



Article

Serendipita indica: A Promising Biostimulant for Improving Growth, Nutrient Uptake, and Sugar Accumulation in *Camellia oleifera*

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Abstract: *Serendipita indica* is a very promising root-associated endophytic fungus that is widely used on various plants; however, whether it affects the growth and physiological activity of an oilseed crop (*Camellia oleifera*) under field conditions remains unclear. In this study, we analyzed the effects of *S. indica* inoculation on root colonization rate, growth rate, photosynthetic parameters, mineral element concentrations and related gene expression, and sugar concentrations and expression of their transporter genes in four-year-old *C. oleifera* trees in the field. The results showed that the root colonization rate of *C. oleifera* increased from 3.37% to 9.42% following being inoculated with *S. indica*. Inoculation with *S. indica* significantly increased the plant height (46.81%), net photosynthetic rate (69.16%), nitrogen balance index (14.44%), chlorophyll index (21.08%), leaf K (7.4%), leaf Ca (13.52%), root P (17.75%), root K (12.80%), soil NH₄⁺-N (17.78%), available K (26.66%), Olsen-P (184.30%), easily extractable glomalin-related soil protein (39.26%), and soil organic carbon (16.25%) concentrations compared to the uninoculated treatment. Inoculation with *S. indica* also significantly up-regulated the expression of *CoHKT1;1* and *CoCAX1;2* in the leaves and roots and *CoPht1;1*, *CoPht1;2*, and *CoPht1;3* in the leaves. Plants inoculated with *S. indica* also presented significantly higher leaf glucose, fructose, and sucrose concentrations, accompanied by up-regulated expression of *CoSWEET2a*, *CoSWEET7*, *CoSWEET9b*, *CoSWEET17a*, and *CoSWEET17b*. These results suggest that *S. indica* has significant potential as a biostimulant for enhancing the growth and nutritional profile of *C. oleifera*, thereby contributing to sustainable oilseed production.

Keywords: *Camellia oleifera*; endophytic fungi; SWEET; sucrose



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1. Introduction

Serendipita indica (formerly *Piriformospora indica*) is a root-associated endophytic fungus belonging to the family Thelephoraceae of the phylum Basidiomycota that can colonize the root epidermal cells, cortical cells, and intracellular space of many plants to establish symbiotic relationships with plant hosts [1]. The fungus can be cultured on artificial medium [2]. The effects of *S. indica* on plants are manifested in promoting plant growth and accelerating the uptake of mineral elements such as nitrogen and phosphorus [3] in different plants, including peach [4], corn [5], and *Artemisia annua* [6]. *S. indica* can initiate a signaling cascade involving the activation of 3-phosphoinositide-dependent protein kinase 1, oxidative signal inducible 1, and mitogen-activated protein kinases 3/6

to promote the growth of host plants [7]. Moreover, *S. indica* significantly induces plant resistance to biotic and abiotic stresses [1], such as flooding [4], salinity [5], drought [3], and heavy metals [6]. These characteristics make *S. indica* very promising for agricultural applications in the field, especially for improving nutritional absorption and reducing pesticide use [8].

Camellia oleifera is a significant woody plant cultivated for edible oil production globally, with China being the largest producer and having a cultivation area covering 4.5×10^6 hm² [9,10]. Additionally, *C. oleifera* is a deep-rooted species that effectively mitigates soil erosion [8,11], while its fallen leaves and withered branches contribute to increased soil organic matter [12]. The oil of *C. oleifera* is known for its moisturizing, antioxidant, and anti-inflammatory effects and is widely used in skin and hair care products as well as an adjunctive treatment for cardiovascular disease and inflammation [13,14]. However, in China, *C. oleifera* is mainly planted in mountain areas with poor soil, which results in limited fruit yield and tree growth [14]. Liu et al. [15] reported that the rhizosphere soil of *C. oleifera* in the field was abundant in arbuscular mycorrhizal (AM) fungi populations. The inoculation of AM fungi and *S. indica* enhanced the growth and phosphorus uptake of potted *C. oleifera* plants [16]. *S. indica* possesses a high-affinity phosphate transporter, known as PiPT, which is classified within the phosphate:proton symporter family, a subgroup of the Major Facilitator Superfamily [16,17]. AM fungi are not yet able to be propagated in vitro on a large scale, while *S. indica* is able to be cultured in vitro on artificial media [18].

In *C. oleifera*, a lot of gene identifications and their functional analyses have been carried out around the stress response and seed development, such as the Aux/IAA gene family, the CCCH-type zinc finger protein gene family, and the SWEET gene family [19–21]. Liu et al. [22] also revealed that *S. indica* partly improved the plant growth of trifoliolate orange, associated with high expression of IAA synthetic and transporter genes. Since *S. indica* has AM-fungus-like functions, we therefore hypothesized that *S. indica* can promote the growth as well as regulate the physiological activity of *C. oleifera* in the field. To confirm this hypothesis, this study inoculated *S. indica* on field *C. oleifera* trees to investigate its potential effects on tree growth, photosynthetic characteristics, nutrient acquisition, and soil properties, with a focus on the molecular mechanisms of P, K, Ca, and sugars.

2. Materials and Methods

2.1. Preparation of *S. indica*

S. indica was provided by the Institute of Root Biology, Yangtze University. The fungal blocks stored at -75 °C were activated at 30 °C for 72 h in dark. The 1×1 cm² size mycelial blocks on PDA solid medium were cut and inoculated into PDB liquid medium for proliferation culture at 150 r/min and 30 °C for 7 days. Subsequently, the mycelium was filtered and rinsed three times with distilled water. The 1 g of fresh mycelium was combined with 100 mL of distilled water and pulverized with a juicer, and the concentration of the propagule suspension was 1.77×10^9 CFU/mL. A flow chart of *S. indica* proliferation is presented in Figure 1a.

2.2. Experimental Set-Up and Design

Four-year-old *C. oleifera* plants with essentially uniform growth (average height of 66.3 cm, stem diameter of 7.7 mm, and leaf number of 74) were inoculated on 21 March 2023, in Xianhedian, Huangpi, Wuhan, Hubei, China ($114^{\circ}21'56''$ E, $31^{\circ}06'27''$ N). The soil nutrient levels were: NH_4^+ -N of 34.15 mg/kg, Olsen-P of 34.8 mg/kg, available K of 47.05 mg/kg, organic carbon of 6.28 g/kg, and pH of 5.6. The orchard was located in a subtropical monsoon climate with an altitude of 52 m, an average annual temperature of 15 °C, and an average annual precipitation of about 800 mm.

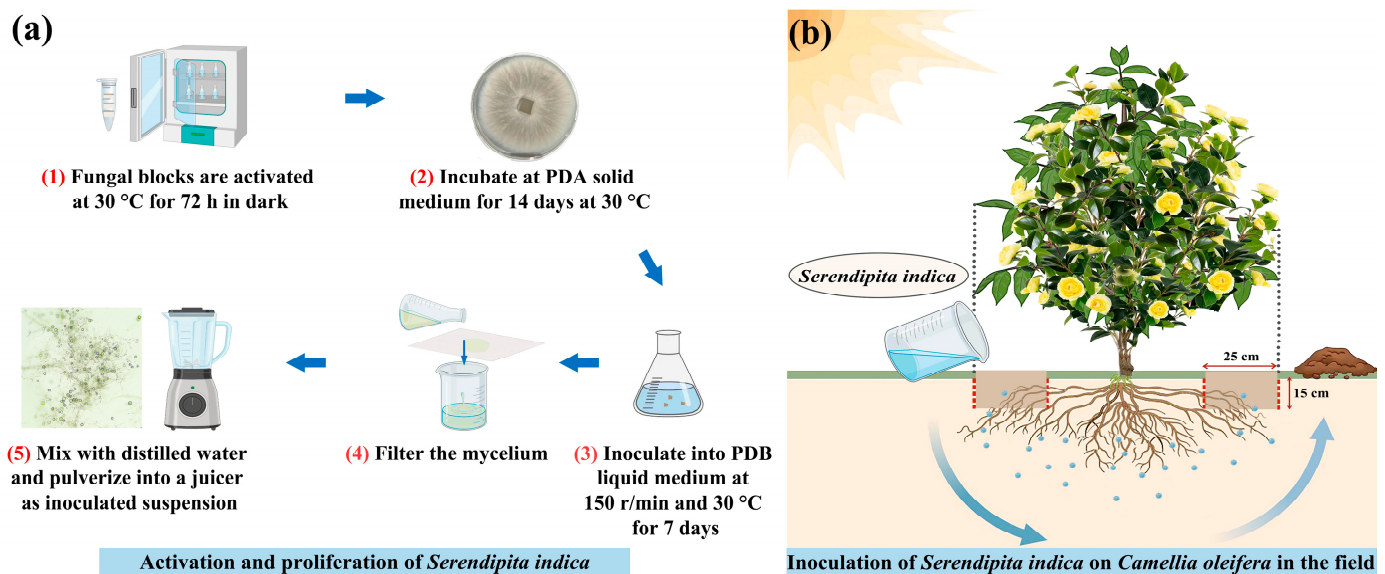


Figure 1. A flow chart of *Serendipita indica* proliferation (a) and field inoculation (b).

The inoculation dosage of *S. indica* was 2 L of propagule suspension per plant. A ring groove with 25 cm wide and 15 cm deep was dug under the crown of *C. oleifera* plants to expose the roots. The *S. indica*'s suspension was shaken well and evenly poured into the ring groove so that it directly colonized the roots (Figure 1b). The no-inoculation with *S. indica* (Control) was applied with an autoclaved propagule suspension of *S. indica*.

The experiment was a one-way experimental design with two treatments, including *S. indica* inoculation and Control. Each treatment was replicated four times for a total of 16 trees (2 trees in one replication). The experiment was terminated on 18 October 2023.

2.3. Determination of Growth Behavior and Root Fungal Colonization Rate

Seven months after the inoculation of *C. oleifera*, the fourth fully expanded leaf at the top of the current year's branch and the current year's roots from the ring groove were collected. In the field, they were immediately frozen using liquid nitrogen and brought back to the laboratory for subsequent analysis. Plant height, stem diameter (10 cm from the ground), and leaf number were determined before the inoculation and 7 months after the inoculation, and their rate was estimated on a monthly basis on the change. Root fungi were stained as per the trypan blue staining method [23]. Root fungal colonization rate (%) = root length of *S. indica* colonization/total length of examined root segments $\times$ 100.

2.4. Measurement of Photosynthetic Characteristics

Measurements of the net photosynthetic rate, intercellular CO₂ concentration, stomatal conductance, and transpiration rate were made on the 5th fully expanded leaf from the top at 9:00 a.m. on 18 October 2023, using a Li-6400 photosynthesizer (Li-COR Inc., Lincoln, NE, USA). The nitrogen balance index (Nbi), chlorophyll index (Chl), and flavonoid index (Flav) were measured on the 4th fully expanded leaf from the top using a portable plant polyphenol meter (Force-A, Orsay, France).

2.5. Determination of P, K, Ca, and Sugar Concentrations in Leaves and Roots

Root and leaf P, K, and Ca concentrations were measured using an ICP spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) after the plant samples were dried, ground, sieved, and digested. The fructose, glucose, and sucrose concentrations

in the leaves and roots were determined using the colorimetric method described by Wu et al. [13].

2.6. Determination of Soil Properties

The soil adhering to the surface of the roots was collected for analysis of the soil properties. The soil available nutrient levels, including $\text{NH}_4^+\text{-N}$, Olsen-P, and available K were determined using a high-precision Soil Ntrient Detector Shandong (Shandong Hengmei Electronic Technology Co., Ltd., Weifang, China). Easily extractable glomalin-related soil protein (EG) and difficultly extractable glomalin-related soil protein (DG) were extracted from the air-dried soil using the protocol of He et al. [24], and the sum of the two was the total glomalin-related soil protein (TG). The protein concentration was determined using the Bradford [25] method. The soil organic carbon (SOC) content was determined via the wet oxidation method [26].

2.7. Transporter Genes Expression

The total RNA from the leaves and roots was extracted, verified for integrity and purity, and then reversed to cDNA. Six phosphorus transporter genes (*CoPht1;1*, *CoPht1;2*, *CoPht1;3*, *CoPht1;4*, *CoPHO1;1*, and *CoPHO1;3*), one potassium transporter gene (*CoHKT1;1*), two calcium transporter genes (*CoCAX1;1* and *CoCAX1;2*), and ten SWEET genes [27] were chosen, and their specific primer sequences (Table 1) were designed by Primer Premier 5.0 software. qRT-PCR was performed on a real-time quantitative PCR instrument (Bio-Rad Laboratories, Hercules, CA, USA). The quantitative results were calculated using the $2^{-\Delta\Delta C_t}$ method of Livak and Schmittgen [28]. For *SWEET* genes, *GAPDH* was selected as the internal reference gene; for the mineral element transporter genes, *EF-1 α* was selected as the internal reference gene.

Table 1. Specific primer sequences of selected genes in qRT-PCR.

Genes	Forward Sequence (5' → 3')	Reverse Sequence (5' → 3')
<i>CoPHT1;1</i>	GTTCTTGGCGGAGTCAATTTC	CATCCTCATCTTCCTCGTTCTC
<i>CoPHT1;2</i>	TCCCTTTGCTTCTTCCGATT	TCCCTTTGCTTCTTCCGATT
<i>CoPHT1;3</i>	GAGTCAGAGCAGCAGAAAGTAG	TGTAGTCCCAAGCAAGTGAAG
<i>CoPHT1;4</i>	CCGTTACACCGCCCTTATC	CTGGGTCTTCAGCCATCTT
<i>CoPHO1-1</i>	AGCAGCCCTTGAAGTCATTAG	GAAGTTGCCCGCATTGTTTATG
<i>CoPHO1-3</i>	GAGCTTTCAGTGGCCTAACA	GTCGCCTCACCGAGTTTATC
<i>CoCAX1;1</i>	ATCAGCGATGGCTCACCTGTTATC	GGAGGAAGTGGGAGAGAGGGAAAT
<i>CoCAX1;2</i>	CCGCCGCCAACATCTCTTCT	CCGTAATTGGAAGTGAGGGAGCAA
<i>CoHKT1;1</i>	TGGGTACTTGGCTTTGAAGGTTT	GCAGACACAGAGGTGAAGAACAA
<i>EF-1α</i>	AGACTGTGGCTGTTGGTGTT	ATCCAAACCCGCACAGTTCA
<i>CoSWEET1a</i>	GGTGAAGCCAAGAAACCTCCT	CTACTTGCTCGATCGCTTCTCT
<i>CoSWEET1b</i>	TTGGAGGAAGAGAAGCTATCTG	ATGATTCTTGACATGCCATTGAG
<i>CoSWEET2a</i>	GCAGAGAACGCGAAAAAGGT	ATATCCAACGAAGATCTGTGCGATT
<i>CoSWEET2b</i>	ATGGTTTCTTTGGCTTTGTCCC	AGATATGAGTGGCGTGCCGT
<i>CoSWEET7</i>	CCAAGCTCCGGTCACTCATT	ATTAGCACAGGAAGCCAGGG
<i>CoSWEET9a</i>	AGGTTCTACCAGAAAGTCAAGCTG	TGGATTGCTCGGTTTGATA
<i>CoSWEET9b</i>	AAGAGCGTGGAGTACATGCC	GCAAATCCCGAGAGACGACA
<i>CoSWEET10</i>	TGGAGGAAGAGAAGCTATCTGAAT	TGAGGCATGACTGGAATGATTCT
<i>CoSWEET17a</i>	ATGTTGGGTTTCTTGGGGCA	GACAGCTAGAGGTGCTGCAT
<i>CoSWEET17b</i>	GCAAGGCCTCGATGACCAC	TTTGTCGATGCTGTATTGCCG
<i>CoGAPDH</i>	GGTGCCAAGAAGGTGGAATA	GTTGTGCAGCTTGCATTAGAG

2.8. Statistical Analysis

The analysis of variance for the experimental data was performed using SAS 8.1 software, and significant differences were calculated using Duncan's new multiple range tests ($p < 0.05$). For percentages, an arcsine conversion was performed to achieve homogeneity. Correlation analyses were performed using Origin (v2022; OriginLab Corporation,

Northampton, MA, USA), and Sigmaplot (v10.0; SigmaPlot Software Inc., Chicago, IL, USA) was used to draw figures. The homogeneity test of variance and 95% confidence interval analysis of mean value were performed using SPSS (v24.0; IBM Corp., Armonk, NY, USA).

3. Results

3.1. Effect of Inoculation with *S. indica* on the Growth and Root Colonization Rate of Field *C. oleifera*

Inoculation with *S. indica* significantly increased the root fungal colonization rate from 3.37% to 9.42% compared with the Control treatment (Figure 2b; Supplementary Material Table S1). Inoculation with *S. indica* also distinctly increased the plant height (Figure 2c,d; Supplementary Material Table S1) by 46.81% compared with the Control treatment. Inoculation with *S. indica* did not affect leaf number (Figure 2e; Supplementary Material Table S1) or stem diameter (Figure 2f; Supplementary Material Table S1).

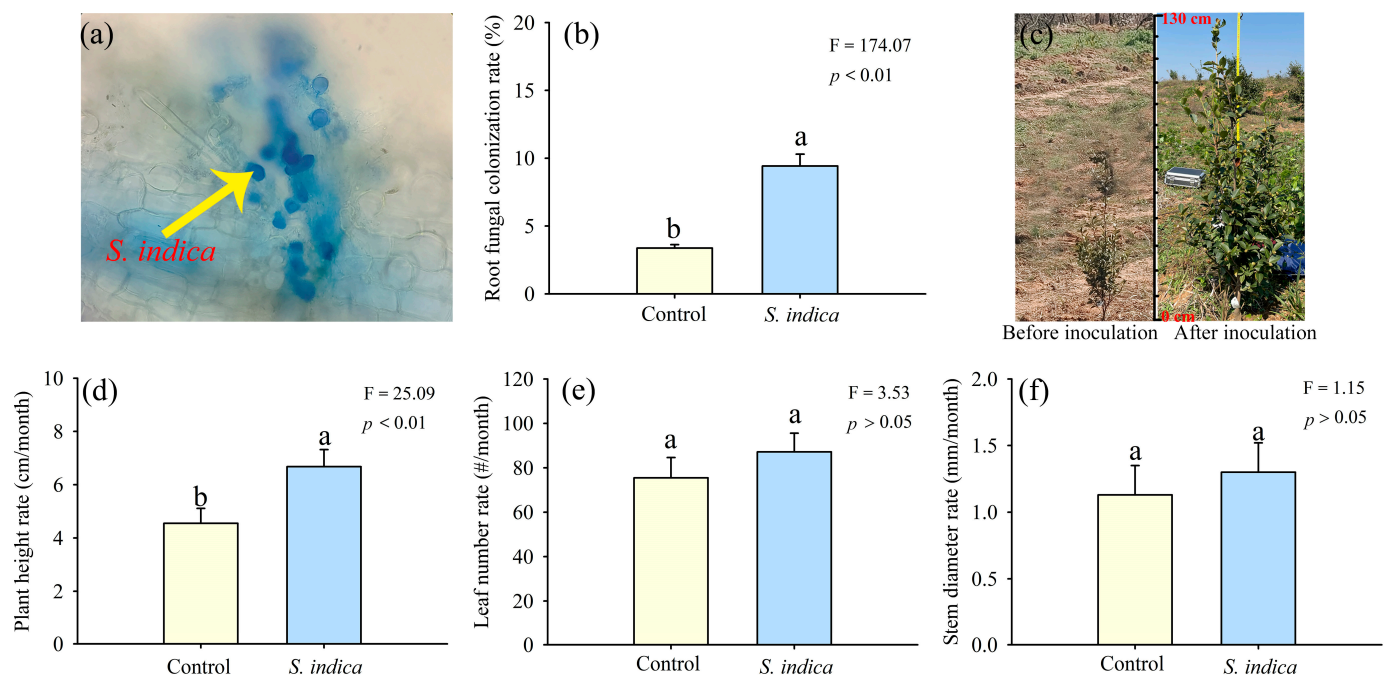


Figure 2. Root colonization of *Serendipita indica* ((a); trypan blue staining) and changes in root fungal colonization rate (b), plant height (c,d), stem diameter (e), and leaf number (f) of field *Camellia oleifera* following *Serendipita indica* colonization for seven months. Data represent means $\pm$ SD ($n = 4$); different letters above the bars indicate significant ($p < 0.05$) differences. The yellow arrow in (a) indicates that pear-shaped spores occur within the root cortex cells. Abbreviations: *S. indica*, *Serendipita indica*.

3.2. Effect of Inoculation with *S. indica* on the Leaf Photosynthetic Characteristics of Field *C. oleifera*

Inoculation with *S. indica* significantly increased the net photosynthetic rate, Nbi, and Chl in the leaves of field *C. oleifera* plants by 69.16%, 14.44%, and 21.08%, respectively, while significantly decreasing the intercellular CO_2 concentration by 17.39% compared with the Control treatment (Figure 3a,c,e; Supplementary Material Table S1). There were no significant changes in leaf stomatal conductance (Figure 3b; Supplementary Material Table S1), transpiration rate (Figure 3d; Supplementary Material Table S1), and Flav (Figure 3e; Supplementary Material Table S1) following *S. indica* inoculation.

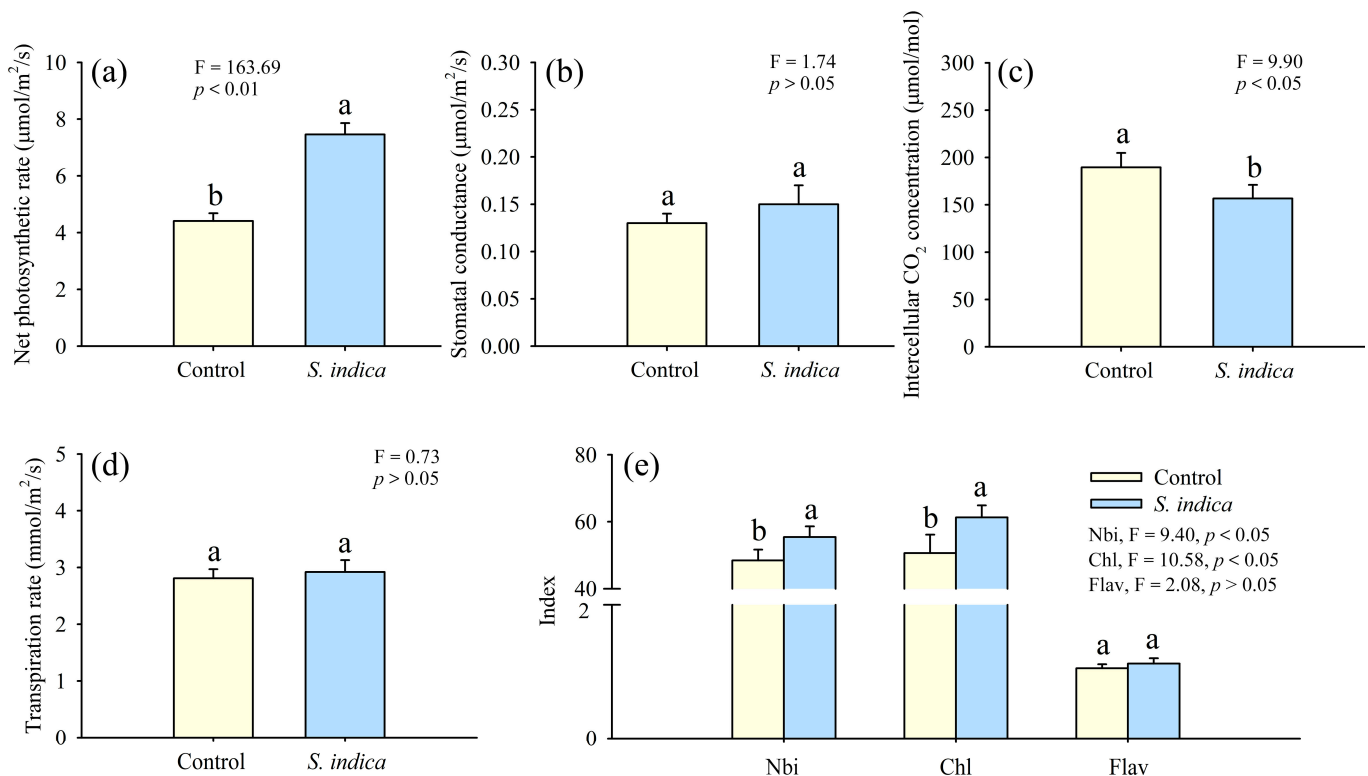


Figure 3. Changes in net photosynthetic rate (a), stomatal conductance (b), intercellular CO_2 concentration (c), transpiration rate (d), nitrogen balance index (Nbi) (e), chlorophyll index (Chl) (e), and flavonoid index (Flav) (e) in the leaves of field *Camellia oleifera* plants following *Serendipita indica* colonization for seven months. Data represent means $\pm$ SD ($n = 4$); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *S. indica*, *Serendipita indica*.

3.3. Effect of Inoculation with *S. indica* on P, K, and Ca Concentrations and Their Transporter Gene Expression in Field *C. oleifera*

Compared with the Control treatment, the treatment with *S. indica* inoculation significantly boosted leaf K (7.40%) and Ca concentrations (13.52%) and root P (17.75%) and K (12.80%) concentrations, respectively, along with no significant difference in leaf P and root Ca concentrations (Figure 4a; Supplementary Material Table S1).

Inoculation with *S. indica* significantly increased the expression of *CoPht1;1*, *CoPht1;2*, *CoPht1;3*, *CoPHO1;1*, *CoPHO1;3*, *CoCAX1;1*, *CoCAX1;2*, and *CoHKT1;1* in leaves by 3.13-, 2.19-, 611.21-, 1.43-, 2.69-, 2.34-, 29.44-, and 32.56-fold, respectively, along with a 1.28-fold down-regulation of leaf *CoPht1;4* expression (Figure 4b; Supplementary Material Table S1). In addition, inoculation with *S. indica* significantly increased the expression of *CoCAX1;1* and *CoHKT1;1* in roots by 2.21- and 2.10-fold, respectively, whereas it decreased the expression of *CoPht1;1*, *CoPht1;2*, *CoPht1;3*, and *CoPht1;4* in roots by 5.56-, 1.22-, 3.78-, and 1.58-fold, respectively.

3.4. Effect of Inoculation with *S. indica* on Sugar Concentrations and *CsSWEET* Gene Expression in Field *C. oleifera*

Inoculation with *S. indica* significantly increased the concentration of sucrose, fructose and glucose in leaves by 28.07%, 29.17%, and 56.01%, respectively, coupled with a significant decrease in root sucrose (21.27%), compared with the Control treatment (Figure 5a; Supplementary Material Table S1). Inoculation with *S. indica* significantly up-regulated the expression of *CoSWEET2a*, *CoSWEET7*, *CoSWEET9b*, *CoSWEET17a*, and *CoSWEET17b* in leaves by 1.34-, 2.96-, 1.52-, 1.50-, and 9.00-fold, respectively, while significantly down-regulating the expression of *CoSWEET1b* and *CoSWEET10* in the leaves by 1.68- and 3.88-fold, respectively (Figure 5b; Supplementary Material Table S1). In addition, inoculation

with *S. indica* significantly boosted the expression of *CoSWEET1a*, *CoSWEET1b*, *CoSWEET7*, *CoSWEET9a*, *CoSWEET10*, and *CoSWEET17a* in the roots by 2.68-, 8.54-, 1.75-, 2.11-, 4.28-, and 4.8-fold, respectively, whereas it significantly restricted the expression of *CoSWEET2a* and *CoSWEET2b* in the roots by 2.53- and 4-fold, respectively.

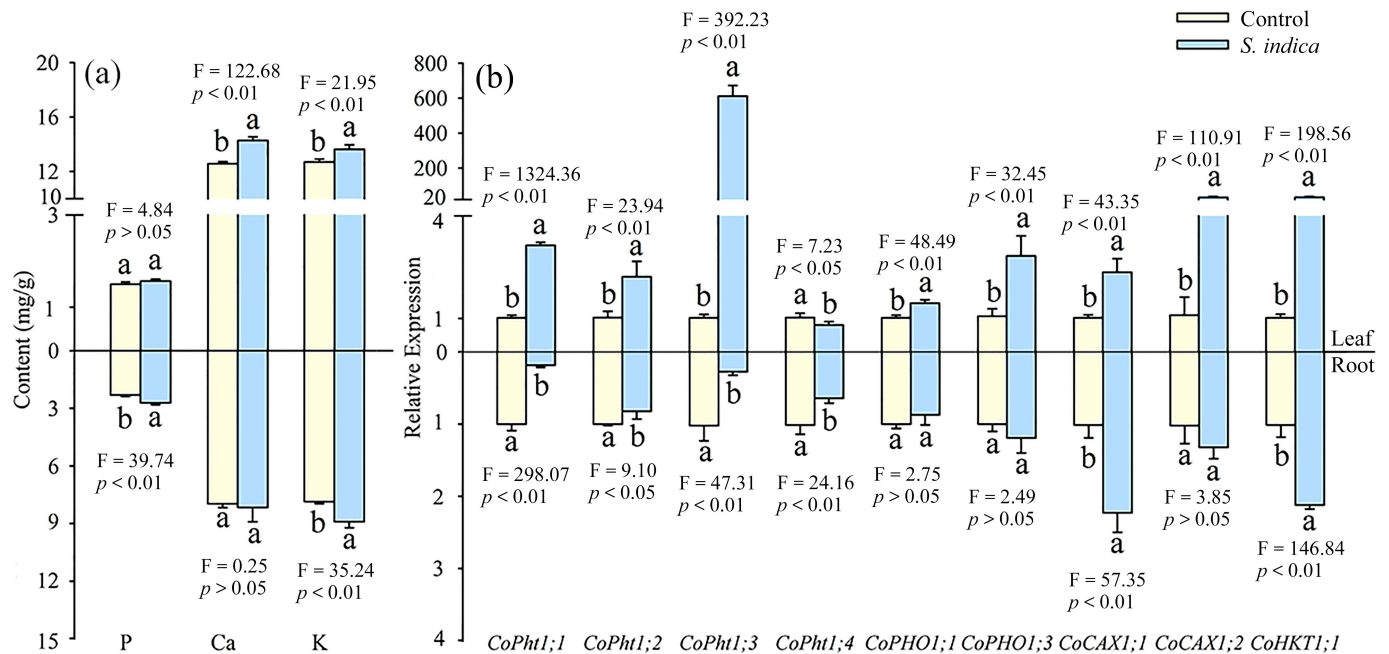


Figure 4. Changes in P, K, and Ca concentrations (a) and the expression levels of their transporter genes (b) in the leaves and roots of field *Camellia oleifera* plants following *Serendipita indica* colonization for seven months. Data represent means $\pm$ SD ($n = 4$); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *S. indica*, *Serendipita indica*.

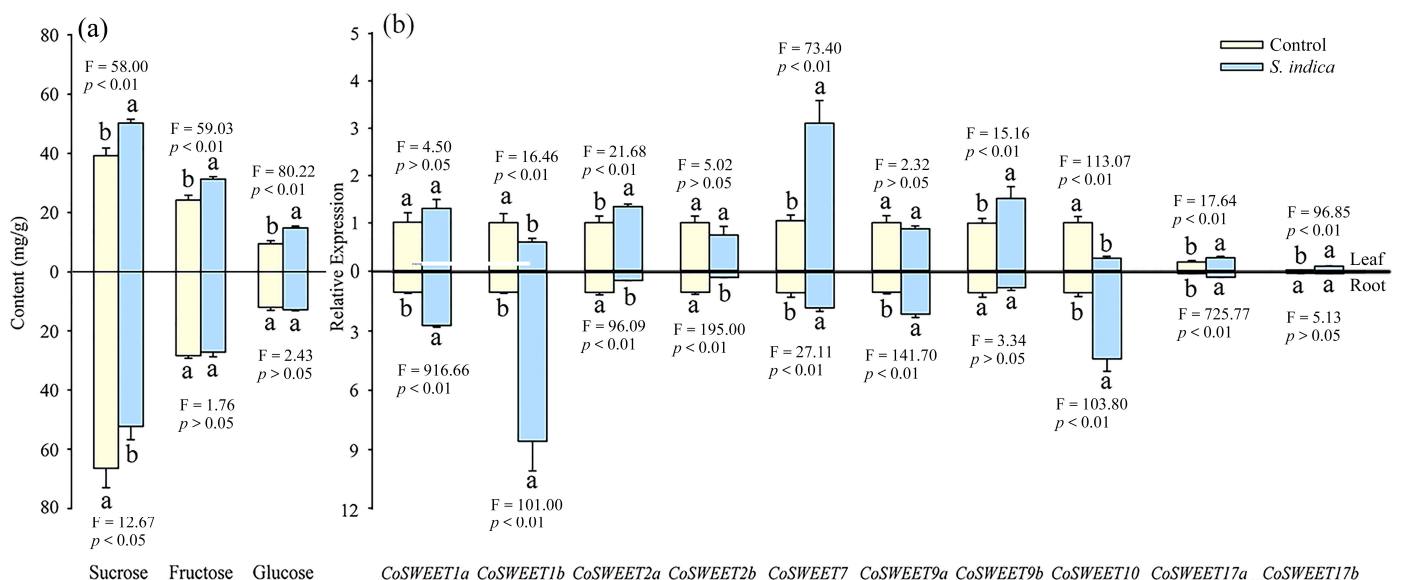


Figure 5. Changes in fructose, glucose, and sucrose concentrations (a) and *CoSWEET* gene expression (b) in the leaves and roots of field *Camellia oleifera* plants following *Serendipita indica* colonization for seven months. Data represent means $\pm$ SD ($n = 4$); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *S. indica*, *Serendipita indica*.

3.5. Effect of Inoculation with *S. indica* on the Soil Properties of Field *C. oleifera*

Compared with the no-inoculation Control, inoculation with *S. indica* significantly boosted the soil $\text{NH}_4^+\text{-N}$, available K, Olsen-P, EG, and SOC concentration by 17.78%, 26.66%, 184.30%, 39.26%, and 16.25%, respectively, followed by no significant change in DG and TG (Figure 6a,b; Supplementary Material Table S1).

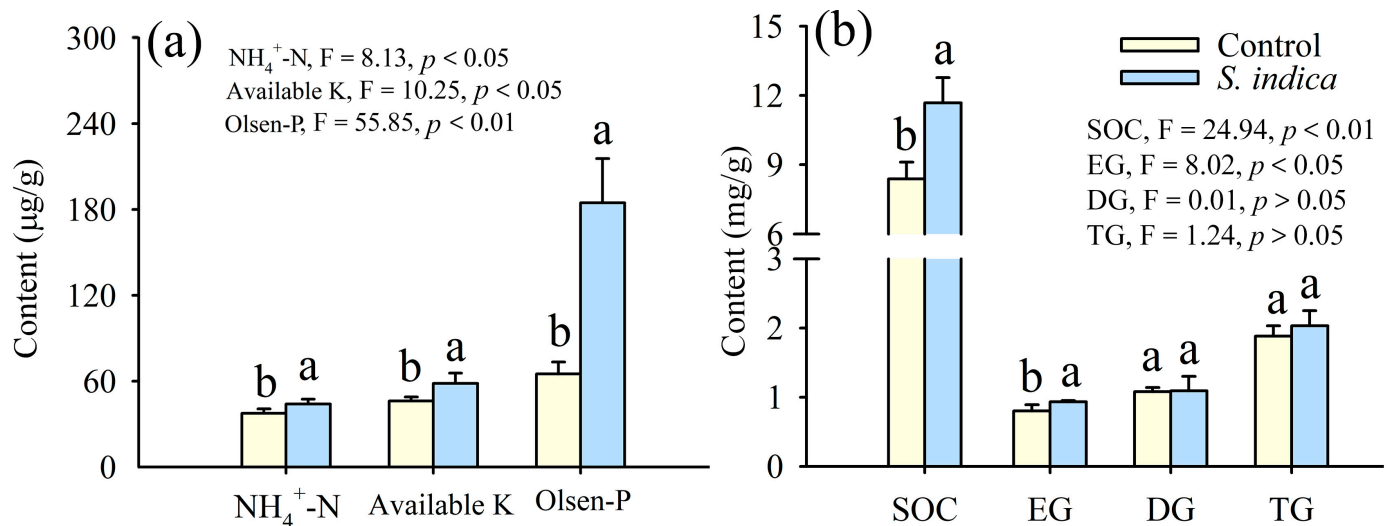


Figure 6. Changes in soil $\text{NH}_4^+\text{-N}$ (a), available K (a), Olsen-P (a), EG (b), DG (b), TG (b), and SOC (b) concentration in field *Camellia oleifera* plants following *Serendipita indica* colonization for seven months. Data represent means $\pm$ SD ($n = 4$); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *S. indica*, *Serendipita indica*.

3.6. Correlation Studies

The leaf sucrose, fructose, and glucose concentrations were significantly positively correlated with *CoSWEET2a*, *CoSWEET7*, *CoSWEET9b*, *CoSWEET17a*, and *CoSWEET17b*, while negatively correlated with *CoSWEET1b* and *CoSWEET10* (Figure 7a). In the roots, sucrose was significantly positively correlated with *CoSWEET2a* and *CoSWEET2b*, whereas it was negatively with *CoSWEET1a*, *CoSWEET1b*, *CoSWEET7*, *CoSWEET9a*, *CoSWEET10*, and *CoSWEET17a* (Figure 7b).

In the leaves, the P concentration was significantly positively correlated with *CoPht1;3* only, and the Ca and K concentrations were significantly positively correlated with the expression of their transporter genes (Figure 7c); in the roots, the P concentration was significantly negatively correlated with *CoPht1;1*, *CoPht1;3*, and *CoPht1;4* and K was significantly positively correlated with *CoHKT1;1* expression (Figure 7d).

The root fungal colonization rate was significantly positively correlated with the leaf net photosynthetic rate, Nbi, Chl, leaf fructose, leaf glucose, and leaf sucrose, while it was negatively correlated with the intercellular CO_2 concentration (Figure 7e). In addition, the root fungal colonization rate was significantly positively correlated with the soil $\text{NH}_4^+\text{-N}$, available K, Olsen-P, SOC, EG, leaf and root P, leaf Ca, and leaf and root K (Figure 7f).

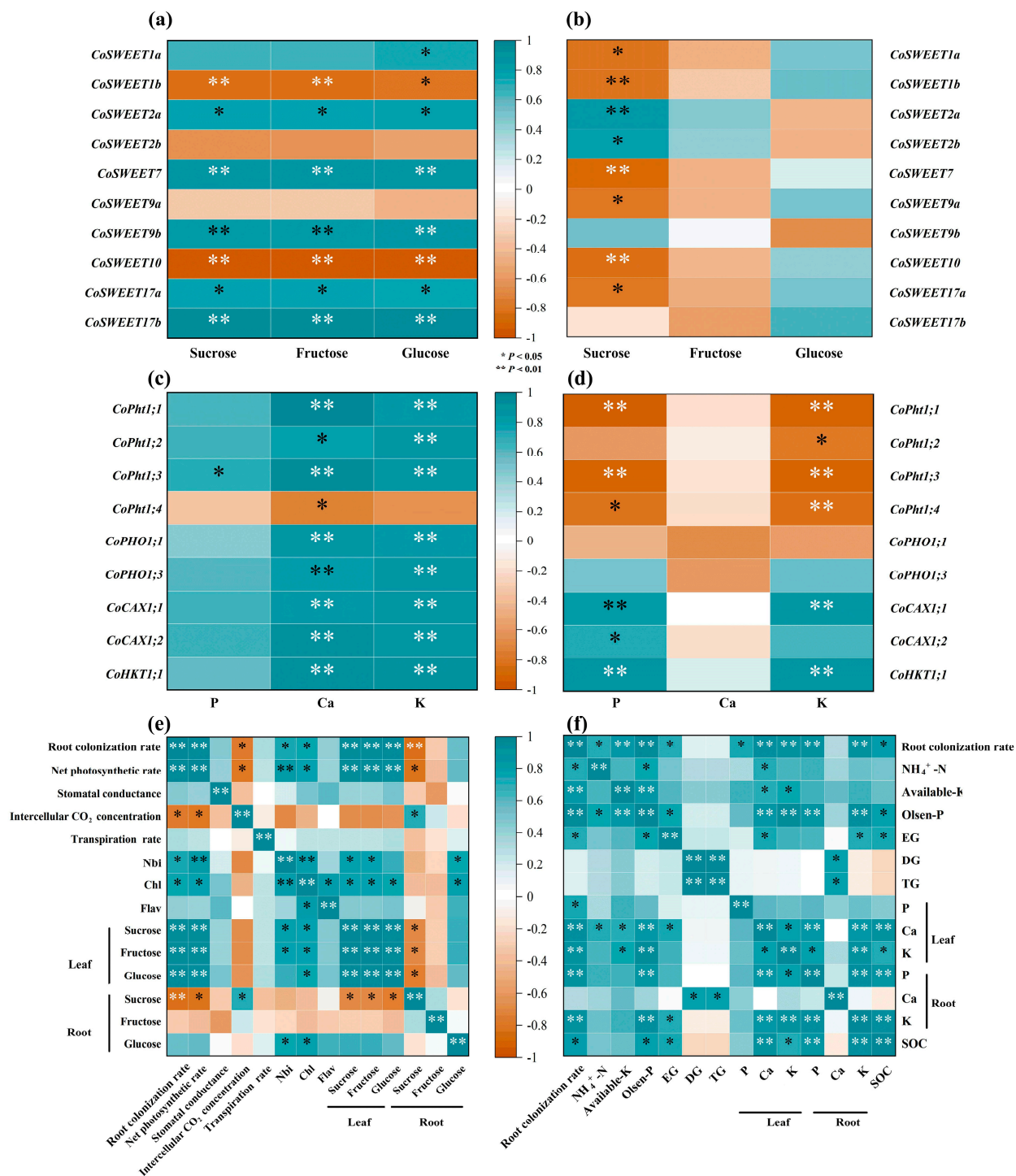


Figure 7. Heat map of the correlation between variables ($n = 8$). (a) The correlation between leaf sugars and corresponding *CoSWEET* expression; (b) the correlation between root sugars and corresponding *CoSWEET* expression; (c) the correlation between leaf P, K, and Ca and their corresponding transporter gene expression; (d) the correlation between root P, K, and Ca and their corresponding transporter gene expression; (e) the correlation between root fungal colonization rate, leaf photosynthetic characteristics, and leaf and root sugars; (f) the correlation between root fungal colonization rate, soil properties, and leaf and root mineral elements. Abbreviations: Nbi, nitrogen balance index; Chl, chlorophyll index; Flav, flavonoid index; EG, easily extractable glomalin-related soil protein; DG, difficultly extractable glomalin-related soil protein; TG, total glomalin-related soil protein; SOC, soil organic carbon.

4. Discussion

S. indica can improve the growth of host plants by colonizing their roots [29]. Our study found, for the first time, that inoculation with *S. indica* promoted the root fungal colonization rate and thus boost the physiological parameters of *C. oleifera* in the field, which is in line with the earlier results in king grass [30], tobacco [31], *Vicia villosa* [24], and peach [4]. Nevertheless, the root fungal colonization rate was relatively low. Trypan blue can stain the colonization of endophytic fungi and AM fungi and it was not entirely clear whether the root colonization was *S. indica* or AM fungi. The use of precise molecular detection techniques is necessary for future work. Additionally, *S. indica* was more effective at promoting the plant height of potted *C. oleifera* than AM fungi [16]. These results demonstrated that *S. indica* is an efficient endophytic fungus that can be used on *C. oleifera*, both in potted and field conditions. However, the results of this study were performed on a small amount of study materials and only one assay was performed, which had some limitations. Therefore, longer and multi-site serial observations are warranted in future studies. In addition, there is an urgent need to investigate whether the effect of *S. indica* inoculation on *C. oleifera* plantations is dependent on plantation management patterns, tree age, and fertilizer application.

Photosynthesis, the process by which plants convert light energy into chemical energy, is influenced by various external factors [32]. This study showed that inoculation with *S. indica* significantly increased the leaf net photosynthetic rate, Nbi, and Chl of *C. oleifera*. The root fungal colonization rate was significantly and positively correlated with the net photosynthetic rate, while it was negatively correlated with the intercellular CO₂ concentration, which indicated that *S. indica* promoted the photosynthetic rate of *C. oleifera* by increasing the chlorophyll concentration to produce more leaf carbohydrates, such as glucose, fructose, and sucrose. Such results are in agreement with the findings of earlier studies on *Panicum miliaceum* inoculated with *S. indica*. Additionally, Shukla et al. [29] demonstrated that *S. indica* improved photosynthesis in rice under arsenic stress by elongating the leaf palisade and spongy tissues, thereby enhancing CO₂ uptake and promoting photosynthesis [33]. The present study also demonstrated that inoculation with *S. indica* significantly increased leaf sucrose, fructose, and glucose concentrations in *C. oleifera* and decreased the root sucrose concentrations, which is consistent with the results obtained from the inoculation of *S. indica* on *Vicia villosa* plants [24]. Sugar is a source of energy to regulate plant growth and metabolic processes [34]. The decrease in root sucrose concentrations suggested that more sucrose was cleaved into the glucose required by root-associated endophytic fungi [35]. The change in sugar allocation may be related to the overall growth strategy of the plant, which allocates more resources to above-ground parts to support growth and defense under abundant photosynthetic product conditions [24].

SWEET proteins are able to transport sugars from intracellular to extracellular areas, or from one cell to another, facilitating intercellular communication and collaboration and ensuring that all parts of the plant have access to carbon sources [36]. SWEET genes play an important role in plant growth and development, including seed germination, pollen development, flower formation, and fruit maturation [21]. In this study, the expression of leaf *CoSWEET2a*, *CoSWEET7*, *CoSWEET9b*, *CoSWEET17a*, and *CoSWEET17b* genes was significantly increased by *S. indica* inoculation and that of leaf *CoSWEET1b* and *CoSWEET10* was decreased. In grapevine, *VvSWEET10* and *VvSWEET15* were highly expressed in the fruits and transported hexose during fruit ripening, thereby affecting the sugar levels of grape berries [36]. In this study, *CoSWEET10* was significantly negatively correlated with the leaf sucrose, fructose, and glucose concentrations. It has been demonstrated that AM fungi reduced the transcription levels of SWEET genes in potato plants [37]. The leaf sucrose levels in *S. indica*-colonized plants remained unchanged, while *SWEET11b* expression was increased, indicating that SWEET genes have tissue-specific expression patterns [38]. Increased expression of SWEET genes may promote efficient distribution and accumulation of sugars among leaf cells, whereas decreased

expression of SWEET genes may be the result of the regulation of sugar accumulation [35]. The expression of *CoSWEET1a*, *CoSWEET1b*, *CoSWEET7*, *CoSWEET9a*, *CoSWEET10*, and *CoSWEET17a* in roots was up-regulated by *S. indica* inoculation, along with a decrease in root sucrose, suggesting that sugars are rapidly translocated to other sites or supplied to the endophytic fungus for its requirement [39]. In citrus fruits, *S. indica* colonization distinctly increased the expression of *CsSWEET* in sarcocarps, followed by no change in fruit glucose and fructose and a significant increase in fruit sucrose [40]. Thus, the symbiotic relationship between plants and root-associated endophytic fungi is effective for sugar allocation [33]. *S. indica* helps host plants to optimize their sugar resources at different growth stages or in different environmental conditions, thereby enhancing survival and adaptation.

Plant development and growth can be affected by beneficial endophytic fungi [41]. In plant–endophytic fungi interaction, endophytic fungi can benefit from root exudation, and, in turn, they benefit the host plant in a variety of ways [33], including increased uptake of mineral elements (e.g., P and K) [42]. P is an essential element for energy metabolism in plant P and is mainly found in ATP and nucleic acids [16]. The increase in P concentrations in the roots but not the leaves suggests that *S. indica* was involved in the acquisition of root P rather than the subsequent transfer of P from the roots to leaves. This is consistent with the findings obtained by Yang et al. [43] in trifoliate orange inoculated with *S. indica*. However, the results of P transporter gene expression showed that inoculation with *S. indica* suppressed root *CoPht1;1*, *CoPht1;2*, *CoPht1;3*, and *CoPht1;4* expression and increased leaf *CoPht1;1*, *CoPht1;2*, and *CoPht1;3* expression, which is in line with the results obtained by Cao et al. [16] when inoculating AM fungi on *C. oleifera* and Bandyopadhyay et al. [44] inoculating *S. indica* on *Oryza sativa*. The Pht1 subfamily belongs to the high-affinity P transporter proteins located in the cell membrane, which are induced by P deficiency stress and are involved in the transport of phosphate from the rhizosphere soil to the root inside [44,45] and the decrease in the expression of *CoPht1* under *S. indica* inoculation conditions may be a result of the high soil Olsen-P levels in the present experimental site. Correlation analysis also revealed that root P was significantly and negatively correlated with root *CoPht1;1*, *CoPht1;3*, and *CoPht1;4* expression and that leaf P was only significantly positively correlated with leaf *CoPht1;3* expression. This suggests that P uptake in *C. oleifera* is a complex process that is not entirely dependent on *S. indica* colonization in the field.

The present study also showed a significant increase in the Ca concentration in the leaves of *C. oleifera* but not in the roots after inoculation with *S. indica*. This is in agreement with the results of Solanki et al. [46] in *Oryza sativa*. Ca plays an important role in cellular structural stability and signaling [47]. Correlation analyses showed a significantly positive correlation for Ca with *CoCAX1;1* and *CoCAX1;2* in the leaves but not the roots. The CAX ($\text{Ca}^{2+}/\text{H}^{+}$ cation exchanger) protein localizes to the vesicles or vesicular membranes that are responsible for the regulation of intracellular Ca^{2+} concentration, as well as being involved in the response to various abiotic stresses [48–50]. Such results imply that *S. indica* is involved in leaf Ca^{2+} transport to increase cytoarchitectural stability and potentially the stress response [46].

K is one of the macrolelements for plant growth and is involved in the osmoregulation and activation of related enzymes [46,51]. The present study showed that leaf and root K concentrations and *CoHKT1;1* expression in the leaves and roots were significantly elevated after *S. indica* colonization, which is in agreement with the results described by Solanki et al. [46] regarding *Oryza sativa*. Correlation analysis also confirmed that K was significantly and positively correlated with *CoHKT1;1* expression both in the leaves and roots. HKT is known to play a key role in maintaining plant survival under saline conditions and in increasing the $\text{K}^{+}/\text{Na}^{+}$ ratio [52]. This suggests that *S. indica* increases the K uptake in plants by up-regulating *HKT1;1* expression, which is critical for enhancing photosynthesis [53]. Moreover, *S. indica* also increased the soil available K levels and the root fungal colonization rate was positively correlated with the soil available K concentration.

This is consistent with the findings of Zhou et al. [54] in field tangor citrus colonized by AM fungi. Endophytic fungi facilitate the release or transformation of available K in the soil [24], but further studies are needed to determine how *S. indica* mineralizes the soil K [55].

The results of this study also showed that *S. indica* increased the soil NH_4^+ -N levels and Olsen-P, SOC, and EG concentrations. Among them, EG, a newly produced component of glomalin-related soil protein, has the function of cementing soil particles and stores organic carbon and mineral elements [13]. In this study, the root fungal colonization rate was significantly and positively correlated with the EG concentration. The increase in EG content in the soil may have the capacity of improved soil structure [54], which is in line with the results of Cheng et al. [55], who inoculated *S. indica* on navel orange. However, whether and how *S. indica* enhances the soil structure by increasing EG needs to be further investigated.

5. Conclusions

In this study, *S. indica* inoculation significantly improved plant height, photosynthetic efficiency, the chlorophyll index, and mineral element absorption through up-regulating the expression of *CoHKT1;1* and *CoCAX1;2* in the leaves and roots and *CoPht1;1*, *CoPht1;2*, and *CoPht1;3* in the leaves. In addition, the soil available nutrient concentrations were also increased following *S. indica* inoculation. *S. indica* inoculation also increased leaf sugar concentrations by up-regulating *CoSWEET2a*, *CoSWEET7*, *CoSWEET9b*, *CoSWEET17a*, and *CoSWEET17b*. Therefore, *S. indica* provides a new strategy for the sustainable production of *C. oleifera* in the field. Further experiments are needed to understand whether the effects of inoculating *S. indica* are dependent on different environments, endophytic fungi, and varieties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae10090936/s1>, Table S1: The 95% confidence interval for the mean of analyzed variables in this study.

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