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Growth, physiological and N, P, K accumulation responses of *Erythropalum scandens* Bl. Seedlings under different substrates

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Abstract

Erythropalum scandens Bl. is a medicinal woody vegetable found in southern China and parts of Southeast Asia. Studies have shown improper substrate hindered *E. scandens* seedling growth, causing water accumulation and nutrient deficiency. In pursuit of an ideal growth medium for *E. scandens* seedlings during the early stages, this study conducted a pot experiment to identify a mixed substrate with optimal water permeability and fertility. In this study, pure Alfisols soil treatment as the control (CK), and two soilless substrates (peat soil and perlite) were combined with Alfisols soil into different volume ratios, in order to better use soil resources from understory space and balance the texture of mixed substrates. The growth, physiological characteristics and nutrient status of 24-month-old *E. scandens* seedlings were determined after planting in different mixed ratios. The results showed that as the proportion of peat soil increased in the mix, most indexes exhibited an initial increase followed by a decline, while soluble protein content decreased consistently. Conversely, an increasing perlite ratio resulted in a general decline in most growth and physiological indexes. Root growth, biomass accumulation and chlorophyll content, peaked in the 66.67% Alfisols soil + 33.33% perlite (T4) treatment. Notably, T3 (66.67% Alfisols soil + 33.33% peat soil) showcased the best above-ground growth, while T1 (50.00% Alfisols soil + 50.00% peat soil) excelled in element content accumulation. In conclusion, the cultivation substrate should primarily consist of Alfisols soil, constituting at least 50%. The addition of peat soil enhances above-ground growth and nutrients accumulation, while perlite contributes to robust root development. One third of peat soil and a small amount of perlite can be added to the substrate during *E. scandens* seedling cultivation, and proper fertilization should also be used in order to increase nutrient accumulation in aboveground and underground parts. This research provides valuable insights into maximizing the potential of *E. scandens* seedlings through precise cultivation methods.

Keywords Medicinal plant, Peat soil, Perlite soil, Root morphology, Nutrients uptake

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Introduction

Optimal cultivation conditions play a vital role in achieving plant growth and yield increments during seedling stage and outdoor environment of many plant species (such as timber trees, crops, vegetables and so on) [1, 2]. Soilless substrates, including peat soil, coconut husk, perlite, and vermiculite, have been traditionally employed in the cultivation of flowers, fruits, and vegetables, offering potential advantages in production efficiency and cost reduction [3–5]. In the 1970s, the research explored the cultivation of lettuce (*Lactuca sativa* L.), perennial ryegrass (*Lolium perenne* L.) and other vegetables using peat soil, perlite and similar nutrient-rich soilless substrates [6]. Then some other soilless substrates (such as wood chips, de-inked paper sludge, chitosan, wheat straw, banana leaves and cotton stalk, etc.) were used as potential light additive substrates or substrate supplements, which improve the soil enrichment and crop growth status [7–10]. Studies have shown that incorporating light additive substrates can significantly boost the growth, yield and quality of vegetables [11, 12]. For example, the combination of perlite and peat in a 1:1 (v/v) ratio can effectively be used for the cultivation of vegetables (such as Leek, etc.) [13]. A mixed volume ratio of 30% peat+30% compost+30% coir+10% perlite has been found suitable for the cultivation of various vegetables including kale (*Brassica napus* L. 'Red Russian'), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.) and mustard (*Brassica juncea* L. 'Ruby Streaks') [14]. So peat soil is a great substrate to improve the growth and yield of various vegetables. Besides, proper additions of peat soil would also improve the nutrient status of mixed substrates. For lettuce, peat soil was a suitable substrate for its cultivation [15], and the mixed substrate [(mushroom residue: vermiculite: perlite=6:2:2 (v/v)] was superior to other treatments in terms of plant yield and leaf growth, which has high contents of total phosphorus, alkali-hydrolytic nitrogen, available potassium and organic matter [16]. Besides, the study of Cicek et al. [17] showed that under the ratio of 94% peat/perlite+6% bat manure, basil plants had the best comprehensive performance of various agronomic traits and yield. Peat soil: perlite=3:1 (v/v) can be used to plant wild rocket salad (*Diplotaxis tenuifolia* (L.) DC) seedlings [18]. In short, rational use of light additive substrates can improve the growth condition and photosynthetic capacity of plants, and also have a strong promoting effect on the improvement of the yield and quality of leafy vegetables. However, it is worth noting that some studies have reported adverse effects of certain light additive substrates on specific crops. For example, Beaulieu et al. [19] observed a decrease in plant biomass when crops like tomatoes and chrysanthemums were grown in a Hydra Fiber-peat mix ("fiber") matrix compared with bark and peat soil. Pure light additive substrate substrates have been associated with unstable root growth and a tendency for seedlings to lodge. Besides, some researches about leafy vegetables (such as *Acer truncatum*, mallow and so on) [20, 21] showed that proper substrates would improve the growth and quality of vegetables seedlings. Therefore, soil and light additive substrate are often employed to provide better root stability and enhance their resistance to wind and lodging [22]. It is evident that the appropriate use and proportion of soil and light additive substrate necessitate extensive testing and investigation for verification.

The resources of leafy vegetable are abundant around the world, especially in Asia, Africa, South America and some other areas, including sorts of wild vegetables (and also known as "forest vegetables", etc.) with satisfactory taste and high nutrient values. *Erythropalum scandens* Bl. (called "Chicangteng" in Chinese) is a woody leafy vegetable commonly found in China, Vietnam and several Southeast Asian countries. It has the function of soil and water conservation, and often grows in limestone mountains and tropical rainforests. The young stems and leaves are rich in nutrition, and have high edible and medicinal value [23, 24]. With its unique flavor, *E. scandens* is widely introduced and cultivated in Guangxi, China, including about 67 hectares in Daxin County of Chongzuo City and nearly 100 hectares in Nanning Arboretum. It commands a market price ranging from 40 to 100 yuan per kilogram, presenting a promising economic opportunity with an average annual income of over 2660–6650 yuan per hectare [25]. Compared with other leafy vegetables, the edible parts of *E. Scandens* had better flavor and taste, which has been introduced in many areas of Guangxi and improved local economic development. So it is significant to do some researches about its cultivation and harvests. There have been many studies in the early stage of the cultivation measures and some systematic studies on fertilization. Guo [26] showed that 24-month-old cutting seedlings of *E. scandens.* had the best yield and contents of edible carbohydrates, amino acids, total flavonoids under 0.8 kg/ plant of chicken manure+1.2 kg/ plant of cow manure; for 18-month-old cutting seedlings, 2.14 g/ plant urea+4.44 g/ plant potassium per phosphate+1.33 g/ plant potassium chloride treatment was the most beneficial to their growth, while the growth indexes such as the number of new leaves per plant and the length of new branches were the best, and the nutrient status was the most balanced. The demand for nutrients was very strong during the growth of *E. scandens* seedlings, and N, P, K all have important effects [27, 28]. In addition, there were also studies on the ratio of nitrogen form in *E. scandens*, indicating that NH_4^+-N was preferred by *E. scandens* seedlings [25]. It can be seen from the above studies that *E. scandens* has a strong demand for nutrients, and the substrates mixed with soil, fine sand, perlite and peat soil were suitable for its growth. In Guo [26] study, pure Alfisols soil was used as the cultivation medium. However, the initial nutrient contents of pure Alfisols soil was low in this experiment, causing growth inhibition of *E. scandens* after planting for a period of time. Hence, fertilizers were needed to improve their yield and quality. Besides, the mixed substrate of Alfisols soil: sand : perlite peat=6:2:1 (v/v/v) was used in the Ma et al. [27, 28] and Ma et al. [25] tests. This mixed substrate was better than pure Alfisols soil, with looser texture and richer nutrient contents (Supplementary Table S2). Studies have shown that when Alfisols soil was used for cultivation alone, the matrix was prone to slow water permeation and water accumulation, which caused root rot and other phenomena and affected roots normal growth. Besides, if pure Alfisols soil or other pure soil collected directly from understory space were used as substrates to cultivate *E. Scandens* seedlings, the growth status, yield and quality of those seedlings were strongly inhibited by nutrient restriction, waterlogging and some other factors related to substrates texture. At the same time, pure soil would become far more sticky after irrigation. Therefore, it is of great significant to balance the texture of the substrates, avoid its excessive viscosity and improve the content of the available nutrients. Some researchers found that the addition of some soilless subatrates (perlite, peat soil, bark, etc.) could make the soil looser, which was conducive to the spread and growth of the root, and promote the increase of the yield of the above-ground part. However, the experiments of Ma et al. [27, 28] had a relatively fine ratio of cultivation substrate, and the mixture of perlite and peat soil used in the experiment was provided by Guiyuxin Agricultural Technology Company (Supplementary Table S2). The volume proportion of perlite and peat soil in this mixed matrix was unknown, and it was difficult to promote it in mass production. Therefore, it is essential to explore mixtures of Alfisols soil and other substrates (peat soil, perlite, etc.) in order to explore a reasonable ratio volume for the cultivation and production of *E. scandens* seedlings.

In summary, proper additions of soilless substrates have become important methods during vegetables cultivation, which can improve the growth status, yield and quality of vegetables. Based on the premise that there is no systematic study on the cultivation medium of *E. scandens* at present, this study mixed Alfisols soil with perlite and peat soil in different volume proportions, in order to study the growth, physiological condition and the macro-nutrient (NPK) status in the above-ground and underground parts of *E. scandens* seedlings under different mixed substrates. The study's objectives were (1) To identify suitable mixed substrates for promoting growth, physiological status, and macro-nutrients accumulation in *E. scandens* seedlings (2) To evaluate the roles of peat soil, perlite, and Alfisols soil in influencing the growth and development of *E. scandens.* The findings of this study hold the potential to establish a solid foundation for the mass cultivation and promotion of *E. scandens* production.

Materials and methods

Test site and materials

(1) Test site: The experiment was conducted at the teaching base of the College of Forestry, Guangxi University, Nanning City, Guangxi Zhuang Autonomous Region (108° 22'E, 22° 48'N). The climate of the experimental site was subtropical monsoon, with an average annual temperature and precipitation of 21.6℃ and 1304 mm, respectively. The climate environment was suitable for the growth of *E. scandens* seedlings. To control the light conditions for the experiment, the test site was covered with a layer of black blackout net (The net was 2.5 m high from the ground and was made of a black nylon plastic sheet), which has a light transmittance rate of 67.50% of natural light.

(2) Plant materials: The 24-month-old (the time from propagation) cutting seedlings of *Erythropalum scandens* Bl. in this study were propagated at the teaching base of the College of Forestry, Guangxi University. During April to June in 2021, we pruned the upper tender branches of 48-month-old plant shoots from Nanning Arboretum to create 15–20 cm cuttings (it is a kind of local plant in Guangxi Zhuang Autonomous Region, China, and the parent trees had been planted for 4 years). These cuttings were briefly soaked in a 1000 mg/L IBA solution for 10 s, and then placed in disinfected fine sand to stimulate root growth (the fine sand used for cutting should be soaked and disinfected with 0.2% $KMnO_4$ solution for 24 h and then washed with clean water). In April 2022, the bare roots of the same size seedlings were transplanted into their respective substrates, with an initial cutting of 6.53 mm in ground diameter and 10.25 cm in length.

(3) Substrates: Alfisols soil was collected from a 120-month-old eucalyptus forest at Nanning Arboretum with the following physical and chemical properties: pH 5.65, cation exchange capacity (CEC) 12.5 cmol $_{(+)}$ kg^{-1}, volumetric weight 1.29 g/cm^3, total carbon 12.37 g/kg, total nitrogen 0.72 g/kg, total phosphorus 0.28 g/kg, total potassium 4.37 g/kg, ammonium-nitrogen content 10.04 mg/g, nitrate nitrogen content 12.33 mg/g, available phosphorus content 12.21 mg/g, available potassium content 70.13 mg/g. Changchun Yinong Saishi Peat Development Co., LTD provided peat soil, with the following physical and chemical properties: pH 6.00, cation exchange capacity (CEC) 9.43 cmol $_{(+)}$ kg^{-1}, volumetric weight 0.25 g/cm^3, total carbon 20.35 g/kg, total nitrogen 2.15 g/kg, total phosphorus 2.43 g/kg, total potassium 5.36 g/kg. Nanning Guiyuxin Agricultural Technology

Co., LTD., provided Perlite with a particle size of 3 ~ 6 mm. All the above indexes were determined by lab analysis. The bags used in the experiment was white non-woven bag with a diameter of 25 cm and a height of 25 cm, and 1 plant was planted in each bag with a cultivation medium of different proportions.

Experimental design

A completely randomized experiment was conducted with a total of 8 treatments, including 1 control (CK, 100% Alfisols soil) and 7 treatments (T1-T7) (Supplementary Table S1 and Figure S1). Each group consisted of 10 biological replicates, with 1 seedling per replicate, totaling 80 seedlings. For a comprehensive analysis, 3 seedlings from each treatment were randomly selected and used for destructive sampling to determine root growth indexes, physiological indexes, biomass, and nutrient element contents. Growth and physiological indexes were determined during and after the experiment. The aboveground growth indexes were measured every 60 days from June 2022, while physiological indexes, root growth indexes, biomass and nutrient content for each plant part were measured after 180 days of the experiment (October 2022) (Supplementary Figure S2).

Indexes determination

Growth indexes

Aboveground growth The aboveground growth indexes were measured every 60 days after seedling planting and were measured 3 times in total. Among them, the length of new branches and new internodes were measured using a steel tape within 0.01 cm. The ground diameter and the thickness of the new branches were measured by using electronic Vernier calipers within 0.01 mm. In cases where a plant featured multiple new branches, growth indexes of all branches were measured. The length of new shoots and the number of internodes were summed, and the average value of internode length and new shoot thickness were calculated. All seedlings were measured, with 10 biological replicates in each group.

Underground Growth At 180 days after transplantation, we randomly selected three healthy seedlings from each treatment to assess their root attributes. The plants were removed from the bags and their roots were meticulously cleansed thoroughly with tap water and deionized water (until all the substrates and other impurities were removed from the roots). Subsequently, the roots were carefully seperated by scissors from the junction of fibrous root and root neck, and it was needed to ensure the integrity of the fibrous root structure for subsequent scanning. After thorough cleaning, the roots were subjected to scanning using the Epson Expression 10,000 XL system. The analysis of various root parameters such total root length, total root surface area, total root projection area, total root volume, and the average diameter, was conducted using the WinRHIZO system.

Biomass accumulation

At 180 days after planting, three healthy seedlings were randomly selected for each treatment, washed with clean water and deionized water, blot-dried, divided into three parts of roots, stems and leaves, and placed in the oven for drying. After sterilizing at 105 ℃ for 30 min, drying at 75℃ to constant weight within 0.01 g [24].

Physiological and biochemical indexes

To determine the physiological and biochemical indexes of mature functional leaves at 180 days after planting, the 2nd ~ 5th mature functional leaves were collected from the top buds of the upper branches of 3 seedlings with similar growth status. The content of chlorophyll, soluble sugar and soluble protein content in leaves of *E. scandens* seedlings were determined respectively, Referring to Ma et al., 2023 [25]. There were 3 biological replicates per index.

Leaf total nitrogen, phosphorus and potassium contents

Three seedlings were randomly selected from each treatment at 180 days after planting to determine nutrients contents. The 2nd ~ 5th mature functional leaves from the top bud of the upper branch were taken. The procedure of leaf drying was the same as Sect. 2.3.2 (Biomass accumulation). After drying, the leaf samples were ground into powder and passed through a 60-mesh sieve for the determination of N, P and K content. Total nitrogen (TN) content was determined using a continuous flow analyzer (AA3; Bran + Luebbe, Hamburg, Germany) following the previous method described by Xue et al. [29]. Furthermore, the molybdenum-antimony resistance colorimetric method was used to determine total phosphorus (TP) content, as documented by Lyu et al. [30]. Whereas total potassium (TK) content was assessed by using a flame photometer [31].

Statistical analysis

The data was summarized using Microsoft Excel 2016. IBM SPSS 18.0 was used for variance analysis, Duncan's new complex range method was used for multiple comparisons among the treatments ($P = 0.05$), and Microsoft Excel 2016 was used to draw data charts (all the datas in this study met the assumptions for ANOVA: normality, homogeneity of variance. We used SPSS 18.0 software to check these). Besides, correlation heat map was created by using R4.2.2 software.

Table 1 Growth status of aboveground parts of *Erythropalum Scandens* seedlings under different ratios of substrates

Treatment	Ground diameter increment/△mm	New Branches thickness/mm	Total Length of new branches/cm	Number of new branches	Number of new leaves	Number of internodes	Average length of internodes/cm
CK	0.89 ± 0.31a	1.81 ± 0.41abc	80.83 ± 40.92abc	2.89 ± 1.05bc	16.22 ± 5.85abc	16.78 ± 6.76a	4.67 ± 0.60a
T1	1.26 ± 0.76a	2.31 ± 0.62a	103.74 ± 53.06ab	3.33 ± 1.00abc	17.56 ± 6.50abc	19.89 ± 7.51a	5.03 ± 0.82a
T2	0.90 ± 0.26a	1.45 ± 0.60bc	58.13 ± 39.02bc	2.80 ± 1.03bc	12.80 ± 6.21bc	13.90 ± 7.45a	3.91 ± 1.29a
T3	1.08 ± 0.42a	2.13 ± 0.54a	129.74 ± 76.65a	4.50 ± 1.58a	22.00 ± 9.72a	22.70 ± 10.33a	5.30 ± 1.43a
T4	0.97 ± 0.31a	1.89 ± 0.64abc	85.03 ± 55.24abc	3.70 ± 1.77ab	18.10 ± 10.77ab	19.20 ± 12.18a	4.63 ± 1.36a
T5	0.74 ± 0.27a	2.28 ± 0.43a	115.52 ± 39.44a	4.00 ± 0.87ab	19.67 ± 4.80ab	20.33 ± 4.69a	5.61 ± 1.17a
T6	0.78 ± 0.27a	1.36 ± 0.44c	45.39 ± 22.36c	2.30 ± 0.95c	9.80 ± 4.29c	11.70 ± 5.40a	4.16 ± 1.58a
T7	1.00 ± 0.39a	1.94 ± 0.64ab	105.36 ± 80.73ab	3.30 ± 2.00abc	17.30 ± 11.20abc	19.90 ± 13.09a	4.79 ± 1.21a

Note: Values are mean ± standard deviation (n = 10); different lowercase letters indicate a significant difference between treatments (P < 0.05). Same below.

Table 2 Growth status of underground parts of Erythropalum Scandens seedlings under different ratios of substrates

Treatment	Total root length/cm	Total root surface area/cm^2	Total root projection area/cm^2	Total root volume/cm^3	Root average diameter/mm
CK	940.46 ± 284.35b	53.46 ± 15.70a	17.02 ± 5.00c	0.24 ± 0.07b	0.18 ± 0.01a
T1	1374.27 ± 324.67b	76.52 ± 17.47a	24.36 ± 5.56bc	0.34 ± 0.07b	0.16 ± 0.04a
T2	1318.28 ± 233.00b	62.48 ± 37.73a	31.04 ± 7.33ab	0.35 ± 0.07b	0.18 ± 0.01a
T3	1100.22 ± 276.07b	61.50 ± 14.86a	19.58 ± 4.73c	0.27 ± 0.06b	0.16 ± 0.04a
T4	2045.13 ± 187.07a	122.01 ± 23.78a	36.08 ± 2.96a	0.55 ± 0.11a	0.19 ± 0.02a
T5	1207.40 ± 558.56b	68.69 ± 32.39a	21.86 ± 10.31bc	0.31 ± 0.15b	0.18 ± 0.01a
T6	1370.63 ± 298.52b	75.52 ± 15.59a	24.04 ± 4.96bc	0.33 ± 0.07b	0.18 ± 0.01a
T7	1471.44 ± 103.39b	82.30 ± 4.63a	26.20 ± 1.48abc	0.37 ± 0.02b	0.17 ± 0.01a

Results

Growth indexes

Aboveground growth

The growth indexes of the above-ground parts were significantly affected by different treatments except for ground diameter, the number of internodes, and the average internode length (Table 1 and Supplementary Table 3). New Branches thickness resulted higher in T1 (33.33% Alfisols soil + 66.67% peat soil), T3 (66.67% Alfisols soil + 33.33% peat), and T5 (33.33% Alfisols soil + 66.67% peat soil) by 25%, 17% and 27%, respectively, compared to the control. Furthermore, compared to the control, T3 (66.67% Alfisols soil + 33.33% peat) improved the total length of new branches, number of new branches, and number of new leaves by 60.51%, 55.71%, and 35.64%, respectively. There are no significant differences between T3 and T5 for the total length of new branches. The treatments, T2, T4, T6, and T7, also improved the above-ground indexes compared to CK, following T3 and T5. Overall, the results showed that excessive addition of perlite could inhibit the growth of the aboveground part of the plant, while appropriate addition of peat soil could promote its lengthening and coarser growth, but excessive addition of peat soil could also lead to coarser growth restriction.

When T3→T1→T5 (with the increase of the proportion of peat soil and the decrease of the proportion of Alfisols soil, Supplementary Figure S1, same below), the increment of ground diameter and the thickness of new branches increased first and then decreased, while the remaining indexes decreased first and then increased. When T4→T2→T6 (with the increase of perlite proportion and the decrease of Alfisols soil proportion, Supplementary Figure S1, same below), all the indexes showed a decreasing trend, and the growth of *E. scandens* seedlings was even weaker than CK when too much perlite was added. When T6→T7→T5 (with peat soil gradually replaces perlite, Supplementary Figure S1, the same below), except that the increment of ground diameter increased first and then decreased, the remaining indexes increase gradually with the increase of peat soil.

Underground growth

Data on root attributes of *E. scandens* seedlings showed that total root length, total root projection area and total root volume were significantly influenced by different treatments, while there was no significant effect of various treatments on root surface area and average root (Table 2 and Supplementary Table 4). T4 (66.67% Alfisols soil + 33.33% perlite) exhibited enhanced total root length, root surface area, root projection area, root volume, and root mean diameter with values of 2045.13 cm, 122.01 cm^2, 36.08 cm^2, 0.55 cm^3 and 0.19 mm, respectively. Furthermore, the average root diameter exhibited an initial increase, followed by a decrease, in the order of treatments T3 → T1 → T5, while the remaining indexes continued to increase steadily. Most indexes were improved in T1, T3 and T5 treatments than CK. When T4→T2→T6, all indexes decreased first and then increased, but the increase was not obvious; When T6→T7→T5, the remaining indexes except the average diameter of the root increase first and then decrease. The root growth indexes of most treatments were better than CK (pure Alfisols soil). Thus, properly adding peat soil can promote the lengthening and thickening growth of the root, but excessive addition of peat soil has a negative effect on root growth. Appropriate addition of perlite can loosen the substrates and promote root growth, but excessive perlite addition will also adversely affect root growth.

Biomass

Different substrates treatments significantly affected the fresh weight of stems and leaves, while not impacted the fresh weight of roots and dry weight of any part (Fig. 1A-B and Supplementary Table 5). Among fresh weights, the root and leaf reached their peak (7.25 g and 18.39 g) under T4 (66.67% Alfisols soil+33.33% perlite) and T3 (66.67% Alfisols soil+33.33% peat) treatment, respectively. Meanwhile, stem and total fresh weights reached the maximum value (16.21 g and 40.29 g) under T7 treatment. In terms of dry weight, except for the leaf dry weight (5.95 g) in T3 treatment, the root dry weight, stem dry weight and total dry weight achieved the highest values in T4 treatment, measuring 3.56 g, 5.11 g and 13.64 g, respectively. Regarding trends, when considering T3→T1→T5, root fresh weight initially increased and then decreased, while stem, leaf and total fresh weight showed the opposite pattern. Root dry weight exhibited an initial increase followed by a decrease, while stem weight followed the reverse trend and leaf and total dry weight decreased gradually. Conversely, when examining T4→T2→T6, fresh weight decreased initially and then increased, except for stems, while the dry weight showed a continuous decrease, excluding leaves. Finally, for T6→T7→T5, all indexes of fresh weight and dry weight increased first and then decreased. Overall, most treated plants exhibited improved biomass accumulation compared to the control . In conclusion, the addition of light additive substrates in Alfisols soil can promote the biomass accumulation of *E. scandens* seedlings, the addition of peat soil is better than that of perlite, but the excessive addition of both will cause the biomass of *E. scandens* seedlings to decrease.

Physiological characteristics

Chlorophyll and soluble sugar contents in leaves were significantly affected by different substrate proportions, while soluble protein content was not significantly affected (Fig. 2A-C and Supplementary Table 6). T4 treatment (66.67% Alfisols soil+33.33% perlite) greatly enhance the Chl *a*, Chl *b* and Chl *a*+*b* contents with values of 2.11 mg/g, 1.18 mg/g and 3.29 mg/g, respectively; T3 (66.67% Alfisols soil+33.33% peat soil) and T7 (33.34% Alfisols soil+33.33% peat soil+33.33% perlite) treatments greatly enhanced soluble sugar and soluble protein contents (14.71% and 21.61 mg/g). In the order of treatments T3→T1→T5, chlorophyll content decreased first and then increased, while soluble sugar and soluble protein content increased and decreased, respectively; For T4→T2→T6, the content of chlorophyll and soluble protein decreased, while the content of soluble sugar increased first and then decreased; For T6→T7→T5, chlorophyll content decreased first and then increased, soluble sugar and soluble protein content increased first and then decreased. Thus, peat soil can promote the synthesis of chlorophyll and sugar, while the excessive addition of perlite will affect the synthesis of chlorophyll, sugar and protein. The addition effect of peat soil is better than that of perlite.

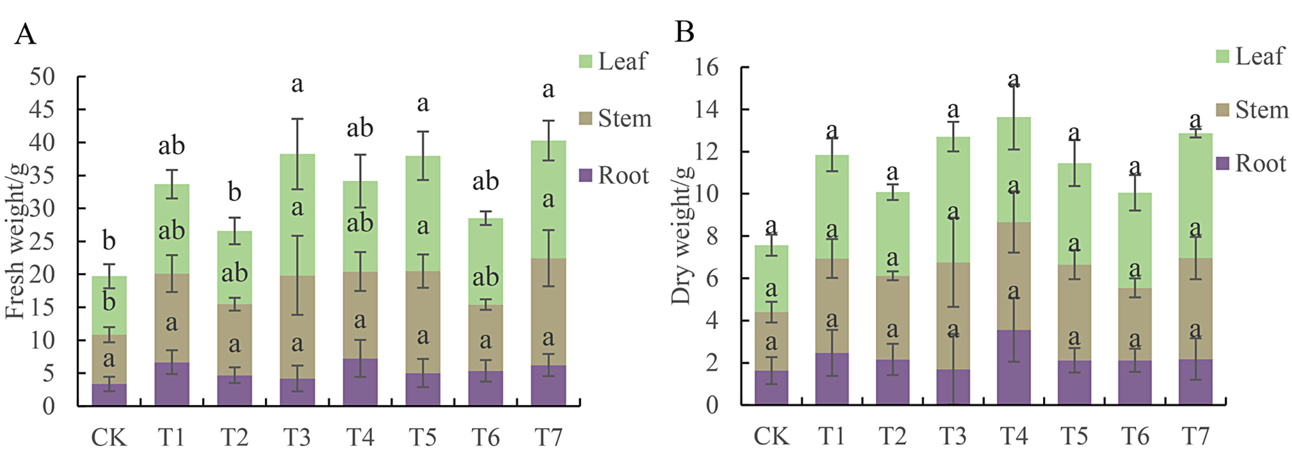

Fig. 1 Biomass accumulation of each part of the *Erythropalum scandens* seedlings under different ratios of substrates (**A**: fresh weight; **B**: dry weight)

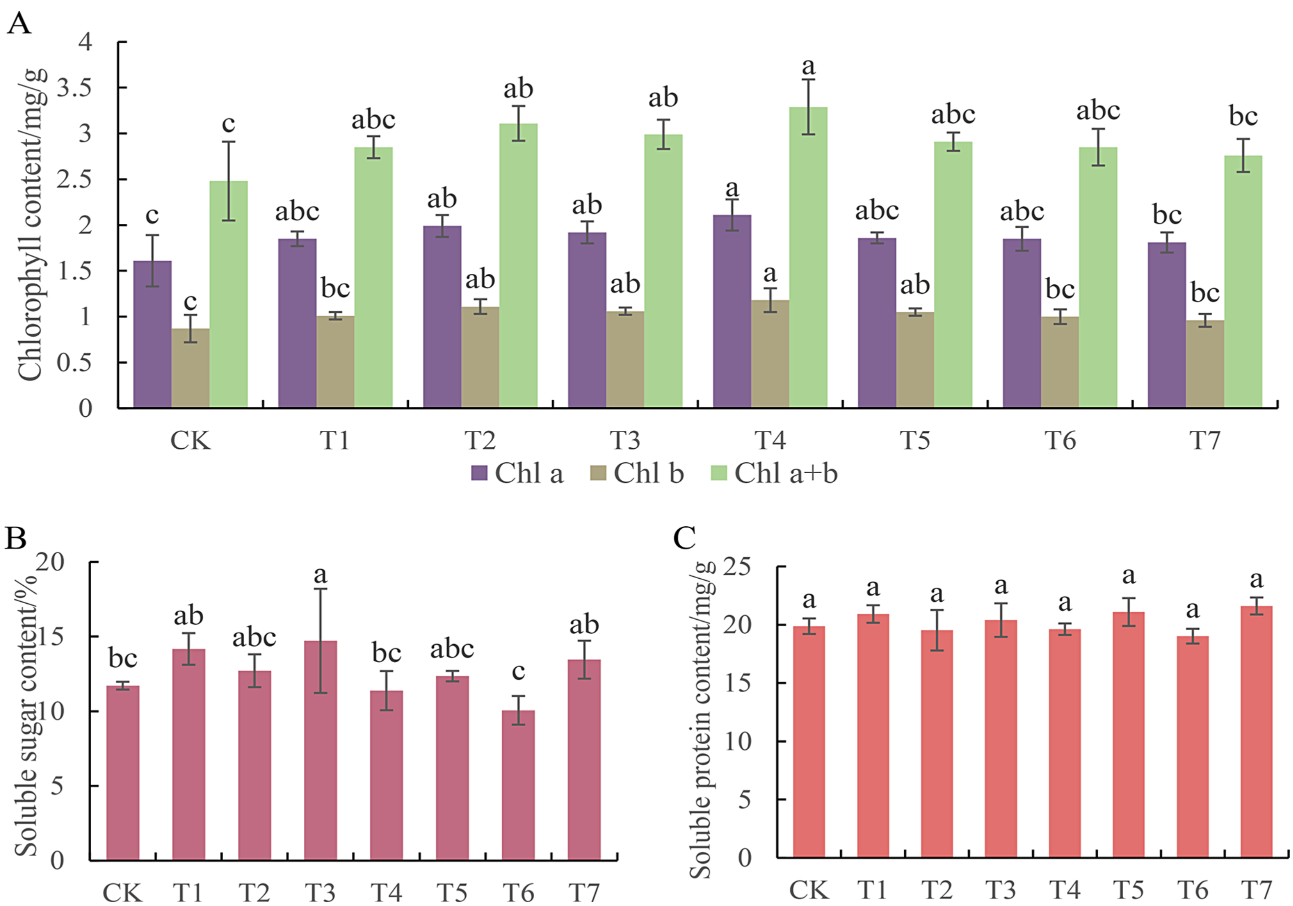

Fig. 2 Physiological indexes of leaves of *Erythropalum scandens* seedlings under different ratios of substrates (**A**: chlorophyll content; **B**: soluble sugar content; **C**: soluble protein content)

Contents of total nitrogen (TN), phosphorus (TP) and potassium (TP)

TN, TP and TK in each part of the seedlings were significantly affected by different substrates (Fig. 3A-C and Supplementary Table 7). TN contents of root, stem and leaf in T1 treatment (50% Alfisols soil+50% peat soil) had the maximum (14.26 g/kg, 11.50 g/kg and 15.81 g/kg); TP contents of root, stem and leaf in T1, T7, T1 treatments had the maximum (1.41 g/kg, 1.55 g/kg, 0.97 g/kg); TK contents of root, stem and leaf in T1, T5, T5 treatments had the maximum (17.11 g/kg, 19.09 g/kg, 15.43 g/kg). In the order of treatments T3→T1→T5, the TN and TP contents in roots and leaves increased first and then decreased, while the contents of TK in roots and stems and leaves decreased first and then increased; For T4→T2→T6, except TN content in roots, TN contents in stems and leaves and TK contents in roots, stems and leaves increased first and then decreased; For T6→T7→T5, TN and TP contents in roots, stems and leaves increased first and then decreased; For TK contents, roots increased first and then decreased, while stems and leaves increased continuously. It can be seen from the accumulation of the three elements that the TN contents of leaf>root>stem, the TP content of stem>root>leaf, and the TK contents of root>leaf>stem. It can be seen that the excessive addition of peat soil and perlite is not conducive to the accumulation of nitrogen, phosphorus and potassium in the body of *E. scandens* seedlings, and the addition effect of peat soil is slightly better than that of perlite. Nitrogen is more easily accumulated in leaves, while phosphorus and potassium are more easily accumulated in stems and roots.

Correlation analysis

The results of the correlation analysis indicate significant relationships among plant growth, nutrient uptake, root traits, and chlorophyll contents under various treatments (Fig. 4). Notably, total fresh weight exhibited strong positive correlations with N uptake ($R=0.64$), soluble sugar content ($R=0.68$), P uptake ($R=0.63$), chlorophyll content ($R=0.40$), dry weight ($R=0.89$), number of new branches ($R=0.68$), and average internode length ($R=0.61$). Similarly, total dry weight displayed significant positive correlations with chl-a ($R=0.40$), chl-b ($R=0.63$), total root surface area ($R=0.68$), total root length ($R=0.66$), and

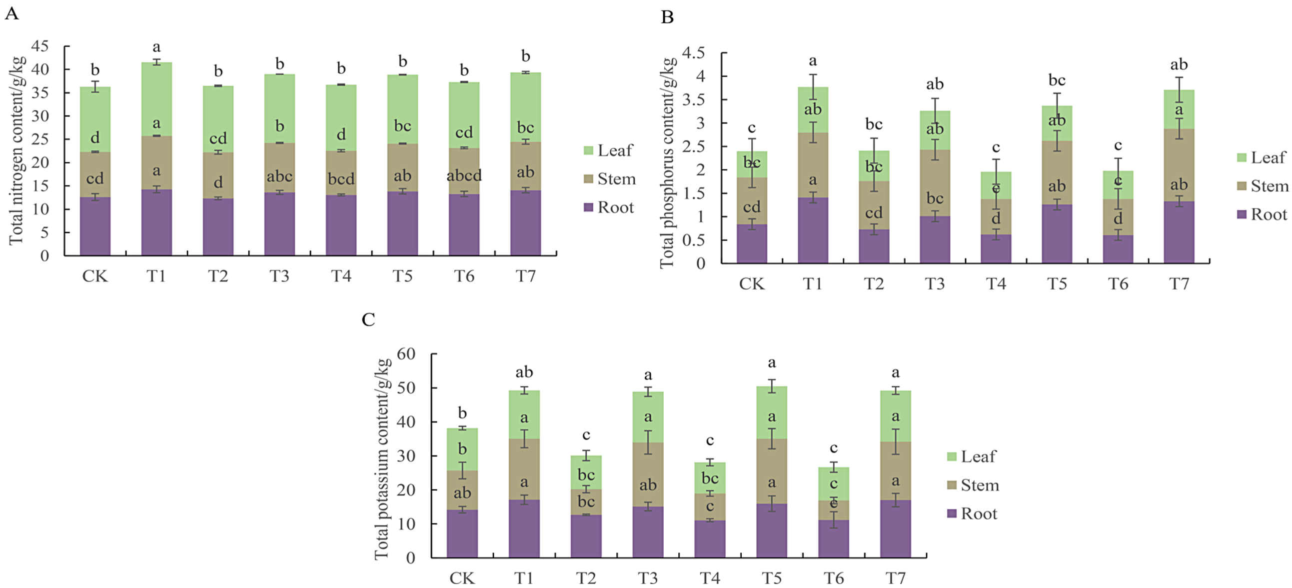

Fig. 3 Contents of total nitrogen, total phosphorus and total potassium of different parts of *Erythropalum scandens* seedlings under different ratios of substrates (**A**: total nitrogen content; **B**: total phosphorus content; **C**: total potassium content)

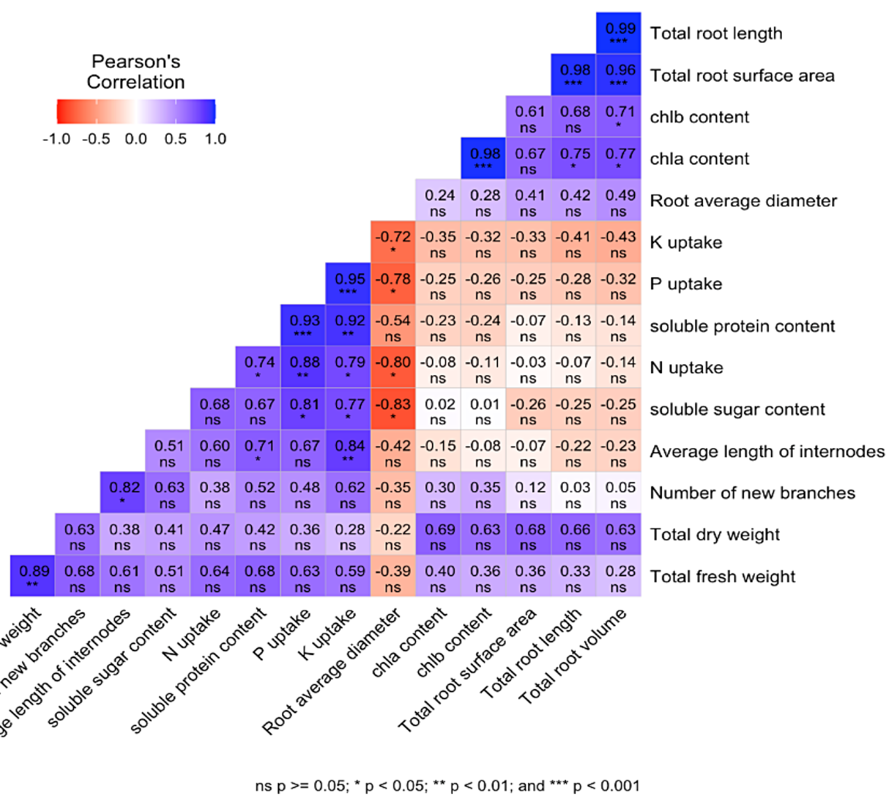

Fig. 4 Correlation analysis between different indexes

total root volume ($R=0.63$). Furthermore, the root average diameter negatively correlated with nutrient (N, P, and K) uptakes, soluble sugar, and protein content. These findings underscore the potential of suitable substrates to enhance soil health, promoting increased plant dry and fresh weights and enhancing overall plant productivity.

Discussion

The substrates used in agricultural practices can be broadly categorized into two main groups: soil and light additive substrates. Notably, light additive substrates such as coconut husk, peat soil, perlite, among others, have gained prominence in various cultivation applications. When incorporated into soil, nutrition-rich substrates like coconut husk and peat soil exert a transformative impact on soil properties, including pH value, conductivity, bulk density and porosity along with other crucial physical and chemical indexes. Moreover, the decomposition of these substrates can provide necessary nutrients for plants and improve the micro environment of roots. It has been used in the cuttings and cultivation of various plants [32, 33]. The effects of the addition of peat soil and perlite on the growth and physiological characteristics of *E. scandens* seedlings will be analyzed in the following aspects.

Effects of peat soil on the growth and physiological characteristics of *E. Scandens*

Peat soil, typically sourced from swamps, lakes and mountain valleys, is the product of incomplete decomposition of plant remains, characterized by strong water retention and rich nutrient content. In this study, the results showed that as the proportion of peat soil in the mixed substrates increased, the growth, biomass accumulation, and soluble sugar content of both the aboveground and underground parts of the seedlings were improved—up to an optimal threshold. These findings are in line with Haghighi et al. [34], who found that adding peat soil significantly enhanced tomato plant growth and biomass accumulation compared to perlite. Similarly, Meng et al. [35] reported that a mixture of dairy manure biogas residue and peat soil could promoted the growth and yield of tomatoes and peppers. In addition, Chrysargyris et al. [36] documented that the ratio of the 5% olive-mill wastes (OMW) and 10% grape-mill wastes (GMW) can promote the growth of the Olive tree and grapevine. This aligns with our findings, which indicated that moderate amounts of organic amendments, like peat soil, can positively influence plant growth. Alsmairat et al. [37] further highlighted that strawberries achieved their highest yield when a turf: perlite=4:1 (v/v), while coconut husk: perlite=4:1 (v/v) had the strongest photosynthesis and the highest fruit firmness. Similarly, in our study, moderate peat soil addition improved the overall growth status of plants, including new branch thickness and root biomass. Previous research on blueberry showed that the yield of blueberry plants, fruit growth status, and the contents of total phenols and flavonoids in fruits could be well improved by rationally mixing peat soil with coconut husk, pine bark and other substrates [38–40]. Our results parallel these findings, where the appropriate addition of peat soil enhanced plant biomass and nutrient uptake. However, consistent with the results of Xiao et al. [41] on melons—where the best volume ratio for the substrate was turf, coconut husk, and vermiculite at 6:3:1 (v/v/v), and excess peat soil reduced melon yield. The stress caused by high peat soil content in our study can be attributed to its strong water retention capacity, which leads to excessive moisture in the substrate [41, 42]. The stress caused by high peat soil content in our study can be attributed to its strong water retention capacity, which leads to excessive moisture in the substrate, as noted by Xiao et al. [41] and other studies [42]. The waterlogged environment may have inhibited root development, similar to the findings in water-sensitive plants like *E. scandens*, which thrives in well-drained conditions [43]. This aligns with previous studies indicating that the drainage characteristics of the substrate are crucial for optimal plant growth, particularly for species native to sandy or well-drained soils, such as those in the limestone regions of southwest Guangxi [43].

In terms of nutrient content, peat soil generally has higher concentrations of essential elements like N, P, and K compared to typical soil [44]. While this nutrient richness can benefit plants, it can also cause nutrient stress when excessive. Bestic-Pennings et al. [45] and Lin et al. [46] reported similar outcomes, where peat soil released enough minerals, such as iron and manganese, to meet plant needs but could also pose a risk of nutrient overload. This supports our observation of nutrient stress in plants with high peat soil content, leading to reduced soluble protein levels. Furthermore, Thakur et al. [47] found that substrates like vermiculite and coconut husk in excessive proportions could lead to toxicity, a risk we also noted with high peat soil concentrations. Overall peat soil positively affects plant growth by providing moisture and nutrients, its proportion must be carefully managed to avoid waterlogging and nutrient toxicity.

Effects of perlite on the growth and physiological characteristics of *E. Scandens*

Perlite is an aqueous form of volcanic glass devoid of nutrients, is characterized by its low bulk density [48, 49] and approximately 65% water-holding capacity, which helps ameliorate soil structure, mitigate waterlogging [50]. Adding perlite to mixed substrates has been shown to loosen the soil, improving root growth. In this study, we found that incorporating perlite into loose soil promoted the growth of *E. scandens* seedlings, particularly enhancing root development. These findings are consistent with those of Kostas et al. [51], who demonstrated that in *Rosmarinus officinalis* L., a perlite-to-peat soil ratio of 2:1 in the substrate yielded the best root growth, with an 85% survival and rooting rate for cuttings. Similarly, Cesonien˙ e et al. [52] observed that replacing 15–45% of perlite soil with perlite in mixed substrates significantly promoted the growth of blueberry young trees, reducing peat usage and contributing to environmental protection. This further supports our results, where perlite contributed to better root growth. Moreover, Guo et al. [53] found that a perlite-to-peat soil ratio of 2:1 (v/v) resulted in the highest net photosynthetic rate and optimal root growth in *Pholidota chinensis* Lindl., with biomass accumulation in leaves and pseudobulbs also improved. This aligns with our findings where perlite's contribution to loosening the substrate promoted root growth, subsequently enhancing the aboveground biomass of *E. scandens*. Li et al. [54] also confirmed that adding perlite and peat soil to garden soil benefited root growth in alfalfa, which resonates with our findings for *E. scandens*, a species native to the sandy soils of the limestone mountain areas of southwest Guangxi. The addition of perlite helped loosen the substrate, promoting root development, while also preventing water accumulation, a key factor for root health. However, as Jahan et al. [49] showed in their study on wheat plants, excessive perlite addition, although beneficial for loosening the soil and improving water stress resistance, can lead to growth restrictions. We observed similar trends in our study, where excessive perlite addition led to declines in growth and physiological indices such as chlorophyll and soluble protein content in both aboveground and underground parts of the vine. This phenomenon may be attributed to the fact that while perlite improves soil structure, it lacks nutrients, and excessive amounts reduce the overall nutrient content of the substrate. Ma et al. [27, 28] highlighted the significant demand for nitrogen, phosphorus, and potassium during the seedling stage of *E. scandens*, especially nitrogen, which is critical for its growth and development. Our findings corroborate this, as treatments with perlite (T2, T4, T6) showed poorer growth and physiological performance compared to those supplemented with peat soil (T1, T3, T5). In fact, the excessive addition of perlite resulted in weaker performance than even the control (pure Alfisols soil). Samartzidis et al. [4] also demonstrated that while adding zeolite and perlite improved the matrix's porosity and enhanced photosynthetic capacity, it did not significantly increase yield. Similarly, Zabaleta et al. [55]found that arugula plants cultivated with perlite alone had inferior growth and yield compared to treatments where biochar was added to the substrate. These findings further emphasize that while perlite improves soil structure, its quantity must be carefully controlled to avoid nutrient deficiencies and ensure optimal growth conditions.

Effects of the addition of different substrates on element content of *E. Scandens*

The reasonable addition of cultivation medium significantly influences the accumulation of nutrient elements in plants. This study revealed that the accumulation patterns of the major nutrients—nitrogen (N), phosphorus (P), and potassium (K)—in *E. scandens* followed distinct trends: N was more easily accumulated in the leaves (leaf > root > stem), P primarily accumulated in the stems (stem > root > leaf), and K was enriched in the roots (root > leaf > stem). This study revealed that the accumulation patterns of the major nutrients—N, P and K in *E. scandens* followed distinct trends: N was more easily accumulated in the leaves (leaf > root > stem), P primarily accumulated in the stems (stem > root > leaf), and K was enriched in the roots (root > leaf > stem). These findings are consistent with those of Ma et al. [27, 28], who observed similar nutrient accumulation patterns in the fertilization of *E. scandens* seedlings. Furthermore, the nutrient distribution results in our study are also comparable to those observed in *Phalaenopsis* spp [56]. and *Sinocalycanthus chinensis* [57], as well as other plant species, where N plays a critical role in leaf growth, while P and K are more integral to the development of roots and stems. This aligns with the general principles of plant physiology regarding nutrient uptake and distribution. In addition, our results demonstrated that the appropriate addition of peat soil positively impacted nutrient accumulation (N, P, K) in the roots, stems, and leaves of *Acanthophora acanthophora*. However, excessive peat soil and perlite additions negatively affected nutrient accumulation in *E. scandens*, similar to the trends observed in previous studies on soil structure and nutrient uptake. Excessive peat soil causes substrate to retain too much water, leading to a sticky consistency that can hinder plant growth and nutrient absorption. This finding echoes previous observations, where over-saturation of substrates can disrupt root oxygen availability, thereby limiting nutrient uptake [49, 50]. Similarly, the overuse of perlite results in unstable soil structure and insufficient nutrient supply, ultimately affecting nutrient absorption. Our results are also in line with studies like those of Jahan et al. [49], who found that the excessive addition of perlite in combination with other substrates, while improving soil structure, reduced the nutrient uptake efficiency in plants. This phenomenon highlights the importance of balancing substrate components to avoid hindering plant growth, as also noted by Guo et al. [53] in their study on *Pholidota chinensis*, where an optimal mix of perlite and peat soil promoted the best growth performance. While peat soil is known for its high water retention and porosity, which play crucial roles in enhancing the soil environment, its high cost, poor sustainability, and non-regenerable nature present limitations in practical applications [58–60]. Similarly, perlite, despite being a readily available substrate used extensively in horticultural studies, lacks inherent nutrients, which limits its effectiveness in promoting plant growth when used in excess. These observations are corroborated by previous studies that suggest the need to balance substrates with nutrient-rich alternatives like coconut coir or biochar. Both substrates not only loosen the soil but also provide the necessary nutrients, enhancing both plant growth and substrate sustainability.

Correlations

One notable finding is the strong positive correlation observed between total fresh weight and several key parameters, including nitrogen (N) uptake, soluble sugar content, phosphorus (P) uptake, and chlorophyll content. This suggests that an increase in these factors corresponds to a significant boost in the plant's fresh weight. Similarly, the positive correlations identified between total dry weight and parameters such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total root surface area, total root length, and total root volume highlight their collective influence on plant dry weight. Interestingly, we noted a negative correlation between the root average diameter, nutrient uptake (N, P, and K), and soluble sugar and protein content. This suggests that as the root diameter decreases, the plant's ability to absorb these essential nutrients and accumulate certain compounds like soluble sugar and protein may increase. Overall, these findings emphasize the pivotal role of suitable substrates in improving soil health. By optimizing soil conditions, we can positively impact plant dry and fresh weights, which, in turn, can significantly enhance overall plant productivity. These results provide valuable insights for agricultural practices and may contribute to more effective strategies for maximizing plant growth and yield.

Conclusions

In this study, the growth, physiological characteristics and accumulation of N, P and K nutrients of 24-month-old cutting seedlings were investigated under different cultivation substrates. It was found that more than 50% of Alfisols soil should be used to cultivate *E. scandens* seedlings. Suitable addition of peat soil and perlite in Alfisols soil was beneficial to the growth of the young plants. Among all treatments, T3 treatment (66.67% Alfisols soil+33.33% peat soil) had the best above-ground growth, T4 treatment (66.67% Alfisols soil+33.33% perlite) had the best root growth and biomass accumulation, and the highest chlorophyll content. T1 treatment (50.00% Alfisols soil+50.00% peat soil) had the best accumulation of element content. The addition effect of peat soil is better than perlite. However, it should be noted that peat soil and perlite can not be added excessively.

Supplementary Information

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Supplementary Material 1

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by D.M. and B.Y. Experiments were done by D.M., B.Y., J.S., Y.L. and J.Y. The first draft of the manuscript was written by D.M. and X.T. provided the seedlings and materials in this work. W.T., I.A., Y.W. and L.W. revised the original manuscript and I.A. polished the language and expressions. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. D.M and B.Y contributed equally to this work.

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Data availability

All data will be available on reasonable demand from corresponding author.

Declarations

Competing interests

The authors declare no competing interests.
This article does not contain any studies with human participants or animals performed by any of the authors.

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