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Mitigating ammonium toxicity in strawberry cultivation: effective fertilization practices for sustainable crop production

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Abstract

Nitrogen is crucial for plant growth, but deficiency and excess can harm plants. Fertilizers like Diammonium Phosphate (DAP), which releases ammonium (NH_4^+), are common, yet over-application can cause NH_4^+ toxicity, resulting in stunted roots and leaf damage. This study investigated the impact of NH_4^+ toxicity on strawberry growth, yield, and fruit quality to inform better fertilization practices. The experiment was conducted at The Islamia University of Bahawalpur, Pakistan. Five treatments with varying DAP rates (0 g, 4 g, 7 g, 10 g, and 13 g per plant) were applied to strawberry plants in a completely randomized design with four replications. Photosynthetic pigments, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), electrolyte leakage (EL), and yield parameters were measured. The 4 g DAP treatment yielded the highest chlorophyll-a (0.5775 mg/g FW) and total chlorophyll content (0.705 mg/g FW). However, increasing DAP doses led to a decline in chlorophyll-a, chlorophyll-b, and total chlorophyll content, with the 13 g DAP treatment exhibiting the lowest levels. H_2O_2 content increased with higher DAP doses, with the 13 g DAP treatment showing the highest value (75 μmol/g FW). Higher DAP doses also increased MDA content and EL, indicating oxidative stress and membrane damage. The 4 g DAP treatment showed minimal changes in H_2O_2 and MDA content. Moderate DAP levels (4 g per plant) enhanced strawberry growth, yield, and photosynthetic activity, while higher doses caused significant stress, leading to reduced growth and yield. Managing NH_4^+ levels in fertilization is crucial for optimizing strawberry production. Therefore, moderate doses of DAP (ammonium ion) should be used to avoid ammonium toxicity.

Keywords Ammonium toxicity, Diammonium phosphate, Electrolyte leakage, Fertilization management, Nutrient homeostasis, Oxidative stress, Strawberry yield

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Introduction

Nitrogen is an essential nutrient for plant growth and productivity, but its availability can be both limiting and toxic to plants [1]. To optimize nitrogen utilization efficiency, fertilizers containing ammonium (NH_4^+) are widely used. However, excessive NH_4^+ application can lead to its rapid accumulation in plant tissues, causing root stunting and leaf damage [2, 3]. NH_4^+ toxicity has long been a significant challenge in agriculture, negatively affecting plant growth and yield [4]. While NH_4^+ plays a crucial role in amino acid and protein synthesis, excessive levels are harmful to plant cells [5]. Even plants that prefer NH_4^+ as a nitrogen source can suffer toxicity when exposed to high concentrations [6].

NH_4^+ toxicity manifests in several physiological impairments, including reduced shoot biomass and chlorosis, particularly in young plants under hydroponic conditions [5]. Additionally, NH_4^+ stress leads to shortened roots, a common phenotype observed across various plant species [7]. Strawberries (*Fragaria* × *ananassa*), in particular, prefer nitrate over ammonium. An increased ammonium ratio in fertilizer solutions has been shown to significantly reduce flower production in long-day strawberry cultivars such as 'Albion,' while still promoting daughter plant formation. Previous studies suggest that elevated ammonium levels may inhibit flowering due to reduced nitrate uptake and metabolic imbalances. Furthermore, ammonium-based nutrition has been reported to impair photosynthesis in strawberries compared to nitrate-based nutrition [8, 9]. Direct exposure of root tips to NH_4^+ is both necessary and sufficient to inhibit primary root growth, with approximately 70% of this inhibition attributed to reduced cell elongation [10]. Auxin, a key regulator of root development [11, 12], has been implicated in NH_4^+-mediated root elongation inhibition, as auxin-resistant mutants exhibit reduced sensitivity to NH_4^+ [13]. Additionally, NH_4^+ influences root gravitropism, with moderate concentrations enhancing and high concentrations suppressing this response in *Arabidopsis* [14]. Given the broad impact of NH_4^+ stress, strawberries warrant special attention in this context.

Strawberries are a highly nutritious fruit, rich in dietary fiber, fructose, essential fatty acids, and micronutrients such as vitamin C and folate [15–17]. Additionally, they contain diverse phenolic compounds, including flavonoids, hydrolyzable tannins, phenolic acids, and condensed tannins, contributing to their strong antioxidant capacity and health benefits [18, 19]. Studies indicate that strawberries possess greater antioxidant activity than other fruits such as apples, peaches, and oranges, largely due to their high vitamin C and phenolic content [20]. Compared to various fruits, vegetables, and cereals, strawberries consistently rank among the highest in total antioxidant capacity and phenolic content [21]. As a result of their nutritional significance, global strawberry production has increased by 142% over the past two decades, reaching 7.7 million tonnes in 2013. Major producers include China, the United States, Mexico, Turkey, and Spain [22].

To meet increasing demands, farmers extensively apply fertilizers to enhance strawberry yield. Diammonium phosphate (DAP) [$(NH_4)_2HPO_4$] is one of the most widely used phosphorus fertilizers, providing 18% nitrogen and 46% phosphate [23]. In 2019, global DAP consumption in agriculture reached 17.2 million tonnes, with India being the largest consumer, followed by the United States, Pakistan, Bangladesh, and Turkey. Together, these countries account for over 92% of global DAP use. Due to its high solubility and favorable physical properties, DAP is an effective source of nitrogen and phosphorus for plants [24].

However, excessive DAP application can lead to NH_4^+ stress in strawberries, potentially reducing growth and yield. While NH_4^+ toxicity has been extensively studied in other crops, its specific effects on strawberries remain underexplored. This study aims to investigate the impact of NH_4^+ concentration on strawberry growth, yield, and fruit quality. It is hypothesized that moderate DAP levels will enhance plant performance, while excessive NH_4^+ will induce oxidative stress and suppress growth. By optimizing nitrogen management in strawberry cultivation, this research seeks to promote sustainable agricultural practices, mitigate environmental pollution from over-fertilization, and improve overall crop productivity. The findings will benefit strawberry growers and contribute to a broader understanding of plant nutrition and stress physiology across multiple crop species.

Materials and methods

Experimental site

The study was conducted at the Green Belt, The Islamia University of Bahawalpur (IUB), Pakistan, (29°22'11.5" N, 71°45'49.0" E) during November 2018-19. The meteorological conditions during the study period are given in Fig. 1.

Treatments

The levels of fertilizer (DAP) applied in the study were selected based on previous research and practical considerations to assess the effects of varying ammonium concentrations on strawberry plant growth. The treatments included a control (0 g/plant) and four levels of DAP application (4 g/plant, 7 g/plant, 10 g/plant, and 13 g/plant). The application of these treatments was repeated three times during the study period. The first treatment was applied after three weeks of transplantation of plants followed by another treatment after three weeks and last treatment applied after two weeks of second treatment.

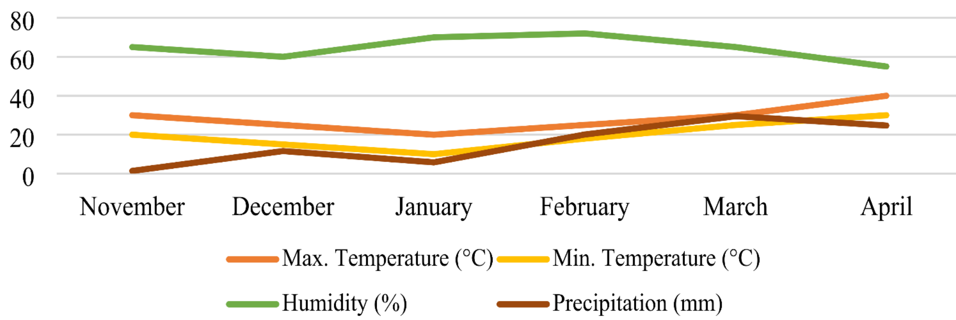

Fig. 1 Monthly average maximum and minimum temperatures (°C), humidity (%), and precipitation (mm) in Bahawalpur during the study period (November 2018 to April 2019)

Table 1 Physicochemical properties of the soil used in the study

Depth (cm)	Electrical conductivity mS/m	pH	Organic matter (%)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Saturation (%)	Texture
0–6	260	7	0.91	24.7	179	34	Loam

Table 2 Chemical analysis of the irrigation water used in the study

EC (mS/m)	Calcium + magnesium (mmol/L)	Sodium (mmol/L)	Carbonate (mmol/L)	Bicarbonate (mmol/L)	Chloride mmol/L)	Sodium adsorption ratio	Residual sodium carbonate (mmol/L)
80	2.1	3.8	Nill	4.1	0.21	2.62	Nill

These levels reflect common concentrations used in agricultural practices for strawberry cultivation and were designed to assess the impact of ammonium toxicity and its effects on plant health. The selection of these fertilizer levels was also influenced by the soil's nutrient status and the potential for plant tolerance to ammonium, as well as by previous studies on fertilizer application rates for strawberries under similar conditions.

Soil preparation

The soil mixture was prepared using two parts silt, one part peat moss, and 5–10% perlite. Each pot was filled with 1 kg of this prepared soil mixture. The physicochemical properties of the soil are detailed in Table 1. Soil and water analyses, as presented in Tables 1 and 2, were conducted by a certified laboratory at the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur (IUB), Pakistan.

Plant transplantation, irrigation, and growth conditions

Uniform strawberry plants (cv. Chandler) were sourced from a commercial field nursery in Upper Dir District, Kohat, Pakistan, and disinfected before transplantation. The plants were immersed in a 0.1% solution of sodium hypochlorite for 5 min, followed by thorough rinsing with distilled water to remove any residual disinfectant. The disinfected plants were then transplanted into plastic pots on November 23, 2018, containing the prepared soil mixture (Fig. 2).

The plants were maintained in a controlled environment in the Green Belt of The Islamia University of Bahawalpur (IUB), Pakistan, with the following growth conditions: average day/night temperatures of 25 °C/18°C, relative humidity ranging from 60 to 80%, and natural light exposure for 12 h per day. The plants were irrigated twice weekly or as needed and fertilized with Phostrogen, a balanced nutrient solution, on December 7, 2018, and January 11, 2019. Phostrogen comprises potassium nitrate, ammonium phosphate, urea, calcium, magnesium sulfates, and trace elements. A 0.5% Phostrogen solution was uniformly applied to each pot. Weeds were removed regularly, and hoeing was performed five weeks after fertilization to mix the soil and ensure adequate irrigation and aeration. Harvesting commenced on March 25, 2019, approximately four months after transplantation.

Photosynthetic pigments

To determine chlorophyll content, 0.5 g of fresh leaves were extracted with 10 mL of 80% acetone. The resulting solution was then analyzed using a spectrophotometer, and the concentrations of chlorophyll a and chlorophyll b were calculated [25]. This method allows for the precise measurement of chlorophyll pigments, which is essential for understanding plant growth and development.

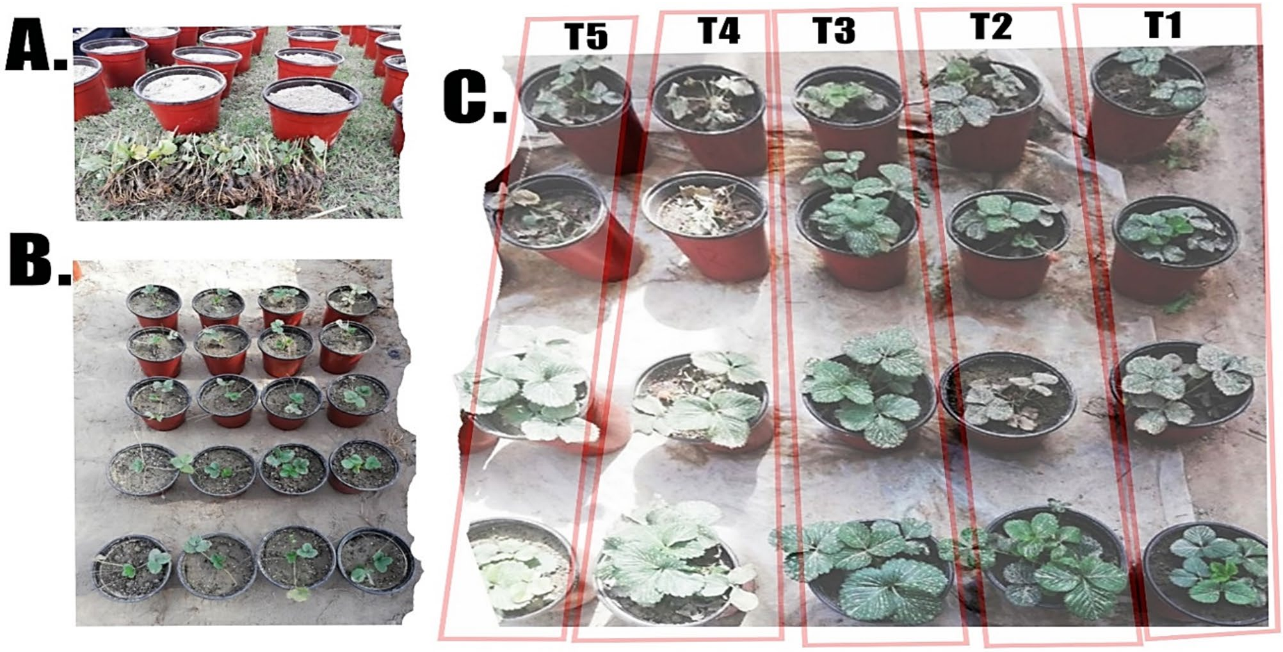

Fig. 2 Experimental setup for evaluating the effects of ammonium toxicity on strawberry plants, illustrating the stages of plant development from seedling to maturity, along with treatment application and its impact on growth. (**A**) Seedlings before planting, (**B**) seedlings two weeks after transplantation, and (**C**) plants three weeks after the application of DAP treatments. T1 = Control, T2 = 7 g DAP/pot, T3 = 4 g DAP/pot, T4 = 13 g DAP/pot, T5 = 10 g DAP/pot

Hydrogen peroxide (H^2O^2), malondialdehyde (MDA), and electrolyte leakage (EL)

Lipid peroxidation was assessed by measuring MDA content. A 0.5 g leaf sample was homogenized in 10 mL of 0.1% TCA, centrifuged, and the supernatant collected. One mL aliquot was mixed with 4 mL of 0.5% TBA in 20% TCA, heated at 95 °C for 30 min, and then cooled. After centrifugation, the absorbance was measured at 532 nm, subtracting the non-specific absorption at 600 nm. MDA content was calculated using its extinction coefficient and expressed as nmol/g fresh weight [26]. Additionally, H_2O_2 content was measured spectrophotometrically at 390 nm using KI and an H_2O_2 standard curve [27].

Electrolyte leakage was measured in leaves using the method of Lin et al. [28]. Leaf segments were submerged in distilled water for 2 h, and initial electrical conductivity (EC1) was recorded. Then, samples were heated to 120 °C for 20 min, cooled, and final electrical conductivity (EC2) was measured. EL was calculated as (EC1/EC2) × 100, providing a percentage value.

Data analysis

A one-way analysis of variance (ANOVA) was performed to evaluate variability across treatment groups, followed by Tukey's Honest Significant Difference (HSD) test for post hoc mean comparisons at a 5% significance level. The assumptions of ANOVA, including normality and homogeneity of variances, were validated before analysis. Additionally, Pearson correlation analysis was applied to determine relationships between ammonium concentration, chlorophyll content, oxidative stress markers (H_2O_2, MDA), and electrolyte leakage. Pearson correlation was chosen because it measures the strength and direction of linear relationships between continuous variables, providing insights into how increasing ammonium levels affect physiological parameters in strawberry plants. This analysis helps to identify key associations, such as whether higher ammonium concentrations correspond to increased oxidative damage or decreased chlorophyll content. All statistical computations were performed using OriginPro software (version 2024b), and results are presented as means ± standard error (SE).

Results

Growth and morphological parameters

The 4 g DAP treatment showed optimal growth, with a notable increase in the mean number of leaves (7.25) compared to the control (5.25) (Fig. 3A). This treatment also resulted in the widest leaf width, indicating a positive response to moderate DAP levels. Additionally, the 4 g DAP treatment showed the longest leaf length (3.05 cm), suggesting enhanced cell elongation and division. In contrast, higher DAP doses led to progressive declines in these parameters. The 7 g DAP treatment resulted in a decrease in leaf width (2.94 cm) and leaf length (2.73 cm) (Fig. 3B and C), while the 10 g DAP treatment showed further reductions in these parameters. The 13 g DAP treatment showed the lowest values for leaf width

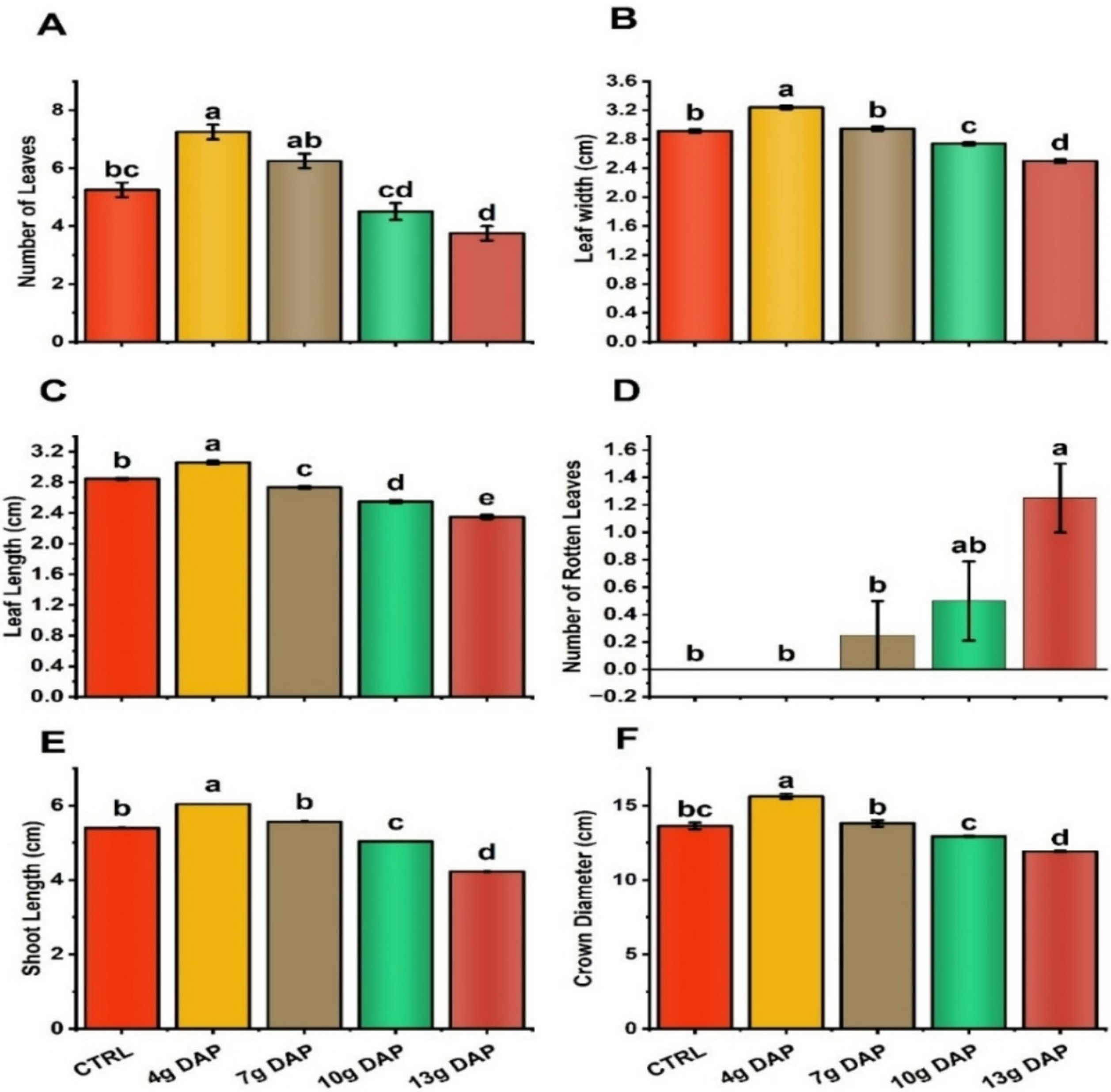

Fig. 3 Comparison of parameters at harvesting stage: (**A**) number of leaves, (**B**) leaf width (cm), (**C**) leaf length (cm), (**D**) number of rotten leaves, (**E**) shoot length (cm), and (**F**) crown diameter (cm). Means with different letters (e.g., 'a', 'b', 'c') indicate statistically significant differences ($p \leq 0.05$)

(2.49 cm) and leaf length (2.35 cm), indicating significant stress-induced damage.

Moreover, the number of rotten leaves increased with increasing DAP doses, indicating stress-induced damage. The control group showed no rotten leaves, while the 7 g DAP treatment had a mean of 0.25 rotten leaves, the 10 g DAP treatment had a mean of 0.5 rotten leaves, and the 13 g DAP treatment had a mean of 1.25 rotten leaves (Fig. 3D). Furthermore, the 4 g DAP treatment resulted in the longest shoot length, indicating enhanced stem elongation (Fig. 3E). However, higher DAP doses led to progressive declines in shoot length, with the 13 g DAP treatment showing the shortest shoot length. Regarding crown diameter, the 4 g DAP treatment resulted in the largest crown diameter, indicating enhanced root growth. However, higher DAP doses led to progressive declines in crown diameter, with the 13 g DAP treatment showing the smallest crown diameter (Fig. 3F).

Photosynthetic parameters

The 4 g DAP treatment showed a notable increase in chlorophyll-a content compared to the control, indicating enhanced photosynthetic activity (Fig. 4A). Chlorophyll b content also showed a similar trend, with the 4 g DAP treatment resulting in the highest value and the 13 g DAP treatment showing the lowest value (Fig. 4B). The control group had a moderate chlorophyll b content (0.1025 mg/g FW). This treatment also resulted in the highest total chlorophyll content, suggesting optimal photosynthetic capacity (Fig. 4C). In contrast, higher DAP doses led to progressive declines in chlorophyll-a and total chlorophyll content, with the 13 g DAP treatment showing the lowest values (0.1225 and 0.1625 mg/g FW, respectively).

Hydrogen peroxide (H^2O^2), malondialdehyde (MDA), electrolyte leakage (EL)

The accumulation of oxidative stress markers and membrane damage indicators increased with higher DAP concentrations. Hydrogen peroxide levels were significantly elevated in the 13 g DAP treatment, reaching 75 μmol/g FW, compared to 20 μmol/g FW in the control (Fig. 5A). Similarly, malondialdehyde content, an indicator of lipid peroxidation, was highest in the 13 g DAP treatment (7.5 nmol/g FW) and lowest in the control (2.8 nmol/g FW) (Fig. 5B). Electrolyte leakage, a measure of membrane integrity, followed the same trend, with the highest value recorded at 51% in the 13 g DAP treatment, whereas the control exhibited the lowest leakage at approximately 15% (Fig. 5C). These results indicate that excessive DAP application induces oxidative stress and membrane damage, whereas moderate DAP levels help maintain cellular stability.

Yield related parameters

The 4 g DAP treatment showed optimal yield, with the highest number of buds (2.75) and fruit biomass (9.71 g), indicating enhanced reproductive growth and productivity. This suggests that moderate ammonium ion stress can stimulate strawberry plant yield. In contrast, higher DAP doses led to progressive declines in yield. The 13 g

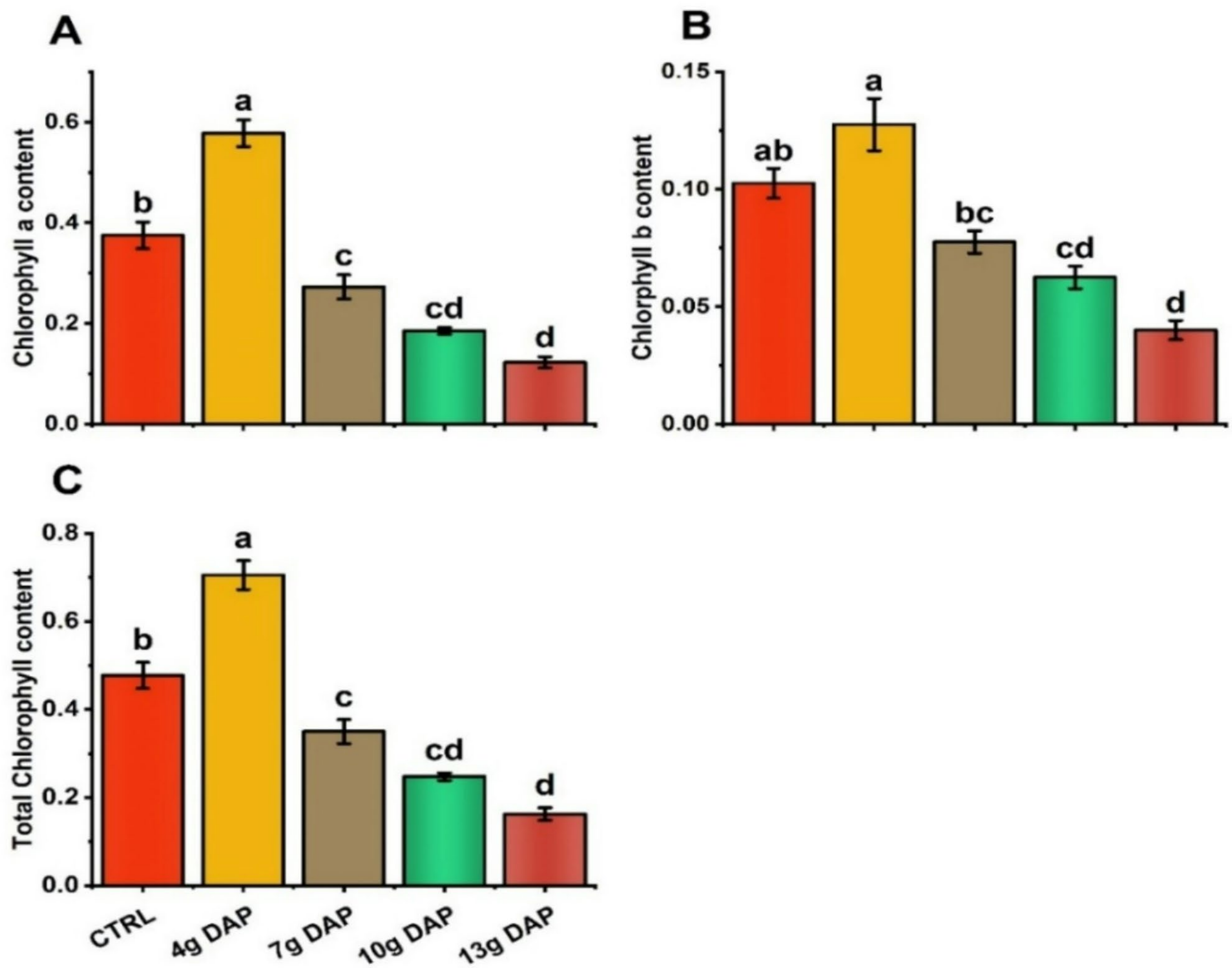

Fig. 4 Comparison of parameters at harvesting stage: (**A**) chlorophyll a content (mg/g FW), (**B**) chlorophyll b content (mg/g FW), and (**C**) total chlorophyll content (mg/g FW). Means with different letters (e.g., 'a', 'b', 'c') indicate statistically significant differences ($p \leq 0.05$)

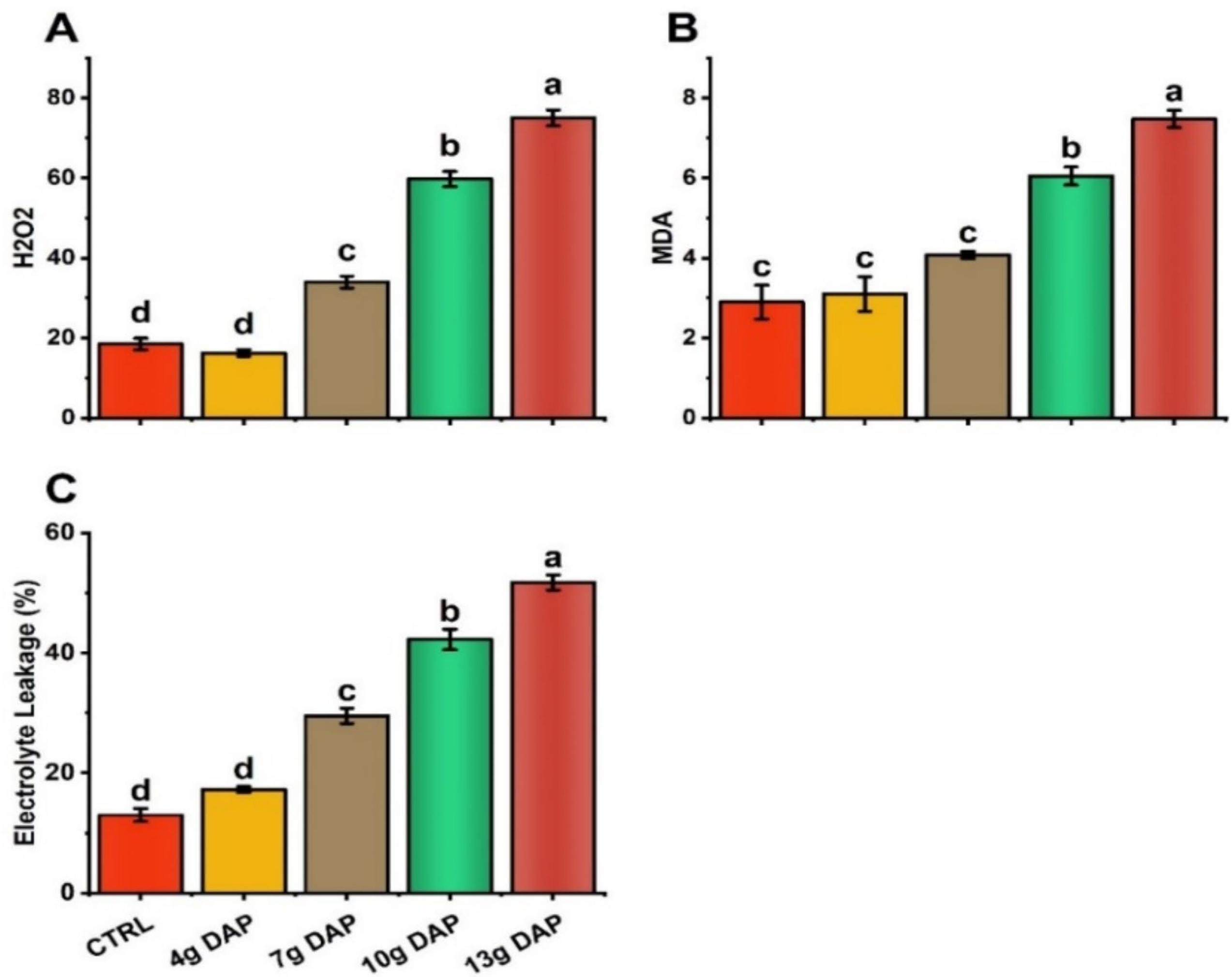

Fig. 5 Comparison of parameters at the harvesting stage: (**A**) H_2O_2 (μmol/g FW), (**B**) MDA (nmol/g FW), and (**C**) electrolyte leakage (%). Means with different letters (e.g., 'a', 'b', 'c') indicate statistically significant differences ($p \leq 0.05$)

DAP treatment showed the lowest number of buds (0.5) and fruit biomass (3.83 g), indicating reduced reproductive growth and productivity. The control group showed average values for both parameters, indicating normal yield in the absence of ammonium ion stress. Notably, the number of buds was significantly higher in the 4 g DAP treatment compared to the control, indicating enhanced flower initiation and development under moderate stress. However, excessive stress (higher DAP doses) led to a decline in bud formation and fruit production. The results suggest that moderate ammonium ion stress can stimulate strawberry plant yield, while excessive stress leads to reduced yield. These findings have implications for optimizing fertilizer application and stress management strategies in strawberry cultivation to maximize yield and productivity (Fig. 6A and B).

Agronomic parameters at the harvesting stage

The application of 4 g DAP resulted in significant improvements in various growth parameters at the harvesting stage compared to the control and higher DAP treatments. Shoot length was highest in the 4 g DAP treatment (7.2 cm), followed by the control (6.1 cm), while the 13 g DAP treatment showed the lowest value (4.3 cm) (Fig. 7A). A similar trend was observed for root length, where the 4 g DAP treatment recorded the maximum value (4.8 cm), whereas the 13 g DAP treatment had the shortest roots (3.9 cm) (Fig. 7B). Plant length was also significantly enhanced under the 4 g DAP treatment (12.5 cm), whereas the 13 g DAP treatment exhibited the lowest plant length (8.1 cm) (Fig. 7C). Shoot biomass reached its peak at 5.6 g in the 4 g DAP treatment, while it declined to 2.6 g in the 13 g DAP treatment (Fig. 7D). Similarly, root biomass was highest at 1.2 g under 4 g DAP but was significantly reduced under 10 g and 13 g DAP (0.1 g and 0.08 g, respectively) (Fig. 7E). Total plant

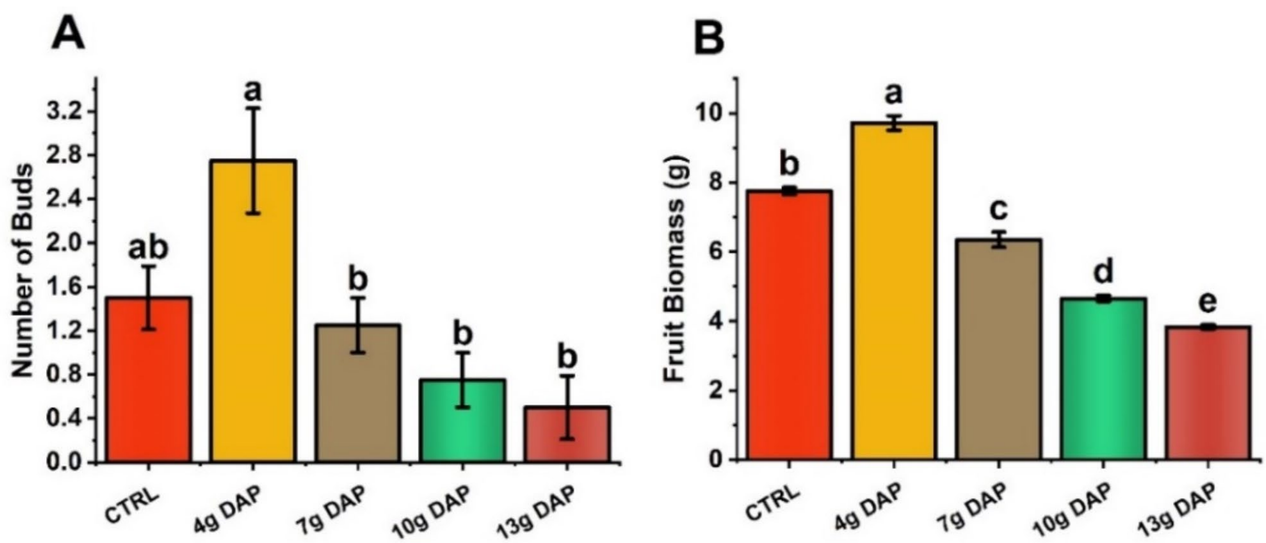

Fig. 6 Comparison of parameters at the harvesting stage: (**A**) number of buds and (**B**) fruit biomass (g). Means with different letters (e.g., 'a', 'b', 'c') indicate statistically significant differences ($p \leq 0.05$)

biomass followed the same trend, with the 4 g DAP treatment exhibiting the highest value (7.1 g), whereas the 13 g DAP treatment had the lowest (3.8 g) (Fig. 7F). These findings suggest that a moderate DAP dose (4 g) enhances growth and biomass production, whereas higher concentrations exert adverse effects, likely due to ammonium toxicity (see Fig. 8).

The Pearson correlation reveals strong positive correlations between growth traits (e.g., leaf area, shoot length, and chlorophyll content) and yield parameters like fruit and plant biomass, indicating their vital role in strawberry productivity. In contrast, stress markers such as H_2O_2, MDA, and EL show strong negative correlations with photosynthetic pigments and biomass. The number of rotten leaves negatively correlates with key growth traits, further reflecting stress impacts.

Discussion

Nitrogen (N) is an essential macronutrient for plant growth and development, playing a pivotal role in various physiological and metabolic processes. As a core component of proteins, enzymes, and nucleic acids, nitrogen is integral to plant structure and function [29]. However, when N supply exceeds crop demand, it disrupts physiological homeostasis, negatively impacting plant health and yield quality [30]. One of the major concerns associated with excessive nitrogen, particularly in the form of ammonium (NH_4^+), is ammonium toxicity, which leads to metabolic imbalances and physiological stress [31]. This study examined the effects of ammonium stress induced by varying Diammonium Phosphate (DAP) concentrations on the growth, development, and yield of strawberry plants.

Results revealed that moderate DAP application (4 g/pot) significantly enhanced strawberry plant growth, reflected in increased leaf number, leaf width, leaf length, shoot length, and crown diameter. These findings align with previous studies, which have shown that nitrogen at optimal levels improves plant biomass accumulation by supporting protein synthesis, chlorophyll formation, and enzymatic activities essential for growth [32–34]. The improved growth at this DAP level can be attributed to a balanced nitrogen supply, which promotes cell division and expansion while ensuring efficient nutrient assimilation. Additionally, moderate nitrogen levels facilitate the synthesis of phytohormones such as auxins and cytokinins, which regulate cell differentiation and shoot elongation [35].

Conversely, higher DAP doses (7 g, 10 g, and 13 g/pot) led to a progressive decline in these growth parameters, suggesting ammonium-induced stress. The observed increase in the number of rotten leaves at elevated DAP levels further supports this, as excessive NH_4^+ can cause nutrient imbalances, leading to chlorosis, necrosis, and overall leaf senescence. High ammonium concentrations have been reported to disrupt nitrogen metabolism, leading to excess accumulation of free amino acids and toxic nitrogenous compounds, which impair cellular function and retard growth [36–38]. The reduction in shoot length and crown diameter at high DAP doses may also be attributed to root damage caused by ammonium toxicity, which hinders water and nutrient uptake, leading to reduced turgor pressure and growth inhibition.

Similarly, moderate DAP application (4 g/pot) enhanced photosynthetic activity, as indicated by increased chlorophyll-a and total chlorophyll content. This improvement in chlorophyll content can be linked

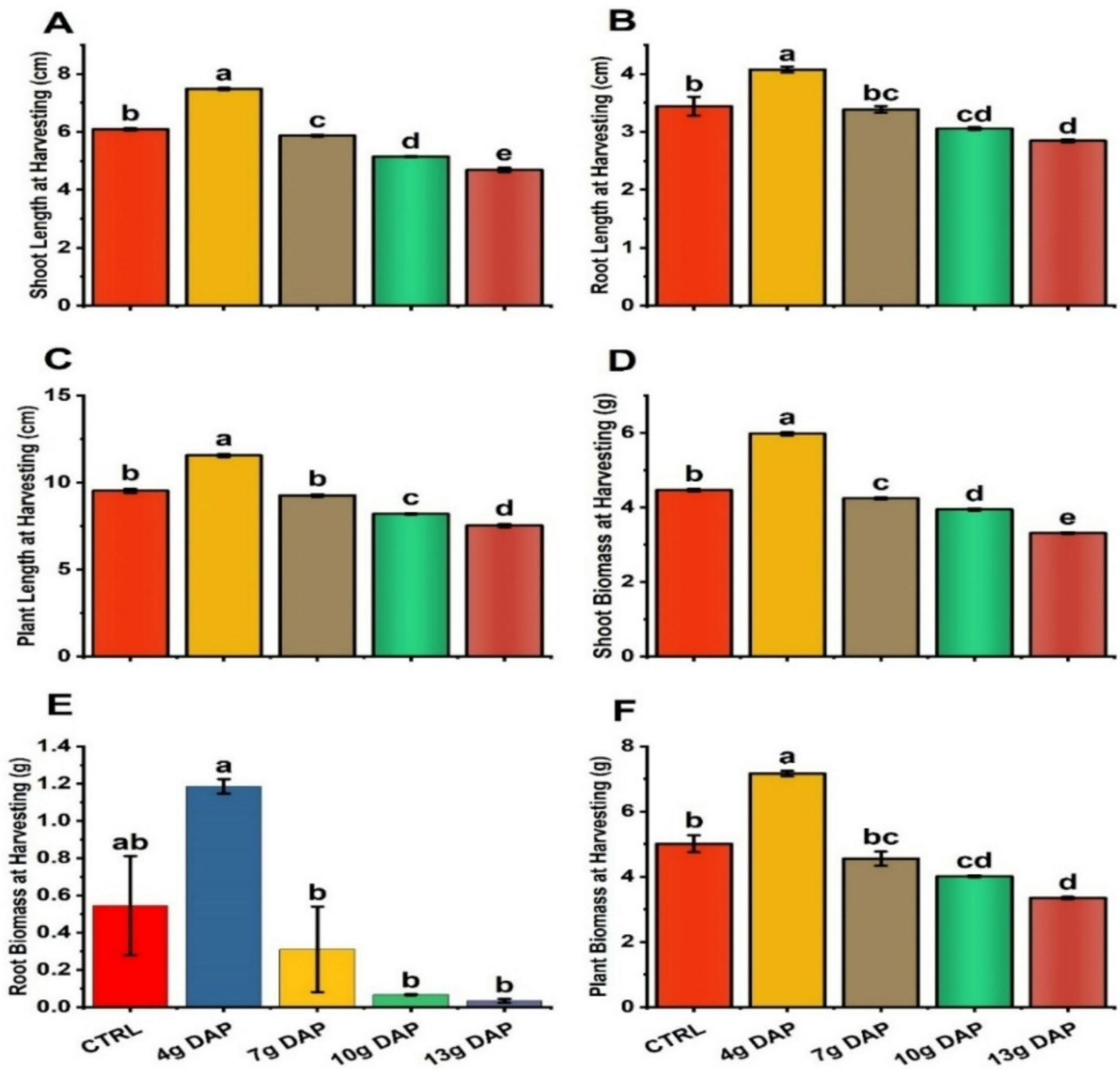

Fig. 7 Comparison of parameters at the harvesting stage: (**A**) shoot length (cm), (**B**) root length (cm), (**C**) plant length (cm), (**D**) shoot biomass (g), (**E**) root biomass (g), and **F**) plant biomass (g). Means with different letters above bars indicate statistically significant differences ($p \le 0.05$)

to the adequate availability of nitrogen, a crucial component of chlorophyll molecules, which ensures efficient light capture and energy conversion for photosynthesis. These findings are in agreement with studies on other crops, where optimal nitrogen levels have been shown to enhance photosynthetic efficiency and overall plant productivity [39, 40]. However, at higher DAP doses, chlorophyll-a and total chlorophyll content exhibited a significant decline, indicating stress-induced damage to the photosynthetic apparatus. One possible explanation is that excessive NH_4^+ disrupts the uptake of essential nutrients such as magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^+), which are vital for chlorophyll biosynthesis and chloroplast stability. Additionally, NH_4^+ stress can impair stomatal function, reducing CO_2 uptake and photosynthetic efficiency, further contributing to growth inhibition. This phenomenon has been previously reported in ammonium toxicity studies on various crops, including cauliflower, broccoli, and citrus [41–43].

The observed trend in chlorophyll-b content, where the 4 g DAP treatment resulted in the highest values while the 13 g DAP treatment exhibited the lowest, suggests that excessive ammonium inhibits accessory pigment synthesis [44]. Chlorophyll-b plays a vital role in light absorption and energy transfer within the photosynthetic system, and its reduction under high ammonium stress

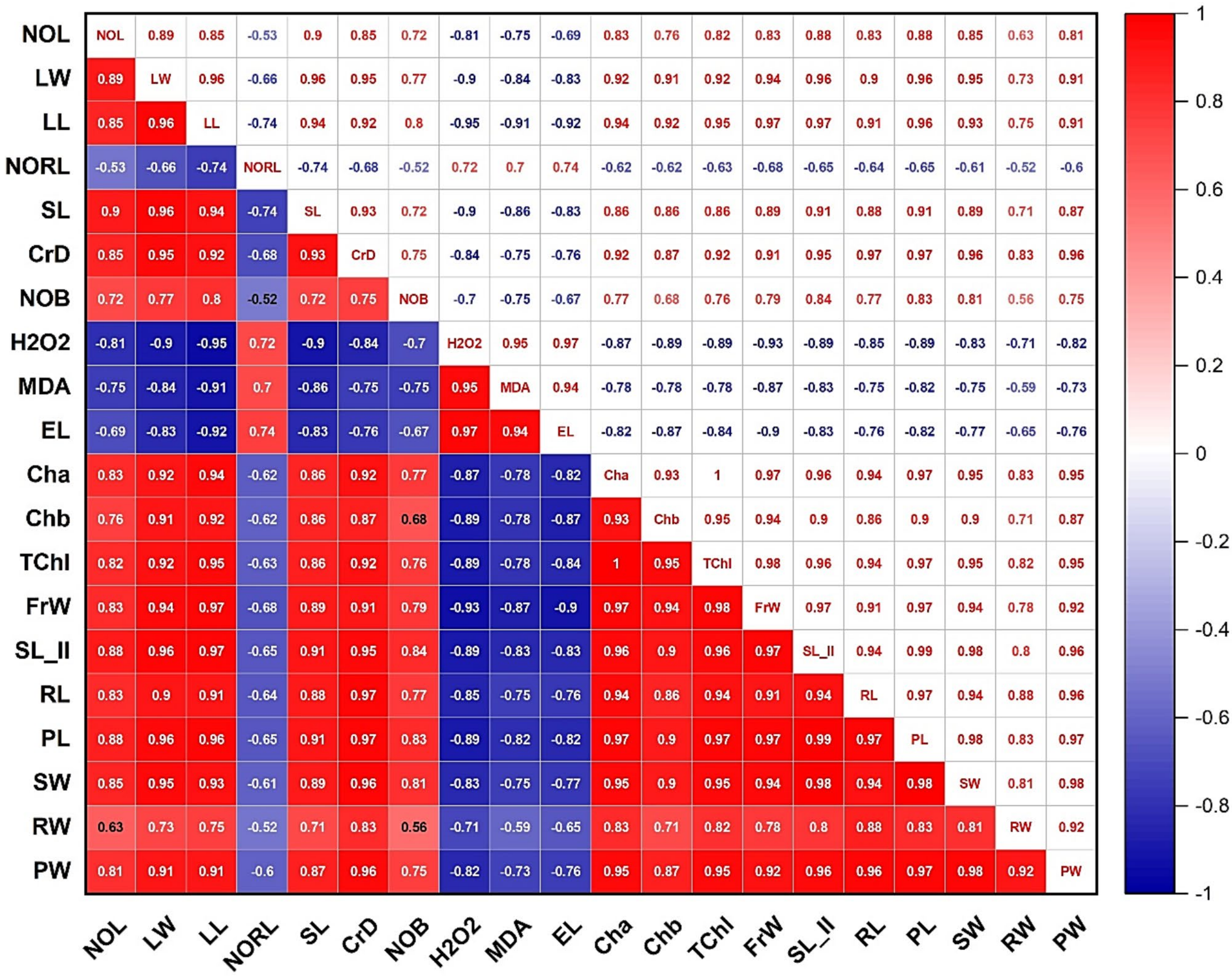

Fig. 8 Pearson correlation for different growth attributes of strawberry plants. The intensity of the blue color shows a negative correlation while the intensity of the red color shows a positive correlation. NOL = number of leaves; LW = leaf area; LL = leaf length; NORL = number of rotten leaves; SL = shoot length; CrD = crown diameter; NOB = number of buds; H_2O_2 = hydrogen peroxide content; EL = electrolyte leakage; Cha = chlorophyll a content; Chb = chlorophyll b content; Tchl = total chlorophyll; FrW = fruit biomass; SL-II = shoot length at harvesting; RL-II = root length at harvesting; PL-II = plant length at harvesting; SW-II = shoot biomass at harvesting; RW-II = root biomass at harvesting; PW-II = plant biomass at harvesting

indicates compromised light-harvesting efficiency [45]. This is in line with previous research demonstrating that ammonium toxicity disrupts pigment stability, leading to photoinhibition and reduced photosynthetic capacity [46–47].

Furthermore, a significant increase in H_2O_2 content with increasing DAP doses indicates that excessive ammonium induces oxidative stress. NH_4^+ toxicity has been shown to disrupt nitrogen metabolism, leading to an energy imbalance that affects mitochondrial and chloroplast electron transport chains, thereby generating excessive reactive oxygen species [48–50]. The concurrent increase in malondialdehyde levels suggests that lipid peroxidation and membrane damage occur under excessive NH_4^+ stress, further compromising cell integrity. The marked increase in electrolyte leakage at higher DAP doses reinforces this notion, as excessive ROS production leads to oxidative damage of membrane lipids, proteins, and ion channels, ultimately causing membrane destabilization and ion leakage. These findings are consistent with previous reports indicating that ammonium stress accelerates ROS accumulation, resulting in oxidative damage and reduced plant performance [51–54].

In contrast, the 4 g DAP treatment exhibited minimal changes in H_2O_2 and MDA content, along with a moderate increase in EL. This suggests that a moderate ammonium supply induces a controlled stress response, potentially activating antioxidant defense mechanisms without causing severe cellular damage [55–58]. Plants have evolved adaptive strategies to manage mild stress, such as upregulating antioxidant enzyme activity (e.g., catalase, superoxide dismutase) to detoxify ROS and maintain cellular homeostasis [59–62]. However, beyond a critical threshold, excessive NH_4^+ overwhelms these defense systems, leading to oxidative stress, membrane degradation, and overall growth suppression [63].

These findings collectively suggest that strawberry plants have a specific tolerance threshold for ammonium ions, beyond which physiological and metabolic disruptions become severe. The optimal growth and development observed at 4 g DAP/pot indicate that moderate ammonium levels support plant productivity, whereas excessive DAP application induces oxidative damage, membrane instability, and reduced growth performance. This highlights the importance of precise nitrogen management in strawberry cultivation to optimize yield while avoiding ammonium toxicity-related stress. Future studies should explore the role of ammonium detoxification pathways and antioxidant enzyme activities in mitigating ammonium toxicity in strawberries.

Conclusion

This study establishes that excessive ammonium ions, resulting from high DAP doses, induce stress in strawberry plants by disrupting physiological and biochemical processes. These results underscore the critical role of ammonium ion management in strawberry cultivation. Implementing moderate DAP doses and regular monitoring of soil ammonium levels can prevent toxicity and optimize nutrient efficiency. We recommend the use of moderate levels of DAP fertilizer to avoid ammonium toxicity. Applying DAP in split doses, monitoring soil ammonium levels regularly, and timing fertilization during active growth phases can help optimize nutrient uptake and prevent toxicity. Additionally, considering ammonium-tolerant cultivars may enhance resilience under varying fertilization conditions. This work emphasizes the importance of balancing ammonium-based fertilization to minimize stress and maximize crop performance, contributing to the broader understanding of nitrogen management in horticultural crops.

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Author contributions
GS, TA, HQ: Methodology, supervision, writing and drafting, and research design RN, FK: Experimentation and data Curation; MY, MWH, MSR: Validation and Software, writing, Investigation, drafting, statistical analysis, and validation; MTN, GA: writing, Software, Resource, research design, validation, data collection, drafting, statistical analysis; MJA, RTA: writing, funding, statistical analysis, Resource, software, validation. All authors reviewed the manuscript.

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Data availability
The author confirms that all data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable. Plant collection was done after getting proper permission.

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Study protocol must comply with relevant institutional, National, and international guidelines and legislation
Our experiment follows the relevant institutional, national, and international guidelines and legislation.

Competing interests
The authors declare no competing interests.

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References

1. Leghari SJ, Wahocho NA, Laghari GM, HafeezLaghari A, MustafaBhabhan G, HussainTalpur K, Lashari AA. Role of nitrogen for plant growth and development: A review. Adv Environ Biol. 2016;10(9):209–19.
2. Gooding MJ, Davies WP. Foliar Urea fertilization of cereals: A review. Fertilizer Res. 1992;32:209–22.
3. Phillips SB, Mullins GL. Foliar burn and wheat grain yield responses following topdress-applied nitrogen and sulfur fertilizers. J Plant Nutr. 2004;27(5):921–30.
4. Gerendás J, Zhu Z, Bendixen R, Ratcliffe RG, Sattelmacher B. Physiological and biochemical processes related to ammonium toxicity in higher plants. Z Pflanzenernähr Bodenkd. 1997;160(2):239–51.
5. Britto DT, Kronzucker HJ. NH_4^+ toxicity in higher plants: A critical review. J Plant Physiol. 2002;159(6):567–84.
6. Balkos KD, Britto DT, Kronzucker HJ. Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. Cv. IR-72). Plant Cell Environ. 2010;33(1):23–34.
7. Li LB, Shi SW. Effects of elevated NH4 + on Arabidopsis seedlings different in accessions.
8. Li Q, Li BH, Kronzucker HJ, Shi WM. Root growth Inhibition by NH_4^+ in Arabidopsis is mediated by the root tip and is linked to NH4 + efflux and GMPase activity. Plant Cell Environ. 2010;33(9):1529–42.
9. Shi X, Hernández R, Hoffmann M. Impact of nitrate and ammonium ratios on flowering and asexual reproduction in the everbearing strawberry cultivar *Fragaria*× *Ananassa* Albion. Horticulturae. 2021;7(12):571.
10. Overvoorde P, Fukaki H, Beeckman T. Auxin control of root development. Cold Spring Harb Perspect Biol. 2010;2(6):a001537.
11. Cao Y, Glass AD, Crawford NM. Ammonium Inhibition of Arabidopsis root growth can be reversed by potassium and by auxin resistance mutations aux1, axr1, and axr2. Plant Physiol. 1993;102(3):983–9.

12. Zou N, Li B, Dong G, Kronzucker HJ, Shi W. Ammonium-induced loss of root gravitropism is related to auxin distribution and TRH1 function and is uncoupled from the Inhibition of root elongation in Arabidopsis. J Exp Bot. 2012;63(10):3777–88.
13. Halvorsen BL, Carlsen MH, Phillips KM, Bøhn SK, Holte K, Jacobs DR Jr, Blomhoff R. Content of redox-active compounds (i.e., antioxidants) in foods consumed in the united States. Am J Clin Nutr. 2006;84(1):95–135.
14. Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B, Battino M. The Strawberry: composition, nutritional quality, and impact on human health. Nutrition. 2012;28(1):9–19.
15. Vauzour D, Vafeiadou K, Rendeiro C, Corona G, Spencer JP. The inhibitory effects of berry-derived flavonoids against neurodegenerative processes. J Berry Res. 2010;1(1):45–52.
16. Etminan M, Takkouche B, Caamano-Isorna F. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol Biomarkers Prev. 2004;13(3):340–5.
17. Tulipani S, Romandini S, Suarez JMA, Capocasa F, Mezzetti B, Battino M, Novembrino C. Folate content in different strawberry genotypes and folate status in healthy subjects after strawberry consumption. BioFactors. 2008;34(1):47–55.
18. Määttä-Riihinen KR, Kamal-Eldin A, Törrönen AR. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). J Agric Food Chem. 2004;52(20):6178–87.
19. Aaby K, Skrede G, Wrolstad RE. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). J Agric Food Chem. 2005;53(10):4032–40.
20. Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition. 2005;21(2):207–13.
21. Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Blomhoff R. A systematic screening of total antioxidants in dietary plants. J Nutr. 2002;132(3):461–71.
22. FAO. Food and agriculture data. FAOStat. 2013. Available at: http://faostat.fao.org/beta/en. Accessed 14 July 2013.
23. Nadarajan S, Sukumaran S. Chemistry and toxicology behind chemical fertilizers. In: Lewu FB, Volova T, Thomas S, Rakhimol RK, editors. Controlled release fertilizers for sustainable agriculture. Academic; 2021. pp. 195–229.
24. Maqsood MA, Naqsh-e-Zuhra, Ashraf I, Rasheed N, Shah Z. -u-H. Sources of nitrogen for crop growth: Pakistan's case. In: Aziz T, Wakeel A, Watto MA, Watto MS, Maqsood MA, Kiran A, editors. Nitrogen assessment. Academic; 2022. pp. 13–28.
25. Arnon DI. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 1949;24:1–15.
26. Heath RL, Packer L. Photoperoxidation in isolated Chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125:189–98.
27. Loreto F, Velikova V. Isoprene produced by leaves protects the photosynthetic apparatus against Ozone damage, quenches Ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 2001;127:1781–7.
28. Lin M-J, Nosaka K, Ho C-C, Chen H-L, Tseng K-W, Ratel S, Chen TC-C. Influence of maturation status on eccentric Exercise-Induced muscle damage and the repeated bout effect in females. Front Physiol. 2018;8:1118. https://doi.org/10.3389/fphys.2017.01118.
29. Zayed O, Hewedy OA, Abdelmoteleb A, Ali M, Youssef MS, Roumia AF, Seymour D, Yuan ZC. Nitrogen journey in plants: from uptake to metabolism, stress response, and microbe interaction. Biomolecules. 2023;13(10):1443. https://doi.org/10.3390/biom13101443.
30. Farhan M, Sathish M, Kiran R, Mushtaq A, Baazeem A, Hasnain A, Hakim F, Naqvi SAH, Mubeen M, Iftikhar Y, Abbas A, Hassan MZ, Moustafa M. Plant nitrogen metabolism: balancing resilience to nutritional stress and abiotic challenges. Phyton-International J Experimental Bot. 2024;93(3):581–609. https://doi.org/10.32604/phyton.2024.046857.
31. Ali R, Nagalli S. Hyperammonemia. [Updated 2023 Apr 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557504/
32. Noor H, Ding P, Ren A, Sun M, Gao Z. Effects of nitrogen fertilizer on photosynthetic characteristics and yield. Agronomy. 2023;13(6):1550. https://doi.org/10.3390/agronomy13061550.
33. Shah IH, Jinhui W, Li X, Hameed MK, Manzoor MA, Li P, Zhang Y, Niu Q, Chang L. Exploring the role of nitrogen and potassium in photosynthesis: implications for sugar accumulation and translocation in horticultural crops. Sci Hort. 2024;327:112832. https://doi.org/10.1016/j.scienta.2023.112832.
34. Fathi, Fathi A. Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: A review. Agrisost. 2022;28:1–8. https://doi.org/10.5281/zenodo.714358.
35. Abualia R, Riegler S, Benkova E. Nitrate, auxin and Cytokinin-A trio to Tango. Cells. 2023;12(12):1613. https://doi.org/10.3390/cells12121613.
36. Britto DT, Kronzucker HJ. NH_4^+ toxicity in higher plants: A critical review. J Plant Physiol. 2002;159(6):567–84. https://doi.org/10.1078/0176-1617-0774.
37. Auron A, Brophy PD. Hyperammonemia in review: pathophysiology, diagnosis, and treatment. Pediatr Nephrol. 2012;27(2):207–22. https://doi.org/10.1007/s00467-011-1838-5.
38. Qiang B, Zhou W, Zhong X, Fu C, Cao L, Zhang Y, Jin X. Effect of nitrogen application levels on photosynthetic nitrogen distribution and use efficiency in soybean seedling leaves. J Plant Physiol. 2023;287:154051. https://doi.org/10.1016/j.jplph.2023.154051.
39. Guo P, Ren J, Shi X, et al. Optimized nitrogen application ameliorates the photosynthetic performance and yield potential in peanuts as revealed by OJIP chlorophyll fluorescence kinetics. BMC Plant Biol. 2024;24:774. https://doi.org/10.1186/s12870-024-05482-x.
40. Hu J, Zheng Q, Dong C, Liang Z, Tian Z, Dai T. Enhanced stomatal conductance supports photosynthesis in wheat to improved NH_4^+ tolerance. Plants. 2024;13(1):86. https://doi.org/10.3390/plants13010086.
41. Chen H, Jia Y, Xu H, Wang Y, Zhou Y, Huang Z, Yang L, Li Y, Chen L-S, Guo J. Ammonium nutrition inhibits plant growth and nitrogen uptake in citrus seedlings. Sci Hort. 2020;272:109526. https://doi.org/10.1016/j.scienta.2020.109526.
42. Drath M, Kloft N, Batschauer A, Marin K, Novak J, Forchhammer K. Ammonia triggers photodamage of photosystem II in the Cyanobacterium Synechocystis Sp. strain PCC 6803. Plant Physiol. 2008;147(1):206–15. https://doi.org/10.1104/pp.108.117218.
43. Yang S, Hao D, Jin M, et al. Internal ammonium excess induces ROS-mediated reactions and causes carbon scarcity in rice. BMC Plant Biol. 2020;20:143. https://doi.org/10.1186/s12870-020-02363-x.
44. Liu J, Wang Y, Li Y, Peñuelas J, Zhao Y, Sardans J, Wu J. Soil ecological stoichiometry synchronously regulates stream nitrogen and phosphorus concentrations and ratios. CATENA. 2023;231:107357. https://doi.org/10.1016/j.catena.2023.107357.
45. Lin N, Luo X, Wen J, Fu J, Zhang H, Siddique KHM, Zhao Y. Black biodegradable mulching increases grain yield and net return while decreasing carbon footprint in rain-fed conditions of the loess plateau. Field Crops Res. 2024;318:109590. https://doi.org/10.1016/j.fcr.2024.109590.
46. Yang X, Xia X, Zhang Z, Nong B, Zeng Y, Wu Y, Li D. Identification of anthocyanin biosynthesis genes in rice pericarp using PCAMP. Plant Biotechnol J. 2019;17(9):1700–02. https://doi.org/10.1111/pbi.13133.
47. Ma X, Liu Y. A modified geometrical optical model of row crops considering multiple scattering frame. Remote Sens. 2020;12(21):3600. https://doi.org/10.3390/rs12213600.
48. Akpor OB, Ezekudo EO, Sobajo OA, Edoh PA, Mabayoje SO. Optimization and antimicrobial properties of biosurfactant production by four Indigenous soil bacterial species. Asian J Agric Biol. 2023;2022146. https://doi.org/10.35495/ajab.2022.146.
49. Bui VH, Nguyen HC, Ngo QH. Establishment of rice yield prediction model using soil compaction. Asian J Agric Biol. 2023;202109327. https://doi.org/10.35495/ajab.2021.09.327.
50. Fatemi R, Yarnia M, Mohammadi S, Vand EK, Mirashkari B. Screening barley genotypes in terms of some quantitative and qualitative characteristics under normal and water deficit stress conditions. Asian J Agric Biol. 2023;2022071. https://doi.org/10.35495/ajab.2022.071.
51. Fatmawati U, Sari DP, Santosa S, Wiraswati SM. IAA-producing and phosphate solubilizer of rhizosphere actinobacteria consortium to promote plant growth in soybean (*Glycine max* L). Asian J Agric Biol. 2023;2021402. https://doi.org/10.35495/ajab.2021.402.
52. Ijaz M, Afzal A, Shabbir G, Iqbal J, Rafique M. Breeding wheat for leaf rust resistance: past, present and future. Asian J Agric Biol. 2023;2021426. https://doi.org/10.35495/ajab.2021.426.
53. Ishaq L, Simamora AV, Bako PO, Benggu YI, Airthur MM, Roefaida E, Nguru ESO. Abundance of arbuscular mycorrhizal fungi in the rhizosphere of healthy and declining citrus in East Nusa Tenggara, Indonesia. Asian J Agric Biol. 2023;2023011. https://doi.org/10.35495/ajab.2023.011.
54. Ismail HN, Noor NM, Ahmad Z, Wan Anuar WNH. Algal composition in the ecosystem of rice fields under the application of herbicides and insecticides. Asian J Agric Biol. 2023;202106254. https://doi.org/10.35495/ajab.2021.06.254.

55. Li C, Ahmad S, Cao C. Mitigation of climate crisis from rice paddy field by tillage combination in central China. Asian J Agric Biol. 2023;2022122. https://doi.org/10.35495/ajab.2022.122.
56. Osei AF, Jin X, Abdullah WZBW, Sidique SNM. Silicon improves strawberry plants' nutrient uptake and epicuticular wax formation in a rhizosphere cooling system. Asian J Agric Biol. 2023;2022060. https://doi.org/10.35495/ajab.2022.060.
57. Pangaribuan DH, Widagdo S, Hariri AM, Siregar S, Sardio MI. The effect of rice straw mulch and cow urine on growth, yield, quality on sweet corn and pest population density. Asian J Agric Biol. 2023;202103123. https://doi.org/10.35495/ajab.2021.03.123.
58. Safdar ME, Ehsan A, Maqbool R, Ali A, Qamar R, Ali H. Assessing the critical period of weed competition in direct-seeded rice (*Oryza sativa* L). Asian J Agric Biol. 2023;2022190. https://doi.org/10.35495/ajab.2022.190.
59. Sennoi R, Ruttanaprasert R, Chinaworn S, Puttha R. Effects of hormone and cold treatments on dormancy breaking of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Asian J Agric Biol. 2023;2021422. https://doi.org/10.35495/ajab.2021.422.
60. Shaukat N, Farooq U, Akram K, Shafi A, Hayat Z, Naz A, Hakim A, Hayat K, Naseem S, Khan MZ. Antimicrobial potential of banana Peel: A natural preservative to improve food safety. Asian J Agric Biol. 2023;202003188. https://doi.org/10.35495/ajab.2020.03.188.
61. Sindesi OA, Ncube B, Lewu MN, Mulidzi AR, Lewu FB. Cabbage and Swiss Chard yield, irrigation requirement, and soil chemical responses in zeolite-amended sandy soil. Asian J Agric Biol. 2023;202111387. https://doi.org/10.35495/ajab.2021.11.387.
62. Taratima W, Kunpratum N, Maneerattanarungroj P. Effect of salinity stress on physiological aspects of pumpkin (*Cucurbita moschata* Duchesne 'Laikaotok') under hydroponic conditions. Asian J Agric Biol. 2023;202101050. https://doi.org/10.35495/ajab.2021.01.050.
63. Victoria O, Idorenyin U, Asana M, Jia L, Shuoshuo L, Yang S, Okoi IM, Ping A, Egrinya EA. Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress. Asian J Agric Biol. 2023;2022076. https://doi.org/10.35495/ajab.2022.076.

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