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Lanthanum nitrate enhances aroma precursors of tobacco leaves via influencing chemical components and photosynthetic characteristics

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Aroma precursors are key biochemical factors determining the aroma quality of flue-cured tobacco (*Nicotiana tabacum L*.). While rare earth elements (REEs) are known to enhance tobacco growth, their role in the biosynthesis of aroma precursors and overall quality remains insufficiently understood. In this study, a controlled pot experiment was conducted using flue-cured tobacco treated with a gradient of lanthanum nitrate [$La(NO_3)_3$] solutions (0, 25, 50, 90, 130, and 170 mg L^{-1}) applied via foliar spraying to systematically evaluate dose-dependent effects. The impacts of these treatments on aroma precursors, photosynthetic characteristics, chemical components, and agronomic traits were assessed. A hormetic concentration-dependent response showed: 25–90 mg L^{-1} lanthanum nitrate significantly increased the content of polyphenols, plastid pigments, and chemical components, while improving photosynthetic efficiency and promoting shoot and root growth. However, supraoptimal concentrations above 130 mg L^{-1} exhibited inhibitory effects, significantly reducing growth parameters, polyphenol content, plastid pigment accumulation, and photosynthetic efficiency, particularly at 170 mg L^{-1}. Partial least squares path modeling (PLSPM) revealed that the promotion of aroma precursor biosynthesis was indirectly mediated through improvements in chemical components and photosynthetic characteristics. Based on comprehensive optimization, foliar application of 90 mg L^{-1} lanthanum nitrate is proposed as the optimal concentration, achieving 33.16% and 22.20% enhancements in total phenol and total chlorophyll content, respectively, while maintaining an optimal growth-physiology balance. These findings provide novel insights into REE-mediated quality improvement mechanisms and establish a scientific basis for the precision application of lanthanum in premium tobacco production. Future studies need to employ molecular techniques such as transcriptomics and metabolomics to further elucidate how rare earth elements influence biosynthetic pathways associated with phenolic and carotenoids metabolism.

Keywords Rare earth element, Lanthanum nitrate, Plastid pigments, Polyphenols, Photosynthetic characteristics

Aroma quality is a critical determinant of commercial value in flue-cured tobacco (*Nicotiana tabacum L.*), largely shaped by the presence of aroma precursors that accumulate during leaf growth and development. These scentless and stable precursors, including plastid pigments (chlorophylls, carotenoids), polyphenols, carbohydrates, amino acids, and alkaloids[1], undergo complex transformations during curing, aging, and combustion, yielding a diverse spectrum of aromatic compounds through enzymatic, oxidative, and pyrolytic pathways[2]. For instance, chlorophyll derivatives degrade in flavor-active micromolecules compounds such as neophytadiene and furans through phytol degradation[3], while carotenoids serve as major terpene precursors, generating numerous aroma compounds that define the sensory characteristics of tobacco[4,5]. Polyphenols, which are the secondary metabolites comprising over 280 types including tannins (e.g., chlorogenic acid, caffeic

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acid), coumarins (e.g., scopoletin, scopoline), and flavonoids (e.g., rutin), contribute direct aromatic properties and secondary aromas formed through distillation, oxidation, hydrolysis, and pyrolysis[6]. Notably, chlorogenic acid, rutin, and scopoletin play critical roles in leaf development and final product quality[7], further influencing color, flavor, and taste of flue-cured tobacco[7–9].

Rare earth elements (REEs), including the 15 lanthanides plus scandium (Sc) and yttrium (Y), are lustrous, silvery-white, and soft metals with similar chemical properties[10], have been applied in agriculture since the 1980 s to enhance crop performance[11]. REEs have demonstrated trace-element-like effects on plants[12], like promoting seed germination in tomatoes and the growth of medicinal plants[13,14], improving chloroplast ultrastructure and chlorophyll biosynthesis in rice[15], increasing ROS-related enzymes activity in cucumber[16], and bolstering stress resistance[17–19]. These effects often exhibit hormesis, where low concentrations stimulate metabolic and growth responses while higher levels become inhibitory[20,21]. In tobacco specifically, low dose lanthanum promoted Hill reaction activity, Mg^{2+}-ATPase activity, and photophosphorylation in chloroplasts, whereas high concentrations suppress these processes[22].

Despite established knowledge regarding REEs' roles in plant growth and chlorophyll metabolism[15,16,23–25], their impact on tobacco quality, particularly the biosynthesis of aroma precursors, remains inadequately explored. This gap is especially salient given the economic importance of aroma characteristics in flue-cured tobacco. To bridge this knowledge gap, the present study systematically evaluates the concentration-dependent effects of foliar-applied lanthanum nitrate on aroma precursors, photosynthetic performance, chemical components, and agronomic performance in flue-cured tobacco. Our specific objectives were to (1) identify the optimal lanthanum nitrate concentration for tobacco quality enhancement, (2) quantify its effects on key physiological and biochemical traits, and (3) elucidate the mechanistic pathways through which REEs application influences aroma precursor accumulation. We hypothesized that optimal concentrations of lanthanum nitrate would improve root development, photosynthetic efficiency, and chemical profiles, thereby stimulating the biosynthesis of aroma precursors and offer insights into REE-mediated quality improvement in flue-cured tobacco.

Materials and methods

Plant material and soil

Flue-cured tobacco *Nicotiana tabacum cv.* Yunyan 87, a common commercial cultivar, used in this study were provided by the Guizhou Tobacco Science Research Institute. All experimental research on the plant material complied with relevant institutional, national, and international guidelines. Soil for the pot experiment was collected from Shiban Town, Guiyang, China (26°26′N, 106°37′E) (Fig. 1). This yellow soil with a clay loam texture had the following physiochemical properties: pH 6.5, organic carbon 20.9 g kg^{-1}, total nitrogen 1.89 g kg^{-1}, available nitrogen 160.9 mg kg^{-1}, total phosphorus 0.71 g kg^{-1}, olsen-phosphorus 27.1 mg kg^{-1}, total potassium 5.47 g kg^{-1}, and available potassium 197.7 mg kg^{-1}.

Experimental design and cultivation

A completely randomized pot experiment with three biological replicates was conducted. Lanthanum nitrate [$La(NO_3)_3$] was applied via foliar spray at six concentrations (0, 25, 50, 90, 130, and 170 mg L^{-1}, pH 5.6 ± 0.1), which were determined based on literature reports on the application of rare earth elements in tobacco and related plants. A total volume of 140 mL $La(NO_3)_3$ per plant was delivered in four split doses (20, 30, 40, and 50 mL) at 7-day intervals, starting at the 30th day after transplanting. Applications ensured complete foliar absorption without runoff.

Tobacco seedling at the five-leaf stage were transplanted into plastic pot (35 cm diameter × 26 cm height) which was filled with 15 kg of prepared soil. Plants were cultivated for 145 days under ambient temperature of approximately 28 °C. Soil moisture was maintained at 70% of field capacity by watering every two days. Conventional fertilization was applied in three stages: a base fertilizer of $N–P_2O_5–K_2O$ (9–10–27) at 750 kg ha^{-1} incorporated during pot preparation, and two top-dressing of $N–P_2O_5–K_2O$ (14–19–20) at 225 kg ha^{-1} on the 22nd and 37th day after transplanting.

Sampling and pretreatment

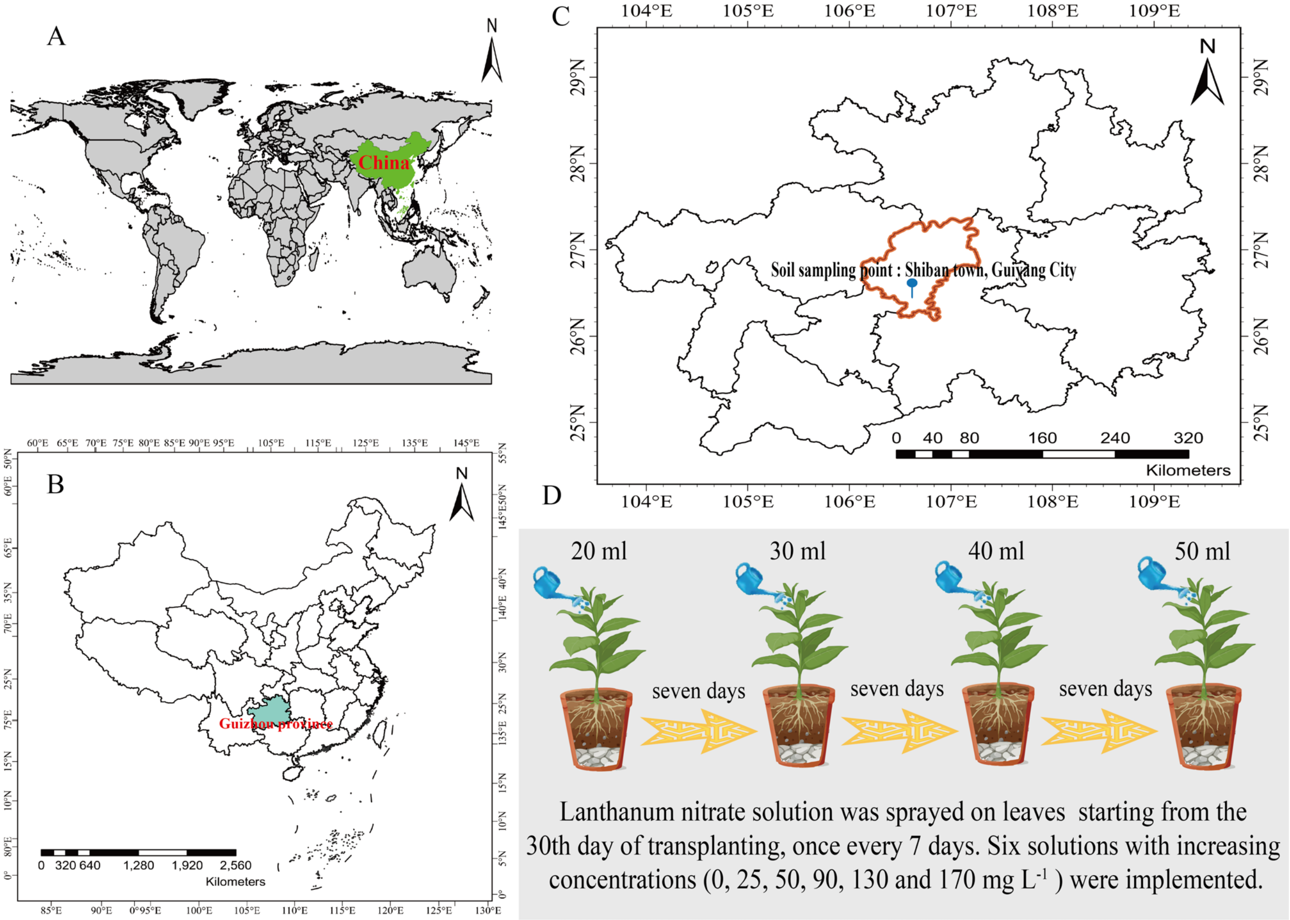
At maturity (day 145), middle tobacco leaves (positions 7th −12th from the base) were sampled and partitioned into three parts: (1) the midrib halves of the 10th leaf were stored at −80 °C for plastid pigment analysis; (2) the 11th leaf without veins was freeze-dried at −45 °C for 48 h, crushed, and stored at 4℃ in the dark for polyphenol analysis; (3) the remaining leaves were dried, grounded, and stored at room temperature for chemical component analysis.

Agronomic traits

Agronomic traits (plant height, stem girth, and length and width of the largest leaf) were measured at the rosette stage (before the second spraying), the vigorous growth stage (before the last spraying), and the maturity stage (before the harvest), according to the *Tobacco Industry Standard of the People's Republic of China: Investigating and Measuring Methods of Agronomical Characteristics of Tobacco* (YC/T 142–2010). The maximum leaf area was calculated using the following formula:

$$Maximum\, leaf\ area\ \left(cm^2\right) = Leaf\ length \times\ Leaf\ width \times\ 0.6345$$

Root morphology was digitized via Win RHIZO (Regent Instruments Ins. Canada) to analyze total length (cm), surface area (cm^2), mean diameter (cm), volume (cm^3) and number of root tips.



Lanthanum nitrate solution was sprayed on leaves starting from the 30th day of transplanting, once every 7 days. Six solutions with increasing concentrations (0, 25, 50, 90, 130 and 170 mg L^{-1}) were implemented.

Fig. 1. Soil sampling point and experimental implementation scheme. Panel (**A**) displays the location in China, panel (**B**) shows the location in Guizhou Province, panel (**C**) indicates the location of the sampling site, and panel (**D**) displays the experimental implementation scheme. The maps were created by ArcGIS Pro 3.3 (https://pro.arcgis.com/).

Chemical components

Dry ground samples of leaves were digested with H_2SO_4-H_2O_2 to analyze the content of total N, P, and K using Kjeldahl's method, vanadate-molybdate yellow colorimetric method, and flame spectrophotometry, respectively[26]. Chlorine and nicotine contents were determined using the silver nitrate volumetric method and the ultraviolet spectrophotometric method, respectively[27]. Total sugar and reducing sugar contents were estimated with the microplate colorimetric method and the 3,5-dinitrosalicylic acid colorimetric method[28,29].

Photosynthetic characteristics

Photosynthetic characteristics of fresh tobacco leaves, including transpiration rate (Tr), stomatal conductance (Gs), net photosynthetic rate (Pn), and internal CO_2 concentration (C_i), were measured using GFS-3000 Portable Photosynthesis System (WALZ GmbH, Germany). The measurements were conducted under the following controlled conditions: photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1}, ambient CO_2 concentration, leaf chamber temperature set at 25 °C, airflow rate at 750 μmol s^{-1}, and relative humidity maintained at ambient levels.

Aroma precursors

The contents of plastid pigments (chlorophyll a, chlorophyll b and carotenoid) were determined using the colorimetric method with 95% acetone extraction[30]. The total chlorophyll content was calculated as the sum of chlorophyll a and chlorophyll b.

The contents of polyphenols (neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, scopoletin and rutin) were quantified via high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II, America) under gradient elution conditions (Table S1)[31]. Separation was achieved using a ZORBAX Eclipse Plus C18 Analytical column (4.6 mm × 250 mm, 5 μm; Agilent) maintained at 30 °C. The flow rate was 1 mL min^{-1}, injection volume was 10 μL, and detection was performed at 340 nm.

For sample preparation, 1.7000 g of freeze-dried tobacco leaves were weighed into a 100 mL conical flask. Then, 20 mL of 50% HPLC-grade methanol (TEDIA) was added, and the mixture was sonicated (40 kHz) for

30 min for extraction. An additional 20 mL of 50% methanol was added, followed by another 30 min of sonication. The extract was quantitatively transferred and filtered into a culture dish. The filtrate was concentrated to less than half of its original volume in a 60 °C water bath and further reduced to 2–4 mL using vacuum freeze dryer. The concentrate was diluted to 10 mL with 50% methanol. A 2 mL aliquot was filtered through a 0.22 µm aqueous phase membrane. All steps were performed under light-protected conditions. The standard reference solutions were prepared according to the YC/T 202–2006 protocol. Total phenol content was the sum of the five measured polyphenols. A representative chromatogram of the standard compounds of five tested polyphenols is shown in Figure S1.

Statistical analysis

Data were processed using Microsoft Excel 2019 and the Data Processing System (v18.10, China)[32]. Treatments differences were assessed by one-way ANOVA (LSD, $P < 0.05$). Graphs were generated with GraphPad Prism 10. The PLS-PM model, random forest model, and correlation heatmaps were generated by *plspm*, *randomForest*, and *corrplot* packages in R (4.4.1), respectively.

Results

Root architecture

The application of $La(NO_3)_3$ exerted a concentration-dependent influence on the root growth of flue-cured tobacco (Fig. 2). Relative to the control, low to moderate concentrations (25–90 mg L^{-1}) significantly enhanced all root morphometric parameters ($P < 0.05$), with the greatest stimulation observed at 90 mg L^{-1}. At this optimal concentration, root length, root diameter, and root tip number increased by 14.42%, 31.08%, and 18.62%, respectively ($P < 0.05$). Root volume and surface area also exhibited increases, though these were not statistically significant. In contrast, higher concentrations (130–170 mg L^{-1}) progressively inhibited root growth, with the strongest suppression occurring at 170 mg L^{-1}, where root tip number decreased by 50.76% ($P < 0.05$). Overall, the 90 mg L^{-1} treatment produced the most substantial improvement in root development, confirming it as the optimal concentration for promoting root development in flue-cured tobacco.

Chemical components

Foliar lanthanum nitrate supplementation significantly altered leaf chemical components (Table 1). With the exception of nicotine and chlorine, both the absolute contents and coordination ratios of the measured chemical constituents increased significantly following lanthanum nitrate treatment ($P < 0.05$). Treatments with 50–130

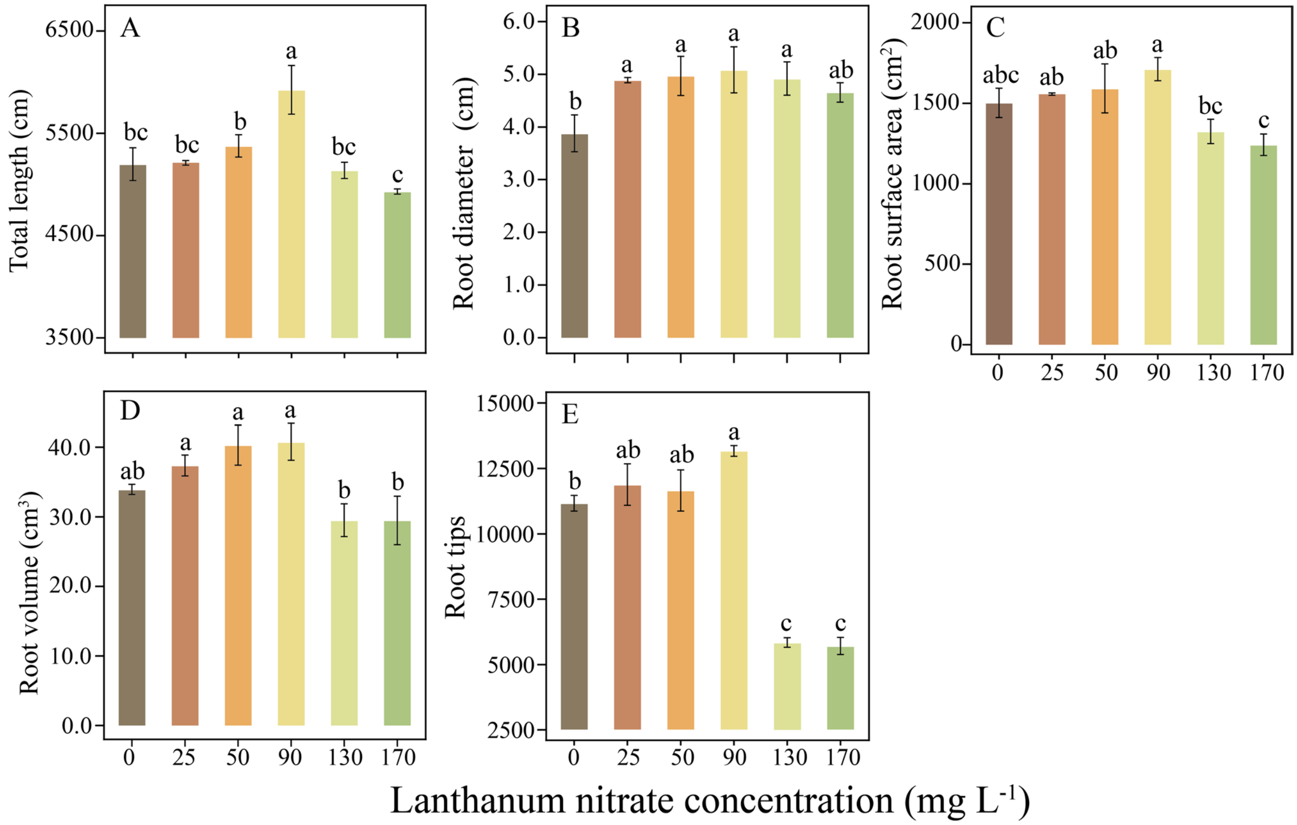


Fig. 2. Effect of lanthanum nitrate on the root architecture of flue-cured tobacco. The error bar represents standard error of 3 individual plants ($n = 3$). Different lowercase letters on the bars indicate statistical difference between treatments (LSD, $P < 0.05$).

Lanthanum nitrate dose	Total *N*	Total *P*	Total K	Nicotine	Chlorine	Reducing sugar	Total sugar	K/Chlorine	*N*/Nicotine
(mg L^{-1})	(%)	(%)	(%)	(%)	(%)	(%)	(%)		
0	4.06 ± 0.10c	0.30 ± 0.01 cd	1.91 ± 0.05d	1.50 ± 0.07a	0.96 ± 0.02a	2.53 ± 0.01c	3.31 ± 0.05c	1.99 ± 0.01b	2.73 ± 0.21b
25	4.53 ± 0.08b	0.32 ± 0.01bc	2.10 ± 0.02c	1.70 ± 0.15a	0.90 ± 0.02abc	2.92 ± 0.09bc	4.26 ± 0.33b	2.34 ± 0.04a	2.73 ± 0.30b
50	4.56 ± 0.11b	0.34 ± 0.02bc	2.33 ± 0.07a	1.44 ± 0.08a	0.90 ± 0.03ab	3.06 ± 0.18ab	4.20 ± 0.18b	2.43 ± 0.23a	3.20 ± 0.26ab
90	4.96 ± 0.13a	0.40 ± 0.00a	2.16 ± 0.03bc	1.42 ± 0.20a	0.83 ± 0.01c	3.37 ± 0.20a	5.45 ± 0.13a	2.62 ± 0.08a	3.59 ± 0.37a
130	4.93 ± 0.15a	0.35 ± 0.00b	2.28 ± 0.03ab	1.67 ± 0.14a	0.86 ± 0.03bc	2.98 ± 0.12ab	4.45 ± 0.12b	2.57 ± 0.02a	2.99 ± 0.24ab
170	4.54 ± 0.13b	0.27 ± 0.01d	2.20 ± 0.07abc	1.47 ± 0.06a	0.85 ± 0.01bc	2.96 ± 0.14abc	4.42 ± 0.24b	2.48 ± 0.14a	3.10 ± 0.20ab

Table 1. Effect of lanthanum nitrate on the chemical components of flue-cured tobacco leaves. Data represents means ± standard error of 3 individual plants ($n = 3$). Different lowercase letters indicate statistical difference between treatments (LSD, $P < 0.05$). K/Chlorine: ratio of total K content to nicotine content; N/Nicotine: ratio of total N content to nicotine content.

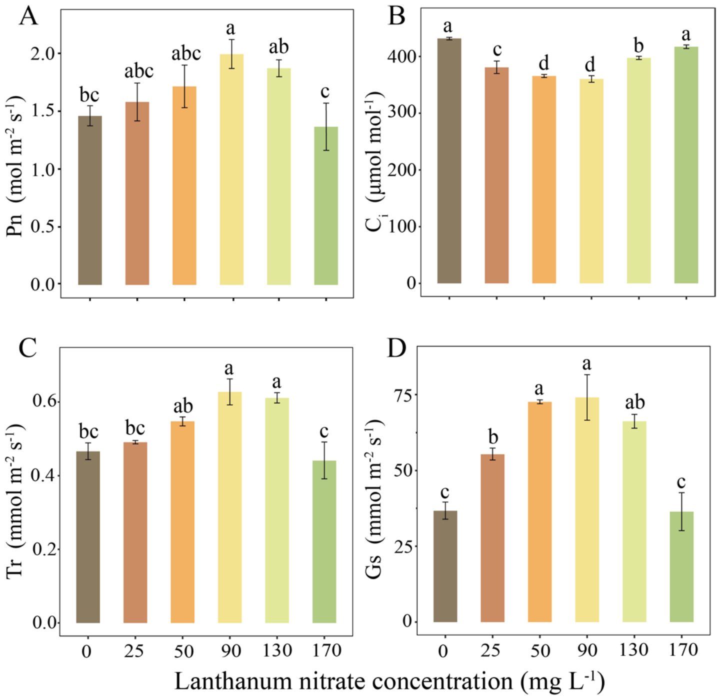


Fig. 3. Effect of lanthanum nitrate on photosynthetic characteristics of flue-cured tobacco. Pn: net photosynthetic rate, C_i: internal CO_2 concentration, Tr: transpiration rate, Gs: stomatal conductance. The error bar represents standard error of 3 individual plants ($n = 3$). Different lowercase letters on the bars indicate statistical difference between treatments (LSD, $P < 0.05$).

mg L^{-1} lanthanum nitrate exhibited significantly higher chemical contents and coordination ratios compared to the control. Based on the established optimal ranges for high-quality tobacco chemical components[33], all lanthanum nitrate treatments improved tobacco quality, with the most pronounced effect observed at 90 mg L^{-1}. Notably, the changes in chemical components paralleled those observed in root architecture. Correlation analysis demonstrated a strong positive relationship between root length and the content of key leaf chemical compounds, including total nitrogen, total potassium, total sugar, and reducing sugar (Fig. 7). These findings suggest that root morphological traits, particularly length and diameter, are positively associated with the accumulation of beneficial chemical compounds in tobacco leaves, like total nitrogen, total potassium, total sugar, and reducing sugar.

Photosynthetic characteristic

Lanthanum nitrate significantly affected the photosynthetic performance of flue-cured tobacco (Fig. 3). A clear concentration-response relationship was observed for key photosynthetic parameters, including the net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs). In the 25–130 mg L^{-1} range, all three parameters showed significant increase, with the 90 mg L^{-1} treatment producing the most pronounced enhancements compared to the control: Pn, Tr, and Gs increased by 36.60%, 34.70% and 101.84%, respectively. At 170 mg L^{-1}, these parameters decreased slightly by 6.39%, 5.33%, and 0.80%, respectively, indicating inhibitory effects at supraoptimal concentrations. The intercellular CO_2 concentration (C_i) displayed an inverse trend, decreasing significantly in the 25–130 mg L^{-1} range, with the lowest value recorded at 90 mg L^{-1}, but returning to values similar to the control at 170 mg L^{-1}. Overall, these results demonstrate that lanthanum nitrate concentrations between 25 and 130 mg L^{-1} enhance photosynthetic efficiency in flue-cured tobacco, with 90 mg L^{-1} identified as the optimal concentration for maximizing photosynthetic performance.

Aroma precursor content

The application of lanthanum nitrate significantly altered plastid pigments content in flue-cured tobacco (Fig. 4). Compared to the control, treatments of 90 mg L^{-1} and 130 mg L^{-1} lanthanum nitrate resulted in significant increases in chlorophyll a content by 23.63% and 18.77%, respectively ($P < 0.05$). Carotenoids content also increased notably in the 50–130 mg L^{-1} range, showing increases of more than 30%. Furthermore, the 90 mg L^{-1} treatment significantly enhanced both chlorophyll a + b and chlorophyll b levels by 22.20% and 18.66%, respectively, while other concentrations had no statistically significant effect. Overall, lanthanum nitrate markedly influenced plastid pigments, with 90 mg L^{-1} yielding the most pronounced enhancement.

Lanthanum nitrate elicited distinct concentration-response patterns across polyphenols in flue-cured tobacco leaves (Fig. 5). Except for scopoletin, all measured polyphenols exhibited significant concentration-dependent changes. Treatments in the 25–90 mg L^{-1} range significantly increased chlorogenic acid, rutin, and total phenol compared with the control, with peak increases of 64.96%, 27.17%, and 33.16%, respectively, at 90 mg L^{-1} ($P < 0.05$). In contrast, cryptochlorogenic acid and total phenol of the 170 mg L^{-1} treatment decreased by 78.93% and 46.70%, respectively. Additionally, lanthanum nitrate application significantly inhibited scopoletin accumulation, with increasing inhibition observed at higher concentrations, with a 50.06% reduction occurring at 170 mg L^{-1}. These results indicate that lanthanum nitrate modulates polyphenol biosynthesis in a concentration-dependent manner, with optimal enhancement observed at 25–90 mg L^{-1}, particularly at 90 mg L^{-1}.

Factors influencing aroma precursors

Random forest modeling highlighted the relative contributions of tobacco root architecture, photosynthetic characteristics, and chemical components to the accumulation of polyphenols (Fig. 6A–F) and plastid pigments (Fig. 6G-I). Among the evaluated parameters, K/chlorine, total P, chlorine, net photosynthetic rate (Pn) and total sugar were identified as primary determinants of plastid pigments, whereas polyphenol synthesis was most strongly associated with intercellular CO_2 concentration (Ci), root tips, root length, total P, and stomatal conductance (Gs).

Correlation analysis (Fig. 7) revealed a positive correlation between lanthanum nitrate concentration and plastid pigments, particularly chlorophyll a and carotenoids ($P < 0.05$). No significant correlation was observed between plastid pigments and root architectural traits. In addition, plastid pigments correlated strongly with leaf chemical components, including total P, chlorine, K/chlorine ratio, and total sugar, as well as with photosynthetic parameters, especially Tr, Pn, and Gs, carotenoids exhibiting the strongest positive correlations

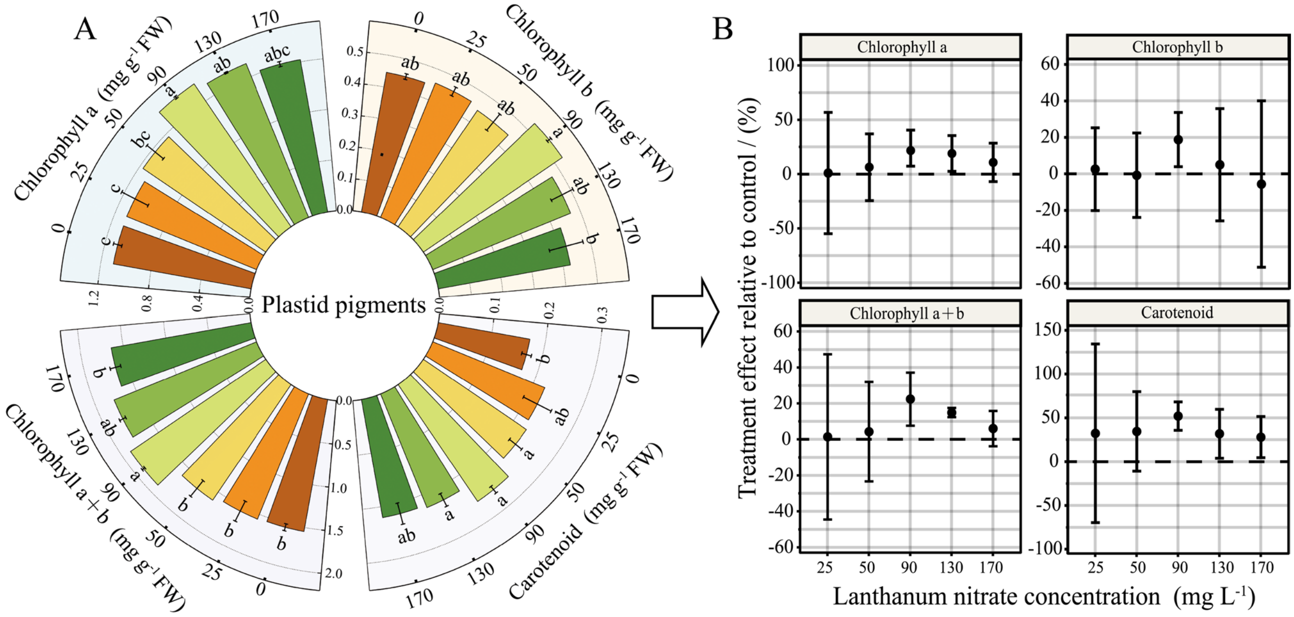


Fig. 4. The plastid pigments of tobacco leaves under different lanthanum nitrate concentrations treatments (**A**) and the changes compared to control (**B**). The error bars in subgraph A represent standard error of 3 individual plants ($n = 3$). Different lowercase letters above the bars indicate significant differences between treatments (LSD, $P < 0.05$). In subgraph B, vertical lines indicate the confidence intervals of the effect size between the treatments and the control.

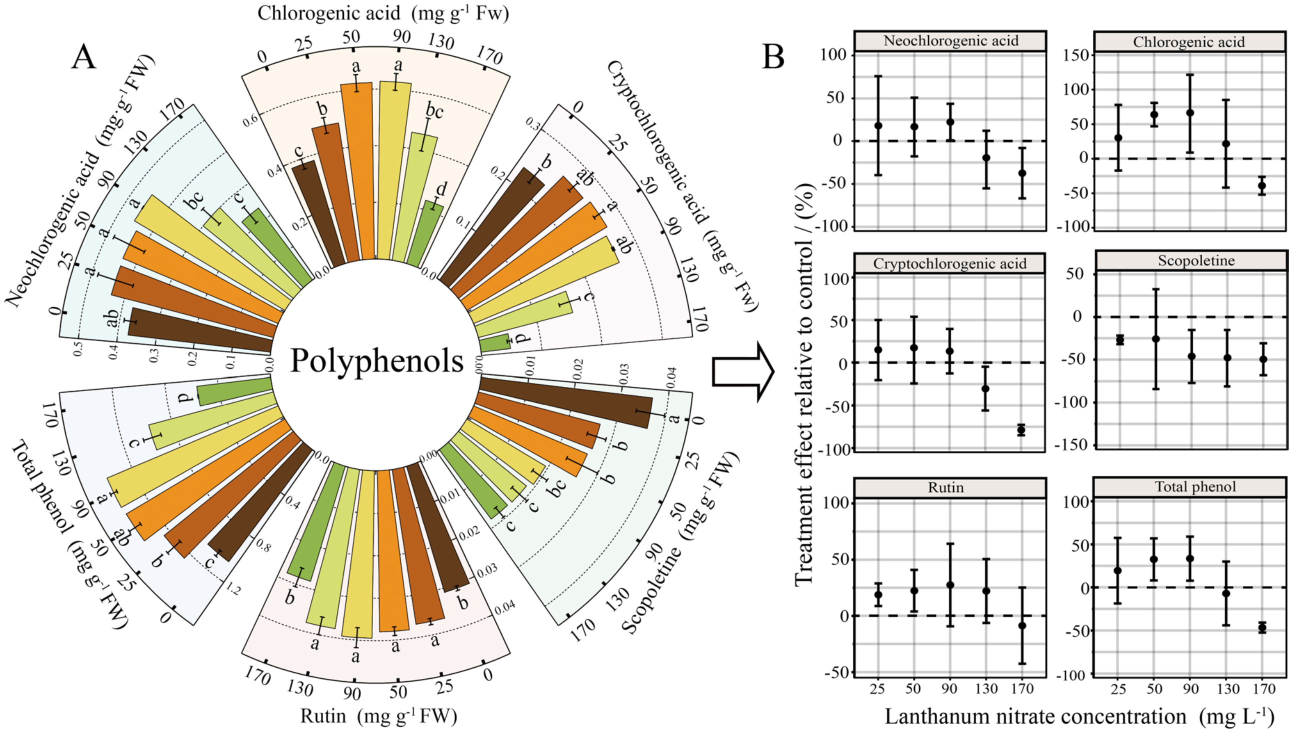


Fig. 5. The polyphenols contents of tobacco leaves under different lanthanum nitrate concentrations treatments (**A**) and the changes compared to control (**B**). The error bars in subgraph A represent standard error of 3 individual plants ($n = 3$). Different lowercase letters above the bars indicate significant differences between treatments (LSD, $P < 0.05$). In subgraph B, vertical lines indicate the confidence intervals of the effect size between the treatments and the control.

($P < 0.05$). Regarding polyphenols, scopoletin was the only compound negatively correlated with lanthanum nitrate concentration. All other polyphenols, except scopoletin and rutin, showed significant positive correlations with root architecture. Furthermore, polyphenol levels were positively correlated with Pn and Gs, negatively correlated with Ci.

Regulatory mechanisms

Partial least squares path modeling (PLSPM) was used to quantify the interactions among variables and identify the key drivers of two aroma precursors, providing mechanistic insights into the positive effects of lanthanum nitrate (0–130 mg L^{-1}) on plastid pigments and polyphenols. The analysis revealed that lanthanum nitrate indirectly promoted the biosynthesis of both polyphenols and plastid pigments by positively influencing chemical components and photosynthetic characteristics (Fig. 8A, C), contributing to total positive effects of 67.1% and 63.6%, respectively (Fig. 8B, D). Among these influencing factors, chemical components and photosynthetic characteristics were confirmed as the primary drivers of the biosynthesis of aroma precursors in flue-cured tobacco.

Discussion

Alteration of aroma precursors in tobacco leaves by lanthanum nitrate

Lanthanum is widely utilized in agriculture due to its ability to regulate metabolism and stimulate crop growth[34–36]. Among various compounds, lanthanum nitrate is one of the most commonly applied forms and has been demonstrated to promote plant development[37]. Plastid pigments, which are key aroma precursors in tobacco, degrade into volatile neutral aroma compounds such as neophytadiene, contributing to the distinctive aroma of flue-cured tobacco[3]. Previous studies have shown that rare earth elements (REEs), including lanthanum, enhance plant cell proliferation, increase chlorophyll content, and accelerate photosynthetic reactions[37–40], particularly at low concentrations[22].

Our findings corroborate these effects, revealing that low concentrations of lanthanum nitrate significantly increased the levels of chlorophyll a, chlorophyll b, and carotenoids, thereby enhancing the photosynthetic capacity[41] and production potential[42] of tobacco leaves. Such results may be primarily attributed to the ability of low-concentration lanthanum nitrate to activate clathrin-mediated endocytosis, induce root uptake, and promote Mg translocation to leaves[43], potentially facilitating plastid pigment biosynthesis. Moreover, lanthanum may also upregulate genes involved in chlorophyll synthesis to stimulate plastid pigment biosynthesis[44]. However, high concentrations of lanthanum may competitively inhibited the uptake of essential elements such as Mg and Ca, disrupted chloroplast ultrastructure, and consequently impaired chlorophyll biosynthesis[45,46], which could

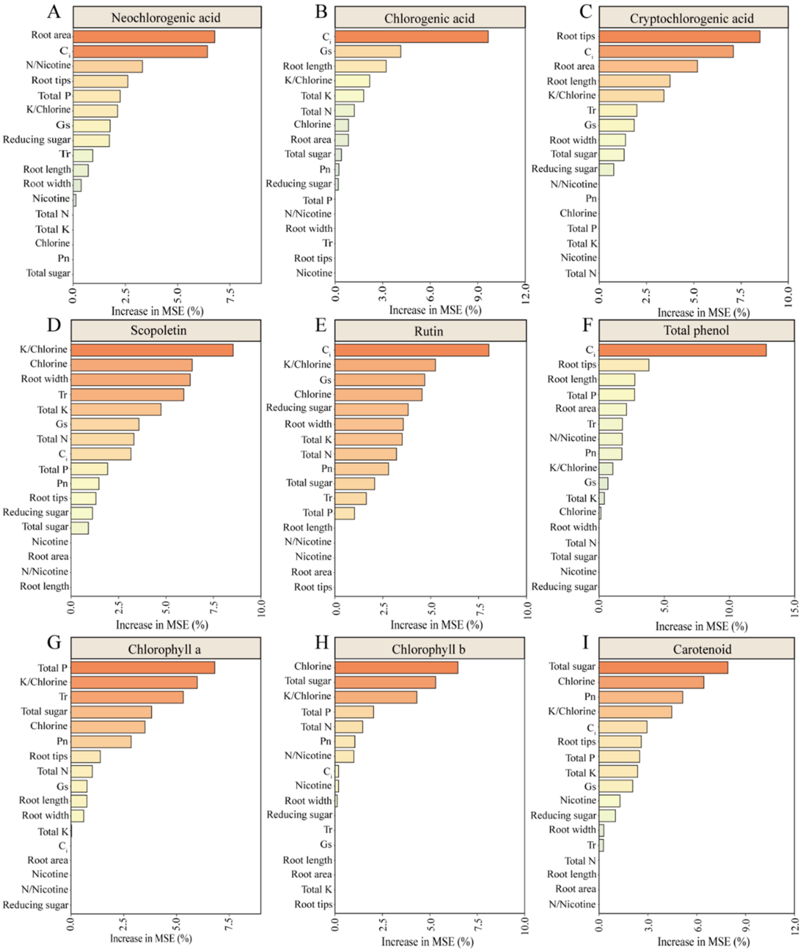


Fig. 6. Variable importance of root architecture, chemical components, and photosynthetic characteristics for estimating polyphenols (**A-F**) and plastid pigments (**G-I**) in flue-cured tobacco based on random forest model. The percentage of increase in MSE is an indicator used for evaluating the importance of a variable. The smaller the percentage of the variable, the less impact it has. Pn: net photosynthetic rate, C_i: internal CO_2 concentration, Tr: transpiration rate, Gs: stomatal conductance, K/Chlorine: Ratio of total K content to nicotine content, N/Nicotine: Ratio of total N content to nicotine content.

explain the observed reduction in plastid pigment content. Notably, the optimum lanthanum concentration may vary depending on the accompanying anion and the cultivation system. In this study, 90 mg L^{-1} lanthanum nitrate (equivalent to 29 mg L^{-1} lanthanum) significantly enhanced pigment levels. Whereas, Chen et al. reported optimal chlorophyll content at 20 mg L^{-1} lanthanum chloride (equivalent to 11 mg L^{-1} lanthanum) under hydroponic conditions[22]. This discrepancy may be explained by (1) the differential ion preferences of tobacco - sensitive to Cl^- but preferring NO_3^-, which may widen the effective concentration range of lanthanum, and (2) the stronger buffering capacity of soil than liquid media, which may allow tobacco plants to tolerate higher lanthanum concentrations.

Polyphenols are essential secondary metabolites and aroma precursors in tobacco, playing a crucial role in enhancing leaf quality due to their oxidizing properties[47]. Representative phenolic aroma precursors, including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, scopoletin, and rutin, constitute over 80% of total polyphenols in tobacco leaves[48]. Similar to plastid pigments, polyphenol content responded to lanthanum nitrate in a concentration-dependent manner (Fig. 5). At concentrations ≤ 90 mg L^{-1}, total phenol levels and most phenolic aroma precursors, except for scopoletin, were significantly enhanced, while higher concentrations inhibited their accumulation. These trends align with previous observations for cerium induced effects on phenolic metabolism[31].

We hypothesize that the stimulatory effects observed at low concentrations may result from the activation of phenylalanine ammonia-lyase (PAL), a crucial enzyme in polyphenol metabolism[49], along with a mild accumulation of reactive oxygen species (ROS) that could initiate defense-related signaling pathways and enhance polyphenol metabolism[50]. Conversely, the inhibition effects at high concentrations are likely attributed to disruption of cellular membrane integrity and damage to organelles involved in polyphenol metabolism[51]. Additionally, lanthanum may competitively bind to essential cofactors such as Ca^{2+} and Fe^{2+} within enzyme active sites, potentially impairing the activity of key enzymes involved in polyphenol production[52].

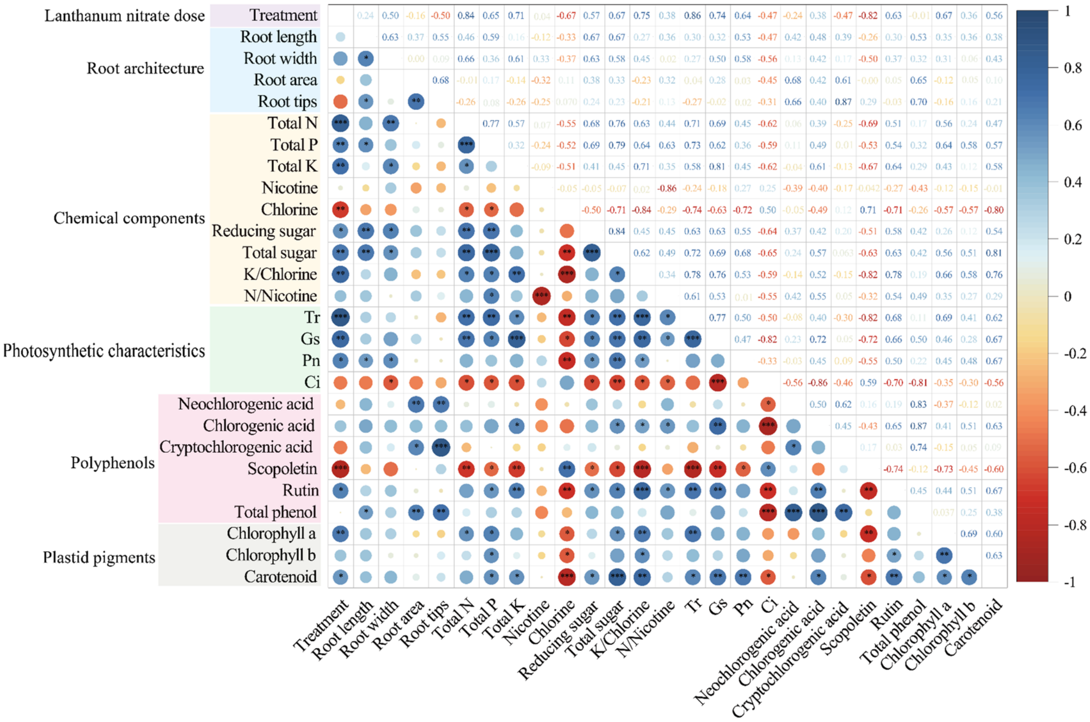


Fig. 7. Correlation analysis between root architecture, chemical composition, photosynthetic characteristics and aroma precursors in flue-cured tobacco at 0–130 mg L^{-1} lanthanum nitrate. *, **, *** represent significant correlations at the 0.05, 0.01, 0.001 level, respectively. Pn: net photosynthetic rate, C_i: internal CO_2 concentration, Tr: transpiration rate, Gs: stomatal conductance, K/Chlorine: Ratio of total K content to nicotine content, N/Nicotine: Ratio of total N content to nicotine content.

Regulation of photosynthetic characteristics in flue-cured tobacco by lanthanum nitrate

Photosynthesis is fundamental to plant growth and metabolism[53]. In this study, a clear concentration-dependent effect of lanthanum nitrate on key photosynthetic parameters was observed (Fig. 3). We propose that at lower concentrations (e.g., 90 mg L^{-1}), lanthanum nitrate likely enhances the photosynthetic rate primarily by improving stomatal conductance, thereby facilitating CO_2 uptake and availability within the chloroplasts. This facilitation may be further supported by more efficient light energy utilization and enhanced electron transport efficiency, collectively contributing to an elevated photosynthetic capacity[54]. Conversely, the suppression of photosynthetic rate and stomatal conductance at higher concentrations (e.g., 170 mg L^{-1}) may be attributed to impaired stomatal opening or structural damage to photosystem II and chloroplast integrity[55]. These findings are consistent with previous studies[37] indicating that excess REEs reduce RuBP carboxylase activity and limit CO_2 assimilation, leading to photosynthetic inhibition[15,39]. Overall, these results suggest that an optimal concentration of lanthanum nitrate can enhance photosynthesis performance in flue-cured tobacco, whereas supraoptimal levels exert detrimental effects.

Modulation of chemical component in flue-cured tobacco leaves by lanthanum nitrate

The aroma quantity, quality, and style of tobacco are largely determined by the composition and coordination of various chemical components. At low concentrations, REEs act as cell cycle inducers, enhancing membrane protease activity, ion transport, and nutrient uptake[56]. Consistent with these effects, our results further confirmed that lower concentrations of lanthanum nitrate significantly increased nitrogen, potassium, and sugar contents, improved the overall coordination of chemical components, and reduced chlorine content, ultimately enhancing tobacco quality (Table 1). In contrast, the negative effects observed at high concentrations of may result from disruption of membrane protein integrity, alterations in cellular microstructure, and impaired membrane permeability[57], which collectively diminish the electrochemical gradient and inhibit active transport of mineral elements[58]. These differential responses are further supported by corresponding variation in agronomic traits (Fig. 2, Table S2, Fig. S2), reinforcing the conclusion that lower concentrations of REEs may improve nutrient balance and tobacco quality, whereas higher concentrations could exert detrimental effects.

Interactions among root architecture, chemical components, photosynthetic characteristics, and aroma precursors in flue-cured tobacco

This interplay between root architecture, chemical components, photosynthetic characteristics, and aroma precursors was systematically analyzed in this study (Fig. 8). Random forest analysis ranked the relative importance of various growth indicators in the formation of aroma precursors (Fig. 6), revealing that these three domains are closely interconnected and essential for the accumulation of aroma precursors, which are critical for

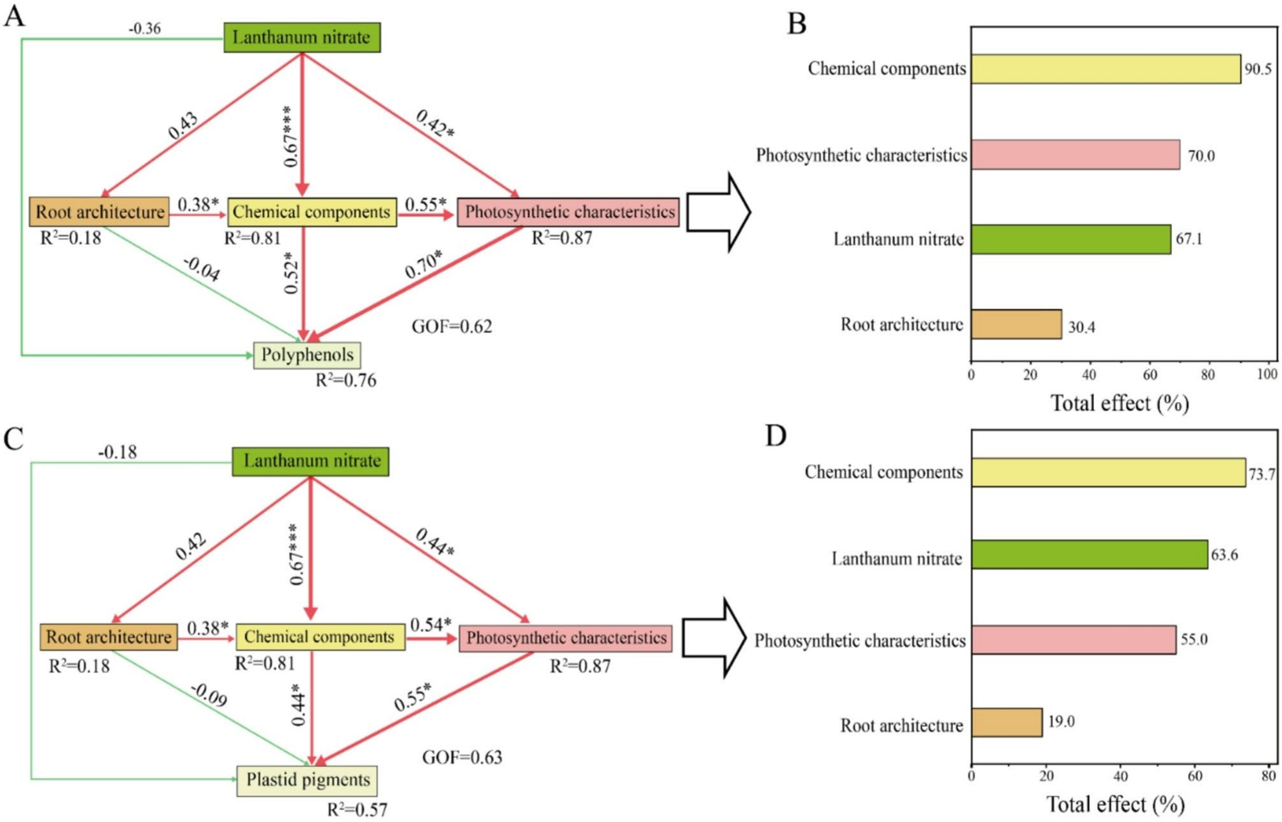


Fig. 8. PLS-PM analysis of polyphenols (**A**, **B**) and plastid pigment (**C**, **D**). Red and green arrows indicated positive and negative correlation, respectively. The thickness of arrow means the magnitude of the path coefficient in the model. Numbers and *, **, *** on the arrows show the path coefficient and the significant differences of 0.05, 0.01, 0.001, respectively. The model was assessed using the Goodness of Fit (GoF).

sensory quality of flue-cured tobacco[59]. The synthesis of aroma precursors depends on the interactions among various chemical components in leaves.

The PLS-PM model demonstrated that lanthanum nitrate influences aroma precursors indirectly through its effects on root architecture, chemical components, and photosynthetic characteristics (Fig. 8). Well-developed roots enhance nutrient absorption[60], which supports transpiration and facilitates nutrient transport to leaves[61], thereby providing the necessary substrates and conditions for the synthesis of plastid pigments and polyphenols. Lanthanum nitrate improves nutrient utilization efficiency and root architecture, collectively promoting the accumulation of related metabolic products.

Pearson correlation analysis (Fig. 7) further supported these findings, showing that photosynthetic characteristics and nutrient accumulation indirectly contribute to the synthesis of plastid pigments and polyphenols. Together, these results suggest a complex regulatory network through which lanthanum nitrate enhances aroma precursor biosynthesis, emphasizing the crucial role of photosynthesis and nutrient dynamics in this process. While this study provides valuable macroscopic insights, the precise molecular mechanisms involved remain to be fully elucidated and warrant further investigation.

Conclusion

Lanthanum nitrate application significantly affected aroma precursors, chemical components, photosynthetic characteristics, and overall growth of flue-cured tobacco in a concentration-dependent manner. Low concentrations, particularly 90 mg L^{-1} here, produced the most pronounced beneficial effects, stimulating the accumulation of plastid pigments and polyphenols, enhancing chemical components and its coordination, boosting photosynthetic characteristics, and stimulating plant growth. In contrast, higher concentrations diminished or even reversed these benefits. PLS-PM analysis indicated that the positive effects of lanthanum nitrate on aroma precursor biosynthesis were primarily mediated indirectly through improvements in chemical components and photosynthetic traits. These findings provide practical guidance for the precision application of lanthanum nitrate in tobacco cultivation, suggesting that moderate dosages can be strategically applied to improve leaf quality and aroma profile, which is of particular value for premium tobacco production. Furthermore, this study highlights the potential of rare earth elements as agronomic regulators, offering opportunities for optimizing fertilization practices in a sustainable and quality-oriented manner.

Data availability

The data supporting the findings of this study are available from the corresponding author (lliu7@gzu.edu.cn; swyang@gzu.edu.cn).

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References

1. Liu, A. et al. Proteomic and metabolomic revealed differences in the distribution and synthesis mechanism of aroma precursors in Yunyan 87 tobacco leaf, stem, and root at the seedling stage. *ACS Omega*. **7**, 33295–33306 (2022).
2. Geng, Z. et al. Aroma precursors of cigars from different tobacco parts and origins, and their correlations with sensory characteristics. *Front. Plant. Sci.* **14**, 1264739 (2023).
3. Wu, Y. et al. Leaf senescence and its relationship with plastid pigment degradation content and the degradation products of different varieties of flue-cured tobacco. *Acta Agriculturae Boreali-Sinica*. **30**, 197–204 (2015).
4. Leng, X. et al. Genome-wide identification and characterization of genes involved in carotenoid metabolic in three stages of grapevine fruit development. *Sci. Rep.* **7**, 4216 (2017).
5. Wahlberg, I. et al. Effects of flue-curing and ageing on the volatile, neutral and acidic constituents of virginia tobacco. *Phytochemistry* **16**, 1217–1231 (1977).
6. Liu, A. et al. Metabolomics and proteomics revealed the synthesis difference of aroma precursors in tobacco leaves at various growth stages. *Plant. Physiol. Biochem.* **192**, 308–319 (2022).
7. Zou, X. et al. Screening of polyphenols in tobacco (nicotiana tabacum) and determination of their antioxidant activity in different tobacco varieties. *ACS Omega*. **6**, 25361–25371 (2021).
8. Li, A. et al. Resources and biological activities of natural polyphenols. *Nutrients* **6**, 6020–6047 (2014).
9. Pandey, K. B. & Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* **2**, 270–278 (2009).
10. Migaszewski, Z. M. & Galuszka, A. The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: a review. *Crit. Rev. Environ. Sci. Technol.* **45**, 429–471 (2015).
11. Wang, Z., Zhang, X. & Mu, Y. Effects of rare-earth fertilizers on the emission of nitrous oxide from agricultural soils in China. *Atmos. Environ. (1994)*. **42**, 3882–3887 (2008).
12. Diatloff, E., Smith, F. W. & Asher, C. J. Effects of lanthanum and cerium on the growth and mineral nutrition of corn and Mungbean. *Ann. Bot.* **101**, 971–982 (2008).
13. Sobarzo-Bernal, O., Gómez-Merino, F. C., Alcántar-González, G. & Saucedo-Veloz, C. Trejo-Téllez, L. I. Biostimulant effects of cerium on seed germination and initial growth of tomato seedlings. *Agronomy* **11**, 1525 (2021).
14. Zhang, C., Li, Q., Zhang, M., Zhang, N. & Li, M. Effects of rare earth elements on growth and metabolism of medicinal plants. *Acta Pharm. Sin B* **3**, 20–24 (2013).
15. Wang, L., Wang, W., Zhou, Q. & Huang, X. Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. *Chemosphere* **112**, 355–361 (2014).
16. Shi, P., Chen, G. C. & Huang, Z. W. Effects of La^{3+} on the active oxygen-scavenging enzyme activities in cucumber seedling leaves. *Russ J. Plant. Physiol.* **3**, 338–342 (2005).
17. Liang, C., Li, L. & Su, L. Effect of lanthanum on plasma membrane H+-ATPase in rice (Oryza sativa) under acid rain stress. *J. Plant. Growth Regul.* **37**, 380–390 (2018).
18. Salgado, O. G. G. et al. Cerium alleviates drought-induced stress in phaseolus vulgaris. *J. Rare Earths*. **38**, 324–331 (2020).
19. Tao, Y. et al. Distribution of rare Earth elements (REEs) and their roles in plant growth: a review. *Environ. Pollut*. **298**, 118540 (2022).
20. Agathokleous, E., Kitao, M. & Calabrese, E. J. The rare Earth element (REE) lanthanum (La) induces hormesis in plants. *Environ. Pollut*. **238**, 1044–1047 (2018).
21. Agathokleous, E., Kitao, M. & Calabrese, E. J. Hormetic dose responses induced by lanthanum in plants. *Environ. Pollut*. **244**, 332–341 (2019).
22. Chen, W. J., Tao, Y., Gu, Y. H. & Zhao, G. W. Effect of lanthanide chloride on photosynthesis and dry matter accumulation in tobacco seedlings. *Biol. Trace Elem. Res.* **79**, 169–176 (2001).
23. Gao, Y., Huang, W., Zhu, L. & Chen, J. Effects of LaCl3 on the growth and photosynthetic characteristics of Fny-infected tobacco seedlings. *J. Rare Earths*. **30**, 725–730 (2012).
24. Lian, H. et al. Foliar-applied lanthanum chloride increases growth and phosphorus acquisition in phosphorus-limited Adzuki bean seedlings. *Plant. Soil.* **442**, 385–399 (2019).
25. Liu, D. et al. The effects of cerium on the growth and some antioxidant metabolisms in rice seedlings. *Environ. Sci. Pollut Res. Int.* **19**, 3282–3291 (2012).
26. Bao, S. *Soil and Agricultural Chemistry Analysis* (China Agriculture, 2000).
27. Wang, R. *Tobacco Chemistry* (China Agriculture, 2003).
28. Lindsay, H. A colorimetric Estimation of reducing sugars in potatoes with 3,5-dinitrosalicylic acid. *Potato Res.* **3**, 170–176 (1973).
29. Wang, Y. et al. Establishment and application of a method for rapid determination of total sugar content based on colorimetric microplate. *Sugar Tech.* **19**, 424–431 (2017).
30. Gao, M. et al. Effect of cadmium on polystyrene transport in parsley roots planted in a split-root system and assessment of the combined toxic effects. *Sci. Total Environ.* **924**, 171633 (2024).
31. Yang, S. et al. Effects of cerium on aroma precursors and growth of tobacco. *J. Chin. Soc. Rare Earths*. **42**, 138–148 (2024).
32. Tang, Q. Y. & Zhang, C. X. Data processing system (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. *Insect Sci.* **20**, 254–260 (2013).
33. Hu, W. et al. Flue-cured tobacco (Nicotiana tabacum L.) leaf quality can be improved by grafting with potassium-efficient rootstock. *Field Crops Res.* **274**, 108305 (2021).
34. Martinez, R. E., Pourret, O., Faucon, M. & Dian, C. Effect of rare Earth elements on rice plant growth. *Chem. Geol.* **489**, 28–37 (2018).
35. Tyler, G. Rare Earth elements in soil and plant systems – a review. *Plant. Soil.* **267**, 191–206 (2004).
36. Xu, Y., Zhang, G., Wang, Y. & Guo, G. Effect of $La(NO_3)_3$ and $Ce(NO_3)_3$ on shoot induction and seedling growth of in vitro cultured Anoectochilus Roxburghii. *J. Plant. Biol.* **59**, 105–113 (2016).
37. Ma, Y. et al. Stimulatory effect of lanthanum nitrate on the root tuber yield of Pseudostellaria heterophylla via improved photosynthetic characteristics. *J. Rare Earths*. **35**, 610–620 (2017).
38. Chen, A., Shi, Q., Xie, X., Huang, X. & Chen, Y. Research progress of rare Earth elements biological effect on algae. *Chin. Rare Earths*. **35**, 103–109 (2014).
39. Cui, W. et al. Lanthanum chloride improves maize grain yield by promoting photosynthetic characteristics, antioxidants enzymes and endogenous hormone at reproductive stages. *J. Rare Earths*. **37**, 781–790 (2019).

40. Song, G., Zhang, P., Shi, G., Wang, H. & Ma, H. Effects of $CeCl_3$ and $LaCl_3$ on callus and root induction and the physical response of tobacco tissue culture. *J. Rare Earths* **36**, 440–448 (2018).
41. Croft, H. et al. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Glob Chang. Biol.* **23**, 3513–3524 (2017).
42. Li, Y. et al. Variation in leaf chlorophyll concentration from tropical to cold-temperate forests: association with gross primary productivity. *Ecol. Indic.* **85**, 383–389 (2018).
43. Cheng, M. et al. Lanthanum (III) triggers AtrbohD- and jasmonic acid-dependent systemic endocytosis in plants. *Nat. Commun.* **12**, 4327 (2021).
44. van Doan, C. et al. The rare earth element lanthanum (La) accumulates in brassica Rapa L. and affects the plant metabolism and mineral nutrition. *Plants (Basel)* **14**, 692 (2025).
45. Song, K. et al. Experimental and theoretical study of the effects of rare earth elements on growth and chlorophyll of alfalfa (medicago sativa L.) seedling. *Front. Plant. Sci.* **12**, 731838 (2021).
46. Jiang, D., Gao, W. & Chen, G. Toxic effects of lanthanum (III) on photosynthetic performance of rice seedlings: combined chlorophyll fluorescence, chloroplast structure and thylakoid membrane protein assessment. *Ecotoxicol. Environ. Saf.* **267**, 115627 (2023).
47. Zhao, S., Wu, Z., Lai, M., Zhao, M. & Lin, B. Determination of optimum humidity for air-curing of cigar tobacco leaves during the Browning period. *Ind. Crops Prod.* **183**, 114939 (2022).
48. Li, Y. et al. Metabolome of flue-cured tobacco is significantly affected by the presence of leaf stem. *BMC Plant. Biol.* **23**, 89 (2023).
49. Xin, P., Shuang-Lin, Z., Jun-Yao, H. & Li, D. Influence of rare earth elements on metabolism and related enzyme activity and isozyme expression in tetrastigma hemsleyanum cell suspension cultures. *Biol. Trace Elem. Res.* **152**, 82–90 (2013).
50. Sahu, B., Sahu, A. K., Thomas, V. & Naithani, S. C. Reactive oxygen species, lipid peroxidation, protein oxidation and antioxidative enzymes in dehydrating Karanj (Pongamia pinnata) seeds during storage. *S Afr. J. Bot.* **112**, 383–390 (2017).
51. Yang, G. et al. Living target of Ce (III) action on horseradish cells: proteins on/in cell membrane. *Biol. Trace Elem. Res.* **150**, 396–402 (2012).
52. Ouyang, J., Wang, X., Zhao, B., Yuan, X. & Wang, Y. Effects of rare earth elements on the growth of Cistanche deserticola cells and the production of phenylethanoid glycosides. *J. Biotechnol.* **102**, 129–134 (2003).
53. Ambavaram, M. M. et al. Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. *Nat. Commun.* **5**, 5302 (2014).
54. Xie, Y. F. et al. Effects of cerium nitrate on the growth and physiological characteristics in cyclocarya Paliurus seedlings. *J. Rare Earths.* **33**, 898–904 (2015).
55. Wen, K., Liang, C., Wang, L., Hu, G. & Zhou, Q. Combined effects of lanthanumion and acid rain on growth, photosynthesis and chloroplast ultrastructure in soybean seedlings. *Chemosphere* **84**, 601–608 (2011).
56. Wang, L., Huang, X. & Zhou, Q. Effects of rare Earth elements on the distribution of mineral elements and heavy metals in horseradish. *Chemosphere* **73**, 314–319 (2008).
57. Yang, G., Chu, Y., Lv, X., Zhou, Q. & Huang, X. Interaction between La(III) and proteins on the plasma membrane of horseradish. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **92**, 42–45 (2012).
58. Gao, J. et al. Dysfunction of rice mitochondrial membrane induced by Yb^{3+}. *J. Membr. Biol.* **248**, 1159–1165 (2015).
59. Yan, T. et al. Analysis of the relationship between chemical composition and sensory comfort of tobacco leaves in the main tobacco-producing areas in China. *Chin. Tob. Sci.* **42**, 60–65 (2021).
60. Takahashi, C. A. & Mercier, H. New insights into the role of the root system of epiphytic bromeliads: comparison of root and leaf trichome functions in acquisition of water and nutrients. *Ann. Bot.* **134**, 711–724 (2024).
61. Cramer, M. D., Hoffmann, V. & Verboom, G. A. Nutrient availability moderates transpiration in ehrharta Calycina. *New. Phytol.* **179**, 1048–1057 (2008).

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Author contributions

Song Guo, Shengzhu Yang and Li Liu conceived and designed the study. Song Guo, Shengzhu Yang and Jianbo Li performed the experiments. Song Guo, Shengzhu Yang and Xiaolie Su analyzed the data. Song Guo wrote the paper. Shiping Deng, Yingang Lu and Li Liu reviewed and edited the manuscript. Sanwei Yang and Li Liu provided project funding support. The final version was read and approved by all authors.

Declarations

Competing interests

The authors declare no competing interests.

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Additional information

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