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Differences in eco-physiological responses to the removal of adventitious roots between *Syzygium nervosum* A. Cunn. ex DC. and *Syzygium cumini* (L.) Skeels saplings under waterlogging

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Abstract

Key message The production of adventitious roots partially counteracts the negative effects of waterlogging on the growth of *Syzygium nervosum* A. Cunn. ex DC. and *Syzygium cumini* (L.) Skeels. *S. cumini* was more responsive and suffered from larger negative effects than *S. nervosum* after the removal of adventitious roots.

Context Adventitious roots contain gas channels and functionally replace or compensate for the loss of primary roots that usually decay during waterlogging. However, the importance of adventitious roots on growth in waterlogged woody plants varies with species. Therefore, there has been some controversy about whether adventitious roots have beneficial effects on the growth of waterlogged plants.

Aims We assessed whether *S. nervosum* and *S. cumini* differentially responded to the ablation of adventitious roots during waterlogging and whether compensatory responses occurred in the primary roots in both species.

Methods *S. nervosum* and *S. cumini* saplings were subjected to waterlogging and adventitious root removal for 120 days, and morphological, physiological, biochemical parameters, and biomass were recorded.

Results All plants survived waterlogging, and produced adventitious roots at the shoot base. Waterlogging had negative effects on the growth of both species, but the effect was more severe in *S. cumini* than in *S. nervosum* as seen from the values of comprehensive evaluation and total biomass. However, *S. nervosum* compensated for the ablation of adventitious roots with increased primary root dry mass, primary root activity, total root length, root tip number, and peroxidase activity.

Conclusions *S. nervosum* with a high proportion of adventitious roots would be at an advantage during waterlogging. The removal of adventitious roots was detrimental to the growth of both species, but *S. nervosum* exhibited less damage than *S. cumini* due to its compensatory physiological responses and its primary roots.

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1 Introduction

According to the Intergovernmental Panel on Climate Change, the global average temperature is predicted to increase by 1.4 °C to 5.8 °C over the period from 1990 to 2100 (IPCC 2013), which will lead to increased intensity and frequency of extreme precipitation events at the global scale (Tabari et al. 2019). Therefore, understanding how plant growth responds to a future wet-ter water environment is crucial, particularly in tropical and subtropical regions. Waterlogging negatively affects the diffusion of O_2 into the soil and reduces the O_2 content in waterlogged soils (Drew 1983), thus inhibiting root respiration and resulting in decreased levels of plant growth and photosynthesis rates, and even death (Kozlowski 1986, 1997).

Plants have evolved morphological, physiological, and biochemical responses to cope with waterlogging-induced hypoxia (Insausti et al. 2001; Vashisht et al. 2011; Zhang et al. 2015). Changes in the biomass allocation can be an important mechanism to improve plant performance under environmental stress (Bazzaz et al. 1987). However, controversial results on the effects of waterlogging on biomass allocation are still observed. For example, some studies have demonstrated that waterlogging led to either higher or lower allocation to roots in plants (Lenssen et al. 2003; Ye et al. 2003), while Wu et al. (2018) reported that waterlogging had no effect on the root/shoot ratio in *Triticum aestivum*. Waterlogging causes a remarkable increase in the storage of non-structural carbohydrates in many species (Limpinuntana and Greenway 1979; Daugherty and Musgrave 1994). This storage serves as a carbon buffer, allowing plant survival in hypoxic conditions when aerobic ATP production cannot meet the demands of a plant (Voesenek and Bailey-Serres 2015). Meanwhile, waterlogging can also lead to rapid accumulation or degradation of plant hormones and regulates the response of plants to waterlogging via complex signaling (Pan et al. 2021). In addition, plants possess a suite of antioxidant enzyme systems and produce some osmoregulatory substances, like free proline, to alleviate membrane lipid peroxidation caused by the accumulation of reactive oxygen species (ROS) under waterlogging (Yang et al. 2011). On the other hand, plant responses to waterlogging frequently involve the production of adventitious roots, which could functionally replace or compensate for the loss of primary roots that usually decay during waterlogging. In turn this can improve the hydraulic conductivity, leaf water

potential, and photosynthetic capacity of waterlogged crops and herbaceous plants (Muhammad 2012).

The removal of adventitious roots had negative effects on photosynthesis, leaf initiation and expansion, growth rates, and dry weight in some crops and herbaceous plants like *Alternanthera philoxeroides* (Mart.) Griseb., *Cotula coronopifolia* L., *Meionectes brownii* Hook. f. (syn. *Haloragis brownii*), *Platanus occidentalis* L., and *Solanum dulcamara* L. (Tsukahara and Kozlowski 1986; Rich et al. 2012; Ayi et al. 2016; Zhang et al. 2017). However, in contrast to these results, relatively few documents reported that the removal of adventitious roots did not adversely affect *Epilobium hirsutum* L. and *Alnus glutinosa* (L.) Gaertn. and even increased the biomass and total shoot height of *Lythrum salicaria* L. (Gill 1975; Stevens and Peterson 2007). Therefore, there has been some controversy about whether adventitious roots have beneficial effects on the growth of waterlogged plants or whether they are merely non-functional expressions of waterlogging injury. Since the waterlogging tolerance and the occurrence of injuries is strongly species-dependent, and knowledge on physiological and molecular aspects of waterlogging tolerance in woody plants is far behind that of herbaceous species (Kreuzwieser et al. 2009; Kreuzwieser and Rennenberg 2014). Thus, quantifying the importance of adventitious roots on the growth of waterlogged woody plants is essential.

S. nervosum (also named as *Cleistocalyx operculatus*) and *S. cumini* are two waterlog-tolerant trees of South China, which play a very important role in the restoration of riparian zones (Jing et al. 2001; Ma et al. 2019; Yang et al. 2022). *S. nervosum* and *S. cumini* actually produced adventitious roots during waterlogging, and *S. nervosum* has higher adventitious root production than *S. cumini* (Li et al. 2022a). We hypothesized that larger negative effects of adventitious root removal happen in the *S. nervosum* saplings with more adventitious roots than in *S. cumini* saplings. We further explored whether compensatory responses in the primary roots would occur in both species. To test our hypothesis, we quantified the changes in morphologic traits, biomass accumulation, and related physiological and biochemical parameters of *S. nervosum* and *S. cumini* saplings caused by the removal of adventitious roots.

2 Materials

2.1 Plant material and experimental design

Two-year-old saplings of *S. nervosum* and *S. cumini* were obtained as previously described (Li et al. 2022a).

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To ensure uniform growth after retillering, each sapling was cut at 5 cm above the soil surface. All saplings without attached soil balls were transplanted into 5-l plastic pots containing sand and red soil (2:1, v/v) and were grown (one sapling planted in each pot) in a greenhouse at Hainan University (20°3′ N, 110°19′ E), which only blocked ambient rainfall but otherwise maintained ambient light and temperature. All saplings were watered regularly and allowed to maintain the soil water content close to field capacity. The sandy soil contained 5.34 mg kg^{-1} ammoniacal nitrogen, 11.78 mg kg^{-1} available phosphorus, and 81.72 mg kg^{-1} available potassium. The annual mean rainfall, temperature, and insulation time in this area are 1715.3 mm, 24.4 °C, and 2000 h, respectively (Li et al. 2022a). After 3 months, saplings with similar growing status were selected for the experiments.

A 2 × 3 factorial design included two species (i.e., *S. nervosum* and *S. cumini*) and three treatments (i.e., control; waterlogging; adventitious root removal during waterlogging). Controls were watered regularly with tap water to maintain the soil water content close to field capacity. For the waterlogging treatment, the saplings were partially submerged in a big plastic bucket filled with tap water (flooded 15 cm above soil surface). For the adventitious root removal treatment, adventitious roots were removed using a razor blade under the water surface as soon as the primordial of the adventitious roots was visible (∼5 mm in length). The solution was entirely replaced every 14 days. Twenty-five saplings were used per treatment (five replicates, and five saplings per replicate). The treatment lasted for 120 days (from June 2019 to October 2019).

2.2 Analyses of plant morphology and biomass

The shoot length of each sapling was measured at the end of the growing season. Then, all saplings were sampled and divided into leaves, shoots, primary roots, and adventitious roots after washing out the soil (Li et al. 2023). The total leaf area per sapling was measured using a leaf area meter (LI-3000C, Li-Cor, Inc., Lincoln, NE, USA). The primary roots of each sapling were scanned with a double-lamp bed scanner (Epson 12000XL, Los Alamitos, CA, USA) at 400 dpi. The total root length and number of root tips number were analyzed with Win-RHIZO (Regent Instruments, Inc., Neplean, ON, Canada). Finally, all tissues were oven-dried at 70 °C to a constant mass, and weighed. Whole-plant relative growth rate (*RGR*) was calculated as $RGR = (b_t − b_0)/t$, where b_0 represents the initial, b_t is the final total biomass of each sapling, and t is the treatment time in days. Leafarea ratio was calculated as the ratio of total leaf area to total plant dry weight. The belowground/aboveground ratio was calculated as the ratio between the total root dry mass (the sum of primary roots and adventitious root dry mass) and the aboveground dry mass (the sum of leaf and stem dry mass).

2.3 Determination of net photosynthetic rate and chlorophyll content

The fourth fully expanded and exposed leaves were selected from the apex of each sapling to determine the net photosynthetic rate and stomatal conductance by using a portable photosynthesis system (TP-3051D, Zhejiang, China): temperature, 30 °C; light intensity, 1400 µmol photons·m^{-2}·s^{-1}; relative humidity, 60%; and ambient CO_2, 350 ± 5 µmol mol^{-1}. The net photosynthetic rate and stomatal conductance were measured from 8:30 to 11:30 a.m. on 29 and 30 September 2019. The content of chlorophyll pigments was measured in the same leaves. Leaf samples (∼0.2 g of fresh sample) were extracted in 10 mL of 80% chilled acetone. After centrifugation at 3000 rpm and 4 °C for 3 min, the supernatant was used for the determination of chlorophyll content. The absorbance of supernatant was recorded at 663 nm, 645 nm, and 470 nm, respectively. Chlorophyll *a*, chlorophyll *b*, and carotenoid concentration were calculated as described by Lichtenthaler (1987). The sum of chlorophyll *a* and chlorophyll *b* was defined as the total chlorophyll.

2.4 Determination of peroxidase, free proline, and malondialdehyde (MDA)

The peroxidase activity was measured spectrophotometrically at 470 nm as previously described (Li et al. 2022a), using guaiacol as substrate. One unit of peroxidase activity was defined as the amount of enzyme that caused an increase of 0.01 in the absorbance per minute under standard conditions. The content of free proline (µg·g^{-1} FW) was measured using the acid ninhydrin method given by Bates et al. (1973). The absorbance was recorded at 520 nm. Malondialdehyde (µmol·g^{-1} FW) content was determined using the 2-thiobarbituric acid method as described by Li et al. (2022a).

2.5 Determination of leaf midday water potential, relative water content, and primary root activity

On 29 and 30 September 2019, the 12:00 to 14:00 leaf water potential of each sapling was recorded using a WP4C Dewpoint hygrometer (Decagon Devices, Inc., Pullman, WA, USA) according to the method of Liao et al. (2019). After the measurement of leaf water potential, the leaf samples were collected to record the relative water content. Samples were dried in a forced-air oven at 70 °C till a constant dry weight was obtained. The primary root activity was measured by the triphenyl tetrazolium chloride method according to Ruf and Brunner (2003). In detail, fresh primary root samples (∼0.2 g) from each sapling were selected, cut into

1-cm pieces, and transferred to 10-mL centrifuge tubes containing 2.5 mL of 1 % triphenyl tetrazolium chloride solution and 2.5 mL 0.1 M potassium phosphate buffer (pH 7.5). The samples were incubated for 1 h at 37 °C in the dark and then mixed with 1 mL of l M H_2SO_4. The root pieces were washed twice with 2 mL of distilled water, and their surfaces were dried carefully with absorbent papers. Then, 3 mL of ethyl acetate was added, and the samples were vortexed for 30 s. The samples were centrifuged at 12,000 rpm and 4 °C for 3 min. The precipitate was discarded, and ethyl acetate was added to adjust the final volume to 5 mL. Absorbance was measured at 485 nm. The reactivity of the samples with triphenyl tetrazolium chloride was expressed as absorption of triphenyl tetrazolium formazan per milligram fresh weight ($mg \cdot g^{-1} \cdot h^{-1} \cdot FW$).

2.6 Determination of non-structural carbohydrate of leaves

The dried and fine-ground leaf sample ($\sim$0.05 g) from each sapling were transferred to 10-mL centrifuge tubes, incubated in 80% ethanol at 80 °C for 30 min and centrifuged at 12,000 rpm and 4 °C for 5 min. The ethanolic extracts were used to determine the total soluble sugars by using the anthrone colorimetric method at 620 nm by spectrophotometry (Wang et al. 2020). The residues from the extraction of the total soluble sugars were transferred to a 25-mL test tube and incubated in 20 mL distilled water at 100 °C for 15 min. After incubation, 2 mL 9.2 mol L^{-1} ice-cooled perchloric acid was added. Then, the samples were incubated for 15 min at room temperature, centrifuged at 4000 rpm and 4 °C for 10 min. The residues were dissolved in distilled water (10 mL) and incubated for 15 min at 100 °C. The solutions were extracted with 2 mL 4.6 mol L^{-1} perchloric acid for 15 min and then centrifuged.

The supernatant was collected, and the residues were washed three times with distilled water. The supernatant and the washing water were combined, and distilled water was added to adjust the final volume to 100 mL. The absorbance was measured at 620 nm. The sum of sugars and starch were presented as non-structural carbohydrate.

2.7 Determination of phytohormone contents

After the net photosynthetic rate measurements, the uppermost fully expanded fresh leaf samples ($\sim$0.5 g) from each sapling were carefully collected from 8:30 a.m. to 10:30 a.m. The samples were immediately frozen in
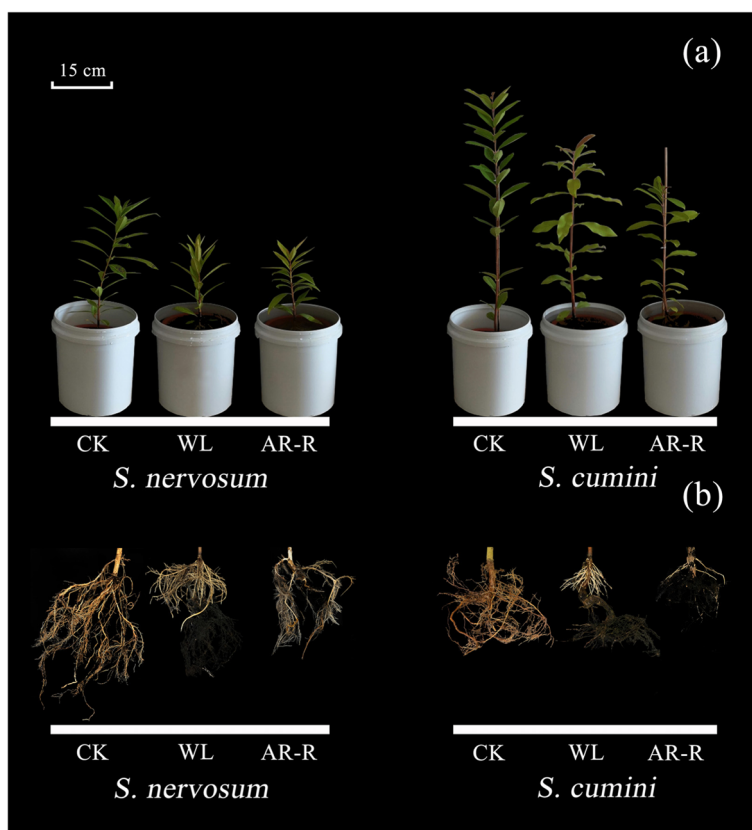

Fig. 1 Aboveground (**a**) and belowground (**b**) growth response of *S. nervosum* and *S. cumini* saplings to waterlogging with or without adventitious root removal. Treatment: CK, well-watered; WL, waterlogging; AR-R, adventitious root removal

liquid nitrogen and extracted in cold 80% (v/v) methanol with butylated hydroxytoluene (1 mmol/L) overnight at 4°C, centrifuged at 10,000 rpm and 4 °C for 20 min. The supernatant was passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA) and dried in N_2. The levels of auxin (IAA), abscisic acid (ABA), gibberellic acid (GA_3) and zeatin riboside (ZR) were determined by enzyme-linked immune sorbent assay (ELISA) according to the method of Yang et al. (2001). All measurements were performed in the Key Laboratory of Molecular Plant Pathology, Ministry of Agriculture, Beijing, China.

2.8 Statistical analysis
All statistical analyses were performed using the SPSS 13.0 (IBM Inc., Chicago, IL, USA). Data for each of the measured traits were tested for normality and homoscedasticity before analysis. Data without normal distribution were transformed logarithmically. Duncan's multiple range test was used to analyze the differences among treatments. An independent-sample *t* test was conducted to determine differences between the two species. Two-factor analysis of variance (ANOVA) with LSD post-hoc tests were used to further determine the effects of waterlogging and adventitious root removal. Differences were considered significant at the $p < 0.05$ level. Additionally, we used a comprehensive evaluation method to evaluate the growth status. Given the large dataset, the principal component analysis was carried out on all the variables (except adventitious root mass) of both species for dimension reduction, and we obtained two eigenvalues (the first two components comprised about 85% of the total variance). Membership function value of each selected eigenvalue was calculated (Yan et al. 2022), and final comprehensive evaluation value was obtained using the membership function value (Xiang et al. 2021). The maximum value was considered as the optimal growth status.

3 Results
3.1 Growth traits
All saplings survived after 120 days of waterlogging treatment, but their growth was considerably inhibited, especially in *S. cumini* (Fig. 1). Adventitious root removal induced the formation of floating roots from the primary roots in *S. nervosum*, but not in *S. cumini* (Fig. 2). In addition, the waterlogging resulted in a decrease of shoot length, total root length, root tip number, whole-plant relative growth rate, and belowground/aboveground biomass (not in *S. nervosum*) of both
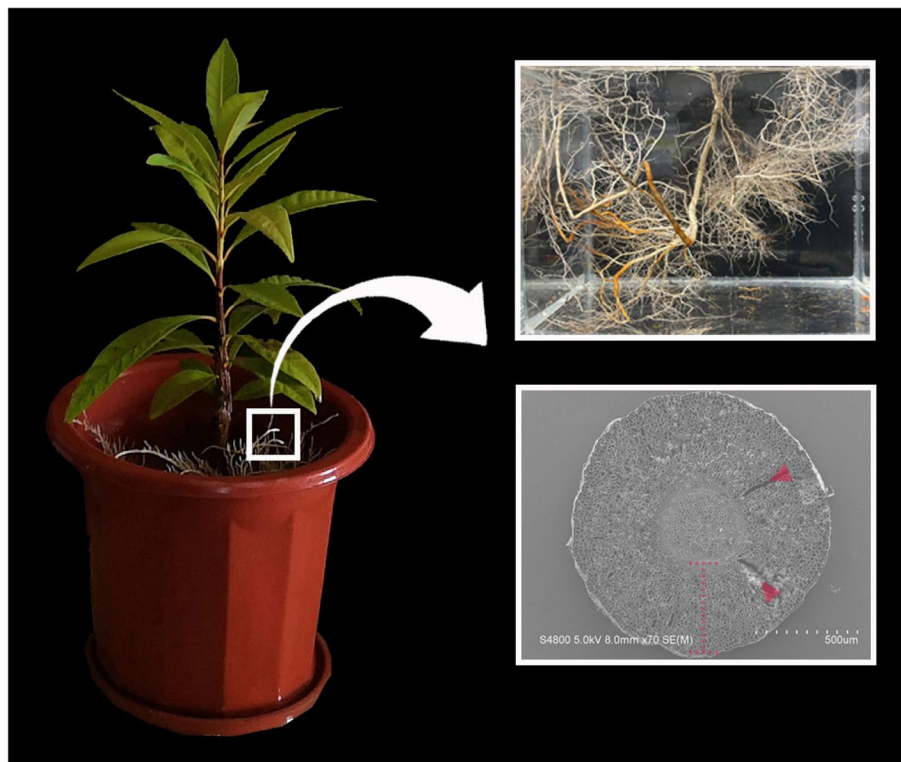

Fig. 2 Compensatory growth in *S. nervosum* after removing the adventitious root. Note: *S. nervosum* responds to adventitious root removal by growing a large number of floating roots with aerenchyma from the primary roots. Cortex cell layers and lysigenous aerenchyma are indicated by red dashed lines and red arrowheads, respectively
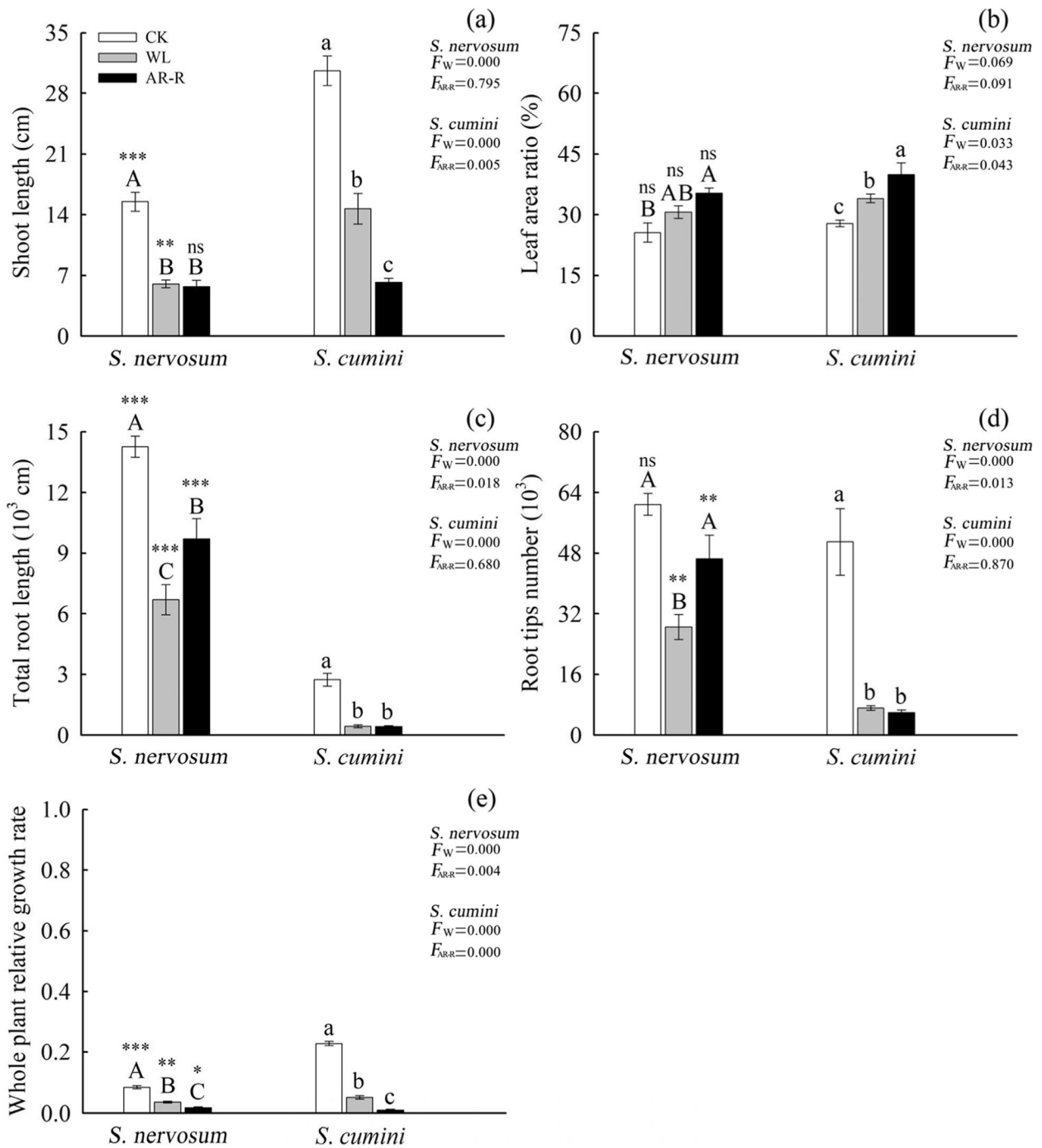
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Fig. 3 Shoot length (**a**), leaf area ratio (**b**), total root length (**c**), root tip number (**d**), and whole-plant relative growth rate (**e**) in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious root removal. Treatment: CK, well-watered; WL, waterlogging; AR-R, adventitious root removal. F_W, waterlogging effect; F_{AR-R}, adventitious root removal effect. Data presented are means $\pm$ SE ($n = 5$). Bars with the different letter within the same species group indicate significant differences at $p<0.05$ by analysis of variance (ANOVA). Asterisks above bars denote statistically significant differences between the species according to independent-samples t test. ns, $p>0.05$; $*p<0.05$; $**p<0.01$; $***p\leq0.001$

species (Fig. 3 and Table 1), and significantly increased the leaf area ratio in *S. cumini* (Fig. 3b). Adventitious root removal also resulted in similar trends as it did in

waterlogging. In addition, compared with the waterlogging treatment, adventitious root removal treatment resulted in decreased belowground/aboveground

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Table 1 Biomass accumulation and allocation in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious root removal

Species	Treatment	ARM (g · plant⁻¹ DM)	PRM (g · plant⁻¹ DM)	SM (g · plant⁻¹ DM)	LM (g · plant⁻¹ DM)	TM (g · plant⁻¹ DM)	BA
S. nervosum	CK	n.a.	5.62±0.17 Ans	7.98±0.54 A***	3.11±0.05 A***	16.7±0.57 A***	0.51±0.03 A***
	WL	1.93±0.17***	1.56±0.08 C***	4.66±0.28 B***	2.81±0.2 AB***	10.96±0.31 B***	0.47±0.02 A***
	AR-R	n.a.	1.98±0.11 B***	4.21±0.37 B***	2.63±0.09 Bns	8.83±0.32 C**	0.29±0.02 B***
	F_W	n.a.	0.000***	0.000***	0.134 ns	0.000***	0.186 ns
	$F_{AR\text{-}R}$	n.a.	0.039*	0.457ns	0.349 ns	0.003**	0.000***
S. cumini	CK	n.a.	4.94±0.35 a	21.76±0.57 a	11.09±0.43 a	37.79±0.84 a	0.15±0.01 a
	WL	0.70±0.13	0.39±0.04 b	10.86±0.7 b	4.67±0.32 b	16.63±0.65 b	0.07±0.01 b
	AR-R	n.a.	0.34±0.04 b	7.44±0.42 c	3.15±0.21 c	10.94±0.38 c	0.03±0.00 c
	F_W	n.a.	0.000***	0.000***	0.000***	0.000***	0.000***
	$F_{AR\text{-}R}$	n.a.	0.862 ns	0.001***	0.007**	0.000***	0.011*

Treatment: *CK* well-watered, *WL* waterlogging, *AR-R* adventitious root removal

Abbreviations: ARM adventitious root mass, *PRM* primary root mass, *SM* stem mass, *LM* leaf mass, *TM* total mass, *BA* the belowground/aboveground ratio. F_W, waterlogging effect; $F_{AR\text{-}R}$, adventitious root removal effect. Data presented are means ± SE (*n* = 5). Different letters indicate significant differences at *p*<0.05 by analysis of variance (ANOVA). Asterisks following capital letters denote statistically significant differences between the species according to independent-samples *t* test. ns, *p*>0.05; *∗p*<0.05; *∗∗p*<0.01; *∗∗∗p*≤0.001; n.a., no adventitious roots

biomass and whole-plant relative growth rate in both species, but increased the total root length and root tip number in the primary roots of *S. nervosum* by 36.7% and 48.1%, respectively. By contrast, adventitious root removal had no effect in *S. cumini* (Fig. 3c and d). Similarly, adventitious root removal treatment significantly decreased shoot length (decreased by 81.3%) and significantly increased leaf area ratio (increased by 15.8%) in *S. cumini*, whereas its effects on *S. nervosum* were non-significant (Fig. 3a and b).

3.2 Biomass allocation

As shown in Table 1, waterlogging and adventitious root removal treatments significantly decreased the dry mass of primary roots, stem, leaf, total plant, and the relative growth rate in both species. A larger decrease of these parameters was found in *S. cumini* than in *S. nervosum*. *S. nervosum* saplings had a significantly higher dry mass of adventitious roots than *S. cumini*. Compared with waterlogging, adventitious root removal resulted in decreased dry mass of stem and leaf in *S. cumini* and total plant dry mass, and relative growth rate in both species but significantly increased the dry mass of primary roots in *S. nervosum*.

3.3 Gas exchange, peroxidase (POD) activities, chlorophyll, MDA, and free-proline contents

As shown in Table 2, compared to controls, waterlogging significantly decreased the chlorophyll *a* and carotenoid contents in *S. cumini* and the net photosynthesis rate, stomatal conductance, and chlorophyll *b* contents in both species. However, the carotenoid/total chlorophyll and POD activities in *S. nervosum* and the MDA contents in both species were increased. Under adventitious root removal, both species presented the smallest net photosynthesis rate and stomatal conductance but the highest POD, MDA, and free proline. In addition, compared with waterlogging, adventitious root removal resulted in an increase in the POD in *S. nervosum* and MDA in *S. cumini*. By contrast, net photosynthesis rate in *S. cumini* and stomatal conductance in *S. nervosum* were significantly decreased.

3.4 Midday leaf water potential, relative water content, and primary root activity

The highest values of midday leaf water potential, relative water content, and primary root activity were detected in controls (Fig. 4). Waterlogging and adventitious root removal decreased the values of midday leaf water potential and relative water content and the primary root activity in both species with respect to controls. Adventitious root removal also resulted in decreased midday leaf water potential and relative water content in *S. cumini* but significantly increased primary root activity in *S. nervosum* compared to waterlogging.

3.5 Non-structural carbohydrates and phytohormone concentrations

Waterlogging significantly increased total soluble sugar, starch, and non-structural carbohydrate in both species

Table 2 Net photosynthesis rate (*Pn*), stomatal conductance (*g*$_s$), pigments, peroxidase (POD), malondialdehyde (MDA), and free proline (Pro) of leaves in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious roots removal

Species	Treatment	Pn (μmol·m⁻²·s⁻¹)	*g*$_s$ (mol·m⁻²·s⁻¹)	Chl *a* (μg·g⁻¹ FW)	Chl *b* (μg·g⁻¹ FW)	Caro (μg·g⁻¹ FW)	Caro/Chl	POD (U·min⁻¹·g⁻¹ FW)	MDA (μmol·g⁻¹ FW)	Pro (μg·g⁻¹ FW)
S. nervosum	CK	8.25±0.99 A**	0.17±0.03 Ans	136.83±11.69 A**	67.65±2.78 Ans	250.44±18.20 A***	1.25±0.13 B**	32.55±3.00 C**	29.01±0.74 B***	14.19±1.15 b
	WL	4.18±0.34 Bns	0.08±0.00 Bns	112.52±3.92 ABns	52.34±1.15 Bns	303.26±18.34 A***	1.84±0.11 A***	53.02±0.74 Bns	38.42±1.31 A***	16.81±0.94 ab
	AR-R	2.91±0.47 Bns	0.03±0.00 C*	108.14±8.56 Bns	53.48±1.59 Bns	282.35±19.77 A***	1.75±0.09 A***	66.36±6.36 Ans	40.68±0.95 A**	17.84±0.64 a
	F_W	0.001***	0.001***	0.071ns	0.000***	0.070ns	0.003**	0.004**	0.000***	0.070ns
	F_{AR-R}	0.442ns	0.035*	0.727ns	0.690ns	0.446ns	0.585ns	0.039*	0.145ns	0.448ns
S. cumini	CK	14.24±0.29 a	0.10±0.01 a	206.92±3.45 a	64.64±1.48 a	49.58±3.34 a	0.18±0.01 a	53.78±1.99 a	11.51±0.66 c	30.89±3.48 B**
	WL	5.14±0.36 b	0.06±0.01 b	126.96±7.00 b	52.22±0.78 b	36.82±3.00 b	0.20±0.01 a	63.45±5.77 a	22.59±1.03 b	44.78±6.50 AB**
	AR-R	3.66±0.51 c	0.05±0.01 b	137.31±11.44 b	53.25±1.61 b	40.44±1.81 b	0.22±0.02 a	68.65±5.68 a	30.34±2.76 a	48.39±4.89 A**
	F_W	0.000***	0.006**	0.000***	0.000***	0.007**	0.350ns	0.181ns	0.001***	0.079ns
	F_{AR-R}	0.022*	0.237ns	0.378ns	0.597ns	0.378ns	0.568ns	0.460ns	0.008**	0.627ns

Abbreviations: Chl a chlorophyll *a, Chl b* chlorophyll *b, Caro* carotenoid, *Caro/Chl* ratios of carotenoid to total chlorophyll. For abbreviations explanation of treatments and data description and statistics are the same as shown in Table 1
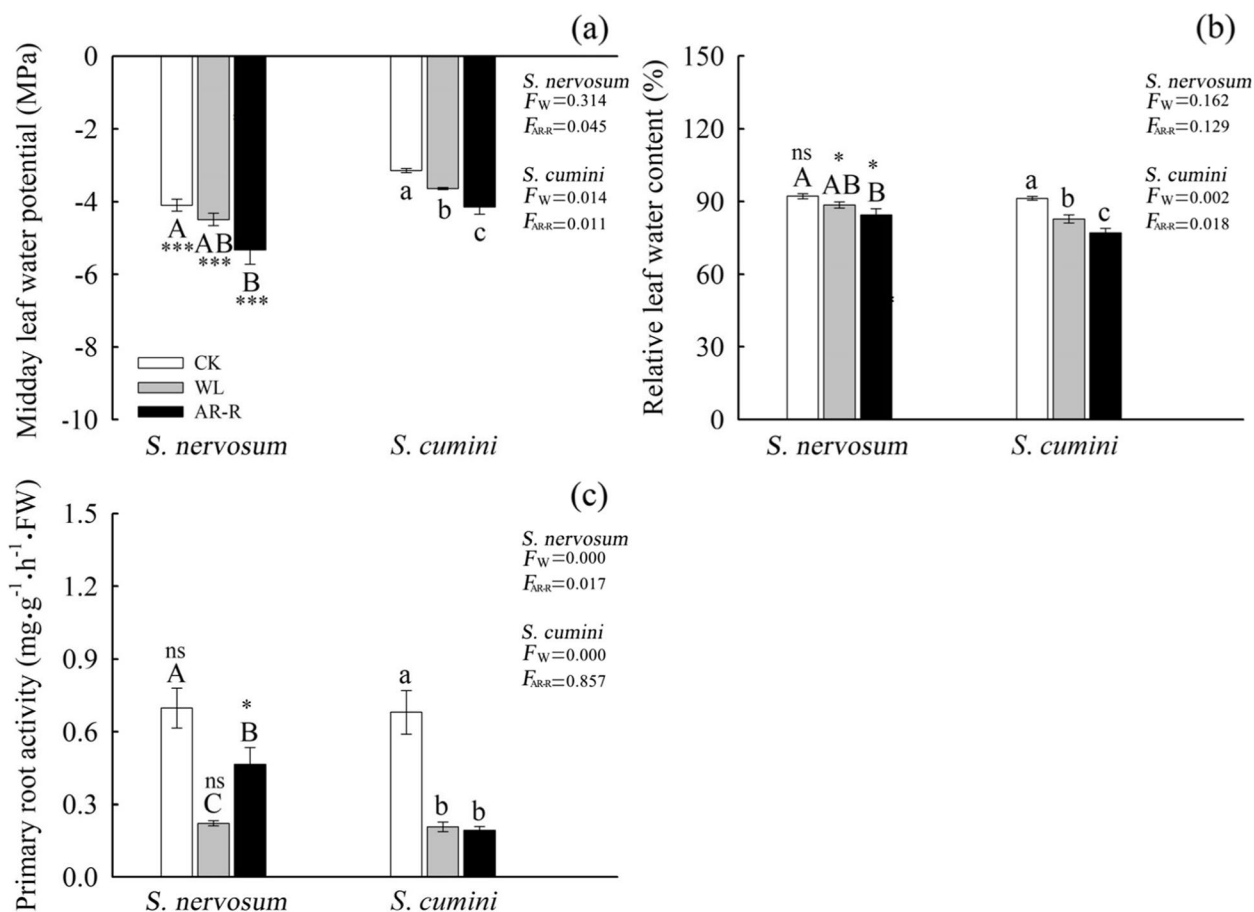
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Fig. 4 Midday leaf water potential (**a**), relative leaf water content (**b**), and primary root activity (**d**) in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious root removal. The abbreviations, explanation of treatments, and data description and statistics are the same as shown in Fig.3

and GA$_3$, ZR, IAA, and ABA contents in *S. cumini* but significantly decreased the GA$_3$, IAA, and ABA contents in *S. nervosum* (Figs. 5 and 6). Moreover, compared to waterlogging, adventitious root removal resulted in an increase in the total soluble sugar (slightly increased in *S. nervosum*), starch, and non-structural carbohydrate in both species and the ZR, IAA, and ABA contents in *S. cumini* but significantly decreased the GA$_3$, IAA, and ABA in *S. nervosum*.

3.6 Comparative comprehensive evaluation among different treatments

As shown in Table 3, two groups of eigenvalues were obtained after dimensional reduction. The final comprehensive evaluation values of *S. nervosum* and *S. cumini* saplings were sequentially reduced in the controls, waterlogging, and adventitious root removal treatments. In other words, both species from the controls possessed the best growth status. In addition, *S. nervosum* always had higher comprehensive evaluation values than did *S. cumini* in all treatments.

4 Discussion

4.1 *S. nervosum* exhibits superior adaptation to waterlogging than *S. cumini*

Relative growth rate and comprehensive evaluation values are important indicators in identifying waterlogging tolerance in plants (Gibberd et al. 2001; Ye et al. 2003; Zhao et al. 2022). This study clearly demonstrated that *S. nervosum* was more tolerant to waterlogging than *S. cumini*. Javier (1987) stated that root aeration is critical in waterlogged soil, thus any plant species with a high proportion of adventitious roots would be at an advantage. Consequently, *S. nervosum* with a larger adventitious root system can maintain a higher rate of minerals uptake and water and O$_2$ transportation to meet the specific resource demands associated with waterlogging (Gill 1975; Rich et al. 2012). This observation is strengthened by the larger decline in chlorophyll *a* content observed in *S. cumini*. Meanwhile, the greater relative waterlogging tolerance of *S. nervosum* appears to also depend on the degree of
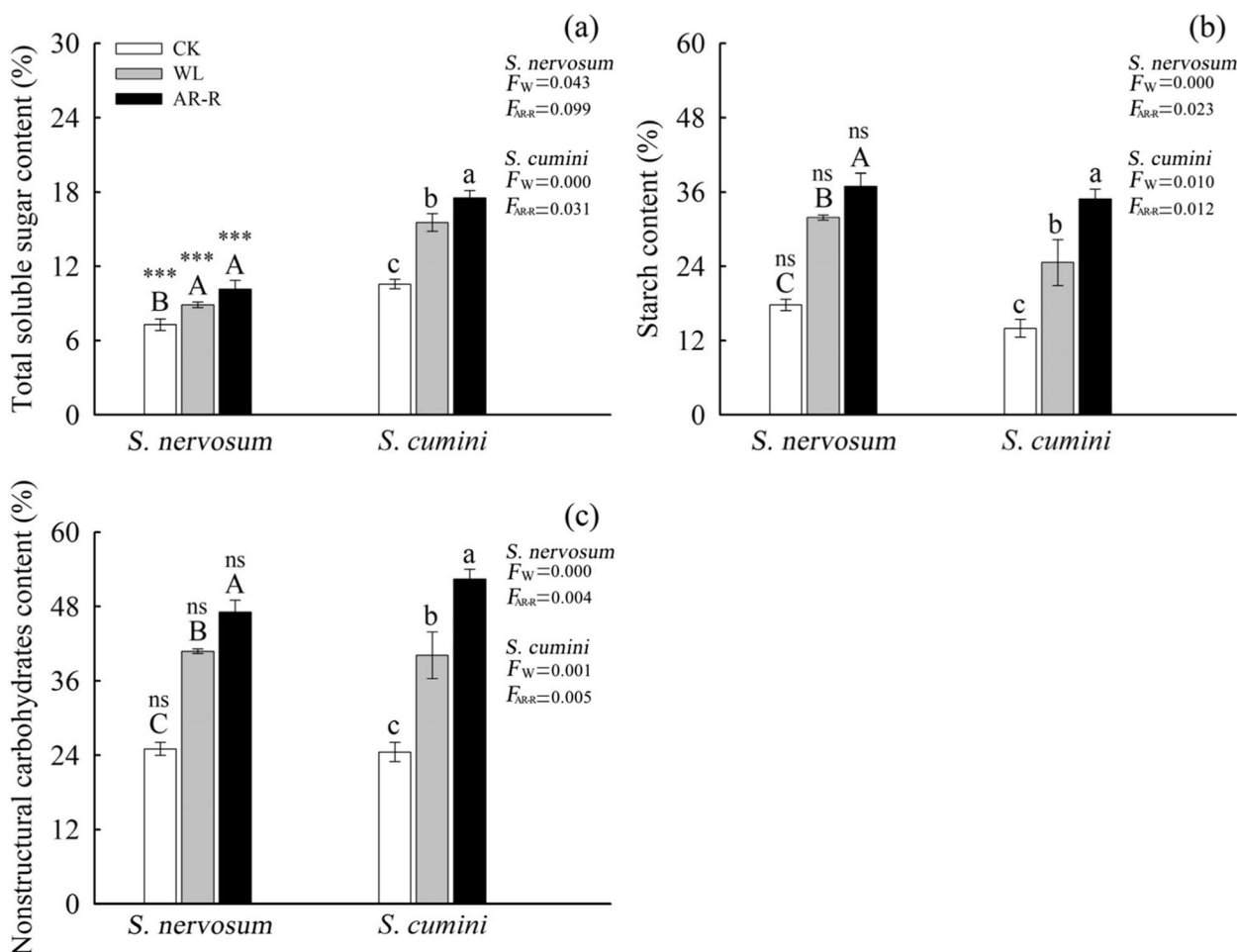
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Fig. 5 Total soluble sugar (**a**), starch (**b**), and non-structural carbohydrates (**c**) contents of leaves in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious root removal. The abbreviations, explanation of treatments, and data description and statistics are the same as shown in Fig.3

development of aerenchyma (Malik et al. 2011; Khan et al. 2014). After waterlogging treatment, *S. nervosum* showed less reduction in belowground/aboveground ratio than *S. cumini*, leading to larger total root length and root tip number in *S. nervosum*. According to the viewpoints of Das and Jat (1977), root length increased with root porosity. Thus, higher total root length represented a larger aerenchyma area, thereby increasing the rate of O_2 diffusion from the shoot to the root, which is essential for the maintenance of aerobic respiration (Yin et al. 2010). This is consistent with our previous work reporting that *Cleistocalyx operculatus* (basionym of *S. nervosum* (DC.) Kosterm.) could maintain a higher porosity of primary roots than *S. cumini* when exposed to waterlogging stress (Li et al. 2022a). Additionally, the fact that *S. nervosum* can better withstand

waterlogging stress might be closely related to its better self-protective ability. Under waterlogging conditions, the accumulation of reactive oxygen species (ROS) can induce lipid peroxidation, chlorophyll degradation, and loss of photosynthetic activity, while the higher peroxidase activity ßand carotenoids can efficiently eliminate the massive amount of H_2O_2 and protecting photosynthetic apparatus from ROS (Li et al. 2012; Liao et al. 2019). On the other hand, the phenomenon may also be attributed to the facts that *S. nervosum* suffered less decrease in midday leaf water potential and relative leaf water content and greater reduction in stomatal conductance. This strategy generally allows for adequate gas exchange while minimizing water loss (Gazal and Kubiske 2004). Therefore, *S. nervosum* exhibits superior adaptation to waterlogging than *S. cumini*.
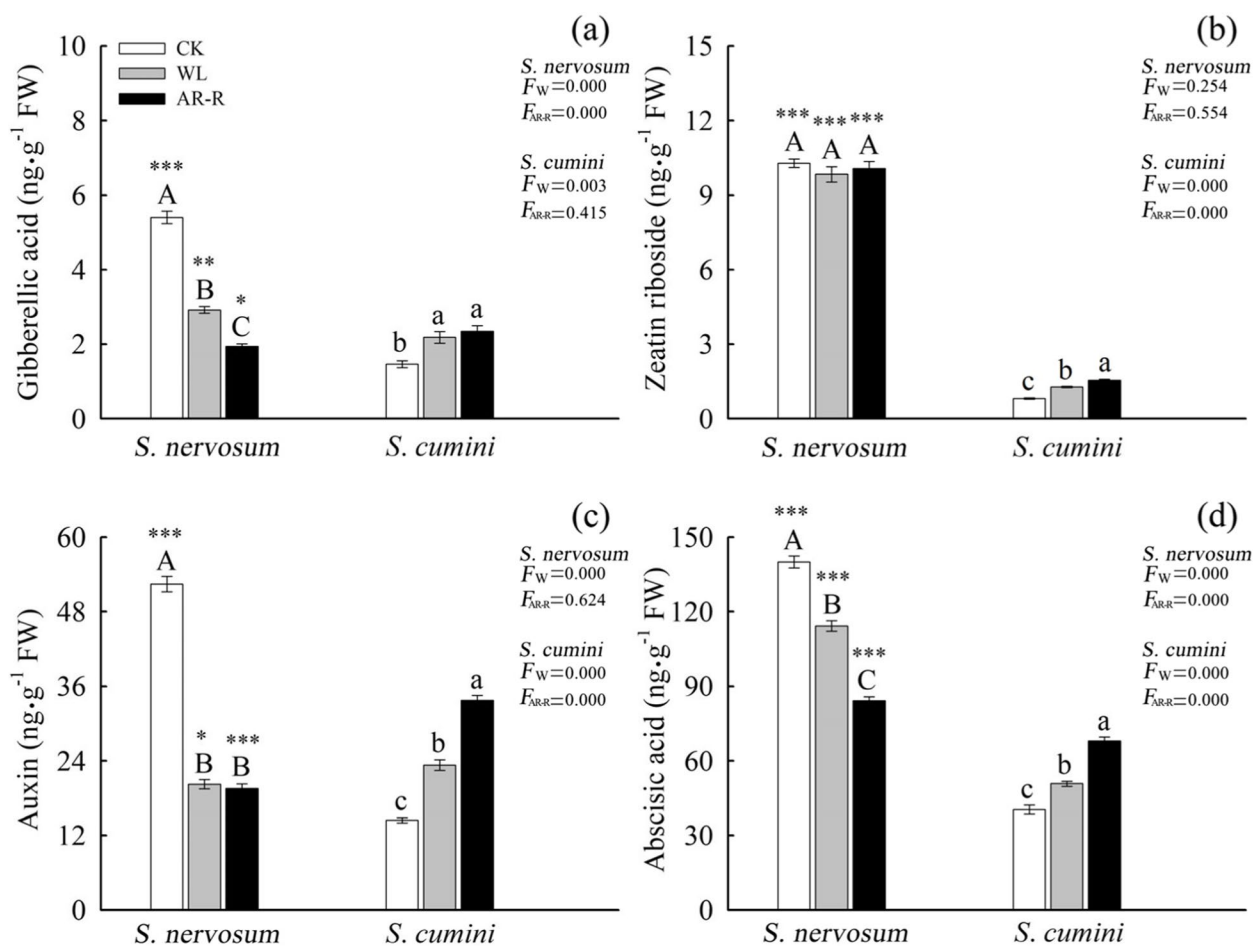
Fig. 6 Gibberellic acid (**a**), zeatin riboside (**b**), auxin (**c**), and abscisic acid (**d**) contents of leaves in *S. nervosum* and *S. cumini* saplings, as affected by waterlogging with or without adventitious root removal. The abbreviations, explanation of treatments, and data description and statistics are the same as shown in Fig. 3

Table 3 Eigenvalue, membership function value, and comprehensive evaluation value in *S. nervosum* and *S. cumini* saplings under different treatments

Species	Treatment	C (1)	C (2)	M (1)	M (2)	CE
S. nervosum	CK	0.667	1.450	0.584	1.000	0.750
	WL	-0.587	0.646	0.086	0.666	0.318
	AR-R	-0.805	0.474	0.000	0.595	0.238
S. cumini	CK	1.718	-0.672	1.000	0.120	0.648
	WL	-0.254	-0.937	0.218	0.010	0.135
	AR-R	-0.738	-0.961	0.027	0.000	0.016

C(μ), eigenvalue; M(μ), membership function value; *CE* comprehensive evaluation value. For abbreviations explanation of treatments are the same as shown in Table 1

4.2 *S. nervosum* had a compensatory response to adventitious root removal, contrary to *S. cumini*

Adventitious root removal was detrimental to the growth of both species. However, growth reduction was more severe in *S. cumini* than in *S. nervosum* as the waterlogging duration was extended. This suggests that *S.* *nervosum* has other mechanisms aside from production of adventitious roots which enable the species to survive under hypoxic conditions and tolerate waterlogging. The compensatory growth in primary roots of *S. nervosum* could be a reasonable explanation. This confirms the results obtained by Matsuura et al. (2016) that *Panicum*

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sumatrense L. exhibits waterlogging tolerance by enhancing root growth. Meanwhile, this is probably due to the ability of *S. nervosum* to develop a large number of floating roots with aerenchyma as a response to waterlogging of the soil, while *S. cumini* did not show significant development of floating roots. This notion is strengthened by the significant decline in soluble sugar (no change), GA_3, and ABA content observed in *S. nervosum*. Because the sugar, GA_3, and ABA derived from leaves were essential for aerenchyma or adventitious root formation under waterlogged soil conditions (Takahashi et al. 2018; Cisse et al. 2022). Moreover, Xie et al. (2009) reported that reduction of root activity in *Deyeuxia angustifolia* (Komarov) Y. L. Chang under waterlogging stress can be compensated for by high total root length. In turn we believe that loss of adventitious roots was compensated by increased primary root activity in *S. nervosum*. This speculation has been indirectly confirmed by Li et al. (2022b), who found that an increase in adventitious root activity in *C. operculatus* [= *S. nervosum*] and *S. cumini*, with a partially damaged adventitious root system, contributes to water and nutrient transport under combined waterlogging and nutrient supply conditions. Consequently, although adventitious root removal was detrimental to the growth of both species, *S. nervosum* showed less damage than *S. cumini* due to the compensatory response of physiology and primary roots.

5 Conclusions and future perspectives

We found that adventitious roots did not prevent injury to the growth of both species when primary roots were waterlogged, but species with a high proportion of adventitious roots would be at an advantage during waterlogging. In addition, when adventitious roots were removed, the growth of both species was impaired, but *S. nervosum* exhibited less damage than *S. cumini* due to the compensatory physiological response and presence of primary roots. Overall, this study provides valuable information for the construction of riparian protective forests. The differences in tolerance to waterlogging between the two species may determine where plants are distributed along a gradient of soil humidity. However, under field conditions, it is unusual for soil moisture conditions to remain constant. Soil may experience fluctuating water regimens ranging from waterlogging to drought. Thus, further work is needed urgently to investigate the ability of adventitious roots to survive fluctuating water tables.

Authors' contributions
F Y designed the study, provided funding and revised the manuscript; DD L performed the experiments, data collection, and drafted the manuscript; LF M performed data processing and statistics; MJ T, JJ Z and WZ Y performed part of the experiment. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
The authors declare that the study was not conducted on endangered, vulnerable or threatened species.

Consent for publication
The authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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