2, has been applied exogenously to leaves in previous studies to eliminate hydrogen peroxide and alleviate photooxidative damage[61,62]. Our studies demonstrated that the application of DMTU effectively reduced H_2O_2 accumulation in nutrient-deficient plants,

restoring it to the same level as that of the control group, particularly in Mg-deficient plants (Fig. 7(a)). Crucially, this reduction in oxidative load was correlated with a significant alleviation of photoinhibition, as evidenced by the partial recovery of F_v/F_m in Mg-deficient leaves (Fig. 7(b)). The direct correlation between H_2O_2 scavenging and the increase in a critical photosynthetic efficiency parameter strongly supports our hypothesis that the accumulation of H_2O_2 is an important limiting factor for recovery from nutrient deficiency. The finding that DMTU was unable to fully restore the maximum quantum efficiency of F_v/F_m to the control levels in Mg-deficient plants is consistent with the observed partial recovery of plant growth only after Mg resupply. The potential reason is that under Mg deficiency conditions, an excessive quantity of H_2O_2 causes irreversible damage to the leaf cells of plants. In contrast, the lower H_2O_2 burden under N deficiency conditions enables the recovery of photosynthesis and initiation of growth after N supplementation or DMTU treatment. These results further emphasize the crucial role that the H_2O_2 concentration plays in determining the degree of recovery from nutritional stress.

5 Conclusions

The results of the present study demonstrated that compared with N deficiency, Mg deficiency caused more severe and irreversible damage to leaf structure and photosynthetic function. However, N supplementation effectively alleviated symptoms of N deficiency, and Mg supplementation resulted in a weaker ability to alleviate symptoms of Mg deficiency. This difference can be attributed to the varying accumulation levels of H_2O_2 under nutrient deficiency stress. Recovery from N or Mg deficiency is dependent on the H_2O_2 concentration; excessive accumulation of H_2O_2 can disrupt cellular structure and ultimately lead to irreversible leaf damage. In agricultural production, N deficiency can be mitigated through timely N application, whereas Mg deficiency causes irreversible damage. Collectively, these findings enhance our understanding of the adverse effects of Mg deficiency and provide insights into mitigating the damage caused by Mg deficiency stress. Since plants become considerably more challenging to recover once visible symptoms of Mg deficiency manifest, early diagnosis and supplementation with magnesium fertilizer are of particular significance in modern agriculture.

Acknowledgements

This paper was funded by the National Key Research and Development Program of China (2023YFD1901101) and the National Natural Science Foundation of China (42577134).

Compliance with ethics guidelines

Qi Zhou, Kehao Chen, Ying Wang, Xusheng Meng, Shiyu Wang, Min Wang, and Shiwei Guo declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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