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Effect of Different Substrate Formula on Photosynthesis and Chlorophyll Fluorescence Characteristics of *Koelreuteria Bipinnata* Seedlings

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Abstract

Applying the statistical method of Simplex Centre and according to the volume ratio=2:1 of organic and inorganic matrix, 15 treatments were mixed by different proportions of four mixed-substrates including S_A(Vcorn-cob: Vchicken manure=1:1), S_B($V_{bagasse}$: $V_{chicken\ manure}$=1:1) , S_C($V_{coconut\ fibre}$: $V_{chicken\ manure}$=1:1) and S_D($V_{peanut\ shells}$: $V_{chicken\ manure}$=1:1), with inorganic medium composed of vermiculite and perlite at the volume ratio of 1:1. Taking latosol, latosol plus compound fertiliser and V_{peat}:$V_{vermiculite}$:V_{perlit}e=4:1:1 as the control groups. The hotosynthesis and chlorophyll fluorescence characteristicsof *Koelreuteria bipinnata* seedling were investigated to study the the effects of different substrate formulations on the growth of *Koelreuteria bipinnata* seedling, and then to screen the best culture medium formula for *Koelreuteria bipinnata*. The results showed that total content chlorophyll of *Koelreuteria bipinnata* seedlings on treatment 13 suhstrate was more than other substrates. The photosynthetic parameters such as *Pn*((10.89±1.27)μmol•m^{-2}•s^{-1}), *Gs*((0.160±0.035)mol•m^{-2}•s^{-1})and the chlorophyll fluorescence parameters such as *Fv/Fm*(0.97±0.01), *Fv'/Fm'*(0.67±0.03), *ΦPSII* (0.34±0.07) and *ETR*(146.29±14.26)of *Koelreuteria bipinnata* seedlings on treatment 13 suhstrate were higher than other substrates, but the parameters of Ls(0.18±0.97)and *NPQ*(2.19±0.37)were lower. However, The Photosynthetic and chlorophyll fluorescence parameters such as *Pn*, *Gs* and, *Fv/Fm*, *Fv'/Fm'*, *ΦPSII*, and *ETR* of *Koelreuteria bipinnata* seedlings on treatment 14 and 15 suhstrate were lower. We can draw the conclusion that Treatment 13 is the best substrate for the growing of *Koelreuteria bipinnata* seedlings, which can substitute non-renewable resources of peat.

Keywords

Organic Matrix; *Koelreuteria Bipinnata*; Substrate Formula; Photosynthesis; Chlorophyll Fluorescence Characteristics.

1. Introduction

Substrate is the foundation for nutrient and water supply during plant growth and development [1]. Substrate cultivation is a simple form of soilless culture that not only offers advantages such as low cost, simple techniques, easy management, and high product quality [2,3], but also helps address problems associated with long-term use of chemical fertilizers and pesticides, including soil compaction, secondary salinization, environmental pollution, and soil continuous cropping obstacles [2]. It has been widely applied in the seedling production of vegetables, flowers, and forest trees [4–7]. The solid substrate used in earlier practices was peat; however, peat is a finite natural resource with uneven distribution and high cost, which increases the raw material cost for seedling production. Excessive extraction also damages the ecological environment of marshlands and leads to resource depletion. Currently, many researchers are

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focusing on developing different substrate formulations using agricultural and forestry wastes to replace the non-renewable peat substrate. Zhu Shenlong et al. [8] found that using a mixed cultivation substrate of garden soil and coconut coir at a volume ratio of 3:1 resulted in higher transplanting survival rates and better seedling growth and quality for olive container seedlings. Hao Zhexia et al. [9] reported that a cultivation substrate composed of vinasse, cow manure compost, and vermiculite at a volume ratio of 65:15:20 was effective for cucumber and tomato seedling production, while a ratio of 70:10:20 was effective for watermelon seedling production. Shao Yangfeng et al. [10] found that a decomposed cow manure – hickory shell – rice husk substrate (volume ratio 7:2:1) significantly improved tomato growth, yield, and quality. It is evident that different substrate formulas have different effects on plant growth, and it is imperative to develop and screen suitable substrate formulas for plant cultivation.

With the increasing awareness of environmental protection and sustainable development, the utilization of agricultural and forestry wastes as alternative substrates has become a research hotspot. The advantages of such organic substrates include low cost, renewable sources, and the ability to recycle wastes, which align with the principles of circular economy and green agriculture [3,7]. However, the physicochemical properties of organic wastes vary greatly depending on the raw materials and decomposition processes, which may result in significant differences in plant growth responses. Therefore, systematic screening and evaluation of different organic waste-based substrates are essential for their practical application in nursery production [11,12].

Koelreuteria bipinnata Franch., commonly known as the Chinese flame tree, belongs to the family Sapindaceae and the genus *Koelreuteria*. It is a deciduous tree native to Yunnan, Guizhou, Sichuan, Hubei, Hainan, Guangxi, and Guangdong provinces in China, growing in sparse mountain forests at altitudes of 400–2500 m [13]. The tree has a tall and elegant shape with dense and beautiful foliage, and possesses strong air pollution resistance and air purification ability, making it an ideal species for courtyard ornamentation and street greening [14]. It has now been introduced to the United States as a landscaping tree [15]. *Koelreuteria bipinnata* also has medicinal and economic value; for example, its roots can be used medicinally to reduce swelling, relieve pain, promote blood circulation, and expel roundworms, and it can also serve as a source of yellow dye, indicating considerable industrial development potential [13]. Photosynthesis is one of the most important anabolic pathways in plants, serving as the fundamental route for synthesizing organic matter and acquiring energy, and directly determining plant growth status and final yield [16]. Currently, there have been numerous studies both domestically and internationally on screening nursery substrate formulations [11,12,17]. However, no report has been found on screening substrate formulations for the commonly used ornamental tree species *Koelreuteria bipinnata*.

Furthermore, photosynthesis is not only the basis for plant growth but also an important indicator of plant health and stress tolerance. Chlorophyll fluorescence techniques, as a non-destructive and rapid method, can effectively evaluate the photochemical efficiency and energy dissipation mechanisms of plant leaves under different environmental conditions [18,19]. Therefore, combining photosynthetic gas exchange parameters with chlorophyll fluorescence parameters can comprehensively evaluate the photosynthetic capacity of *Koelreuteria bipinnata* under different substrate treatments, which is of great significance for identifying suitable cultivation substrates.

Despite the extensive studies on substrate formulation for various horticultural and forestry species [11,12,17], no systematic investigation has been conducted on the substrate formula optimization for *Koelreuteria bipinnata* seedlings. Most previous studies have focused on vegetable and flower seedling production [4–10], while research on ornamental tree species, particularly *Koelreuteria bipinnata*, remains scarce. Furthermore, although photosynthesis and chlorophyll fluorescence parameters have been widely used as sensitive indicators for

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evaluating plant growth and stress responses under different cultivation conditions [18,19,20,21], their application in substrate formula screening for *Koelreuteria bipinnata* has not been reported. Therefore, the present study was designed to: (1) formulate 15 different organic substrate combinations using agricultural wastes (corn cob, bagasse, coconut fiber, and peanut shell) composted with chicken manure, with latosol and peat-based substrates as controls; (2) comparatively evaluate the effects of these substrate formulas on leaf chlorophyll content, photosynthetic gas exchange parameters (net photosynthetic rate *Pn*, stomatal conductance *Gs*, intercellular CO_2 concentration *Ci*, and stomatal limitation *Ls*), and chlorophyll fluorescence parameters (*Fv/Fm, Fv'/Fm', ΦPSII, NPQ*, and *ETR*) of *Koelreuteria bipinnata* seedlings; and (3) screen the optimal organic substrate formula that can serve as a sustainable alternative to the non-renewable peat substrate. The findings of this study will not only provide a scientific basis for the large-scale nursery production of *Koelreuteria bipinnata* but also promote the utilization of agricultural wastes in soilless cultivation systems, contributing to the development of environmentally friendly and cost-effective seedling production practices.

2. Materials and Methods

2.1. Test Materials

The tested substrates were fermented and decomposed organic matrices. The raw materials for decomposition included agricultural waste products such as corn cobs, bagasse, coconut fiber, and peanut shells, all collected or purchased from the surrounding areas of Chikan District, Zhanjiang City. After crushing, these materials were mixed with sterilized chicken manure at a volume ratio of 1:1 and fermented for 4 months to produce the organic substrates. Chicken manure was purchased from a poultry farm in Mazhang District, Zhanjiang City. Peat, perlite, and vermiculite were purchased from a flower market in Chikan District, Zhanjiang City. Latosol was collected from the surface soil layer (0–20 cm) within the biological garden of Lingnan Normal University. The tested potassium sulfate compound fertilizer was produced by Hubei Ezhong Chemical Co., Ltd. ($N:P_2O_5:K_2O$ = 15:15:15). The tested plant material was *Koelreuteria bipinnata* seedlings (stem diameter: 3.5±0.3 cm; plant height: 20±1 cm).

2.2. Experimental Design

The experiment was conducted in a polyethylene plastic greenhouse within the biological garden of Lingnan Normal University. According to the Simplex Centre Design method for substrate formulation and with an organic-to-inorganic matrix volume ratio of 2:1, the four organic substrates decomposed with chicken manure were mixed in different volume proportions with the inorganic substrate ($V_{vermiculite}$: $V_{perlite}$= 1:1) to prepare 15 cultivation substrate formulas. The widely used latosol in the Leizhou area served as Control CK1, latosol + compound fertilizer as Control CK2, and the conventional organic ecological cultivation substrate (V_{peat}: $V_{vermiculite}$: $V_{perlite}$ = 4:1:1) as Control CK3. The specific formulas and physicochemical properties are shown in Table 1 and Table 2, respectively. The experiment used pot cultivation (31.0 cm × 18.5 cm × 26.0 cm), with one seedling planted per pot. After transplanting, the seedlings were shaded with a 60%–70% shading net for 15 days for establishment. After this period, the shading net was removed, and the seedlings were cultivated under full sunlight. Control CK2 received top-dressing of compound fertilizer once every 15 days, with 3 g of fertilizer applied per pot each time. During the growth period, the *Koelreuteria bipinnata* seedlings received appropriate water management and pest and disease control. Each treatment had 6 replicates.

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Table 1. Test substrate formulas (volume ratio)

Treatments	substrate formula
T1	S_A: vermiculite:perlite=4:1:1
T2	S_B:vermiculite:perlite=4:1:1
T3	S_C:vermiculite:perlite=4:1:1
T4	S_D:vermiculite: perlite=4:1:1
T5	S_A:S_B:vermiculite:perlite= 2:2:1:1
T6	S_A:S_C:vermiculite:perlite=2:2:1:1
T7	S_A:S_D:vermiculite:perlite=2:2:1:1
T8	S_B:S_C:vermiculite:perlite=2:2:1:1
T9	S_B:S_D:vermiculite:perlite=2:2:1:1
T10	S_C:S_D:vermiculite:perlite= 2:2:1:1
T11	S_A:S_B:S_C:vermiculite:perlite=1.33:1.33:1.33:1:1
T12	S_A:S_B:S_D:vermiculite:perlite=1.33:1.33:1.33:1:1
T13	S_A:S_C:S_D:vermiculite:perlite=1.33:1.33:1.33:1:1
T14	S_B:S_C:S_D:vermiculite:perlite=1.33:1.33:1.33:1:1
T15	S_A:S_B:S_C:S_D:vermiculite:perlite=1:1:1:1:1:1
CK1	latosol
CK2	latosol)+ compound fertilizer
CK3	peat:vermiculite:perlite=4:1:1

Note: SA: Substrate A(corn cob+chicken manure), SB:Substrate B (bagasse+chicken manure), SC:Substrate C(coconut fibre+chicken manure), SD: Substrate D (peanut shells+chicken manure).

Table 2. Physical and chemical properties of different substrate formulas

Treatment	Bulk density /g·cm^{-3}	Total porosity /%	pH	Organic matter /%	Available N /mg·kg^{-1}	Available K /mg·kg^{-1}	Available P /mg·kg^{-1}
T1	0.45±0.02	70.4±5.41	7.13±0.05	24.33±2.00	1183.0±27.3	12679.3±95.7	892.0±9.7
T2	0.44±0.03	70.7±7.45	7.68±0.04	18.65±1.90	873.8±16.3	10723.0±60.7	668.4±10.9
T3	0.35±0.01	74.8±3.66	7.58±0.03	16.12±2.46	797.5±36.3	8782.6±64.5	716.6±35.9
T4	0.40±0.01	60.2±6.36	7.22±0.05	20.10±0.68	972.7±42.5	12682.2±92.3	672.9±32.4
T5	0.47±0.02	68.1±8.86	7.60±0.02	18.69±1.56	883.2±29.4	8698.0±97.5	1518.7±50.2
T6	0.39±0.01	60.7±4.59	7.64±0.05	13.47±0.85	765.8±53.1	6675.4±77.4	1140.5±20.8
T7	0.44±0.01	78.3±7.61	7.76±0.03	14.56±0.78	777.4±27.8	9086.4±87.6	878.2±20.8
T8	0.38±0.02	59.7±4.14	7.61±0.04	22.46±0.77	999.5±13.7	7869.8±65.8	630.9±45.6
T9	0.45±0.01	62.6±5.39	7.56±0.03	23.53±0.43	997.5±17.3	11261.1±89.5	986.0±42.5
T10	0.37±0.02	73.7±6.01	7.43±0.02	25.05±1.34	1050.8±25.1	9465.1±52.4	569.3±37.8
T11	0.42±0.01	67.8±9.62	7.54±0.04	20.59±1.15	905.5±34.6	7325.5±78.6	1054.1±16.9
T12	0.41±0.02	71.5±8.67	7.10±0.03	23.62±0.84	1038.1±30.2	7836.6±59.4	1119.2±30.6
T13	0.41±0.01	61.2±4.50	7.12±0.02	27.59±2.60	1239.6±40.6	11241.4±50.7	1236.6±30.4
T14	0.45±0.04	69.7±4.78	7.53±0.04	13.45±0.67	746.3±25.7	9662.8±72.3	748.6±25.8
T15	0.41±0.01	76.3±3.36	7.77±0.03	15.41±1.75	785.6±31.9	7751.3±92.7	868.2±15.6
CK1	1.27±0.01	30.3±1.64	6.90±0.04	5.50±0.45	72.5±9.8	80.5±14.6	3.7±0.4
CK2	1.27±0.02	30.3±1.65	6.90±0.04	5.50±0.45	72.5±9.8	80.5±14.6	3.7±0.4
CK3	0.25±0.02	79.6±3.22	6.27±0.04	26.88±1.98	1236.5±11.5	12638.2±75.6	308.3±7.8

Note: Each value in the table represents mean ± standard error; Different lowercase letters in the same column indicate significant difference at the α=0.05 level, the same below.

2.3. Test Methods

2.3.1. Photosynthetic Performance

Between 9:00 and 11:00 a.m., a LI-6400XT portable photosynthesis system (Li-Cor, USA) was used to measure photosynthetic parameters including net photosynthetic rate (Pn), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) of *Koelreuteria bipinnata* leaves. The

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stomatal limitation value was subsequently calculated. The instrument was operated with an open gas path and a built-in light source set at an intensity of 1500 μmol·m^{-2}·s^{-1}. For each treatment, six replicates were measured, and each leaf was measured three times, with the average value taken.

2.3.2. Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters were measured on clear mornings between 9:00 and 11:30 a.m. using pre-marked functional leaves. A LI-6400XT portable photosynthesis system (Li-Cor, USA) was used to determine and calculate the effective photochemical quantum efficiency of PSII (*Fv'/Fm'*), actual photochemical efficiency (*ΦPSII*), non-photochemical quenching coefficient (*NPQ*), and electron transport rate (*ETR*). Between 9:00 and 11:00 p.m., the initial fluorescence (Fo) was measured, after which a saturated pulse (7200 μmol·m^{-2}·s^{-1}) of 0.8 s duration was applied to the leaves to measure the maximum dark-adapted fluorescence (*Fm*). The maximum photochemical efficiency (*Fv/Fm*) was then calculated as $Fv/Fm = (Fm - Fo)/Fm$.

2.3.3. Chlorophyll Content Determination

The ethanol-acetone mixed extraction method [22] was used to determine and calculate the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl). Fresh leaf samples (0.2 g) were ground with 10 mL of ethanol-acetone mixture (1:1, v/v) and extracted in the dark for 24 hours until the leaf tissues became completely white. The absorbance of the extract was measured at 663 nm and 645 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The contents of Chl a, Chl b, and total chlorophyll were calculated according to the equations described by Zeng et al. [22]. Total chlorophyll content was calculated as Chl = Chl a + Chl b.

3. Results and Analysis

3.1. Effects of Different Substrate Formulas on Chlorophyll Content in Leaves of Koelreuteria bipinnata Seedlings

As shown in Figures 1A, 1B, and 1C, Treatment 13 exhibited the highest contents of chlorophyll a (14.01±1.50) mg·g^{-1}, chlorophyll b (4.86±0.48) mg·g^{-1}, and total chlorophyll (18.86±0.56) mg·g^{-1}, which were significantly higher than those of other substrate treatments. Relatively higher contents were also found in Treatments 12, 11, 9, 1, and CK3, while lower contents were observed in Treatments 14 and 15 and Control CK1. These results indicated that substrate formulas T13, T12, T11, and T1 significantly increased the chlorophyll content of *Koelreuteria bipinnata* seedlings.

3.2. Effects of Different Substrate Formulas on Photosynthetic Characteristics of *Koelreuteria bipinnata* Seedlings

As shown in Figure 2A, Treatment 13 had the highest net photosynthetic rate at (10.89±1.27) μmol·m^{-2}·s^{-1}, which was significantly higher than other substrate treatments but not significantly different from CK3 (10.73±1.16) μmol·m^{-2}·s^{-1}. Relatively higher values were also found in Treatments 12, 10, 9, and 1, while lower values were observed in Treatments 14 and 15 and Control CK1. CK1 showed the lowest value at (4.78±0.71) μmol·m^{-2}·s^{-1}, significantly lower than all other treatments.

As shown in Figure 2B, Treatment 13 had the highest stomatal conductance at (0.160±0.035) mol·m^{-2}·s^{-1}, significantly higher than other substrate treatments but not significantly different from Treatments 12, 1, and 7. Relatively higher values were also found in Treatments 11, 8, 6, and CK3. CK1 showed the lowest stomatal conductance at (0.054±0.008) mol·m^{-2}·s^{-1}, significantly lower than other treatments, with lower values also observed in Treatments 14 and 15.

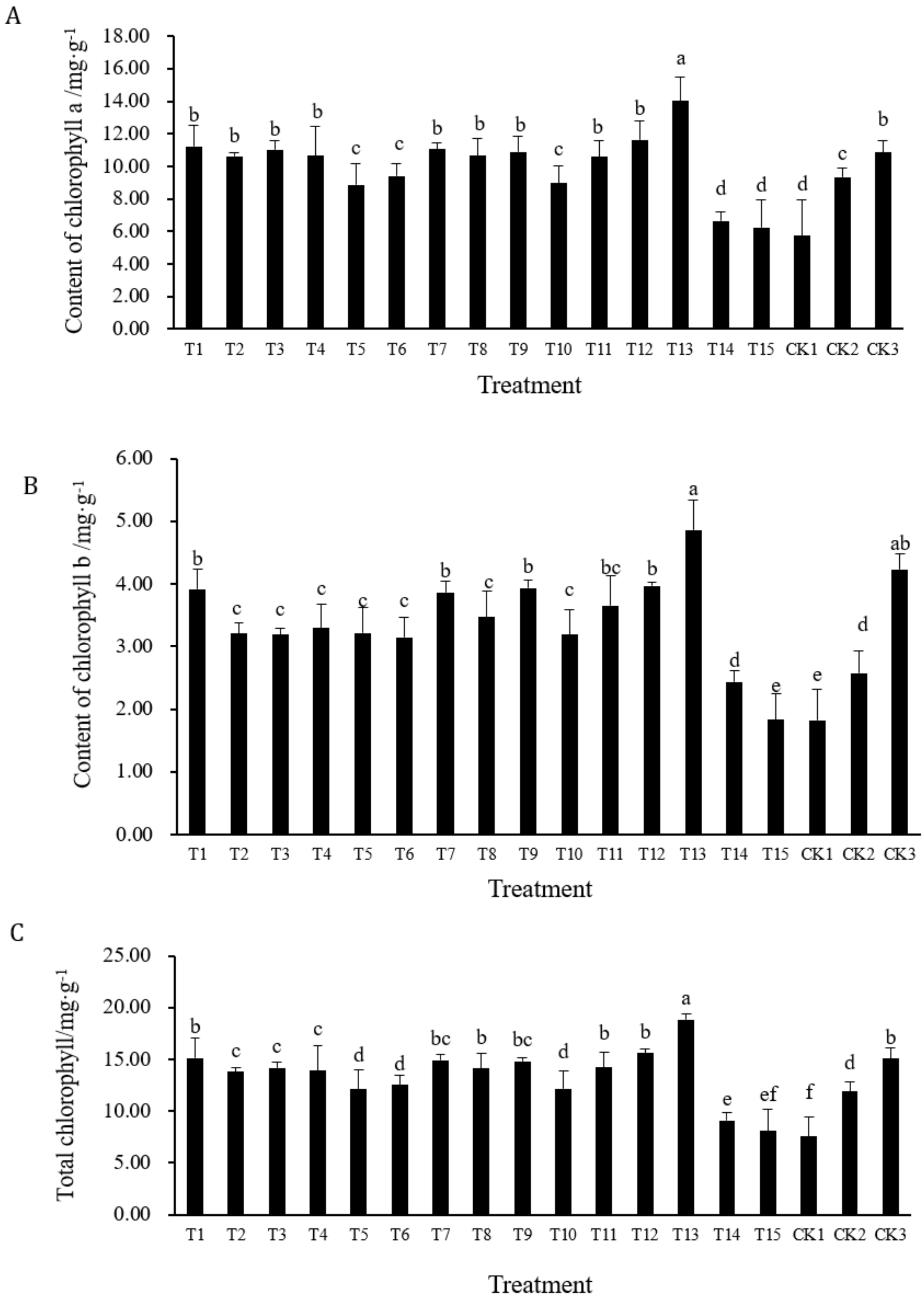

Figure 1. Effect of different substrate formulas on chlorophyll content(A,B,C) in leaves of *Koelreuteria bipinnata* seedlings.

Different letters in the figure indicate significant level.

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As shown in Figure 2C, Treatment 13 had the highest intercellular CO_2 concentration (*Ci*) at (268.81±23.19) μmol·mol^{-1}, significantly higher than other substrate treatments but not significantly different from Treatments 12, 1, 9, and CK3.

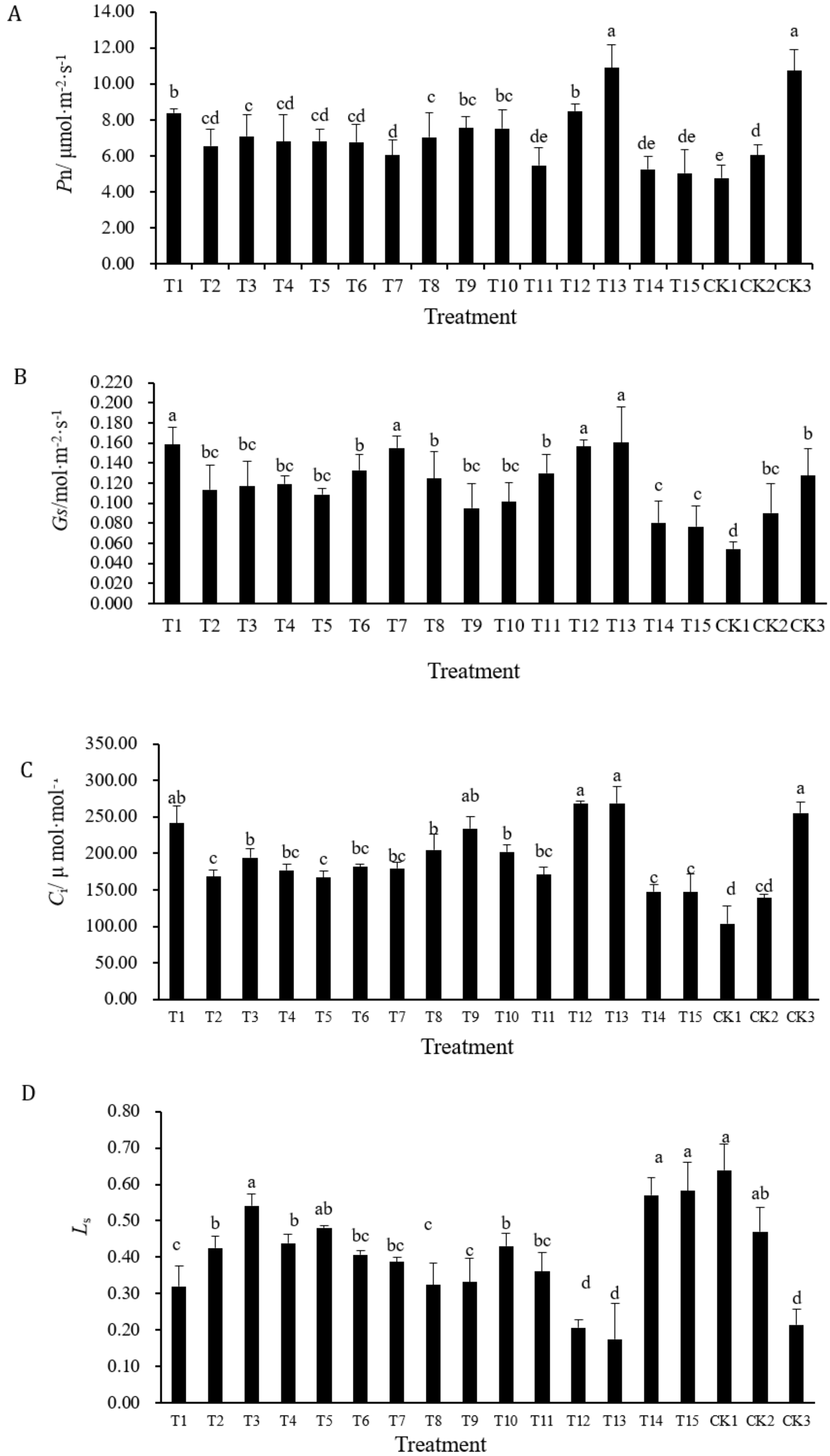

Figure 2. Effect of different substrate formulas on photosynthetic characteristics (A,B,C,D) of *Koelreuteria bipinnata* seedlings

Relatively higher values were also found in Treatments 10 and 8. CK1 had the lowest *Ci* at (213.56±24.71) μmol·mol^{-1}, significantly lower than other treatments, with lower values also observed in Treatments 14 and 15.

As shown in Figure 2D, Control CK1 exhibited the highest stomatal limitation value (*Ls*) at 0.64±0.71, which was not significantly different from Treatments 14, 5, 3, and CK2 but was significantly higher than other substrate treatments. Relatively higher values were also found in Treatments 2, 4, and 10. Treatment 13 had the lowest *Ls* at 0.18±0.97, not significantly different from Treatments 12, 1, and CK3.

In summary, Treatments 13, 12, 1, and CK3 exhibited higher values of net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and intercellular CO_2 concentration (*Ci*), along with lower stomatal limitation values, indicating that substrate formula 13 and related treatments enhanced the photosynthetic capacity of *Koelreuteria bipinnata*. In contrast, Treatments 14 and 15 as well as Controls CK1 and CK2 showed lower *Pn*, *Gs*, and *Ci* values with higher stomatal limitation values, indicating weaker photosynthetic capacity under these substrate formulas.

3.3. Effects of Different Substrate Formulas on Chlorophyll Fluorescence Characteristics of *Koelreuteria bipinnata* Seedling Leaves

Fv/Fm represents the maximum photochemical quantum yield of PSII, reflecting the potential primary light energy conversion efficiency of PSII reaction centers. As shown in Figure 3A, Treatment 13 had the highest *Fv/Fm* at 0.97±0.01, significantly higher than all other treatment groups. Relatively higher values were also found in Treatments 4, 9, 12, and CK3, while lower values were observed in Treatments 5, 6, 14, 15, and CK2. Control CK1 showed the lowest value at 0.39±0.01, significantly lower than all other substrate formulas.

Fv'/Fm' represents the effective photochemical quantum efficiency of PSII under light-adapted conditions, reflecting the primary light energy capture efficiency of PSII reaction centers. Figure 3B shows that Treatment 13 had the highest *Fv'/Fm'* at 0.67±0.03, which was not significantly different from Treatments 1, 12, and CK3 but significantly higher than other treatment groups. Relatively higher values were also found in Treatments 4, 8, and 9. Lower *Fv'/Fm'* values were observed in Treatments 14 and 15 and CK2, with Control CK1 showing the lowest value at 0.1±0.03, significantly lower than all other substrate formulas.

ΦPSII represents the actual photochemical efficiency, reflecting the actual primary light energy capture efficiency of PSII reaction centers under partially closed conditions. Higher *ΦPSII* values indicate higher light energy conversion efficiency. As shown in Figure 3C, Treatment 13 had the highest ΦPSII at 0.34±0.07, not significantly different from Treatment 12 and Control CK3 but significantly higher than other groups. Relatively higher values were also found in Treatments 1 and 5. Lower ΦPSII values were observed in Treatments 14, 15, 11, 8, and 3. Control CK1 showed the lowest value at 0.1±0.03, which was not significantly different from Treatments 14 and 15 but significantly lower than all other treatment groups.

NPQ represents the non-photochemical quenching coefficient, which is caused by thermal dissipation and reflects the plant's ability to dissipate excess light energy as heat, i.e., photoprotective capacity. Figure 3D shows that Control CK1 had the highest *NPQ* at 5.48±0.23, which was not significantly different from Treatments 14 and 15 and Control CK2 but significantly higher than other treatment groups. Relatively higher *NPQ* values were also found in Treatments 2, 5, and 11. Lower *NPQ* values were observed in Treatments 1, 12, 6, 7, 8, 9, 10, and CK3, with Treatment 13 showing the lowest *NPQ* at 2.19±0.37, significantly lower than all other treatment groups.

ETR represents the electron transport rate, reflecting the apparent electron transfer efficiency under actual light intensity. Figure 3E shows that Treatment 13 had the highest *ETR* at 146.29±14.26, not significantly different from Treatment 12 and CK3 but significantly higher than other treatments. Relatively higher *ETR* values were also found in Treatments 1, 5, and 6.

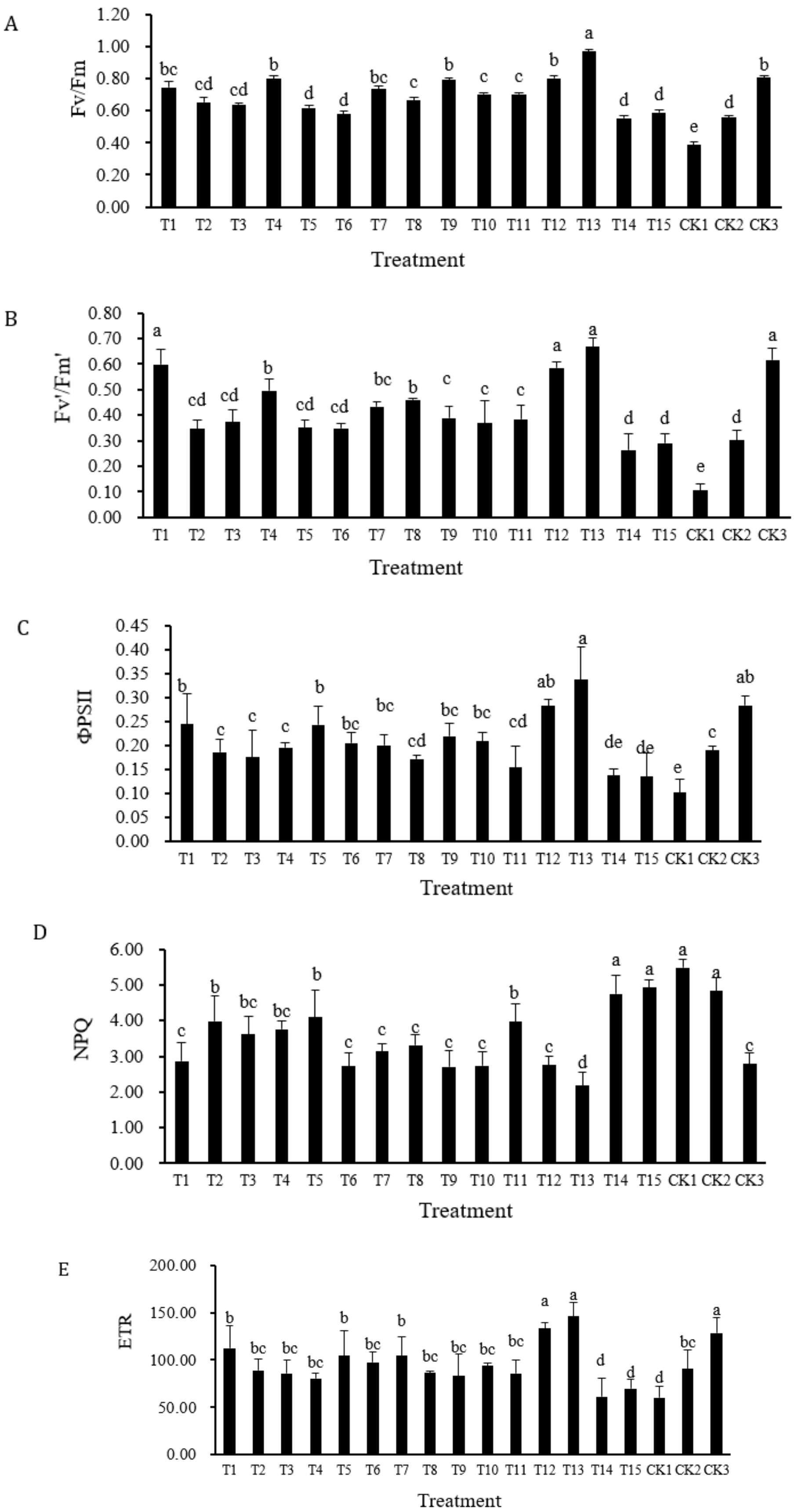

Figure 3. Effect of different substrate formulas on chlorophyll fluorescence parameters(A,B,C,D,E) of *Koelreuteria bipinnata* seedlings

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Lower *ETR* values were observed in Treatments 14 and 15 and CK1, at 61.23±19.12, 69.59±9.83, and 60.36±12.08, respectively, with no significant differences among them but significantly lower than other treatment groups.

In summary, Treatments 13, 12, 1, and CK3 showed higher *Fv/Fm*, *Fv'/Fm'*, *ΦPSII*, and *ETR* values along with lower *NPQ* values, indicating that substrate formula 13 and related treatments enhanced the capture and light energy conversion efficiency of PSII reaction centers in *Koelreuteria bipinnata*, resulting in stronger photosynthetic capacity. In contrast, Treatments 14 and 15 and Controls CK1 and CK2 showed lower *Fv/Fm*, *Fv'/Fm'*, *ΦPSI*I, and *ETR* values with higher *NPQ* values, indicating weaker capture and conversion efficiency of PSII reaction centers and thus lower photosynthetic capacity.

4. Discussion

Chlorophyll is a fundamental substance for photosynthesis in green plants and an important factor affecting photosynthetic rate. Its content directly reflects the growth status and photosynthetic capacity of plants. The results of this study showed that Treatment 13 had the highest contents of chlorophyll a, chlorophyll b, and total chlorophyll, significantly higher than all other substrate formulas. Relatively higher total chlorophyll contents were also found in Treatments 1 and 12, while lower contents were observed in Treatments 14 and 15, with CK1 showing the lowest values. These results indicated that substrate formulas T13, T1, and T12 favorably increased leaf chlorophyll synthesis, enhanced photosynthesis, and promoted plant growth. This may be related to the nitrogen content of these substrates, as nitrogen is a major component of chlorophyll. Higher nitrogen content promotes chlorophyll synthesis, while lower nitrogen content is unfavorable for chlorophyll synthesis, which is consistent with the higher nitrogen levels in T13, T1, and T12 and the lower nitrogen levels in T14, T15, CK1, and CK2.

Net photosynthetic rate directly reflects the plant's utilization of light energy and further reflects its capacity for photosynthetic product accumulation. It is generally considered that the reduction in photosynthetic rate is influenced by both stomatal and non-stomatal limitations. When stomatal conductance and net photosynthetic rate decrease while intercellular CO_2 concentration also decreases, the reduction in net photosynthetic rate is mainly due to the decline in stomatal conductance. When stomatal conductance decreases but intercellular CO_2 concentration increases, the main cause of the reduced net photosynthetic rate is the decline in mesophyll cell photosynthetic capacity [23,24]. In this experiment, when net photosynthetic rate and stomatal conductance were lower, intercellular CO_2 concentration was also lower, indicating that the differences in net photosynthetic rate among substrates were due to stomatal limitations. Furthermore, Treatment 13 exhibited the highest values of net photosynthetic rate ((10.89±1.27) $\mu mol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance ((0.160±0.035) $mol \cdot m^{-2} \cdot s^{-1}$), and intercellular CO_2 concentration ((268.81±23.19) $\mu mol \cdot mol^{-1}$), significantly higher than all other substrate formulas. Relatively higher values of *Pn*, *Gs*, and *Ci* were also found in Treatments 1 and 12, while lower values were observed in Treatments 14 and 15 and Control CK2, with CK1 showing the lowest values. Thus, Treatments 13, 1, and 12 exhibited stronger photosynthetic capacity and higher photosynthetic efficiency, while Controls CK1 and CK2 and Treatments 14 and 15 resulted in weaker photosynthetic capacity and lower efficiency. This may be related to the physicochemical properties of the substrates. Better substrates such as T13, T1, and T12 had higher nutrient content and water-holding capacity, with more favorable physicochemical properties for plant growth (see Table 2). Plants grown in these substrates absorbed adequate nutrients and water, which were sufficient for chlorophyll synthesis and mesophyll cell construction, resulting in higher stomatal conductance and net photosynthetic rate parameters and stronger leaf photosynthetic capacity. In contrast, poorer

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substrates such as CK1, CK2, T14, and T15 had lower nutrient content and water-holding capacity, with less favorable physicochemical properties. Plants grown in these substrates absorbed insufficient nutrients, leading to deficiencies in materials for chlorophyll synthesis and mesophyll cell construction, resulting in lower stomatal conductance and net photosynthetic rate and weaker leaf photosynthetic capacity.

Chlorophyll fluorescence serves as an ideal probe for photosynthesis research, reflecting the plant's photosynthetic capacity and serving as an important indicator for assessing plant growth [20,21]. The results of this experiment showed that *Koelreuteria bipinnata* seedlings under T13, T1, T12, and CK3 treatments exhibited higher *Fv/Fm, Fv'/Fm', ΦPSII, and ETR,* along with lower *NPQ,* indicating that seedlings under T13, T1, T12, and CK3 had higher light energy utilization efficiency during PSII photochemical processes, lower thermal dissipation, higher electron transport rates, and stronger photosynthetic capacity. In contrast, seedlings under T14, T15, CK1, and CK2 treatments showed lower *Fv/Fm, Fv'/Fm', ΦPSII,* and *ETR* but higher *NPQ,* indicating lower light energy utilization efficiency, higher thermal dissipation, lower electron transport rates, and weaker photosynthetic capacity. This may also be related to substrate physicochemical properties. Substrates such as T13 had higher nutrient (e.g., nitrogen) content and more balanced physicochemical properties suitable for plant growth. Plants absorbed sufficient nutrients for chlorophyll synthesis and mesophyll cell construction, leading to higher PSII activity, stronger primary light capture ability of antenna pigments, greater captured light energy, and stronger photosynthetic electron transport capacity. A higher proportion of light energy was used for photochemical reactions, with less surplus energy, resulting in higher fluorescence parameters such as *Fv/Fm, Fv'/Fm', ΦPSII,* and *ETR,* lower *NPQ*, stronger leaf photosynthesis and carbon assimilation, greater accumulation of photosynthetic products, and more vigorous plant growth. Conversely, substrates such as T14 and T15 had lower nutrient content (e.g., nitrogen, see Table 2) and more unbalanced physicochemical properties, which could cause stress and insufficient nutrient supply to plants. This resulted in fewer materials for chlorophyll synthesis and mesophyll cell construction, lower PSII activity, weaker primary light capture ability of antenna pigments, less captured light energy, and weaker photosynthetic electron transport capacity. A smaller proportion of light energy was used for photochemical reactions, leaving more surplus energy, which plants dissipated through photorespiration or heat dissipation as a self-protection mechanism [25,26]. Consequently, fluorescence parameters such as *Fv/Fm, Fv'/Fm', ΦPSII*, and *ETR* were lower, *NPQ* was higher, leaf photosynthesis and carbon assimilation were weaker, accumulation of photosynthetic products was lower, and plant growth was poorer.

The high *ETR* values observed in T13 (146.29) indicated that electrons were efficiently transported from PSII to PSI, supporting $NADP^+$ reduction and CO_2 fixation. This is consistent with the high *Pn* values measured in the same treatment, confirming that the photosynthetic electron transport chain was functioning optimally. The low *NPQ* in T13 suggested that photoprotective energy dissipation was minimal, as most absorbed light energy was utilized for photochemistry rather than being dissipated as heat. This represents an energy-efficient state for plant growth [27].

5. Conclusion

In conclusion, Treatment 13 substrate formula resulted in higher total chlorophyll content ((18.86±0.56) $mg \cdot g^{-1}$), higher photosynthetic parameters including *Pn* ((10.89±1.27) $\mu mol \cdot m^{-2} \cdot s^{-1}$) and *Gs* ((0.160±0.035) $mol \cdot m^{-2} \cdot s^{-1}$), and higher chlorophyll fluorescence parameters including *Fv/Fm* (0.97±0.01), *Fv'/Fm'* (0.67±0.03), *ΦPSII* (0.34±0.07), and *ETR* (146.29±14.26), along with lower values of stomatal limitation *Ls* (0.18±0.97) and *NPQ* (2.19±0.37). Combined with its reasonable and balanced physicochemical properties and good seedling growth indicators, Treatment 13 was identified as the optimal substrate formula in this experiment, suitable as the best organic cultivation substrate for *Koelreuteria bipinnata*. Additionally, Treatments 1 and 12 also showed relatively high photosynthetic and chlorophyll fluorescence parameters including *Pn* and *Fv/Fm*, indicating good photosynthetic performance, while Treatments 14 and 15 performed poorly and should be avoided where possible.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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