

Comparative Effects of Four Seedling Cultivation Methods on Root Architecture, Physiological Characteristics, and Post-Transplanting Growth of Flue-Cured Tobacco Based on Two-Year Field Study

Guo Ting

Hunan Tobacco Company Chenzhou Branch

Li Yan

Hunan Tobacco Company Chenzhou Branch

Tang Xiaoming

Hunan Tobacco Company Zhangjiajie Branch

Zhu Lin

Hunan Tobacco Company Chenzhou Branch

Liu Cailing

China Agricultural University

Hao Shuaihua

China Agricultural University

Guo Songbao

China Agricultural University

He Yinglong

yinglong.he@surrey.ac.uk

University of Surrey

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Abstract

To systematically compare the effects of four representative seedling cultivation methods on root architecture, leaf protective enzyme activities, and post-transplanting growth of flue-cured tobacco, a two-year field experiment was conducted from 2025 to 2026 using paper pots (PB), plastic trays (PT), two-stage seedling cultivation (TS), and floating seedling cultivation (FS). Root architecture was precisely quantified using WinRHIZO, and protective enzyme activities, root activity, dry matter accumulation, and agronomic traits were systematically measured. The results showed that: (1) The four seedling methods differed substantially in root architecture. Paper pots exhibited a pronounced “air-root pruning” effect, with a two-year mean root penetration rate of 63.7%. Penetration rate was highly significantly and positively correlated with root dry weight at 40 days after transplanting ($r = 0.8042$, $P < 0.01$) and significantly and positively correlated with root activity at 40 days after transplanting ($r = 0.6138$, $P < 0.05$), but highly significantly and negatively correlated with pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$). (2) Two-stage seedling cultivation had the highest SOD activity (two-year mean $1828 \text{ U}\cdot\text{g}^{-1}$), while paper pots had the highest CAT activity ($2.844 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) and relatively high SOD activity ($1273 \text{ U}\cdot\text{g}^{-1}$), indicating method-specific activation of the protective enzyme system. (3) At 40 days after transplanting, total dry weight per plant was higher in tray (15.28 g) and floating (14.91 g) treatments, and lower in two-stage (11.58 g) and paper pot (10.50 g) treatments, although differences among treatments were not significant. Paper pots showed the highest dry matter increase multiple during 20–40 days after transplanting (10.73-fold) and the largest increase in root activity (80.4%), reflecting compensatory growth. In 2026, agronomic traits were superior in floating and tray treatments, relatively lower in paper pots, and intermediate in two-stage. In summary, this systematic comparison demonstrates that two-stage seedling cultivation performs best overall, while paper pot seedling cultivation has considerable potential for labor saving, root protection, and green production.

Highlights

- A two-year field study systematically compared four representative seedling cultivation methods—paper pot (PB), plastic tray (PT), two-stage (TS), and floating (FS)—and quantified their differential effects on root architecture, protective enzyme activities, and post-transplanting growth of flue-cured tobacco, with a focus on precise root architecture comparison.
- Paper pot air-root pruning achieved a 63.7% root penetration rate. Penetration rate was highly significantly and positively correlated with root dry weight ($r = 0.8042$, $P < 0.01$) and root activity ($r = 0.6138$, $P < 0.05$) at 40 days after transplanting, but negatively correlated with pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$), revealing a trade-off between root penetration and internal root biomass accumulation.
- Two-stage seedling cultivation exhibited the highest SOD activity ($1828 \text{ U}\cdot\text{g}^{-1}$), while paper pots had the highest CAT activity ($2.844 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) and relatively high SOD ($1273 \text{ U}\cdot\text{g}^{-1}$), demonstrating method-specific activation of the protective enzyme system in response to transplanting stress.

- Paper pot seedlings showed compensatory growth with the highest dry matter increase multiple (10.73-fold) and root activity increase (80.4%) during 20–40 days after transplanting, despite having the lowest total dry weight at 20 days, confirming that potted transplanting provides zero root damage and facilitates rapid field establishment.
- Floating and tray treatments performed better in agronomic traits at 40 days after transplanting, while paper pots had unique advantages in labor saving (33% of two-stage), root protection, and 100% degradability. Two-stage showed the best overall performance, and optimizing paper pot specifications (5.5–6.0 cm aperture, 6.5–7.0 cm depth) is recommended to enhance penetration rate.

1 Introduction

Roots are important organs for water and nutrient uptake in crops, and their architectural characteristics directly affect the efficiency of soil resource acquisition and the ability to adapt to adverse environmental stresses^[1–5]. In tobacco production, root development during the seedling stage is one of the key factors determining transplanting survival rate, the duration of the seedling recovery period, and final leaf yield and quality^[1, 6–8]. Studies have shown that well-developed and vigorous roots not only facilitate rapid water and nutrient uptake after transplanting, thereby shortening the recovery period, but also enhance tobacco plant resistance to stresses such as drought and low temperature^[1–2, 6]. As a physical boundary limiting root growth, seedling cultivation methods profoundly affect the architectural characteristics and physiological functions of tobacco seedling roots by altering growth space, aeration, and water and nutrient supply^[1, 3, 5, 8–9]. Therefore, systematically comparing the effects of different seedling cultivation methods on root development and physiological characteristics is of great significance for optimizing seedling technology and improving seedling quality^[1, 2, 7, 10]. However, although several seedling methods are currently used in production, few studies have simultaneously compared the four representative methods—paper pot, tray, two-stage, and floating—with a particular focus on precise quantification of root architecture^[11–13].

Floating seedling cultivation is currently the most widely used method. The substrate is filled into polystyrene foam trays, seeds are sown into the substrate, and the trays float on a shallow nutrient solution in a greenhouse pond^[1, 8]. This method is convenient to manage, produces uniform seedlings, and facilitates pest and disease control, covering more than 80% of flue-cured tobacco production areas in China^[1, 8, 14]. Studies have shown that floating seedling cultivation can promote vigorous root development, increasing the total number of primary and secondary lateral roots by 13.7% compared with conventional seedling cultivation, increasing main root length by 3 cm, and achieving a transplanting survival rate of over 95%^[1, 14–15]. However, floating seedling cultivation also has prominent disadvantages: roots remain immersed in nutrient solution for extended periods, and insufficient oxygen concentration in the water hinders root cell differentiation, weakening root absorption function, reducing root hairs, and lowering root activity^[13–14, 16]. Seedlings are prone to producing “water roots” and “spiral roots,” resulting in severe transplanting injury and a prolonged recovery period^[13–14]. These coexisting

advantages and disadvantages have prompted researchers to explore alternative methods that can both protect roots and improve seedling efficiency; tray seedling cultivation is one such method ^[17].

Tray seedling cultivation uses rigid plastic trays, with aperture and depth generally larger than those of floating trays, and greater substrate filling volume ^[9–10, 17]. Studies have shown that 74-cell and 51-cell trays with depths of 6–6.5 cm are beneficial for seed germination, seedling growth, and improved seedling quality ^[17]. Tray waterbed seedling cultivation produces rapid seedling growth, with reasonable plant height and leaf number, thick stems, large leaf area, well-developed roots, and high dry matter accumulation ^[17]. However, tray seedling cultivation also involves bare-root transplanting, and root damage during transplanting remains a problem, constraining the full expression of its seedling-raising advantages ^[9–10, 17]. Thus, although tray seedling cultivation is superior to floating seedling cultivation in terms of root growth space, root damage during transplanting has not been fundamentally resolved, providing an impetus for the development of two-stage seedling cultivation ^[18–19].

In two-stage seedling cultivation, seeds are first sown in small-cell trays or trays, and before seedlings reach the transplanting stage, they are temporarily planted into larger nutritional pots or trays for further cultivation ^[18]. After temporary planting in nutritional rings or trays, the main root length of tobacco seedlings is significantly shortened, and the lengths of primary lateral roots, secondary lateral roots, and adventitious roots are also reduced to varying degrees, but root number, fresh root weight, and dry root weight increase ^[10, 18–19]. Studies have shown that measures such as floating + tray two-stage cultivation can effectively improve physiological indicators such as root activity and chlorophyll content compared with conventional floating seedling cultivation, and increase both aboveground and underground fresh and dry weights of seedlings ^[19]. However, two-stage seedling cultivation is cumbersome and labor-intensive, making it unsuitable for large-scale intensive production. Therefore, how to simplify procedures and reduce labor while maintaining seedling quality has become an important direction for seedling technology innovation, drawing attention to paper pot seedling cultivation ^[20].

Paper pot seedling cultivation is a new type of seedling technology developed in recent years, using degradable honeycomb cardboard to make seedling containers ^[20–21]. This technology utilizes the principle of “air-root pruning”: tobacco seedlings grow in paper pots filled with substrate, and the bottom of the paper pot cells is suspended in air. When root tips grow to the paper pot wall, because air humidity cannot meet the water requirements for normal root growth, the exposed main root tips gradually dry and die ^[20–21]. Air-root pruning effectively cuts the main root tip without leaving a wound, thereby promoting lateral root growth ^[20, 22]. Studies have shown that air-root pruning can increase root fresh weight and dry weight by 43.59% and 50%, respectively, compared with the control ^[11, 21]. Compared with floating seedling cultivation, air-root-pruned seedlings have a root-shoot ratio 31% higher, reaching a highly significant level ^[11, 21]. Paper pot seedling cultivation also employs potted transplanting, burying the entire paper pot together with substrate and seedling into the soil, minimizing root damage ^[12–13].

However, systematic research on paper pot seedling cultivation in tobacco is still in its infancy. Key technical parameters such as paper pot aperture, depth, and substrate formulation need optimization, and quantitative differences in root architecture parameters, systematic comparisons of protective enzyme activities, and dynamic characteristics of post-transplanting dry matter accumulation compared with traditional seedling methods lack in-depth data support [22].

In summary, the four seedling cultivation methods each have advantages and disadvantages, but existing studies have mostly focused on improving a single method or simply comparing two methods, lacking systematic comparative research on the four typical methods: paper pot, tray, two-stage, and floating [11–12]. In particular, there are clear research gaps in precise quantification of root architecture, systematic evaluation of protective enzyme activities, and comparison of post-transplanting dry matter accumulation dynamics [11–13]. Specifically, the existing literature has the following deficiencies: first, the extent of the “air-root pruning” effect of paper pots in tobacco lacks quantitative characterization, and the relationships between penetration rate and pre-transplanting root biomass and post-transplanting root function recovery remain unclear; second, the differential regulatory mechanisms of different seedling methods on leaf protective enzyme systems are unclear, and systematic studies linking seedling physiological characteristics with post-transplanting growth dynamics are lacking; third, comparative studies on dry matter accumulation dynamics among paper pot, tray, two-stage, and floating methods are rarely reported, and differences in growth strategies among methods at different post-transplanting stages have not been elucidated; fourth, systematic analysis of differences in agronomic trait responses among different seedling methods and their association with root physiological indicators is lacking.

In view of these gaps, the present study was designed as a systematic comparison of four representative seedling cultivation methods—paper pot (PB), plastic tray (PT), two-stage seedling cultivation (TS), and floating seedling cultivation (FS)—rather than as an evaluation of a single seedling technology. A two-year field experiment was conducted from 2025 to 2026. WinRHIZO root scanning system was used for precise quantification of root architecture, and root architecture parameters, protective enzyme activities, root activity, dry matter accumulation dynamics, and agronomic traits were systematically measured. The specific objectives were: (1) to quantify the differential effects of the four methods on root architecture, with particular emphasis on the air-root pruning effect of paper pots; (2) to compare the physiological responses, especially protective enzyme activities and root activity, among the four methods; and (3) to evaluate post-transplanting growth strategies and agronomic performance. The overall aim was to provide a theoretical basis for scientific selection of flue-cured tobacco seedling methods and for optimization of paper pot seedling technology.

2 Materials and Methods

2.1 Experimental Materials

The flue-cured tobacco (*Nicotiana tabacum* L.) cultivar used was Yunyan 87, and seeds were provided by the Chenzhou Tobacco Company of Hunan Provincial Tobacco Company. The substrates used were a tobacco-specific commercial seedling substrate (composed of peat, perlite, and vermiculite; pH 7.46; organic matter 182.15 g·kg⁻¹; available potassium 2183 mg·kg⁻¹; alkaline hydrolyzable nitrogen 367.0 mg·kg⁻¹). Fire soil ash was prepared by mixing local crop straw and topsoil at a volume ratio of 3:1, burning at low temperature (< 400°C), and passing through a 2-mm sieve. Its basic nutrient contents were total potassium 2.8%, total phosphorus 0.6%, total nitrogen 0.3%, and pH 8.5–9.0.

Paper pots were commercially available B-type honeycomb degradable paper pots (64 cells per tray, aperture 4.8 cm, depth 5.5 cm, single-cell volume approximately 99.5 cm³), made of natural plant fibers, with a longitudinal tensile index ≥ 45.0 N·m·g⁻¹, naturally degradable in soil within 60–90 days. Plastic trays were 51-cell seedling trays (aperture approximately 5.5 cm, depth approximately 6.0 cm, single-cell volume approximately 142.5 cm³). Floating seedling trays were 200-cell polystyrene foam floating trays (aperture 3.5 cm, depth 4.5 cm). For two-stage seedling cultivation, the first stage used 51-cell plastic trays (same specifications as above), and the second stage involved transfer into 10 cm × 10 cm plastic nutritional pots.

2.2 Experimental Design

The experiment was conducted from 2025 to 2026 in a greenhouse at the Chenzhou Tobacco Experimental Station, Hunan Province. The station is located at 25°46' N, 113°02' E, with a subtropical monsoon climate, an annual mean temperature of 17.8°C, annual mean precipitation of 1452 mm, and a frost-free period of approximately 290 days. The soil was red paddy soil; before experiment establishment, the 0–30 cm soil layer had pH 6.23, organic matter 28.6 g·kg⁻¹, total nitrogen 1.52 g·kg⁻¹, available phosphorus 18.4 mg·kg⁻¹, and available potassium 112.5 mg·kg⁻¹.

Four treatments were selected: PB (paper pot + tobacco-specific substrate + 40% fire soil ash), TS (two-stage seedling cultivation), PT (plastic tray + tobacco-specific substrate + 40% fire soil ash), and FS (floating seedling cultivation). For PB and PT, the substrate was a mixture of tobacco-specific substrate and fire soil ash at a volume ratio of 6:4. For TS, the first stage used tobacco-specific substrate (without fire soil ash), and the second stage used the same substrate. For FS, tobacco-specific substrate (without fire soil ash) was used with nutrient solution cultivation. A completely randomized block design was adopted with three replicates. Each plot contained 125 tobacco plants (5 rows × 25 plants), with row spacing of 1.2 m and plant spacing of 0.5 m, and a plot area of approximately 75 m².

Sowing was conducted on December 15 in both 2025 and 2026, with 1–2 seeds per cell at a sowing depth of 0.5 cm, followed by covering with a thin layer of the corresponding substrate. After sowing, unified management was applied in a naturally ventilated greenhouse, with identical water and fertilizer management for all treatments. When seedlings reached the 4–5 leaf stage, they were transplanted to the field on March 24 of the same year. For the paper pot treatment, the entire paper pot together with substrate and seedling was buried into the soil, with the upper edge of the paper pot level with the soil surface. The tray treatment involved bare-root transplanting. Two-stage seedling cultivation involved

bare-root transplanting (nutritional pots removed before transplanting). Floating seedling cultivation involved transplanting with a small amount of substrate. Field management followed local high-quality flue-cured tobacco production standards, with identical field management measures for all treatments.

2.3 Measurement Items and Methods

Samples were collected and measured at 5 days before transplanting (March 19), 20 days after transplanting (April 13), and 40 days after transplanting (May 3). Three representative seedlings were selected per plot, totaling 9 plants per treatment. For agronomic traits, five representative tobacco plants were selected per plot. All samples were selected away from plot edges in a random manner to reduce marginal effects and human selection bias.

2.3.1 Determination of Root Architecture Parameters

Tobacco seedling roots were slowly washed with tap water to remove substrate attachments, and surface water was blotted with absorbent paper. A WinRHIZO root scanning system (Regent Instruments Inc., Canada) was used to scan and analyze roots. Parameters measured included total root length (cm) and root tip number; root number was determined manually by counting primary lateral roots longer than 0.5 cm [5, 20]. Roots and shoots were separated, blanched at 105°C for 30 min, then dried at 70°C to constant weight (approximately 48 h), and weighed with a 0.0001 g analytical balance to calculate root dry weight (g) and root-shoot ratio (root dry weight / shoot dry weight) [6–7, 9].

2.3.2 Determination of Paper Pot Penetration Rate

This was determined only for the paper pot treatment (PB). After completely removing the seedling from the paper pot, the number of roots penetrating the paper pot wall and the total root number were counted. Paper pot penetration rate = (number of roots penetrating the paper pot wall / total root number) × 100% [21–22].

2.3.3 Determination of Protective Enzyme Activities

At 5 days before transplanting, the third fully expanded leaf from the top was collected, rapidly frozen in liquid nitrogen, and stored in a – 80°C ultra-low temperature freezer until analysis.

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) photochemical reduction method [9, 23]. A 0.5 g leaf sample was ground in an ice bath with 5 mL of 0.05 mol·L⁻¹ phosphate buffer (pH 7.8, containing 1% polyvinylpyrrolidone), centrifuged at 4°C and 12,000 × g for 20 min, and the supernatant was used as the crude enzyme extract. The reaction system contained 50 mmol·L⁻¹ phosphate buffer (pH 7.8), 130 mmol·L⁻¹ methionine, 750 μmol·L⁻¹ NBT, 100 μmol·L⁻¹ EDTA-Na₂, 20 μmol·L⁻¹ riboflavin, and an appropriate amount of enzyme extract. The reaction was conducted under 4000 lx light for 20 min, terminated in darkness, and absorbance was measured at 560 nm. One unit of enzyme activity (U) was defined as the amount inhibiting 50% of NBT photoreduction, and activity was expressed as U·g⁻¹ FW.

Peroxidase (POD) activity was determined by the guaiacol oxidation method. The reaction system contained 50 mmol·L⁻¹ phosphate buffer (pH 7.0), 20 mmol·L⁻¹ guaiacol, 40 mmol·L⁻¹ H₂O₂, and an appropriate amount of enzyme extract. Absorbance changes were recorded at 470 nm for 3 min. One unit of enzyme activity was defined as an increase of 0.01 in OD₄₇₀ per minute, and activity was expressed as U·g⁻¹ FW·min⁻¹.

Catalase (CAT) activity was determined by the ultraviolet absorption method. The reaction system contained 50 mmol·L⁻¹ phosphate buffer (pH 7.0), 15 mmol·L⁻¹ H₂O₂, and an appropriate amount of enzyme extract. Absorbance changes were recorded at 240 nm for 3 min. One unit of enzyme activity was defined as a decrease of 0.01 in OD₂₄₀ per minute, and activity was expressed as mg·min⁻¹·g⁻¹.

2.3.4 Determination of Root Activity

Root activity was determined by the triphenyl tetrazolium chloride (TTC) reduction method. A 0.5 g sample of washed root tips was placed in a 10 mL test tube, and 5 mL each of 0.4% TTC solution and 0.1 mol·L⁻¹ phosphate buffer (pH 7.0) were added. The sample was incubated in darkness at 37°C for 3 h. After incubation, 2 mL of 1 mol·L⁻¹ sulfuric acid was added to terminate the reaction. Roots were removed, blotted dry with filter paper, ground in a mortar with a small amount of ethyl acetate and quartz sand until homogenized, transferred to a centrifuge tube, made up to 10 mL with ethyl acetate, and measured at 485 nm. TTC reduction was calculated from a standard curve, and results were expressed as mg TTF·g⁻¹ FW·h⁻¹ [24–25].

2.3.5 Determination of Dry Matter Accumulation

Total dry weight per plant = root dry weight + shoot dry weight. Mean daily weight gain = (final total dry weight – initial total dry weight) / interval days, where stage I was from 5 days before transplanting to 20 days after transplanting (25-day interval), and stage II was from 20 days to 40 days after transplanting (20-day interval). Increase multiple = final total dry weight / initial total dry weight [3].

2.3.6 Determination of Agronomic Traits

At 20 and 40 days after transplanting, five representative tobacco plants were selected per plot, and measurements were performed according to YC/T 142–2010 “Methods for Investigation and Measurement of Tobacco Agronomic Traits” [6]. Items included leaf number (number of effective leaves, length ≥ 10 cm), stem height (from ground to plant top, cm), stem diameter (measured with a vernier caliper at 5 cm above ground, cm), maximum leaf length (from leaf base to tip, cm), maximum leaf width (widest part of the leaf, cm), and single leaf area (cm²) was calculated. Agronomic trait data from 2026 were used for analysis in this paper [26].

2.4 Data Statistical Analysis

Microsoft Excel 2021 was used for data organization and basic calculations. GraphPad Prism 9 was used for analysis of variance and plotting, and treatment differences were tested by Tukey’s multiple comparison test ($\alpha = 0.05$). Data were expressed as “mean ± standard deviation.” Pearson correlation

coefficients were used to analyze the relationships between paper pot penetration rate and various pre- and post-transplanting indicators, with significance levels indicated (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3 Results and Analysis

3.1 Effects of Different Seedling Methods on Root Architecture of Tobacco Seedlings Before Transplanting

Root architecture is a core factor determining nutrient uptake capacity and post-transplanting recovery speed of tobacco seedlings. As shown in Fig. 1 and Tukey's multiple comparison results, the four seedling methods differed in their effects on various root architecture parameters at 5 days before transplanting, with obvious interannual fluctuations.

In terms of total root length (Fig. 1A), in 2025, floating seedling cultivation had the greatest total root length (20.43 cm), significantly higher than two-stage (6.60 cm) and tray (14.03 cm) treatments ($P < 0.05$), but not significantly different from paper pot (13.03 cm). Paper pot was significantly higher than two-stage, and two-stage was significantly lower than tray. In 2026, differences in total root length among treatments were not significant ($P > 0.05$). The two-year mean was in the order floating (14.00 cm) > tray (11.27 cm) > paper pot (10.28 cm) > two-stage (8.10 cm). The slender roots of floating seedlings are related to the absence of mechanical resistance from substrate in nutrient solution, allowing free elongation [1, 8, 16].

In addition to root length, root number further reflects branching capacity [5, 20]. In terms of root number (Fig. 1B), in 2025, floating seedling cultivation had the highest root number (79.33), significantly higher than two-stage (46.33) ($P < 0.01$), with no significant differences among other treatments. In 2026, paper pot had the lowest root number (29.33), significantly lower than two-stage (48.00) ($P < 0.05$), with no significant differences among other treatments. The two-year mean was in the order floating (58.67) > two-stage = tray (47.17) > paper pot (39.08). Although the absolute root number of paper pot was not high, its lateral root initiation sites were more concentrated near the root tip, giving it higher branching density [11].

Differences in root number and length ultimately reflected in root biomass accumulation [5, 20]. In terms of root dry weight (Fig. 1C), Tukey's test in both years showed no significant differences among treatments ($P > 0.05$). The two-year mean was highest in two-stage (0.07422 g), followed by floating (0.04517 g), tray (0.03723 g), and lowest in paper pot (0.02403 g), which was only 32.4% of that in two-stage.

Root dry weight reflects absolute accumulation, while root-shoot ratio reflects the distribution pattern of photosynthates between roots and shoots [20]. In terms of root-shoot ratio (Fig. 1D), Tukey's test in both years also showed no significant differences among treatments ($P > 0.05$). The two-year mean was highest in tray (0.2318), followed by two-stage (0.2132), floating (0.1715), and lowest in paper pot

(0.1358). The smallest root-shoot ratio in paper pot seedling cultivation indicates that it is more conducive to photosynthate allocation to shoots.

Interannual comparison showed that root indicators in 2025 were generally superior to those in 2026, with root length and root number reaching significant differences among some treatments, while root dry weight and root-shoot ratio showed no significant differences among treatments in either year. The interannual difference may be related to the lower temperatures during the seedling period (January–March) in 2026, which inhibited root growth and substrate nutrient mineralization.

Note

PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. A, B, C, and D correspond to root length (cm), root number (number), root dry weight (g), and root-shoot ratio, respectively. Different colored bars represent different years (dark for 2025, light for 2026). ns above bars indicates no significant difference among treatments in the same year, * indicates significant difference, ** indicates highly significant difference ($P < 0.05$, Tukey's multiple comparison test); error bars represent standard deviation. Lines represent the trend of the two-year mean.

3.2 Effects of Different Seedling Methods on Leaf Protective Enzyme Activities of Tobacco Seedlings Before Transplanting

Differences in root architecture are often accompanied by adjustments in leaf physiological metabolism, and the response of the protective enzyme system is an important manifestation [9, 23]. The plant leaf protective enzyme system (SOD, POD, CAT) is an important defense system for scavenging reactive oxygen species and maintaining cell membrane integrity, and its activity directly reflects the stress resistance of tobacco seedlings. As shown in Fig. 2 and Tukey's multiple comparison results, the effects of the four seedling methods on SOD, POD, and CAT activities varied with year and enzyme type. Overall, SOD showed significant differences among some treatments in 2025, while POD and CAT mostly showed no significant differences among treatments in either year.

In terms of SOD activity (Fig. 2A), the two-year mean was highest in two-stage seedling cultivation ($1828 \text{ U}\cdot\text{g}^{-1}$), followed by paper pot ($1273 \text{ U}\cdot\text{g}^{-1}$), floating ($916.7 \text{ U}\cdot\text{g}^{-1}$), and lowest in tray ($637.9 \text{ U}\cdot\text{g}^{-1}$). Tukey's test showed that in 2025, SOD activity in two-stage ($2885 \text{ U}\cdot\text{g}^{-1}$) was significantly higher than in floating ($1160 \text{ U}\cdot\text{g}^{-1}$, $P < 0.001$) and tray ($685 \text{ U}\cdot\text{g}^{-1}$, $P < 0.01$), but not significantly different from paper pot ($1622 \text{ U}\cdot\text{g}^{-1}$). In 2026, there were no significant differences in SOD activity among treatments ($P > 0.05$). SOD activity in two-stage showed the largest interannual fluctuation, decreasing from $2885 \text{ U}\cdot\text{g}^{-1}$ in 2025 to $772 \text{ U}\cdot\text{g}^{-1}$ in 2026. Paper pot maintained relatively high SOD activity in both years ($1622 \text{ U}\cdot\text{g}^{-1}$ in 2025 and $924 \text{ U}\cdot\text{g}^{-1}$ in 2026), possibly related to local oxidative stress induced during the "air-root pruning" process.

POD activity, which acts synergistically with SOD, showed a different pattern^[9, 23]. In terms of POD activity (Fig. 2B), the two-year mean was highest in tray ($349.6 \text{ U}\cdot\text{g}^{-1}$), followed by floating ($321.4 \text{ U}\cdot\text{g}^{-1}$), two-stage ($311.2 \text{ U}\cdot\text{g}^{-1}$), and lowest in paper pot ($301.1 \text{ U}\cdot\text{g}^{-1}$). However, Tukey's test in both years showed no significant differences in POD activity among treatments ($P > 0.05$). POD activity in all treatments was generally lower in 2026 than in 2025, but the ranking among treatments remained basically stable.

In terms of CAT activity (Fig. 2C), the two-year mean was highest in paper pot ($2.844 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), followed by floating ($2.698 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), two-stage ($2.212 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), and lowest in tray ($2.043 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$). Tukey's test showed that in 2025, there were no significant differences in CAT activity among treatments; in 2026, CAT activity in paper pot ($1.933 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) was significantly higher than in tray ($1.357 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$, $P < 0.05$), with no significant differences among other treatments. The high CAT activity in paper pot, together with its high SOD activity, constitutes a relatively complete reactive oxygen species scavenging system.

In terms of interannual differences, SOD activity showed the largest fluctuation, followed by CAT activity, while POD activity was relatively stable. In 2025, SOD and CAT activities in all treatments were generally higher than in 2026, while POD activity showed little interannual variation. These results indicate that different seedling methods differ in their sensitivity to interannual variation, with SOD activity in two-stage being particularly sensitive to year changes.

Note

PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. SOD activity unit: $\text{U}\cdot\text{g}^{-1}$; POD activity unit: $\text{U}\cdot\text{g}^{-1}$; CAT activity unit: $\text{mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$. ns above bars indicates no significant difference among treatments in the same year, * indicates significant difference, ** indicates highly significant difference ($P < 0.05$, Tukey's multiple comparison test); error bars represent standard deviation. Lines represent the trend of the two-year mean.

3.3 Paper Pot Penetration Rate and Its Correlation with Seedling Indicators

In the paper pot treatment, the strength of the "air-root pruning" effect can be quantified by penetration rate; therefore, it is necessary to further analyze the correlations between penetration rate and various indicators at the seedling stage and after transplanting^[11, 21–22]. As shown in Fig. 3A, paper pot penetration rate was highly significantly and negatively correlated with pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$), indicating that the higher the penetration rate, the lower the pre-transplanting root dry weight. This result may be related to the drying and death of penetrating root tips in air, resulting in reduced root biomass accumulation inside the paper pot, indicating that although a higher penetration rate promoted outward root penetration, it did not correspondingly increase total pre-transplanting root dry weight. As shown in Fig. 3B, penetration rate was negatively correlated with pre-transplanting root

activity, but not significantly ($r = -0.3498$, $P = 0.265$), indicating that penetration rate had limited regulatory effect on pre-transplanting root metabolic function.

After entering the field recovery stage, as shown in Fig. 3C, penetration rate was significantly and positively correlated with root dry weight at 20 days after transplanting ($r = 0.578$, $P < 0.05$). Twenty days after transplanting is a critical period for seedling recovery, and a higher penetration rate helps roots adapt more quickly to the field environment and initiate growth after transplanting. As shown in Fig. 3D, penetration rate was positively correlated with root activity at 20 days after transplanting, but not significantly ($r = 0.422$, $P = 0.172$), indicating that the promoting effect of penetration rate on root metabolic function at 20 days after transplanting was not yet stable.

With extended time after transplanting, the positive effect of penetration rate gradually increased. As shown in Fig. 3E, penetration rate was highly significantly and positively correlated with root dry weight at 40 days after transplanting ($r = 0.8042$, $P < 0.01$); as shown in Fig. 3F, penetration rate was significantly and positively correlated with root activity at 40 days after transplanting ($r = 0.6138$, $P < 0.05$). These results indicate that paper pot penetration rate has a sustained positive effect on long-term root development after transplanting. The correlation coefficients of root dry weight and root activity at 40 days after transplanting with penetration rate were both higher than the corresponding values before transplanting and at 20 days after transplanting, indicating that the effect of penetration rate gradually amplified with time after transplanting, showing a positive feedback characteristic of “early penetration → later compensation”.

Combining the six subplots, the correlations between penetration rate and various indicators, from strong to weak, were: root dry weight at 40 days after transplanting ($r = 0.8042$, $P < 0.01$) > root activity at 40 days after transplanting ($r = 0.6138$, $P < 0.05$) > root dry weight at 20 days after transplanting ($r = 0.578$, $P < 0.05$) > root activity at 20 days after transplanting ($r = 0.422$, $P > 0.05$) > pre-transplanting root activity ($r = -0.3498$, $P > 0.05$) > pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$). From the distribution trend of the scatter plots, when penetration rate was in the 60%–70% range, root dry weight and root activity at 40 days after transplanting increased rapidly with increasing penetration rate; when penetration rate exceeded 70%, the increase rate tended to level off. It is therefore speculated that a threshold effect may exist, i.e., when penetration rate reaches approximately 65%–70%, the “air-root pruning” effect has been fully exerted. In this study, the average paper pot penetration rate was 63.7%, still at a moderate level, with considerable room for optimization.

Note

A–F are scatter plots of paper pot penetration rate versus pre-transplanting root dry weight (A), pre-transplanting root activity (B), root dry weight at 20 days after transplanting (C), root activity at 20 days after transplanting (D), root dry weight at 40 days after transplanting (E), and root activity at 40 days after transplanting (F). The x-axis is paper pot penetration rate (%), and the y-axis is the corresponding indicator. ● represents 2025 data points ($n = 6$), ▲ represents 2026 data points ($n = 6$), solid lines are linear fitting trend lines for pooled two-year data, and shaded areas represent 95% confidence intervals.

Pearson correlation coefficients (r) and significance levels are indicated (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Data are from the paper pot (PB) treatment. Penetration rate (%) = (number of roots penetrating the paper pot wall / total root number) $\times$ 100%.

3.4 Effects of Different Seedling Methods on Dry Matter Accumulation of Tobacco Seedlings

Penetration rate may ultimately be reflected in post-transplanting dry matter accumulation through its effects on seedling root traits [3, 11–12]. As shown in Fig. 4A and Tukey's multiple comparison results, total dry weight per plant in all treatments showed a continuous increasing trend with growth progression, but the degree of difference among treatments varied at different stages.

At 5 days before transplanting, two-stage seedling cultivation had the highest total dry weight per plant (0.416 g), significantly higher than paper pot (0.199 g) and floating (0.308 g) ($P < 0.05$), but not significantly different from tray (0.191 g) ($P = 0.0781$); differences among paper pot, floating, and tray were not significant ($P > 0.05$). During this period, dry matter accumulation in two-stage was 109.0% and 117.8% higher than in paper pot and tray, respectively, indicating that "temporary planting hardening" significantly promoted seedling dry matter accumulation.

At 20 days after transplanting, total dry weight in all treatments increased substantially compared with before transplanting. Floating seedling cultivation had the highest total dry weight (2.076 g), but not significantly different from two-stage (1.568 g) and tray (1.553 g) ($P > 0.05$); paper pot had the lowest total dry weight (0.979 g), significantly lower than two-stage ($P < 0.05$), close to significant difference from floating ($P = 0.0812$), and not significantly different from tray ($P > 0.05$). Total dry weight in paper pot was 52.9% lower than in floating, but still 3.9 times higher than before transplanting. Floating seedling cultivation performed best at 20 days after transplanting, possibly because transplanting with substrate reduced root damage and shortened the recovery period; paper pot, due to its small pre-transplanting biomass base, although having a higher increase multiple, still had the lowest absolute dry matter accumulation.

At 40 days after transplanting, total dry weight in all treatments increased further. Tray (15.28 g) and floating (14.91 g) had higher total dry weight, while two-stage (11.58 g) and paper pot (10.50 g) were lower, but Tukey's test showed no significant differences among treatments ($P > 0.05$). Tray had a total dry weight of 1.553 g at 20 days after transplanting, rising to 15.28 g at 40 days after transplanting, with a stage II increase multiple of 9.84-fold, indicating that after passing the bare-root transplanting recovery period, the root biomass advantage accumulated during the seedling stage was fully expressed. Floating seedling cultivation maintained a relatively high dry matter accumulation level, consistent with the good growth foundation established by 20 days after transplanting.

As shown in Fig. 4B, mean daily weight gain in the two stages differed among treatments. Stage I (5 days before transplanting $\rightarrow$ 20 days after transplanting, 25-day interval): no significant differences

among treatments ($P > 0.05$), with floating having higher mean daily weight gain ($0.071 \text{ g}\cdot\text{d}^{-1}$), tray ($0.054 \text{ g}\cdot\text{d}^{-1}$) and two-stage ($0.046 \text{ g}\cdot\text{d}^{-1}$) intermediate, and paper pot the lowest ($0.031 \text{ g}\cdot\text{d}^{-1}$). Stage II (20 days → 40 days after transplanting, 20-day interval): tray had the highest mean daily weight gain ($0.686 \text{ g}\cdot\text{d}^{-1}$), significantly higher than paper pot ($0.476 \text{ g}\cdot\text{d}^{-1}$, $P < 0.01$) and two-stage ($0.501 \text{ g}\cdot\text{d}^{-1}$, $P < 0.05$); floating ($0.642 \text{ g}\cdot\text{d}^{-1}$) was also significantly higher than paper pot ($P < 0.05$) and two-stage ($P < 0.05$); difference between tray and floating was not significant ($P > 0.05$). Mean daily weight gain in this stage was significantly higher than in stage I for all treatments, indicating that 20–40 days after transplanting is a period of rapid dry matter accumulation.

As shown in Fig. 4C, increase multiples in the two stages differed among treatments, but Tukey's test showed no significant differences in any pairwise comparison ($P > 0.05$). Stage I: floating (5.83-fold) and paper pot (4.93-fold) had higher increase multiples, while two-stage (3.82-fold) and tray (3.93-fold) were lower. Although paper pot had the lowest initial biomass, its roots were not damaged during transplanting, allowing rapid growth initiation. Stage II: paper pot (10.73-fold) and tray (9.84-fold) had higher increase multiples, while two-stage (7.38-fold) and floating (6.81-fold) were lower. Paper pot had the highest increase multiple in stage II; combined with its stage I value of 4.93-fold, this indicates that paper pot maintained a relatively high relative growth rate in both stages, showing a “low starting point, sustained high growth rate” compensatory growth characteristic. Tray had a stage II increase multiple of 9.84-fold, indicating that the growth retardation caused by bare-root transplanting was mainly concentrated within 20 days after transplanting, after which the growth rate increased rapidly.

Combining Figs. 4A, B, and C, different seedling methods exhibited differentiated growth strategies in post-transplanting dry matter accumulation. Paper pot seedling cultivation showed a “low starting point, high growth rate” compensatory growth pattern; its total dry weight before transplanting and at 20 days after transplanting were both the lowest, but its stage II increase multiple was relatively high, with the key mechanism being the zero-damage protection of roots by “potted transplanting”. Tray seedling cultivation showed a “lag–burst” growth pattern, with slower growth in stage I and leading mean daily weight gain and increase multiple in stage II. Floating seedling cultivation showed a “steady leading” growth pattern, with higher mean daily weight gain and increase multiple in stage I, but the lowest increase multiple in stage II, possibly related to the relatively weaker adaptability of the “slender” root system of floating seedlings after entering field soil. Two-stage seedling cultivation showed a “steady intermediate” growth pattern, with the highest total dry weight before transplanting, but bare-root transplanting still caused some transplanting injury, so its post-transplanting growth advantage was not sustained and amplified.

Note

PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. In A, ns above bars indicates no significant difference among treatments at the same stage, * indicates significant difference, ** indicates highly significant difference ($P < 0.05$, Tukey's multiple comparison test); error bars represent standard deviation. Total dry weight per plant = root dry weight +

shoot dry weight (blanched at 105°C for 30 min, dried at 70°C to constant weight). In B, mean daily weight gain = (final total dry weight – initial total dry weight) / interval days, where stage I is from 5 days before transplanting to 20 days after transplanting (25-day interval), and stage II is from 20 days to 40 days after transplanting (20-day interval). In C, increase multiple = final total dry weight / initial total dry weight.

3.5 Effects of Different Seedling Methods on Root Activity

Differences in dry matter accumulation are underlain by differences in root absorption function, and root activity is a direct indicator of this function [24–25]. Root activity is an important physiological indicator reflecting root absorption function, metabolic intensity, and stress adaptability [16, 24–25]. As shown in Fig. 5 and Tukey's multiple comparison results, root activity in all treatments showed obvious temporal dynamics before and after transplanting, and differences among time points within each treatment varied with seedling method.

At 5 days before transplanting, floating seedling cultivation had the highest root activity (0.183 mg TTF·g⁻¹·h⁻¹), significantly higher than other treatments ($P < 0.05$), possibly related to more active root metabolism in nutrient solution. Paper pot (0.094) and two-stage (0.062) were intermediate, and tray was the lowest (0.045).

At 20 days after transplanting, root activity in paper pot was significantly higher than at 5 days before transplanting (mean difference = - 0.1448, $P = 0.0337$), while floating, two-stage, and tray treatments showed increases but not significant differences ($P > 0.05$). Among treatments, two-stage seedling cultivation had the highest root activity (0.481 mg TTF·g⁻¹·h⁻¹), significantly higher than paper pot (0.255, $P < 0.05$) and tray (0.292, $P < 0.05$), and 50.8% higher than floating (0.319) ($P < 0.05$). The high root activity in two-stage seedling cultivation is because a strong root system had already been established during the large-pot stage, allowing roots to rapidly adapt to the field environment and initiate vigorous metabolic activity after transplanting.

At 40 days after transplanting, root activity in paper pot was further significantly higher than at 20 days after transplanting (mean difference = - 0.1003, $P = 0.0235$), and highly significantly higher than at 5 days before transplanting (mean difference = - 0.245, $P = 0.0035$); two-stage showed an increase compared with 20 days after transplanting, but not significant (mean difference = - 0.1313, $P = 0.0778$); floating and tray showed no significant differences among time points ($P > 0.05$). Two-stage seedling cultivation had the highest root activity (0.620 mg TTF·g⁻¹·h⁻¹), while paper pot (0.460), tray (0.510), and floating (0.520) were similar. Root activity in paper pot increased by 80.4% from 20 to 40 days after transplanting, the largest increase among the four treatments. This result is consistent with the higher dry matter increase multiple (10.73-fold) of paper pot seedlings during 20–40 days after transplanting, further confirming the protective effect of “potted transplanting” on root function.

Note

PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. Data are mean $\pm$ standard deviation ($n = 3$). Tukey's multiple comparison test was used among time points within the same seedling method; * indicates $P < 0.05$, ** indicates $P < 0.01$, ns indicates no significant difference ($P > 0.05$). Root activity was determined by the TTC reduction method, unit: $\text{mg TTF} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. 5 days before transplanting, 20 days after transplanting, and 40 days after transplanting represent the seedling stage, rosette stage, and vigorous growth stage, respectively.

3.6 Effects of Different Seedling Methods on Agronomic Traits of Tobacco Plants After Transplanting

Root activity and dry matter accumulation are ultimately reflected in aboveground agronomic traits ^[26]. As shown in Fig. 6 and Tukey's multiple comparison results, the effects of different seedling methods on agronomic traits at 20 and 40 days after transplanting in 2026 varied with indicator and stage.

At 20 days after transplanting, there were no significant differences among treatments in leaf number, stem height, stem diameter, maximum leaf width, or single leaf area ($P > 0.05$). Among them, stem height was higher in floating (8.13 cm) and two-stage (7.81 cm), and lower in paper pot (4.03 cm); maximum leaf width was highest in floating (10.89 cm) and lowest in paper pot (7.64 cm); single leaf area was highest in floating (161.4) and lowest in paper pot (81.99). In terms of maximum leaf length, paper pot (16.89 cm) was significantly lower than floating (23.34 cm, $P < 0.01$) and two-stage (21.41 cm, $P < 0.05$), with no significant differences among other treatments; differences among floating, two-stage, and tray (19.78 cm) were not significant ($P > 0.05$).

At 40 days after transplanting, there were no significant differences among treatments in leaf number or maximum leaf width ($P > 0.05$). In terms of stem height, paper pot (18.88 cm) was significantly lower than floating (27.52 cm) and tray (30.22 cm) ($P < 0.05$), with no significant differences among other treatments; two-stage (21.78 cm) was intermediate. In terms of stem diameter, paper pot (6.16) was significantly lower than tray (7.10) ($P < 0.05$), with no significant differences among other treatments. In terms of maximum leaf length, floating (49.92 cm) was significantly higher than paper pot (42.41 cm) and two-stage (42.37 cm) ($P < 0.01$), and tray (47.53 cm) was significantly higher than paper pot and two-stage ($P < 0.05$); paper pot and two-stage were not significantly different; floating and tray were not significantly different. In terms of single leaf area, floating (709.7) was significantly higher than paper pot (540.7, $P < 0.01$) and two-stage (586, $P < 0.05$), with no significant differences among other treatments.

Overall, floating and tray treatments performed better in stem height, maximum leaf length, and single leaf area at 40 days after transplanting, paper pot was relatively lower, and two-stage was intermediate; however, leaf number and maximum leaf width showed no significant differences among treatments, indicating that the effects of different seedling methods on agronomic traits are indicator-specific.

Note

A–F are leaf number (A), stem height (B), stem diameter (C), maximum leaf length (D), maximum leaf width (E), and single leaf area (F) at 20 and 40 days after transplanting. PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. Data are mean $\pm$ standard deviation of three replicates in 2026 ($n = 3$). Within the same indicator and sampling time, * indicates significant difference among treatments ($P < 0.05$, Tukey's multiple comparison test); ns indicates no significant difference ($P > 0.05$). Leaf number unit: number; stem height, stem diameter, maximum leaf length, and maximum leaf width units: cm; single leaf area unit: cm^2 .

4 Discussion

4.1 Differential Regulatory Mechanisms of Different Seedling Methods on Root Architecture of Tobacco Seedlings

Based on the above results, this study found that the four seedling methods produced distinct and characteristic differential effects on various root architecture parameters of tobacco seedlings [1, 9, 11–12, 18]. These differences mainly originate from the different regulatory modes of the methods on root growth space, aeration, and mechanical resistance [1, 3, 5, 8–9].

Floating seedling cultivation had the greatest total root length (two-year mean 14.00 cm), but root dry weight (0.04517 g) and root-shoot ratio (0.1715) were at intermediate levels [1, 8, 15]. This “slender” root architecture is a typical adaptive feature of long-term growth in nutrient solution—roots do not need to penetrate substrate to obtain water and nutrients, thus lacking mechanical stimulation for branching, and root growth is mainly elongation-oriented [1, 8, 16]. Floating seedling roots have obvious functional deficiencies after transplanting: few root hairs and low root activity; after transplanting, roots must undergo transformation from “water roots” to “dry roots,” prolonging the recovery period [13–14, 16].

Unlike floating seedling cultivation, tray seedling cultivation had more roots (two-year mean 47.17) and the largest root-shoot ratio (0.2318). The larger single-cell volume of trays (approximately 142.5 cm^3) provides ample horizontal and vertical growth space for roots, facilitating free branching and biomass accumulation [9–10, 17]. The root architecture of tray seedlings is characterized by “multiple branches and high biomass,” with strong absolute root growth before transplanting. However, tray seedling cultivation also involves bare-root transplanting, during which many root hairs and young lateral roots are damaged. This deficiency is reflected in the dry matter accumulation data at 20 days after transplanting—total dry weight of tray seedlings at 20 days after transplanting (1.553 g) was not significantly different from two-stage (1.568 g), but paper pot was only 0.979 g, indicating that bare-root transplanting had a more obvious impact on small-root-domain seedlings such as paper pot [9, 12, 17].

Two-stage seedling cultivation, developed on the basis of tray seedling cultivation, had the highest root dry weight (two-year mean 0.07422 g), with root-shoot ratio (0.2132) second only to tray. The large-pot

stage (10 cm × 10 cm nutritional pots) provided ample growth space for roots, allowing full horizontal expansion. Moderate inhibition of the main root during temporary planting promoted massive lateral root occurrence, forming high-quality root architecture. However, two-stage seedling cultivation also faces transplanting injury from bare-root transplanting, but its larger root biomass base partially offset the adverse effects of transplanting damage^[18–19].

Compared with the above three methods, paper pot seedling cultivation showed a unique “intermediate” root architecture—root length (10.28 cm) and root number (39.08) were at intermediate levels, but root-shoot ratio was the smallest (0.1358). The core of the paper pot “air-root pruning” principle is that when root tips grow to the bottom opening of the paper pot, because air humidity cannot meet the water requirements for normal root growth, the exposed main root tips gradually dry and die, effectively cutting the main root tip without leaving a wound, thereby promoting lateral root growth^[20–22]. However, in this study, root dry weight in paper pot (0.02403 g) was lower than in tray (0.03723 g) and two-stage (0.07422 g), suggesting that the advantage of the “air-root pruning” effect in promoting lateral root occurrence has not yet fully offset the limitation of its smaller root domain space (single-cell volume approximately 99.5 cm³) on root biomass accumulation^[11–13]. Therefore, appropriately increasing paper pot aperture and depth to enhance penetration rate is a key technical path for further optimizing paper pot seedling effectiveness.

4.2 Essence of the “Air-Root Pruning” Effect and the Key Role of Paper Pot Penetration Rate

After clarifying the root architecture characteristics of paper pots, the core mechanism can be further analyzed through penetration rate. Through correlation analysis, this study quantitatively demonstrated the relationship between paper pot penetration rate and post-transplanting root growth indicators^[11, 13, 21]. Penetration rate was highly significantly and positively correlated with root dry weight at 40 days after transplanting ($r = 0.8042$, $P < 0.01$) and significantly and positively correlated with root activity at 40 days after transplanting ($r = 0.6138$, $P < 0.05$), indicating that penetration rate has a sustained positive effect on post-transplanting root function. This finding reveals the dual effects of penetration rate: the direct effect is that penetrating roots directly enter field soil, achieving damage-free transition of “root-intact transplanting”; the indirect effect is that the penetration process induces massive lateral root occurrence, optimizes root architecture, and enhances root activity^[11, 21–22].

It is noteworthy that penetration rate was highly significantly and negatively correlated with pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$), indicating that the higher the penetration rate, the lower the pre-transplanting root dry weight. This may be related to the drying and death of penetrating root tips in air, resulting in reduced root biomass accumulation inside the paper pot, indicating that although a higher penetration rate promoted outward root penetration, it did not correspondingly increase total pre-transplanting root dry weight^[11, 21–22]. However, penetration rate was not significantly correlated with pre-transplanting root activity ($r = -0.3498$, $P > 0.05$) or root activity at 20 days after

transplanting ($r = 0.422$, $P > 0.05$), indicating that the regulatory effect of penetration rate on root metabolic function at the seedling stage and early post-transplanting stage is not yet stable.

Based on the above correlations, this study proposes a “threshold effect” hypothesis for penetration rate: when penetration rate $< 40\%$, the “air-root pruning” effect is insufficient, and root architecture optimization is limited; when penetration rate $> 60\%$, the root protection effect and architecture optimization reach a relatively optimal balance. In this study, the average paper pot penetration rate was 63.7%, at a moderate to high level, with room for improvement. Optimizing paper pot specifications (aperture increased to 5.5–6.0 cm, depth increased to 6.5–7.0 cm) is expected to further enhance penetration rate.

4.3 Compensatory Growth Induced by the “Root Protection Effect” and Its Physiological Basis

Increased penetration rate not only improves seedling architecture but also affects post-transplanting compensatory growth through the root protection effect^[11–13]. This study found that paper pot seedlings exhibited obvious compensatory growth characteristics after transplanting—the lowest total dry weight at 20 days after transplanting (0.979 g), but a relatively high increase multiple during 20–40 days after transplanting (10.73-fold). This “low starting point, high growth rate” growth characteristic can be explained by the “compensatory growth” theory^[3, 11–12]. Before transplanting, root biomass of paper pot seedlings is limited by paper pot space, but after transplanting, the paper pot is completely buried in soil, and roots are not mechanically damaged during transplanting. Once the paper pot begins to degrade in soil and roots break through the paper pot wall into field soil, they obtain ample growth space and nutrient resources, thereby initiating rapid compensatory growth^[12–13, 22].

The physiological basis of compensatory growth in paper pot seedlings lies in the sustained increase in root activity^[24–25]. In this study, root activity of paper pot seedlings increased from 0.255 mg TTF·g⁻¹·h⁻¹ at 20 days after transplanting to 0.460 mg TTF·g⁻¹·h⁻¹ at 40 days after transplanting, an increase of 80.4%, the largest among the four treatments. This result is consistent with the correlation among penetration rate, root activity, and post-transplanting growth^[11, 13, 21]. It is worth noting that the high protective enzyme activities of paper pot seedlings (SOD 1273 U·g⁻¹, CAT 2.844 mg·min⁻¹·g⁻¹) may provide important physiological protection for their compensatory growth. The local oxidative stress induced by root tip drying and death during “air-root pruning” activated the protective enzyme system, enabling paper pot seedlings to have stronger adaptability to the field environment after transplanting^[11, 21, 23].

4.4 “Quality–Cost–Environment” Trade-Offs Among Different Seedling Methods

In addition to the above physiological and growth effects, trade-offs must be considered from the dimensions of quality, cost, and environment^[1–2, 7]. Integrating multiple indicators such as root

architecture, protective enzyme activities, post-transplanting dry matter accumulation, and agronomic traits, different seedling methods each have their suitable production scenarios^[12]. Two-stage seedling cultivation has relatively superior physiological seedling quality, but is cumbersome (requires temporary planting) and labor-intensive (relative value 1.8), facing a bottleneck of high labor costs in large-scale production^[18–19]. It is suitable for small-scale production or experimental research with extremely high requirements for seedling quality and sufficient labor. Tray seedling cultivation is relatively simple to operate and has relatively high root biomass, but bare-root transplanting injury still exists^[9–10, 17]. It is suitable for large-scale production, but attention must be paid to root protection during transplanting. Floating seedling cultivation is the most convenient to manage (labor relative value 0.8) and is currently the mainstream method, but its root architecture has inherent deficiencies, with the lowest stage II increase multiple after transplanting. It is suitable for large-scale intensive production, but measures such as leaf clipping and hardening should be combined to improve seedling quality^[1, 8, 14]. Although the comprehensive score of paper pot seedling cultivation is relatively low, its advantages in labor saving (labor relative value 0.6, only 33% of two-stage), root protection (zero transplanting damage), and environmental friendliness (100% degradable) are obvious.

The 2026 agronomic trait results showed that floating and tray treatments performed better in stem height, maximum leaf length, and single leaf area at 40 days after transplanting, paper pot was relatively lower, and two-stage was intermediate. This is basically consistent with the dry matter accumulation trend, but the relative disadvantage of two-stage in agronomic traits is not completely consistent with its higher root activity and SOD activity, possibly because its dry matter is more allocated to stem and leaf thickening rather than elongation. In addition, leaf number and maximum leaf width showed no significant differences among treatments, indicating that the effects of different seedling methods on agronomic traits are indicator-specific^[26].

In summary, the selection of different seedling methods should comprehensively consider factors such as seedling quality requirements, production scale, labor costs, and environmental benefits. Under current production conditions, two-stage and tray seedling cultivation remain important choices for ensuring seedling quality; paper pot seedling cultivation, as an emerging technology, is expected to play an important role in labor-saving seedling cultivation after further optimizing specifications and formulations.

5 Conclusion

(1) The four seedling methods differentially regulated tobacco seedling root architecture. Tray and two-stage seedling cultivation had more roots (two-year mean both 47.17), two-stage had the highest root dry weight (0.07422 g), tray had the largest root-shoot ratio (0.2318), followed by two-stage (0.2132), floating had the longest roots (14.00 cm), and paper pot had the smallest root-shoot ratio (0.1358). The “air-root pruning” effect resulted in an average root penetration rate of 63.7% in paper pot seedlings. Penetration rate was highly significantly and positively correlated with root dry weight at 40 days after

transplanting ($r = 0.8042$, $P < 0.01$), significantly and positively correlated with root activity at 40 days after transplanting ($r = 0.6138$, $P < 0.05$), but highly significantly and negatively correlated with pre-transplanting root dry weight ($r = -0.8904$, $P < 0.001$).

(2) Two-stage seedling cultivation had the highest SOD activity (two-year mean $1828 \text{ U}\cdot\text{g}^{-1}$), paper pot had the highest CAT activity ($2.844 \text{ mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), and paper pot also had relatively high SOD activity ($1273 \text{ U}\cdot\text{g}^{-1}$). The protective enzyme system showed a synergistic response to oxidative stress induced by “temporary planting hardening” and “air-root pruning.”

(3) At 40 days after transplanting, total dry weight per plant was higher in tray (15.28 g) and floating (14.91 g), and lower in two-stage (11.58 g) and paper pot (10.50 g), but differences among treatments were not significant. Paper pot seedlings had a higher dry matter increase multiple during 20–40 days after transplanting (10.73-fold) and the largest increase in root activity (80.4%), confirming compensatory growth induced by the root protection effect of “potted transplanting.” In 2026, agronomic traits were superior in floating and tray, relatively lower in paper pot, and intermediate in two-stage.

(4) Considering seedling quality, production cost, and environmental benefits, two-stage seedling cultivation showed the best overall performance, while paper pot seedling cultivation has unique advantages in labor saving (labor input only 33% of two-stage), root protection (zero transplanting damage), and environmental friendliness (100% degradable). It is recommended to further optimize paper pot specifications (aperture increased to 5.5–6.0 cm, depth increased to 6.5–7.0 cm) to enhance penetration rate and fully realize its potential for labor-saving seedling cultivation.

Declarations

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Competing interests The authors declare that they have no conflicts of interest.

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Figures

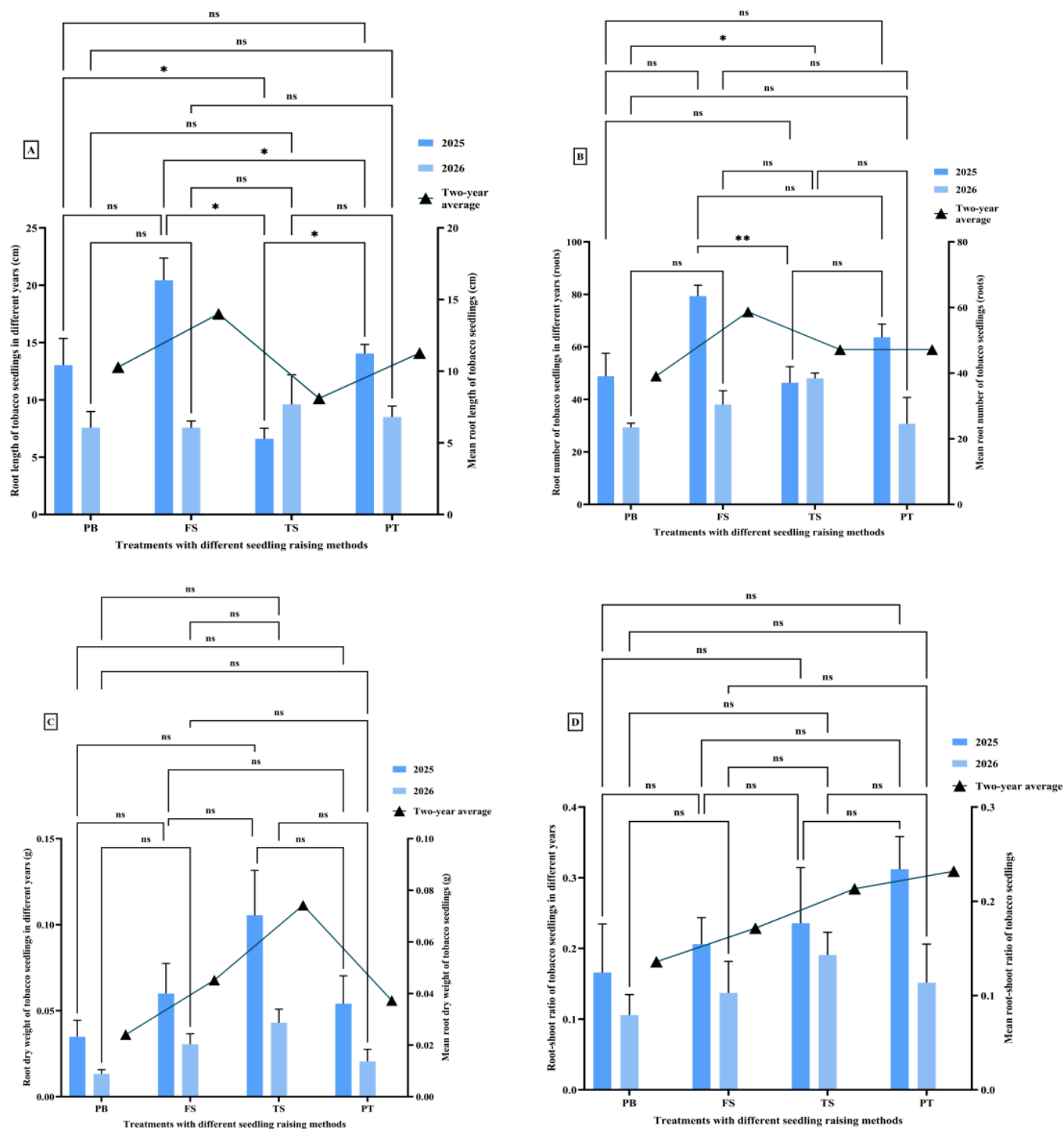


Figure 1

Root architecture parameters of tobacco seedlings before transplanting under different seedling methods in 2025–2026.

Note: PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. A, B, C, and D correspond to root length (cm), root number (number), root dry weight (g), and

root-shoot ratio, respectively. Different colored bars represent different years (dark for 2025, light for 2026). ns above bars indicates no significant difference among treatments in the same year, * indicates significant difference, ** indicates highly significant difference ($P < 0.05$, Tukey's multiple comparison test); error bars represent standard deviation. Lines represent the trend of the two-year mean.

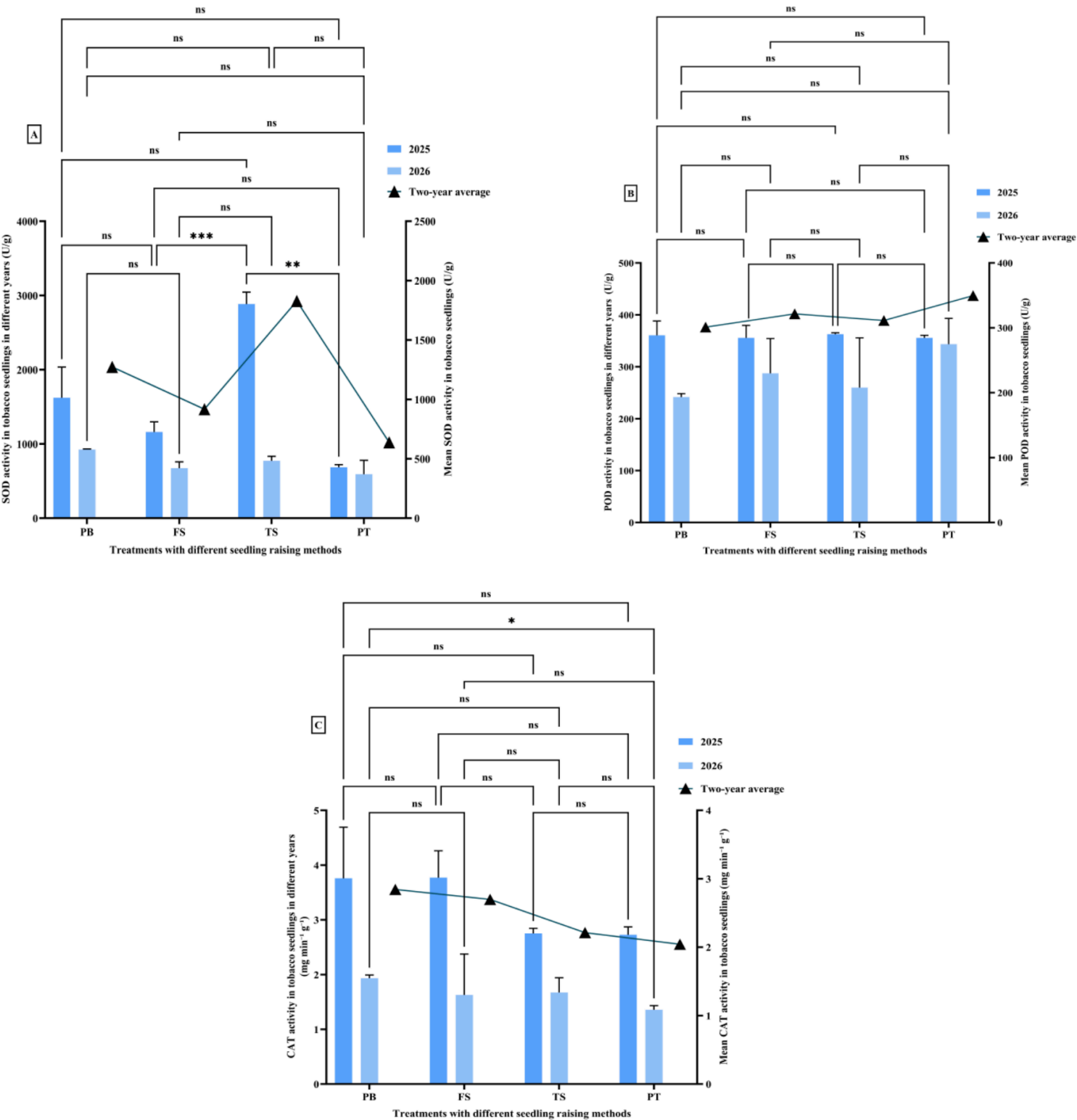


Figure 2

Leaf protective enzyme activities of tobacco seedlings before transplanting under different seedling methods.

Note: PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. SOD activity unit: $\text{U}\cdot\text{g}^{-1}$; POD activity unit: $\text{U}\cdot\text{g}^{-1}$; CAT activity unit: $\text{mg}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$. ns above bars indicates no significant difference among treatments in the same year, * indicates significant difference, ** indicates highly significant difference ($P < 0.05$, Tukey's multiple comparison test); error bars represent standard deviation. Lines represent the trend of the two-year mean.

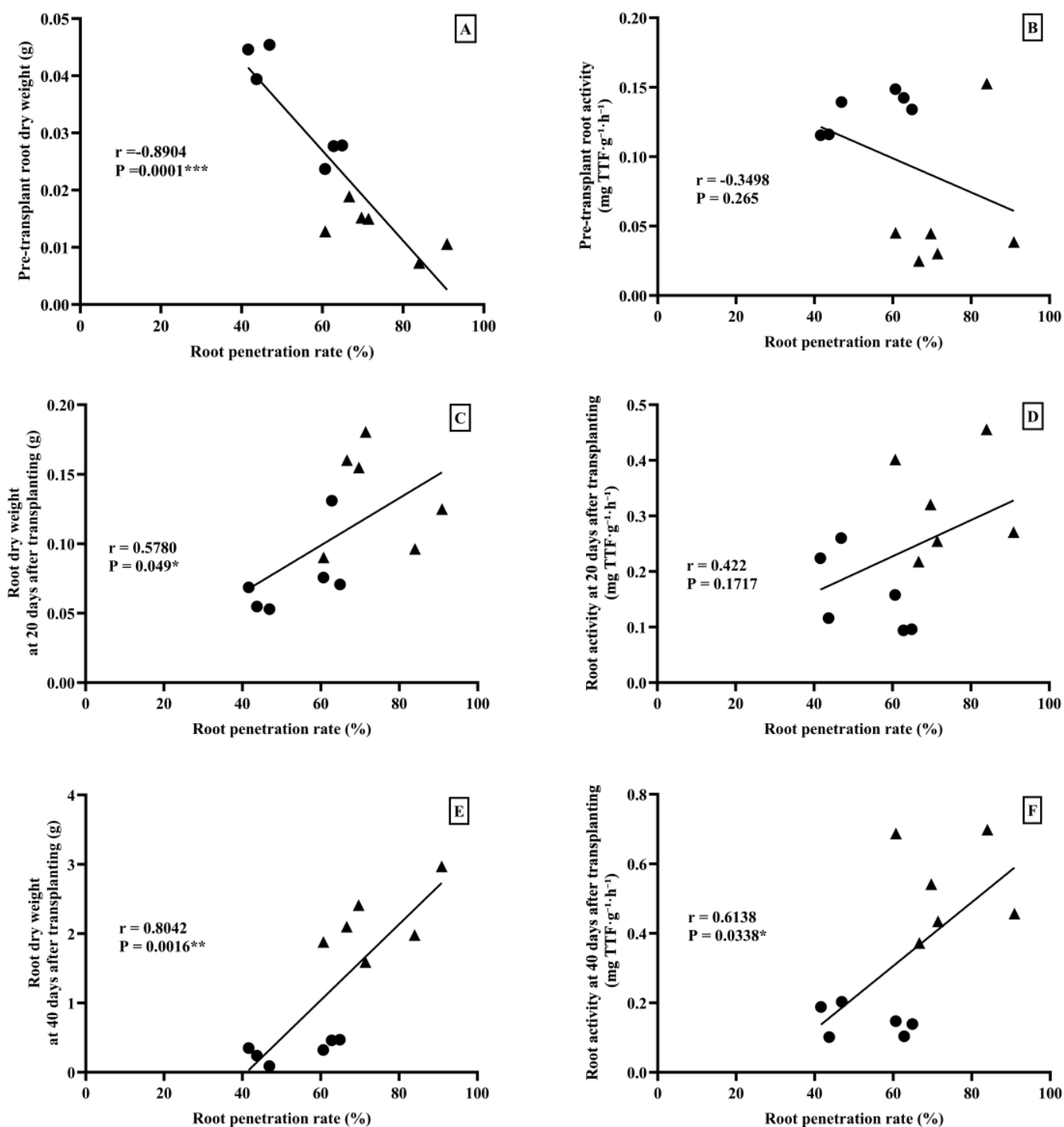


Figure 3

Correlation between paper pot penetration rate and seedling effectiveness indicators.

Note: A–F are scatter plots of paper pot penetration rate versus pre-transplanting root dry weight (A), pre-transplanting root activity (B), root dry weight at 20 days after transplanting (C), root activity at 20 days after transplanting (D), root dry weight at 40 days after transplanting (E), and root activity at 40 days

after transplanting (F). The x-axis is paper pot penetration rate (%), and the y-axis is the corresponding indicator. ● represents 2025 data points (n = 6), ▲ represents 2026 data points (n = 6), solid lines are linear fitting trend lines for pooled two-year data, and shaded areas represent 95% confidence intervals. Pearson correlation coefficients (r) and significance levels are indicated (*P < 0.05, **P < 0.01, ***P < 0.001). Data are from the paper pot (PB) treatment. Penetration rate (%) = (number of roots penetrating the paper pot wall / total root number) × 100%.

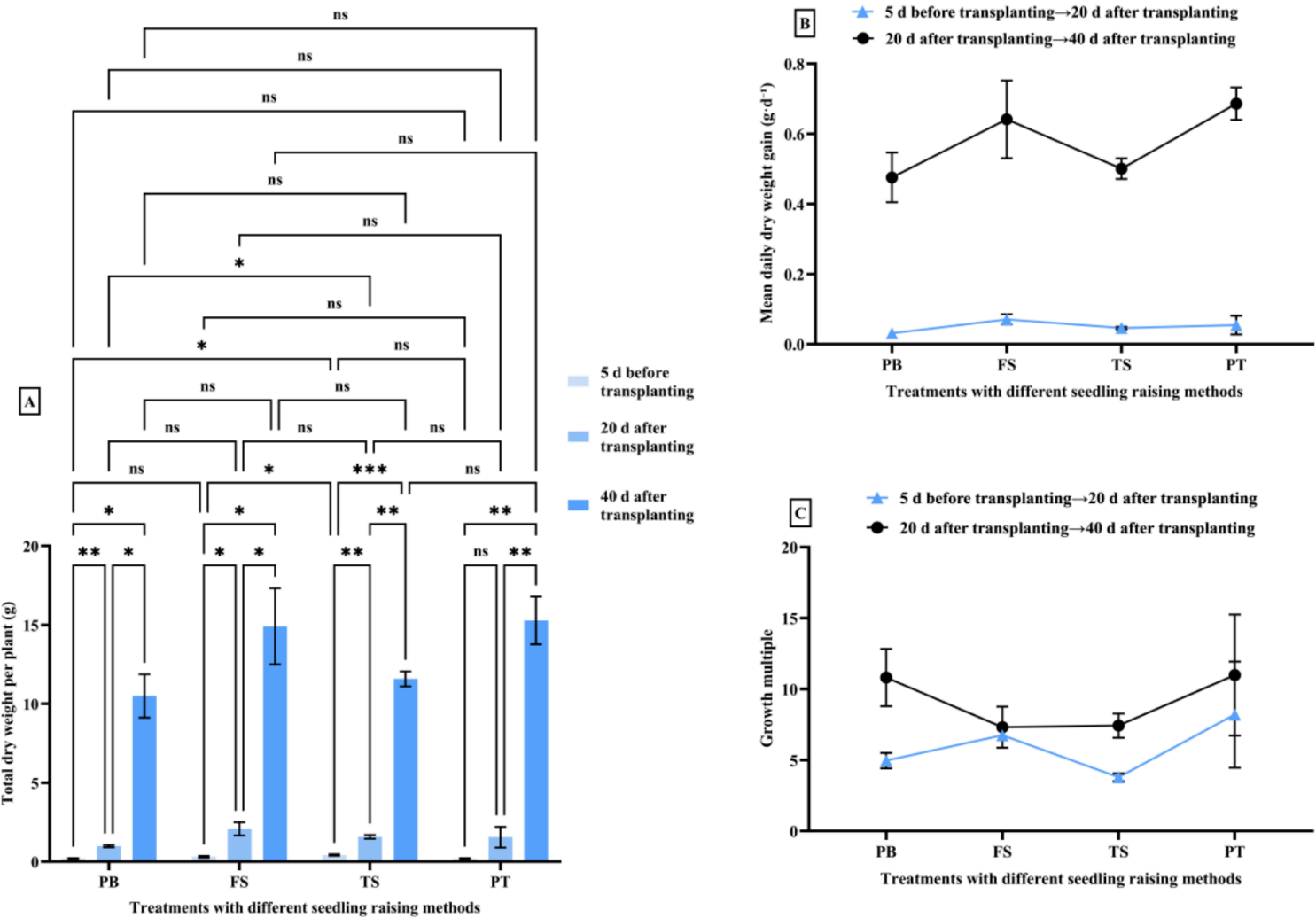


Figure 4

Dynamics of total dry matter accumulation per tobacco seedling before and after transplanting under different seedling methods (two-year mean).

Note: PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. In A, ns above bars indicates no significant difference among treatments at the same stage, * indicates significant difference, ** indicates highly significant difference (P < 0.05, Tukey’s multiple comparison test); error bars represent standard deviation. Total dry weight per plant = root dry weight + shoot dry weight (blanched at 105°C for 30 min, dried at 70°C to constant weight). In B, mean daily weight gain = (final total dry weight – initial total dry weight) / interval days, where stage I is from 5 days

before transplanting to 20 days after transplanting (25-day interval), and stage II is from 20 days to 40 days after transplanting (20-day interval). In C, increase multiple = final total dry weight / initial total dry weight.

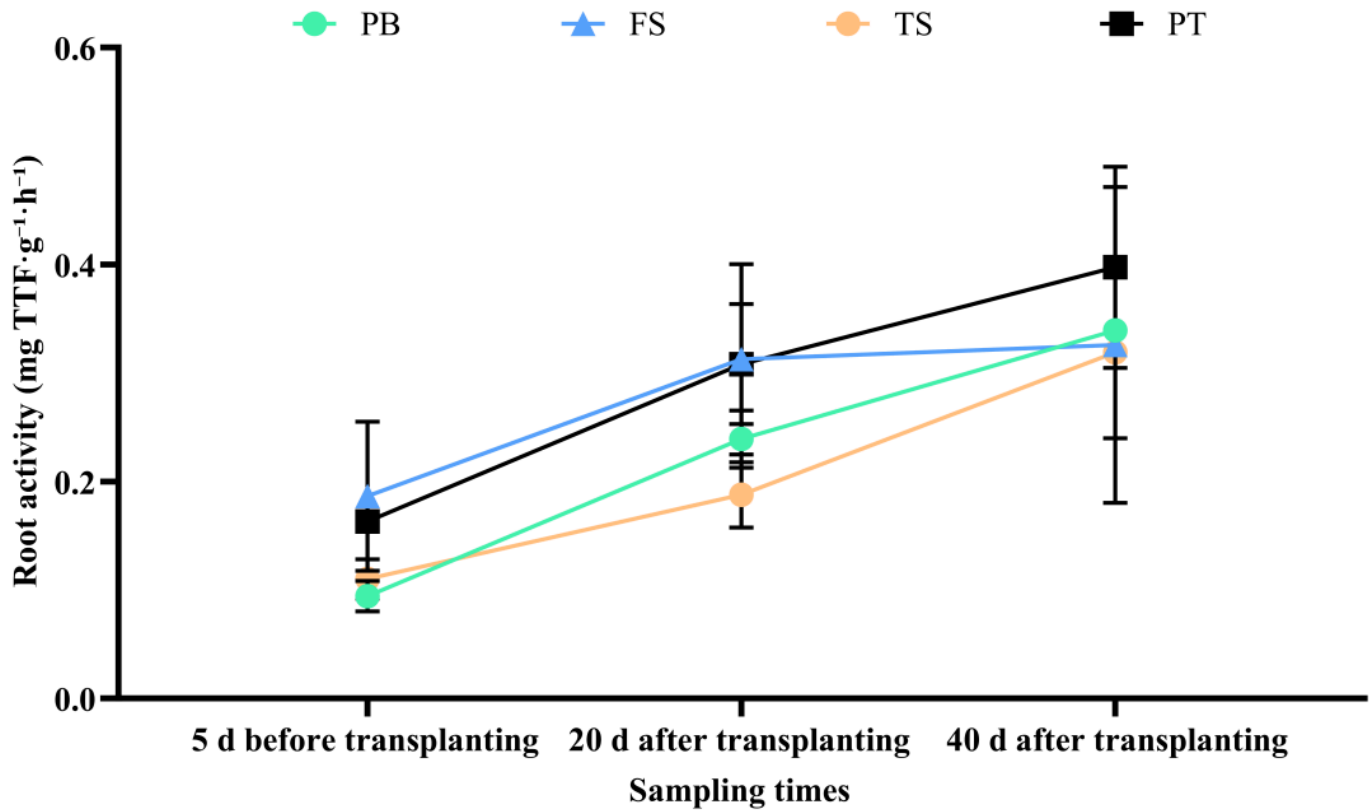


Figure 5

Dynamic changes in root activity of tobacco seedlings before and after transplanting under different seedling methods.

Note: PB: paper pot; FS: floating seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. Data are mean $\pm$ standard deviation ($n = 3$). Tukey's multiple comparison test was used among time points within the same seedling method; * indicates $P < 0.05$, ** indicates $P < 0.01$, ns indicates no significant difference ($P > 0.05$). Root activity was determined by the TTC reduction method, unit: $\text{mg TTF} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. 5 days before transplanting, 20 days after transplanting, and 40 days after transplanting represent the seedling stage, rosette stage, and vigorous growth stage, respectively.

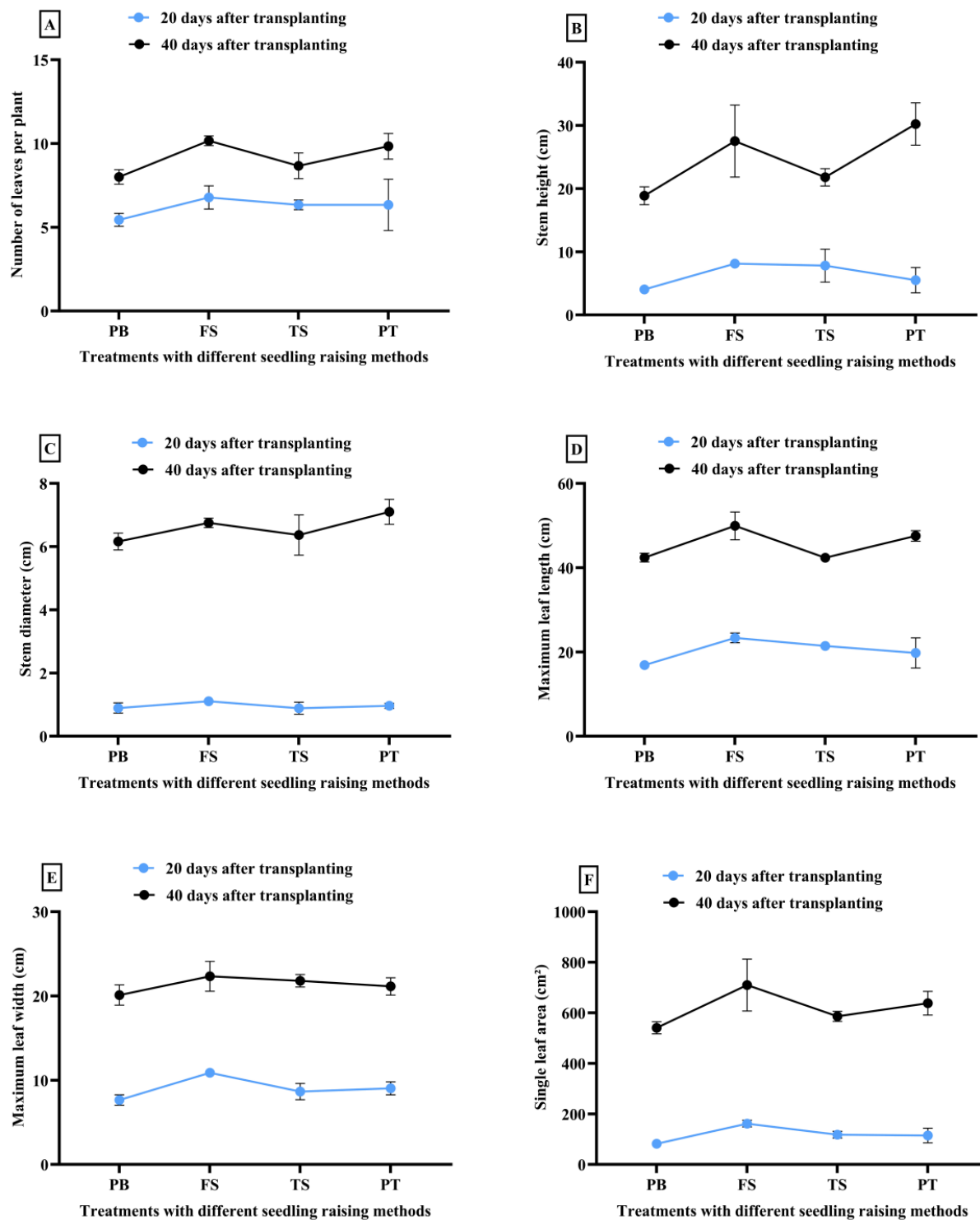


Figure 6

Effects of different seedling methods on agronomic traits of tobacco plants at 20 and 40 days after transplanting.

Note: A–F are leaf number (A), stem height (B), stem diameter (C), maximum leaf length (D), maximum leaf width (E), and single leaf area (F) at 20 and 40 days after transplanting. PB: paper pot; FS: floating

seedling cultivation; TS: two-stage seedling cultivation; PT: tray seedling cultivation. Data are mean $\pm$ standard deviation of three replicates in 2026 ($n = 3$). Within the same indicator and sampling time, * indicates significant difference among treatments ($P < 0.05$, Tukey's multiple comparison test); ns indicates no significant difference ($P > 0.05$). Leaf number unit: number; stem height, stem diameter, maximum leaf length, and maximum leaf width units: cm; single leaf area unit: cm^2 .