

RESEARCH ARTICLE

High atmospheric pressure rescues plant growth under humidity stress: A model for climate-resilient deep underground agriculture

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Abstract

One of the key goals in deep underground science is to better understand and make use of the unique resources found in these environments. Deep underground conditions, such as high atmospheric pressure and stable microclimates, have been shown to potentially benefit various organisms, including in vitro cells, tissues, microbes, and plants. However, the overall experimental results remain inconclusive, as it is uncertain whether the impacts of independent or individual factors on plant physiology counteract or compensate for the effects of abiotic stresses from deep underground. Herein, this study examined whether high atmospheric pressure could serve as an environmental advantage for physiological adaptation under high humidity conditions in the context of deep underground farming systems. Experiments in simulated environmental conditions (Relative humidity [RH] 99%, pressures 96, 110, and 120 kPa) revealed that high humidity impaired *Nicotiana benthamiana* growth by reducing water use, photosynthetic pigments, and phytohormones, while inducing oxidative stress. High atmospheric pressure counteracted these effects by stabilizing water balance, enhancing antioxidative defenses, and improving nutrient assimilation, resulting in up to a 16.13% increase in biomass despite a 52.94% increase in water consumption. Principal component analysis confirmed that high atmospheric pressure mitigated humidity-induced stress and promoted physiological resilience, indicating its potential for developing climate-adaptive crops in underground farming systems. Atmospheric manipulation leveraged high-pressure advantages to overcome humidity limits, benefiting sustainable agriculture and greening in deep underground spaces, especially under increasing climate uncertainty.

KEYWORDS

atmospheric pressure, deep underground environment, environmental stress, humidity, *Nicotiana benthamiana*, oxidative stress

Highlights

- High atmospheric pressure enhances plant resilience under high humidity conditions.
- Increased water consumption and growth observed in *Nicotiana benthamiana* with elevated pressure.
- Mitigative effects of high pressure against humidity-induced stress through physiological adaptations confirmed.
- Potential of subterranean pressures to enhance agricultural productivity in deep underground settings highlighted.
- Strategic utilization of atmospheric pressure proposed for future underground agriculture advancements.

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1 | INTRODUCTION

Underground science has emerged as a pivotal field for deciphering Earth's evolutionary processes and unlocking subterranean resources (Vähäaho, 2014; Xie et al., 2024). Beyond traditional deposits of minerals, fossil fuels, and water (Ghorbani et al., 2023), recent efforts target “environmental resources” unique to deep underground spaces, including ultra-low noise, minimal electromagnetic interference, low background radiation, high atmospheric pressure, and stable microclimates (Wang, Yang et al., 2022). These conditions have spurred strategic innovations such as underground energy storage, ecological habitats, and laboratories (Xie et al., 2024; Yang et al., 2023; Zhang et al., 2023), though practical implementation remains nascent.

Environmental instability severely limits global agricultural productivity (Pareek et al., 2020; Raza et al., 2025), driving demand for innovative approaches such as deep underground farming. This strategy is designed to address a broad spectrum of challenges, including carbon sequestration, pollution abatement, enhancement of climate resilience, and optimization of resource utilization (Calicioglu et al., 2019; Hunter et al., 2017; Liu et al., 2014; Osama et al., 2017; Prost et al., 2017; Xu, 2005; Zabel et al., 2016), and it may additionally be applied to mitigate the adverse effects of catastrophic events such as nuclear strikes or asteroid impacts. A pivotal question emerges: Does high atmospheric pressure, which is costly to replicate at the surface, offer benefits for plant growth that qualify as “deep underground resources” essential for optimizing agricultural benefits? Yet, operationalizing such systems confronts technical barriers, including synergistic stressors like extreme humidity, temperature fluctuations, and reliance on artificial environmental controls (Chen & Shi, 2022; Doyle et al., 2016; Gao et al., 2022; Wang, Yang et al., 2022), necessitating a thorough analysis of their socioeconomic and ecological viability.

Deep underground resource development confronts biological complexities due to unique environmental stressors that trigger multifaceted physiological adaptations. Studies in subterranean laboratories reveal that low background radiation induces stress responses across organisms, including delayed cellular growth, upregulated DNA repair mechanisms, and altered protein synthesis (Liu et al., 2018; Zou et al., 2023). For example, at China's Jiapigou mine (1470 m depth), cells exhibited mitochondrial expansion and enhanced ribosomal pathway activity under low radiation (Liu et al., 2020), while Gran Sasso experiments showed lifespan extension in *Drosophila* alongside accelerated growth in *Caenorhabditis elegans* and fish (Pirkkanen et al., 2020; Van Voorhies et al., 2020). Plant studies further highlight environmental trade-offs: Canola seeds stored underground suffered reduced germination rates (Wang, He et al., 2022), yet short-term exposure at 1410 m increased seedling growth vigor by up to 79% through hormone upregulation (Wang, Yang et al., 2022). Such paradoxical outcomes underscore the need to disentangle adaptive benefits from compensatory stress costs.

To date, findings underscore that deep underground environments exert significant physiological effects on

flora and fauna, with a growing body of evidence indicating that many of these impacts are positive and beneficial. Nevertheless, our understanding of the physiological responses of plants throughout their entire growth cycle under deep underground conditions, as well as the understanding of which independent (or individual) factors impact plant physiology, is hampered by the limited focus of existing studies (Li et al., 2020; Wang, He et al., 2022). Very few investigations derive benefits from controlled variables of individual factors in isolated deep underground environments. Of those studies that relate to this topic, only a modest proportion focus on whether a particular factor plays a positive role, potentially offsetting the negative effects inherent in deep underground environments, given current technological capabilities, a critical issue that facilitates the recognition and utilization of deep underground resources for potential development in plant science and agriculture. We argue that an enhanced understanding of the feasibility of utilizing high atmospheric pressure as a natural resource in deep underground environments will pave the way for capitalizing on the benefits of mitigating high humidity and other adverse effects. This study was conducted to (1) investigate the mechanisms of the combined effects of high humidity and atmospheric pressure on the model plant *Nicotiana benthamiana*, specifically addressing the critical knowledge gap concerning their direct antagonistic interaction, and (2) elucidate the functional pathways through which high atmospheric pressure alleviates high humidity-induced stresses in deep underground environments from a plant physiology perspective.

2 | MATERIALS AND METHODS

2.1 | Plant growth conditions and experimental design

N. benthamiana seeds were germinated and cultivated in plastic pots (15 cm in diameter and height) filled with a mixture of peat and vermiculite (3:1 by volume). The plants were consistently irrigated and grown under fluorescent lighting, providing an intensity of $120 \mu\text{Einstein m}^{-2} \text{s}^{-1}$, within a 16/8 h light/dark cycle at a stable temperature of $(25 \pm 2)^\circ\text{C}$. This growth regimen was maintained in an atmospheric pressure-regulated walk-in chamber. The experimental setup comprised four pressure chambers, each calibrated to simulate atmospheric pressures of 96, 110, and 120 kPa. These pressures were selected based on the gravity-induced increase in atmospheric pressure with depth, following fundamental physical principles. They correspond to the measured depths of 240, 690, and 1410 m, respectively, within the Laboratory of Jiapigou Minerals Limited Corporation of the China National Gold Group Corporation, as determined by in situ measurements (Liu et al., 2018; Wang et al., 2023; Zou et al., 2023). The chamber maintained at 96 kPa with 55% humidity served as the control, replicating surface conditions referred to as “normal” in the study area. Conversely, chambers set at 110 and 120 kPa were configured to replicate the elevated pressure and humidity characteristic of deep underground environments, achieving humidity levels close to 96%.

To exclusively assess the impact of environmental humidity on plant development, an additional setup at 96 kPa with 96% humidity was established. In this investigation, four distinct treatment groups were established, namely: the normal atmospheric pressure and normal humidity (NPNH, 96 kPa + relative humidity [RH] 55%), normal atmospheric pressure and high humidity (NPHH, 96 kPa + RH 95%), medium atmospheric pressure and high humidity (MPHH, 110 kPa + RH 95%), and high atmospheric pressure and high humidity (HPHH, 120 kPa + RH 95%) environments. The growth cycle of *N. benthamiana* was segmented into four stages based on morphological, growth, and developmental markers, with stage I–IV denoting the seedling, vegetative, flowering, and maturity stages, respectively (Table 1).

2.2 | Plant growth and water management

Posttransplantation, as shown in Figure 1, plant height and stem diameter were systematically measured at intervals of 3–5 days. For each measurement, a quarter of the plants within each treatment group's test chamber were randomly selected. Plant height was determined by measuring the vertical distance from the potting soil surface to the apex of the tallest leaves. Stem diameter was gauged approximately 0.5 cm from the plant's cotyledon. At Stage III (flowering stage), leaf area assessments were conducted, selecting the largest and second-largest leaves from each pot, designated as Leaf I and Leaf II, respectively. The dimensions of these leaves were recorded using a ruler, and the leaf area was calculated as the product of each leaf's length and width, taking into account the leaf shape. Water consumption was regularly monitored through plant weigh-ins. Throughout the experiment, all plants received sufficient irrigation to maintain optimal soil moisture levels, with both the irrigation schedule and volume meticulously documented.

Leaf relative water content (RWC) was assessed in the fifth fully expanded leaf from the apex, utilizing the methodology outlined by Bajji et al. (2001):

$$\text{RWC} = \frac{(W_f - W_d)}{(W_t - W_d)} \times 100\%, \quad (1)$$

where, W_f represents the fresh weight, W_d denotes the dry weight after desiccation at 75°C for 48 h, and W_t signifies the turgid weight following leaf rehydration over a 24-h period.

Upon harvesting, shoots were severed at the soil surface using scissors, and roots were carefully extracted

from the soil. Both shoot and root components were then dried, and their weights were recorded to determine the shoot dry weight, root dry weight, and total biomass.

2.3 | Chloroplast pigment and ROS regulatory system analysis

Fresh leaf tissues from leaves were collected for subsequent analyses of chloroplast pigments, ROS regulatory system components, and phytohormones. For each growth stage (Stage III and Stage IV), samples were harvested from five biologically independent plants at 3-day intervals across the stage duration. This longitudinal sampling design captured intrastage temporal dynamics, resulting in a total of $n = 15$ data points per stage derived from five biological replicates sampled at three time points.

To quantify chloroplast pigments in plant leaves, a spectrophotometric method was employed. Fresh leaf samples were collected and immediately submerged in liquid nitrogen to prevent pigment degradation. Approximately 0.1 g of frozen tissue was then homogenized in 80% acetone using a prechilled mortar and pestle. The homogenate was centrifuged at 4°C for 5 min at 10 000 g to separate the supernatant containing the pigments. The absorbance of the supernatant was measured at 649, 665, and 479 nm using a UV-Vis spectrophotometer to determine the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and carotenoids (Caro), respectively. The pigment concentrations were calculated based on the equations established by Lichtenthaler and Wellburn (1983).

For the analysis of the reactive oxygen species (ROS) regulatory system, detection kits for superoxide dismutase (T-SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and proline (PRO) (Nanjing Jiancheng Science Co., Ltd.) were employed to assess their internal levels within the plant.

Images of ROS staining were obtained via immunofluorescence assays on frozen sections of plant tissue, employing a suite of specialized equipment, including a cryostat (Cryotome E, Thermo), an upright fluorescence microscope (NIKON ECLIPSE C1, Nikon Japan), and an imaging system (NIKON DS-U3, Nikon Japan). The procedure utilized a range of reagents, notably PBS buffer (G0002, Servicebio), ROS stain (D7008, SIGMA), DAPI stain (G1012, Servicebio), an autofluorescence quenching agent (G1221, Servicebio), and an anti-fade mounting medium (G1401, Servicebio). Initially, cryosections from thawed leaf tissues were air-dried to minimize moisture.

TABLE 1 Key growth stages and corresponding morphological markers observed during the complete growth cycle, with date markers denoted as days after planting.

Growth stage	Morphological start point	Morphological end point
Stage I (seedling stage)	Seed germination (0 days)	9–10 true leaves fully expanded (23 days)
Stage II (vegetative stage)	10th true leaf expansion (24 days)	Flower buds mature, flowering initiates (43 days)
Stage III (flowering stage)	First flower bud opening (44 days)	Continuous seed development (72 days)
Stage IV (maturity stage)	Partial seed maturation begins (73 days)	Majority of leaves start to senesce (94 days)

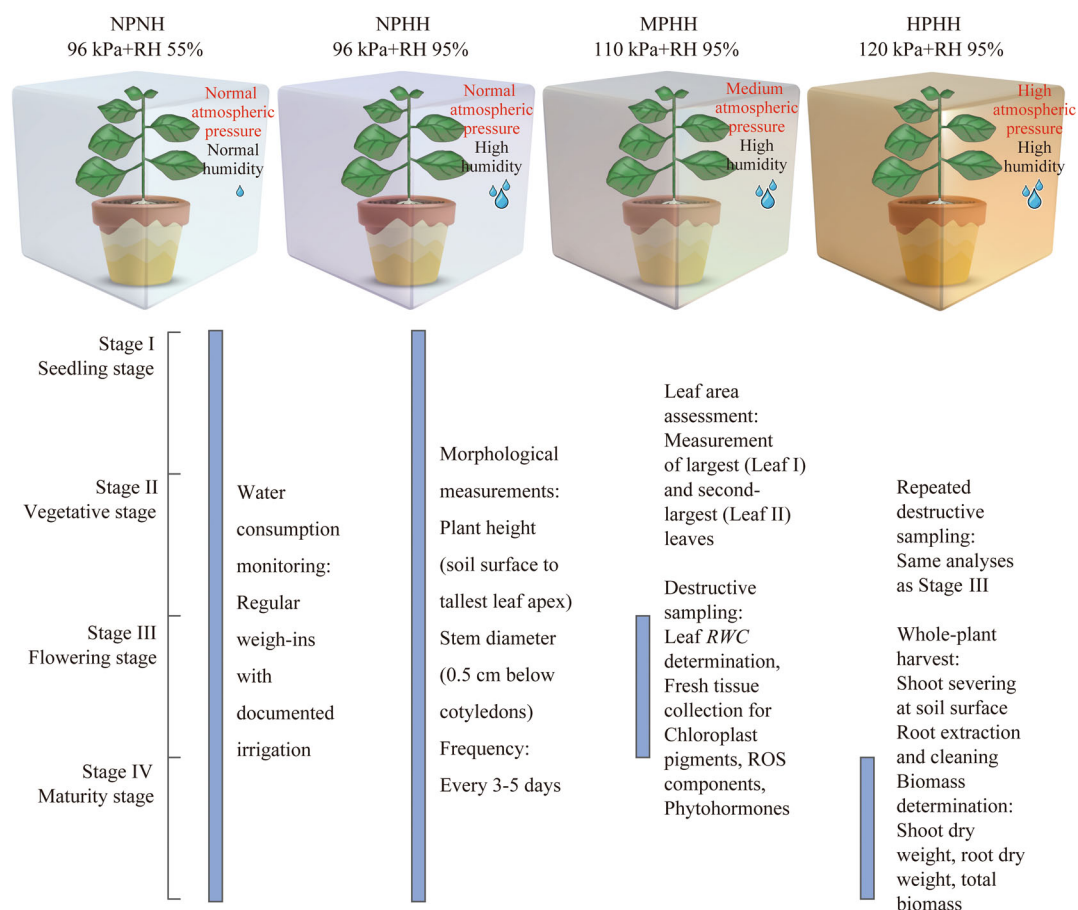


FIGURE 1 Schematic timeline of experimental procedures conducted across *Nicotiana benthamiana* growth stages under simulated subsurface conditions. HPHH, high atmospheric pressure and high humidity; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity; ROS, reactive oxygen species; RWC, relative water content.

A hydrophobic barrier was drawn around the tissue sections using a histological pen to prevent antibody runoff, followed by the application of an autofluorescence quenching agent for 5 min and a subsequent rinse under running water for 10 min. The sections were then incubated with the ROS stain in darkness at 37°C for 30 min, washed three times with PBS (pH 7.4) for 5 min each, and after drying, incubated with DAPI stain in darkness at room temperature for 10 min. This was followed by another series of washes in PBS (pH 7.4), three times for 5 min each. Finally, the sections were lightly dried and sealed with the anti-fade mounting medium before being examined and imaged under the fluorescence microscope, where ROS signals were distinctly marked in red.

2.4 | Phytohormones analysis

The quantification of various phytohormones, including indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellins (GA₃, GA₄, and GA₇), cytokinins such as zeatin (ZT), zeatin riboside (ZTr), isopentenyladenine (IP), and isopentenyl adenosine (IPA), along with salicylic acid (SA), jasmonic acid (JA), and methyl jasmonate (MeJA) was undertaken. The analyses utilized a Thermo Fisher Scientific Vanquish UPLC system coupled with a QE Orbitrap MS. UPLC conditions included a Waters HSS

T3 column (50 mm × 2.1 mm, 1.8 μm) at 40°C, a flow rate of 0.3 mL/min, and a 2 μL injection volume, employing a water (0.1% Acetic acid) and acetonitrile (0.1% acetic acid) solvent system. The gradient was maintained at 90:10 V/V water to acetonitrile for the initial 7 min, briefly altered, and then returned to initial conditions by 9 min. Mass detection was conducted on a Q Exactive hybrid Q–Orbitrap mass spectrometer with a heated ESI source, under parameters: spray voltage of −2.8 kV/3.0 kV, sheath gas pressure at 40 arb, aux gas pressure at 10 arb, capillary temperature at 320°C, and aux gas heater temperature at 350°C. HRMS data, acquired in SIM MS mode, were processed using Xcalibur 4.1 and TraceFinder™ 4.1 Clinical software (Thermo Scientific), aligning with methodologies established in prior research (Balcke et al., 2012; Glauser et al., 2016; Wang et al., 2017).

2.5 | Statistical analysis

To elucidate the multifaceted influences on *N. benthamiana* growth under varying humidity and atmospheric pressure conditions, this study employed principal component analysis (PCA). PCA served to simplify the complex dataset by reducing dimensionality and retaining essential information, thereby facilitating a clearer understanding of the underlying

patterns and relationships. The analysis encompassed 30 variables, encompassing controlled environmental conditions such as atmospheric pressure and humidity, alongside measured plant physiological parameters. These included leaf areas (Leaf I and Leaf II), root dry weight, shoot dry weight, overall biomass, leaf RWC, and plant water consumption. Additionally, phytohormones were incorporated, including IAA, ABA, GA₃, GA₄, GA₇, ZT, ZTr, IP, IPA, SA, JA, and MeJA. Chloroplast pigments such as Chl a, Chl b, total chlorophyll, and Caro, as well as components of the ROS regulatory system, including T-SOD, CAT, GSH, MDA, and PRO were also taken into evaluation. Experimental procedures were rigorously standardized, with each set of measurements replicated at least three times to ensure reliability and reproducibility of results. Data were presented as means $\pm$ standard deviations derived from three biological replicates per treatment group. Statistical analyses were performed using Statistical Analysis Software (SAS) version 9.4 (SAS Inc.). A one-way analysis of variance (ANOVA) was applied to compare differences among the four independent treatment groups: NPNH (96 kPa + 55% RH), NPHH (96 kPa + 95% RH), MPHh (110 kPa + 95% RH), and HPHH (120 kPa + 95% RH). Where ANOVA indicated significant effects ($p < 0.05$) Duncan's multiple range test was used for post hoc comparisons.

3 | RESULTS

3.1 | Plant growth and water consumption

Water consumption varies significantly during the growth process of plants. This research observed that the NPNH group exhibited the highest water usage, with groups under high humidity conditions showing an increase in water consumption correlating with elevated atmospheric pressure. This suggests that under standard atmospheric conditions, an increase in humidity alone leads to a reduction in plant water consumption. Conversely, it is only with a gradual increase in atmospheric pressure that there is an uptick in water usage, which nonetheless fails to reach the levels observed under conditions of normal pressure and humidity. As illustrated in Figure 2, the water consumption of *N. benthamiana* was systematically recorded for each treatment group, and the cumulative water consumption curve over the growth stage was plotted. According to growth observation records, this curve can be divided into four distinct growth stages. The NPNH group demonstrated substantially higher water consumption compared to the other groups, due to its lower environmental humidity. The cumulative water consumption of *N. benthamiana* under different treatments ranked in descending order as follows: NPNH > HPHH > MPHh > NPHH. In various humidity environments, particularly during the transition from the vegetative stage (stage II) to the flowering stage (stage III), the disparity in water consumption between the NPNH group and others gradually increased. Moreover, the water consumption differences among the other three groups were minimal. Notably, the water consumption of *N. benthamiana* progressively increased

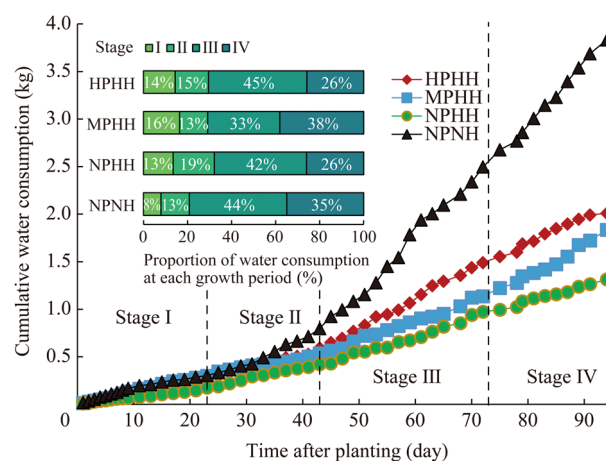


FIGURE 2 Cumulative water consumption and water consumption proportions during distinct growth periods of *Nicotiana benthamiana* in various treatment groups. The figure illustrates the curve of cumulative water consumption for *N. benthamiana*, segmented into four primary growth stages: I—Seedling, II—Vegetative, III—Flowering, and IV—Maturity. Treatments include normal pressure normal humidity (NPNH, 96 kPa + RH 55%), normal pressure high humidity (NPHH, 96 kPa + RH 95%), medium pressure high humidity (MPHh, 110 kPa + RH 95%), and high pressure high humidity (HPHH, 120 kPa + RH 95%).

with an increase in atmospheric pressure, a trend more evident under higher humidity conditions, where increased atmospheric pressure led to heightened water consumption.

The water consumption patterns of *N. benthamiana* during various growth stages are significantly influenced by changes in atmospheric pressure and environmental humidity. Initially, it is evident that the seedling stage exhibits a notable increase in water consumption proportion with rising environmental humidity. Specifically, at a medium atmospheric pressure of 110 kPa, the water consumption at this stage escalates to 16%, doubling the amount observed under NPNH conditions. However, an increase in atmospheric pressure to 120 kPa results in a reduction of water consumption during the seedling stage, aligning with the levels seen in NPHH scenarios. During the vegetative stage, a similar trend is observed where increased environmental humidity leads to heightened water consumption. Yet, the introduction of medium and high atmospheric pressures causes a decline in this proportion, bringing it close to the levels of NPNH. The flowering stage, in contrast, shows a relatively stable water consumption proportion despite increased humidity. Application of medium atmospheric pressure in this stage significantly decreases water consumption by approximately 10%, while higher pressures restore it to NPNH levels. At the maturity stage of *N. benthamiana*, an overall decrease in water consumption proportion is noted with rising humidity. Under medium atmospheric pressure, there is a significant increase in water consumption, equating to the levels of NPNH. Conversely, higher atmospheric pressures lead to a reduction in water consumption, aligning with a high-humidity environment. At the end of the planting experiment, the water consumption of the HPHH group was 52.94% higher than that of the NPHH group.

Notably, although the cumulative water consumption decreased across all high-humidity groups, the data show that the NPHH, MPH, and HPHH groups consumed 65.72%, 52.12%, and 47.59% less water, respectively, compared to the NPNH group. This indicates a trend of gradual recovery in water consumption rates under higher atmospheric pressures.

The plant height and stem diameter of *N. benthamiana* were regularly measured (Figure 3a,b), and plant phenotypes were documented photographically during stages II and III (Figure 3a). The results indicated that plants in the HPHH group were significantly taller than those in other groups, with heights at the end of the planting experiment being 16.13%, 6.71%, and 5.66% higher than those in the NPNH, NPHH, and MPH groups, respectively (Figure 3a). The NPNH group consistently displayed the shortest plant height, while differences between NPHH and MPH were minimal. For stem diameter, NPNH had the smallest values, followed by HPHH, whereas NPHH and MPH exhibited the highest values with no significant difference between them (Figure 3b). Notably, under HPHH correlated with reduced stem diameter compared to other high-humidity groups (NPHH, MPH), a phenomenon that became pronounced in Stage III but was less evident in earlier stages. This morphological response may reflect specific physiological adjustments to combined high-pressure

and high-humidity conditions. Collectively, these data indicated that high atmospheric pressure promoted plant height growth but was associated with altered stem development in *N. benthamiana*.

Stage II is the key period for *N. benthamiana* as it marks the initial stage of differential growth in aspects such as water consumption, plant height, stem diameter, and phenotypes. During this stage, leaf *RWC* significantly increased under NPHH compared to NPNH (Figure 4b). In the MPH condition, *RWC* also showed a significant rise, nearly matching that under NPHH. However, under HPHH, *RWC* decreased to levels not significantly different from those in NPNH. These findings suggest that while high atmospheric pressure affects the leaf *RWC* of *N. benthamiana*, it neutralizes the beneficial impact of high humidity, maintaining the *RWC* within a stable range.

In Stage II, measurements of the largest (Leaf I) and second largest leaves (Leaf II) of *N. benthamiana* were taken to assess leaf area. A notable reduction in leaf area was observed under high humidity conditions (Figure 4a), aligning with the photographic observations depicted in Figure 3c. However, this adverse effect of high humidity on leaf area was mitigated under high atmospheric pressure, leading to no significant difference in leaf area compared to the NPNH condition. This pattern mirrors the observed impact of these

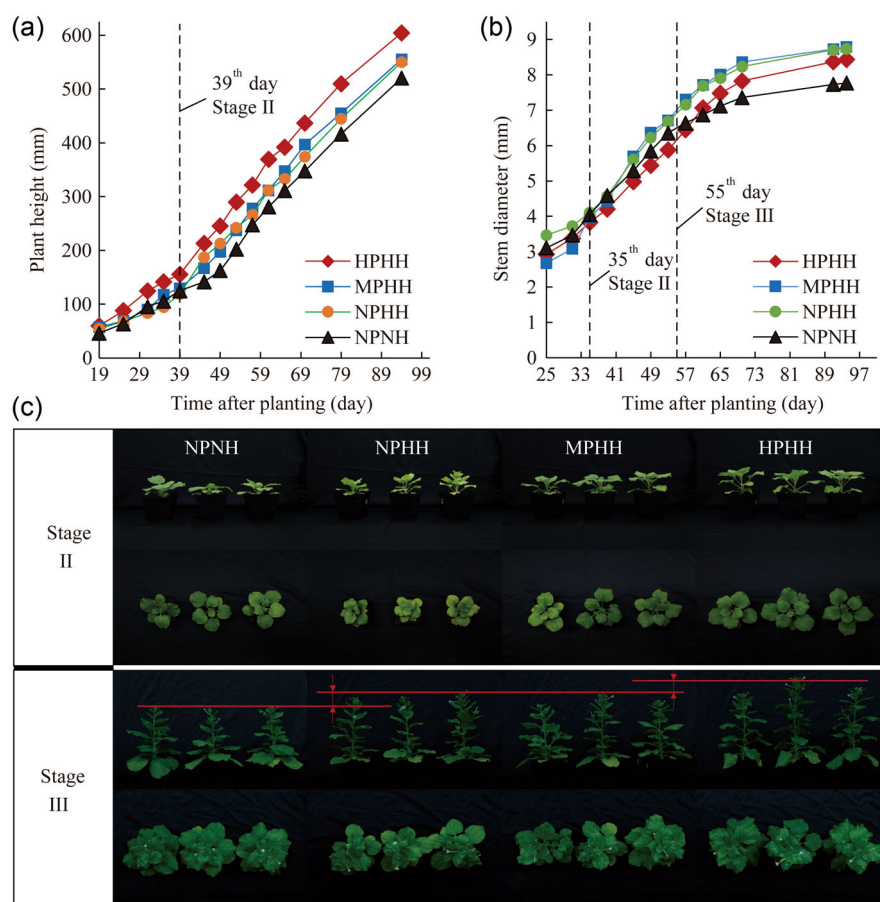


FIGURE 3 The growth of *Nicotiana benthamiana*. (a) Plant height, (b) stem diameter, and (c) phenotypes of *N. benthamiana* from each treatment group. Photographs from front and vertical views were taken to compare morphological differences in the plants. HPHH, high atmospheric pressure and high humidity; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity.

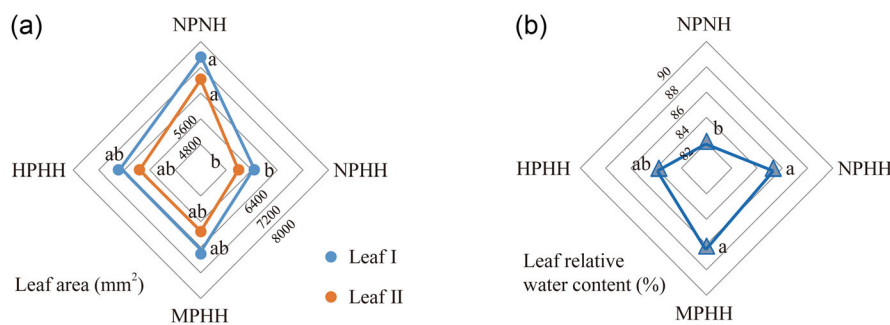


FIGURE 4 Impact of different atmospheric pressure and humidity levels on *Nicotiana benthamiana* leaves. (a) Leaf area comparison, measured during the vegetative period, calculated for the largest (Leaf I) and second largest leaves (Leaf II) in each pot from each treatment group, determined by multiplying leaf length by leaf width. (b) Presents the leaf relative water content of *N. benthamiana* from each treatment group, assessed during the vegetative period; this metric is an established indicator of plant water status, reflecting cellular hydration levels. Different letters indicate significant differences at $p < 0.05$ by Duncan's test. HPHH, high atmospheric pressure and high humidity; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity.

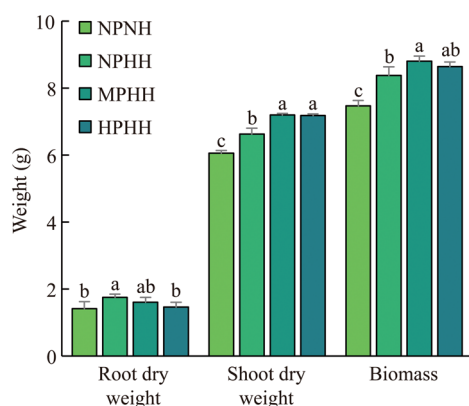


FIGURE 5 Variations in root dry weight, shoot dry weight, and biomass in response to different atmospheric pressures and humidity levels. Error bars indicate the standard deviation of the measurements. Statistical significance is determined by Duncan's test ($p < 0.05$). Bars with different letters (a–c) are significantly different at $p < 0.05$. HPHH, high atmospheric pressure and high humidity; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity.

stressors on leaf *RWC*. In contrast, as depicted in Figure 4b, high humidity generally increased the leaf *RWC*. However, the *RWC* across the four treatment groups ranked as MPH > NPHH > HPHH > NPNH. This suggests that high atmospheric pressure, especially when increased to 120 kPa, counteracts the enhanced water absorption in leaves due to high humidity.

The impact of high atmospheric pressure on the dry weight of roots and shoots in *N. benthamiana* was contrasting, as shown in Figure 5. High atmospheric pressure significantly reduced root dry weight, particularly at 120 kPa, while increasing shoot dry weight. Consequently, the shoot-to-root ratio rose substantially. Although total biomass increased in line with shoot dry weight, this effect diminished at 120 kPa.

3.2 | Enzymatic antioxidants and photosynthetic pigment content

To assess the oxidative damage associated with variations in atmospheric pressure and humidity, several

parameters were evaluated, including T-SOD activity, CAT activity, MDA content, GSH content, PRO content, Caro content, Chl a content, Chl b content, and total Chlorophyll content, as shown in Figure 6. During the flowering stage, the activities of the enzymatic antioxidants T-SOD and CAT significantly increased under elevated atmospheric pressure, as indicated in Figure 6a,b. Specifically, T-SOD activity increased by 42% and 67% at atmospheric pressures of 110 and 120 kPa, respectively, compared to NPNH, and by 24% and 46% compared to high humidity in NPHH. CAT activity similarly rose by 72% and 69% at atmospheric pressures of 110 and 120 kPa, respectively, against NPNH, and by 13% and 11% compared to NPHH. The levels of MDA and the nonenzymatic antioxidant GSH showed no significant differences among these treatment groups, as depicted in Figure 6c,d. PRO, a crucial osmoprotectant that aids plants in coping with oxidative damage from abiotic stress, exhibited a significant decrease of 35% at 110 kPa atmospheric pressure relative to NPNH, as displayed in Figure 6e. However, when the atmospheric pressure was further raised to 120 kPa, PRO content returned to NPNH levels, even showing a 6% increase. Similarly, total chlorophyll content was considerably affected by high atmospheric pressure, with a substantial reduction of 31% at 110 kPa compared to NPNH. As shown in Figure 6f–i, high humidity consistently reduced chlorophyll and carotenoid contents. This suppression was partially reversed by elevated atmospheric pressure in a threshold-dependent manner: at 110 kPa, Caro levels remained statistically indistinguishable from high-humidity controls, whereas at 120 kPa, Caro fully recovered to NPNH levels, and chlorophyll approached but did not fully reach NPNH levels.

As shown in Figure 7, the accumulation of ROS in *N. benthamiana* plants under various pressure and humidity conditions was observed. ROS staining images were obtained from cryosectioned immunofluorescence experiments. In these images, ROS-positive cells are marked in red, while cell nuclei are indicated in blue. A comparison between groups reveals that high humidity conditions alone significantly increase the concentration of ROS within the plant, as evidenced by a denser

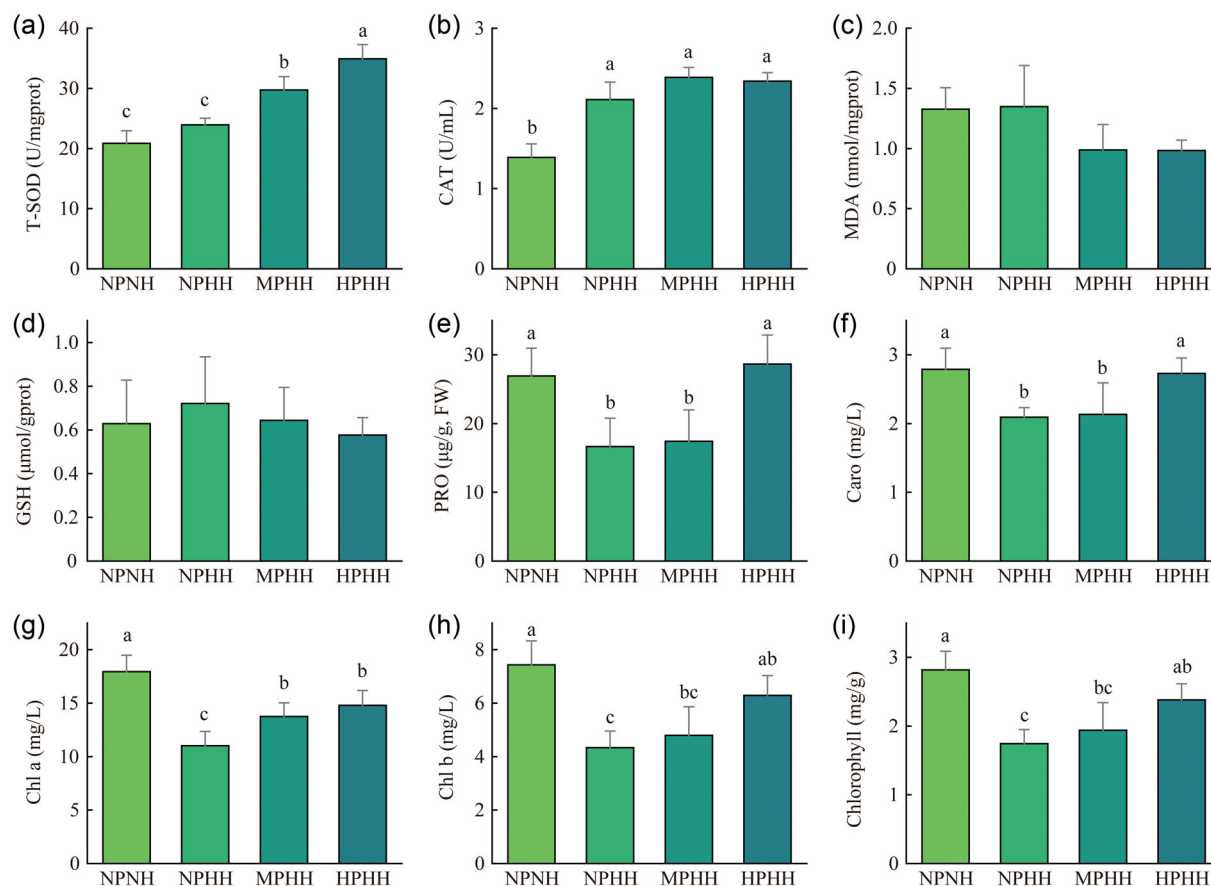


FIGURE 6 Measured biochemical indexes in *Nicotiana benthamiana* plants. (a) T-SOD activity, (b) CAT activity, (c) MDA content, (d) GSH content, (e) proline content, (f) carotenoid content, (g) Chl a content, (h) Chl b content, and (i) Chlorophyll content during period III. Letters above the error bars denote the statistical significance determined by Duncan's test ($p < 0.05$). CAT, catalase; Chl, chlorophyll; GSH, glutathione; HPHH, high atmospheric pressure and high humidity; MDA, malondialdehyde; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity; PRO, proline; T-SOD, detection kits for superoxide dismutase.

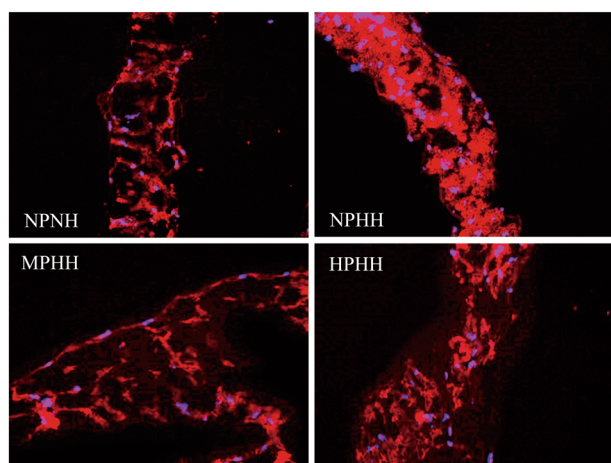


FIGURE 7 Immunofluorescence staining for ROS of *Nicotiana benthamiana* from each treatment group during stage III. Microscopic observation and image collection were performed by fluorescence microscopy. Nucleus is blue by labeling, and ROS-positive cells labelled by fluorescein are red. HPHH, high atmospheric pressure and high humidity; MPH, medium atmospheric pressure and high humidity; NPHH, normal atmospheric pressure and high humidity; NPNH, normal atmospheric pressure and normal humidity; ROS, reactive oxygen species.

distribution of red spots. Upon increasing the atmospheric pressure, there is a noticeable reduction in the concentration of ROS in the plants, indicated by a decrease in the density of red spots.

3.3 | Phytohormone content

Table 2 displayed changes in key phytohormone concentrations in *N. benthamiana* under varying atmospheric pressures. At the flowering stage (III), high humidity suppressed ABA, JA, and GA₄ levels, but elevated pressure reversed this suppression, with recovery intensifying at higher pressures. Conversely, humidity-induced surges in cytokinins (particularly ZTr and IPA) were attenuated by increased pressure, contrasting sharply with the ABA/JA/GA₄ response. At maturation (IV), humidity inhibited ABA secretion, yet medium (110 kPa) and high pressure (120 kPa) consistently weakened this effect. Notably, humidity-enhanced SA and IP concentrations declined under high pressure, with IP dropping below NPNH levels. GA₇ and IPA showed significant increases only under HPHH conditions.

3.4 | Correlation among measured plant physiological and biochemical indicators

Correlation analysis of physiological and biochemical parameters across the growth of *N. benthamiana* revealed interconnected responses to atmospheric pressure and humidity (Figure 8). (1) Humidity showed a significant positive correlation with water retention indicators (leaf relative water content) and antioxidant capacity (T-SOD), but a strong negative correlation with

photosynthetic efficiency markers (chlorophyll, Caro, leaf area) and growth-restricting hormones (ABA, IAA, GA₃); (2) Atmospheric pressure exhibited robust positive correlations with antioxidative defense (T-SOD, PRO) and stress-signaling molecules (MeJA), alongside biomass accumulation. Conversely, it correlated negatively with oxidative damage markers (MDA) and reduced glutathione (GSH). (3) Internal parameter linkages highlighted resource allocation trade-offs. Water consumption positively correlated with photosynthetic

TABLE 2 Contents of various phytohormones in the leaf of *Nicotiana benthamiana* from each treatment during period III and IV.

Stage	Treatment	IAA	ABA	GA ₃	GA ₄	GA ₇	ZT	ZTr	IP	IPA	SA	JA	MeJA
III	NPNH	0.8312	4.5530 ^a	0.4416 ^a	0.0824 ^a	0.0539 ^a	0.0160	0.7542 ^c	0.0876	0.3169 ^d	10.1702	0.2892 ^{a,b}	0.4779 ^{a,b}
	NPHH	0.9786	1.5175 ^c	0.1956 ^b	0.0454 ^b	0.0581 ^a	0.0206	1.3924 ^a	0.0915	0.9394 ^a	10.8481	0.1688 ^b	0.4197 ^{a,b}
	MPHH	1.1152	2.1306 ^{b,c}	0.1629 ^b	0.0572 ^{a,b}	0.0473 ^{a,b}	0.0213	1.0799 ^b	0.0699	0.6582 ^b	11.5722	0.3032 ^{a,b}	0.3784 ^b
	HPHH	0.9706	2.7894 ^b	0.2031 ^b	0.0741 ^a	0.0346 ^b	0.0124	0.6442 ^c	0.0617	0.4359 ^c	9.0229	0.4674 ^a	0.5936 ^a
IV	NPNH	1.1104 ^a	11.5350 ^a	–	0.0613	0.0305 ^b	–	0.1811 ^{a,b}	0.3049 ^b	0.2811 ^b	14.3125 ^b	0.4389	–
	NPHH	0.6687 ^b	2.3817 ^c	–	0.0904	0.0261 ^b	–	0.2623 ^a	0.4924 ^a	0.5007 ^b	32.6210 ^a	0.4767	–
	MPHH	0.6445 ^b	8.0571 ^b	–	0.1057	0.0261 ^b	–	0.2964 ^a	0.1763 ^c	0.5134 ^b	18.7996 ^b	0.2789	–
	HPHH	0.4907 ^b	7.1782 ^b	–	0.1021	0.0480 ^a	–	0.1256 ^b	0.1422 ^c	1.0345 ^a	15.6879 ^b	0.4362	–

Note: The measured plant hormones include indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellins (GA₃, GA₄, and GA₇), different cytokinins (including zeatin [ZT], zeatin riboside [ZTr], isopentenyladenine [IP], and isopentenyl adenosine [IPA]), salicylic acid (SA), jasmonic acid (JA), and methyl jasmonate (MeJA). Different letters within a column represent significant differences at $p < .05$ by Duncan's test between treatments. Unit: ng/g, FW.

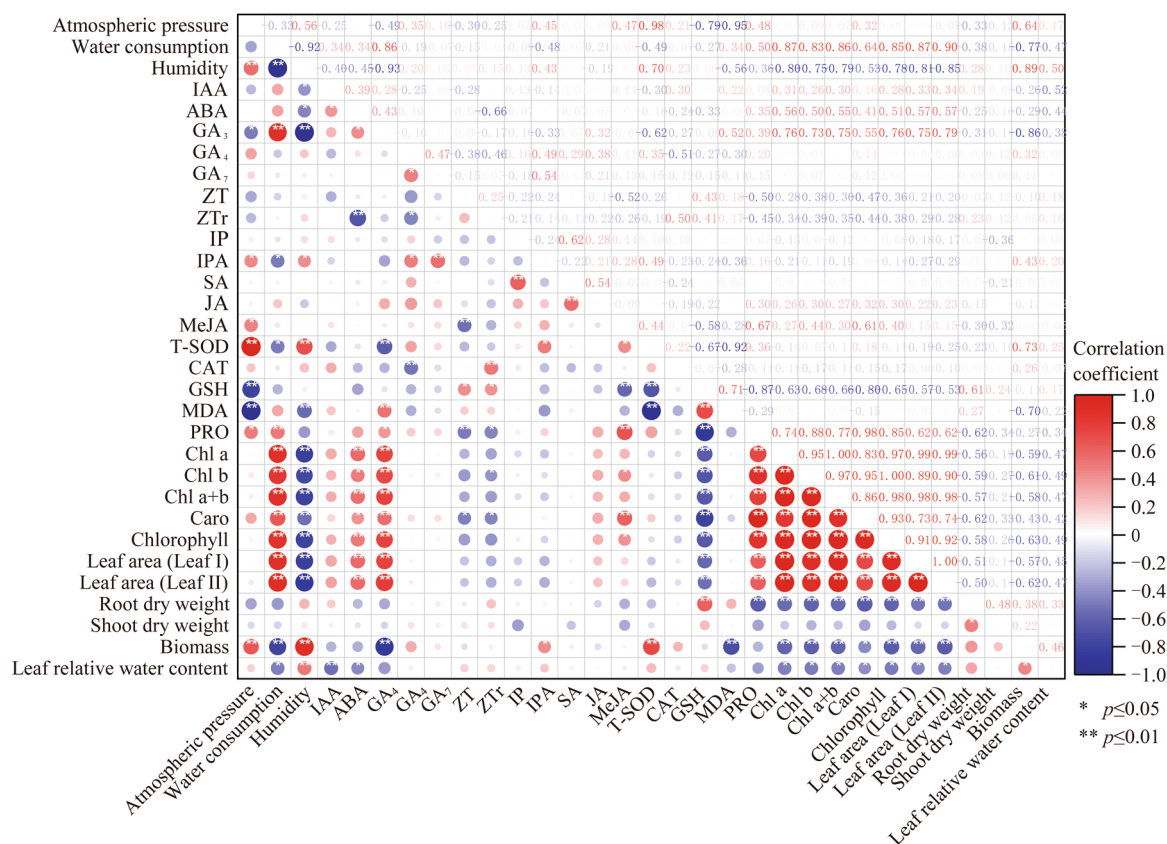


FIGURE 8 Correlation matrix of the physiological and biochemical factors of *Nicotiana benthamiana*. The upper triangular part of the graph is the correlation coefficient matrix, the lower triangular part is the graphical indication, with the diameter of the dots denoting the values of the correlation coefficients; positive and negative correlations are indicated by colors, and significance levels are indicated by asterisks. ABA, abscisic acid; CAT, catalase; Chl, chlorophyll; GA, gibberellins; GSH, glutathione; IAA, indole-3-acetic acid; IP, isopentenyladenine; IPA, isopentenyl adenosine; JA, jasmonic acid; MDA, malondialdehyde; MeJA, methyl jasmonate; PRO, proline; SA, salicylic acid; T-SOD, detection kits for superoxide dismutase; ZT, zeatin; ZTr, zeatin riboside.

investment (chlorophyll, leaf area) but negatively with humidity-adapted biomass. GSH served as a hub, showing positive correlations with root growth (root dry weight) and oxidative stress (MDA, ZT/ZTr), but negative links to leaf antioxidants (T-SOD, PRO) and photosynthesis. These data indicated that pressure and humidity modulated plant physiology through integrated shifts in water management, antioxidant responses, and carbon allocation.

3.5 | Effects of atmospheric pressure and humidity on plant physiological data clustering

In the simulation of deep underground environment, the growth and regulation of plants display complex phenomena, where numerous physiological and biochemical parameters intricately respond to the feedback mechanisms of artificially adjusted atmospheric pressure and humidity. The structural form of a series of parameters measured in this study collectively constitutes the final growth pattern of plants. These parameters are inter-related and may exhibit compensatory and reciprocal feedback characteristics. For example, an increase in humidity leads to a decrease in water consumption, but plants adjust to this through a series of regulations under high-pressure conditions, mitigating some of the impacts of increased humidity, while simultaneously introducing other physiological and biochemical changes.

As shown in Figure 9, each treatment group was closely clustered, enveloped by 95% confidence ellipses for the four clusters, indicating that the influences caused

by increases in atmospheric pressure and humidity were statistically significant ($p < 0.05$) for clustering the data. The eigenvalues for principal component numbers 1 and 2 (PC1 and PC2) were 11.65 and 6.20, respectively, accounting for 40.2% and 17.4% of the variance, respectively, with a cumulative variance of 57.59%. The highest loadings in PC1 were related to factors like plant chlorophyll, PRO, Caro, leaf area, and dry matter accumulation in plants, ranging from 0.17 to 0.29 (absolute value). PC2's composition was more complex, including adjustments in T-SOD, IPA, GA₄, GA₇, MDA, MeJA, GSH, ZT, etc. Therefore, we revealed that the loading scores for PC1 were mainly related to plant growth, while PC2 was primarily influenced by high atmospheric pressure and physiological responses in plants.

It was evident that the effect of humidity differentiated the treatment groups along the PC1 axis, from left to right, linking factors such as plant chlorophyll, PRO, Caro, GA₃, leaf area, and dry matter accumulation in plants to this clustering phenomenon. Regarding PC2, the MDA content and activities of GSH and ZT were negatively correlated with atmospheric pressure, as were the activity of T-SOD and the contents of IPA, GA₄, GA₇, MeJA. This indicates the influence of high atmospheric pressure on physiological and biochemical regulatory processes. As humidity increased, the cluster shifted to the left, leading to unfavorable effects such as the closure of leaf stomata, reduced efficiency in photosynthesis, and limited growth. Conversely, with the increase in atmospheric pressure, the cluster moved in an upward and right direction, which could be interpreted as a physiological manifestation of the mitigating process.

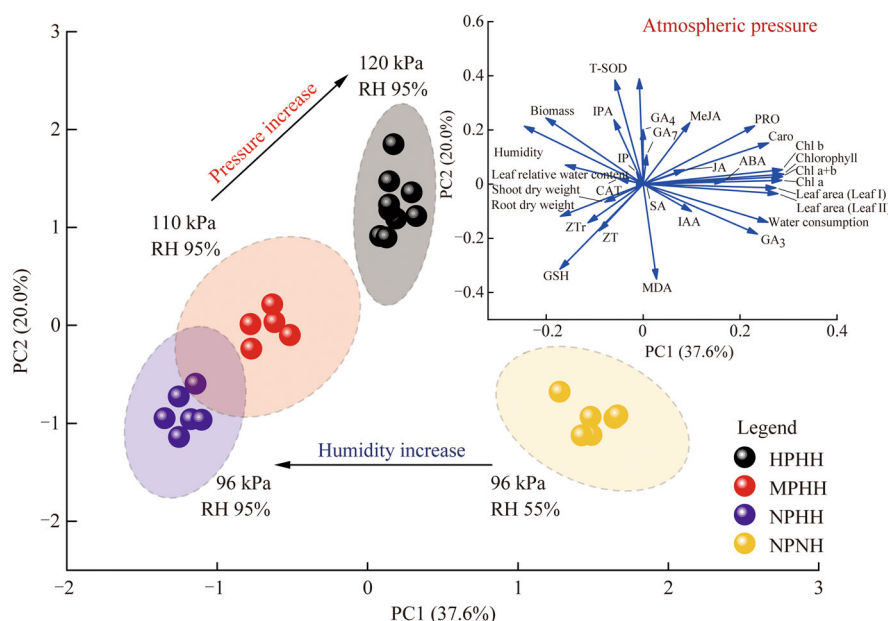


FIGURE 9 Biplot of principal component analysis (PCA) for the factors of the physiology and biochemistry of *Nicotiana benthamiana*, showing the effects of atmospheric pressure and humidity on plant growth and regulation. Blue arrows denote the loadings, which are the linear combination coefficients of the factors that constitute the PCs. Black arrows indicate the shifting process of plant physiology and biochemistry induced by atmospheric pressure and humidity variation. Samples from each plant tissues are clustered and enveloped in their respective 95% confidence ellipse. ABA, abscisic acid; CAT, catalase; Chl, chlorophyll; GA, gibberellins; GSH, glutathione; HPHH, high atmospheric pressure and high humidity; IAA, indole-3-acetic acid; IP, isopentenyladenine; IPA, isopentenyl adenosine; JA, jasmonic acid; MDA, malondialdehyde; MeJA, methyl jasmonate; MPH, medium atmospheric pressure and high humidity; NPH, normal atmospheric pressure and high humidity; PC, principal component; NPNH, normal atmospheric pressure and normal humidity; PRO, proline; SA, salicylic acid; T-SOD, detection kits for superoxide dismutase; ZT, zeatin; ZTr, zeatin riboside.

These findings will be discussed in further detail in the following sections.

4 | DISCUSSION

4.1 | Combined effects of high humidity and atmospheric pressure in deep underground environments

Research findings in deep underground environments indicated that high humidity had varying degrees of negative impacts on both instrumentation and personnel (Liu et al., 2018), which were difficult to eliminate at present. Similarly, for plants, a high humidity level of 95% RH imposed multifaceted constraints on the physiology of *N. benthamiana*. Increased leaf surface moisture under high humidity impeded light penetration to lower plant strata, reducing photosynthetic efficiency by disrupting chlorophyll-mediated light absorption (Alberte et al., 1975; Pettersen et al., 2007). This was compounded by vapor-induced light scattering in enclosed spaces and weakened leaf barriers that facilitated pathogen proliferation (Roberts and Paul, 2006). The suppression of transpiration under high humidity diminished stomatal conductance, limiting CO₂ uptake and nutrient assimilation (McDonald et al., 2002; Merilo et al., 2013), while concurrently reducing levels of growth-promoting hormones (IAA, GA) and elevating oxidative stress through ROS accumulation. Although plants activated stress-responsive genes and antioxidative defenses (e.g., T-SOD and CAT) to counteract ROS (Menezes-Benavente et al., 2004), the overall physiological trade-off, such as reduced leaf expansion, impaired photosynthetic pigment synthesis, and restricted biomass accumulation, highlighted high humidity as a dominant stressor in underground environments.

Comprehensive analysis, as depicted in Figure 10, suggests that high humidity negatively impacted *N. benthamiana* by suppressing water consumption, inhibiting photosynthetic pigment synthesis, and reducing leaf expansion, coupled with decreased levels of growth-related hormones (e.g., IAA, GA) and elevated oxidative stress due to ROS accumulation, ultimately restricting nutrient assimilation and biomass accumulation. These findings demonstrated that deep underground environments impose severe limitations on plant growth. However, in contrast, elevated atmospheric pressure may mitigate humidity stress, potentially through mechanisms such as enhanced turgor pressure supporting water balance and transpiration, alongside the observed boost in antioxidative enzymes (T-SOD, CAT), which could contribute to reduced oxidative damage. It also restored photosynthetic pigments, increased biomass, and rebalanced stress-related (ABA/JA) and growth-promoting hormones, suggesting a compensatory role against humidity-induced physiological constraints (Jiang et al., 2024).

4.2 | High atmospheric pressure as a compensatory modulator

Elevated atmospheric pressure (120 kPa) counteracted high humidity-induced stress through distinct compensatory

mechanisms. By enhancing cellular turgor pressure, high pressure stabilized internal water relations, promoting water uptake and transpiration rates despite humidity-driven stomatal closure. This was evidenced by a 52.94% increase in water consumption under high pressure compared to NPHH conditions. Concurrently, high pressure upregulated antioxidative defenses, with T-SOD and CAT activities increasing by 67% and 69%, respectively, relative to NPNH (Figure 6a,b), effectively scavenging ROS and mitigating oxidative damage (Figure 7). Notably, PRO levels, which were initially suppressed by high humidity, recovered to NPNH-equivalent values under 120 kPa. This recovery reflected PRO's critical role in osmoregulation and membrane stabilization during pressure-mediated stress alleviation (Gholami Zali & Ehsanzadeh, 2018; Iqbal et al., 2015). The restoration of photosynthetic pigment content (Chl a, Chl b, and Caro) and aerial biomass production further underscored pressure-mediated metabolic recovery. This recovery coincided with improved nutrient mobilization, which may have resulted from pressure-induced alterations in membrane thermodynamics that enhanced intracellular transport. A similar phenomenon was observed in both plants and microorganisms such as *Lactobacillus plantarum* (Ulmer et al., 2002).

Physiological rebalancing of hormones played a critical role, as evidenced by the recovery of ABA and JA levels. ABA and JA levels rebounded to NPNH-equivalent values under high pressure (Table 2), reinstating stress signaling while GA₄ recovery supported growth regulation. The transient upregulation of cytokinins (e.g., ZTr, IPA) under high humidity, which resembled responses observed during metal or drought stress (Reguera et al., 2013; Ren et al., 2016; Wu et al., 2014), was reversed by pressure elevation. This reversal suggested restored cellular homeostasis. These adaptive responses collectively shifted resource allocation toward shoot development at the expense of root biomass (Figure 5), reflecting a prioritized survival strategy under compounded environmental stresses. PCA analysis (Figure 9) further corroborated this compensatory interplay, showing that pressure-triggered factors (T-SOD, GA₄, MeJA) clustered along PC2 counterbalanced humidity-driven declines in chlorophyll and leaf area (Al-Zahrani et al., 2022; Azarin et al., 2022; Rajput et al., 2021). However, the antioxidative response exhibited nuanced complexity, as T-SOD activity surged while GSH levels declined marginally. This pattern mirrored drought-stress adaptations, where SOD and APX activities scale with stress severity while CAT and GPX diminish (Sharma & Dubey, 2005). This redox equilibrium shift highlights the trade-offs inherent in pressure-mediated stress compensation, where prioritized ROS scavenging pathways may transiently override others to maintain cellular integrity under multifactorial stress (Zhang et al., 2016).

4.3 | Synergistic or antagonistic interactions?

Previous analyses indicated that the interplay between high humidity and atmospheric pressure revealed antagonistic physiological outcomes. PCA analysis (Figure 9) more

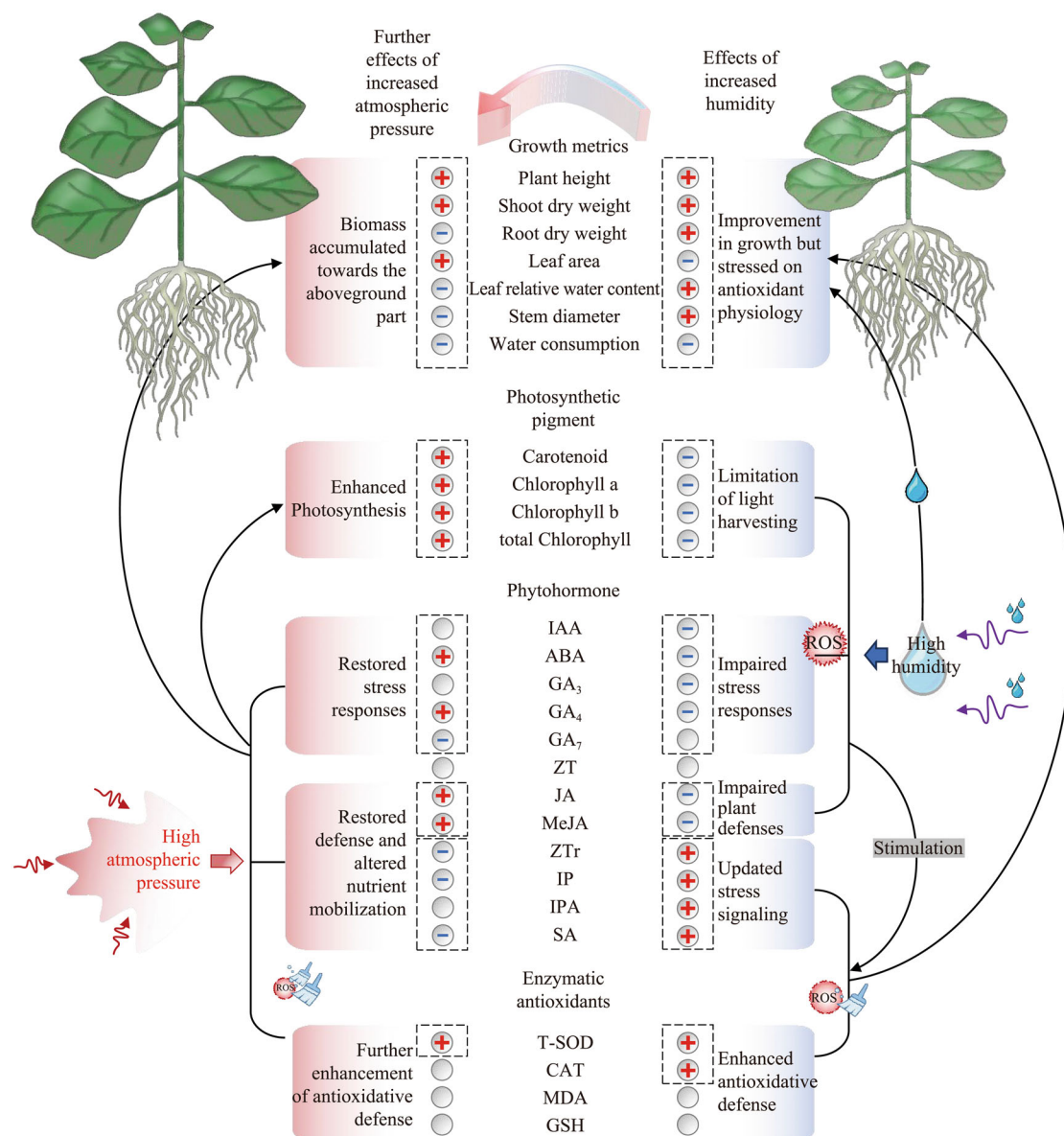


FIGURE 10 Physiological changes and potential mechanisms in *Nicotiana benthamiana* following treatments with high humidity and high atmospheric pressure in simulated deep underground environments. The right side of the figure delineates physiological responses under elevated humidity (relative to normal humidity and pressure conditions), while the left side depicts subsequent adaptations to elevated atmospheric pressure within the context of high humidity (contrasted against high humidity at normal pressure). Arrows indicate processes of influence, with positive red signs denoting an increase or up-regulation, and negative blue signs denoting a decrease or down-regulation. ABA, abscisic acid; CAT, catalase; Chl, chlorophyll; GA, gibberellins; GSH, glutathione; IAA, indole-3-acetic acid; IP, isopentenyladenine; IPA, isopentenyl adenosine; JA, jasmonic acid; MDA, malondialdehyde; MeJA, methyl jasmonate; PRO, proline; SA, salicylic acid; T-SOD, detection kits for superoxide dismutase; ZT, zeatin; ZTr, zeatin riboside.

clearly illustrated the impacts of these two key environmental factors on plant physiology. It demonstrated that humidity-dominated effects (leftward PC1 shift), which were associated with chlorophyll loss, reduced leaf area, and osmotic imbalance, were partially offset by pressure-induced adaptations (upward PC2 shift). These adaptations included enhanced antioxidative capacity (T-SOD) and hormone-mediated stress resilience (ABA, JA). This antagonism was particularly evident in stomatal regulation: while high humidity suppressed transpiration through ABA-mediated stomatal closure (Assmann et al., 2000), elevated pressure forced stomatal aperture enlargement, a response that inversely mirrors low-pressure adaptations where stomatal density increases and apertures narrow (Arce et al., 2021; Kammer et al., 2015). Notably, while

high pressure restored water consumption and photosynthetic parameters to near-NPNH levels, it introduced secondary effects such as stem diameter reduction and cytokinin downregulation (Table 2), suggesting incomplete compensation. The transient cytokinin surge under high humidity, analogous to metal/salt stress responses, was reversed by pressure elevation, indicating restored cellular homeostasis at the cost of growth-regulatory flexibility (Reguera et al., 2013; Zhang et al., 2016).

The threshold-dependent nature of these interactions, exemplified by proline content normalization at 120 kPa but not at 110 kPa, emphasizes the nonlinearity of pressure-mediated stress alleviation. This nonlinearity extended to redox equilibrium: although T-SOD activity scaled positively with pressure (Zhang et al., 2020), GSH

levels declined marginally, a pattern divergent from heavy metal stress where both SOD and GSH increase, highlighting the uniqueness of pressure-humidity antagonism. This antagonism aligns with observations in other systems where combined stressors trigger distinct redox and hormonal cross-talk (Rizhsky et al., 2002), underscoring the need to define pressure-humidity equilibrium points for optimal underground cultivation. Notably, PCA's PC2 dimension (Figure 9), encompassing T-SOD, GA₄, and MeJA, captured this compensatory hierarchy, where pressure-triggered antioxidative and hormonal adjustments (Al-Zahrani et al., 2022; Rajput et al., 2021) counterbalanced humidity-driven photosynthetic impairment.

The complexity of these interactions was further manifested in resource allocation trade-offs. Elevated CO₂ partial pressure under high pressure enhanced nocturnal respiration and biomass production but concurrently exacerbated ROS generation (Nanda et al., 2010). This necessitated energy diversion toward antioxidant synthesis at the expense of root development. This balance between photosynthetic gain and oxidative cost underscores the context-dependency of pressure-mediated compensation, a phenomenon mirrored in drought-stress gradients where mild stress enhances GPX activity but severe stress depletes it (Sharma & Dubey, 2005). Collectively, the high humidity-pressure interplay is predominantly antagonistic rather than synergistic, with pressure partially counteracting humidity-induced stress through compensatory mechanisms that operate within threshold-dependent physiological trade-offs.

4.4 | Integration of water and hormonal signaling underpins dynamic adaptation

N. benthamiana adapted to high humidity and atmospheric pressure through physiological responses. These responses manifested as dynamic shifts in hormone concentrations and water use patterns, prioritizing osmotic stability early and photosynthetic efficiency later, mediated by water relations-hormonal cross-talk. During seedling establishment (Stage I), the abrupt surge in water consumption under medium pressure (110 kPa + RH 95%), doubling NPNH levels, reflects an initial reliance on turgor-driven cell expansion, facilitated by transient cytokinin upregulation (Table 2) to exploit moisture abundance (Reguera et al., 2013). However, as pressure escalates to 120 kPa, PRO-mediated osmotic stabilization (Figure 6e) and stomatal aperture enlargement override this strategy, redirecting resources toward antioxidative defense (T-SOD/CAT surge, Figure 6a,b) to counter humidity-induced ROS (Figure 7) (Hasanuzzaman et al., 2020; Rajput et al., 2021). Transcriptomic studies in plants exposed to abiotic stresses, including hypoxia (which can share signaling components with pressure responses) or osmotic stress, often reveal the upregulation of genes encoding antioxidant enzymes like SODs and CATs, alongside key transcription factors (e.g., members of the WRKY, NAC, or MYB families) that regulate the antioxidant defense network (Chai et al., 2025; Ma & Hu, 2024; Yu et al., 2024). For instance, hypoxia-

responsive ERF-VII transcription factors (like RAP2.12 in *Arabidopsis*) are known to activate genes involved in ROS detoxification (Seok et al., 2022). It is plausible that elevated atmospheric pressure activates similar or distinct signaling cascades, potentially involving calcium signaling, MAP kinase pathways, or ROS themselves as secondary messengers, leading to the transcriptional activation of SOD and CAT genes. Future studies employing transcriptomics or proteomics under controlled high-pressure conditions are crucial to identify the specific genetic regulators and signaling nodes responsible for this enhanced antioxidative capacity.

By the flowering stage (Stage III), hormonal dynamics shifted decisively. ABA and JA rebounded under high pressure (Table 2) (Okamoto et al., 2009), suppressing cytokinin-driven repair to prioritize defense. Concurrently, GA₄ recovery occurred, distinct from the persistent inhibition of GA₃, and drove significant vertical growth (Figure 3a). This stem elongation, however, occurred at the cost of radial stem growth (Figure 3b) and, crucially, root biomass accumulation (Figure 5). This represents a clear physiological trade-off in resource allocation under elevated atmospheric pressure. This integration of water relations signals (water potential gradients) and hormonal cues (ABA/JA-CTK antagonism) culminated in maturation (Stage IV) with aerial biomass dominance (Figure 5). Pressurized CO₂ enrichment sustained nocturnal respiration despite ROS trade-offs, echoing drought-acclimated resource diplomacy (Fanourakis et al., 2013). Crucially, PCA (Figure 9) revealed that the PC2 hierarchy, connecting T-SOD, GA₄, and MeJA, exposed a regulatory axis. This axis gated PRO-dependent osmotic thresholds (120 kPa) to synchronize growth-defense transitions. This mechanism diverged from singular stress-response by embedding environmental memory into phase-specific resource allocation (Al-Zahrani et al., 2022; Merilo et al., 2013). This water-hormone integration thus enables dynamic growth-defense trade-offs across developmental stages under stress.

5 | CONCLUSIONS AND FUTURE PROSPECTS

This study conducted pot cultivation experiments of *N. benthamiana* within a simulated chamber, artificially controlling air humidity and atmospheric pressure to imitate the conditions of high humidity (RH 99%) and varying atmospheric pressures (at 96, 110, and 120 kPa) found in deep underground environments. The findings elucidate the intricate interaction between environmental humidity and atmospheric pressure and their impact on the growth and regulation of *N. benthamiana*. Elevated humidity was found to impair plant growth by decreasing water consumption, disrupting photosynthetic efficiency due to increased leaf surface moisture, and compromising the plant's innate defense mechanisms through the inhibition of crucial phytohormone synthesis or signaling. These adverse effects are compounded by stomatal closure and diminished transpiration efficiency, leading to potential overheating and

reduced nutrient uptake. In contrast, high atmospheric pressure serves as a mitigative agent against the detrimental effects of high humidity. It enhances internal water relations, boosts plant turgor and stress resilience, and induces physiological adaptations, including improved antioxidative defenses, restoration of defensive capabilities, better nutrient mobilization, and increased photosynthesis, thereby optimizing plant growth and resilience in high humidity conditions.

With the advancement of deep underground space development and the continuous improvement of environmental control technologies, such as ventilation and thermohygro-metric systems, strategies for addressing the challenges of subterranean plant cultivation are becoming increasingly refined. Elevated atmospheric pressure has emerged as a potential factor to enhance plant growth, but a thorough understanding of plants' physiological responses to such conditions remains essential. Progress in this area requires interdisciplinary collaboration across agronomy, plant physiology, phytopathology, microbial science, genetics, and longitudinal experimental research. The findings of this study suggest specific engineering implications for underground facility design, particularly the need to integrate controlled atmospheric systems capable of pressure modulation alongside optimized ventilation to counteract humidity stress and enhance plant productivity. Future studies should focus on the effects of elevated atmospheric pressures on gene regulation, adaptive responses, and physiological mechanisms. Furthermore, targeted research steps should include screening diverse plant species for pressure tolerance and mapping species-specific pressure thresholds for optimal growth. Such research studies will provide critical insights into plant acclimatization and adaptation, offering practical applications for optimizing environmental conditions in deep underground spaces.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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