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Article

Beyond rooting: Enhancing post-root growth and vigor in ZZ plant (*Zamioculcas zamiifolia*) through an integrated propagation protocol

Além do enraizamento: aumento do crescimento e do vigor no período pós-enraizamento da planta ZZ (*Zamioculcas zamiifolia*) por meio de um protocolo integrado de propagação

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Abstract
This study aimed to optimize leaf-cutting propagation of ZZ plant (*Zamioculcas zamiifolia*), which typically suffers from slow post-rooting growth. In the first experiment, four leaf cutting types (blade+rachis, blade, basal, and apical segments) were tested on four substrates: cocopeat (CP), cocopeat+perlite (CP+PL), peat moss+perlite (PM+PL), and vermicompost+vermiculite (VC+VM). Rooting success was high (>88%) across all treatments, but post-rooting growth varied significantly. Blade and blade+rachis cuttings on CP and PM+PL showed superior rhizome development, leaf area, and biomass, likely due to higher initial carbohydrate and nitrogen reserves. Based on these results, a second experiment evaluated the effects of naphthalene acetic acid (NAA) at 0, 1000, and 2000 mg L^{-1} on the top-performing cuttings in CP and PM+PL. PM+PL proved optimal for root and rhizome growth due to its balanced water retention and aeration. The highest root volume (6.05 cm^3), leaf area (51.5 cm^2), leaf chlorophyll content (5.80 mg g^{-1}) and plant fresh weight (15.93 g) were obtained by NAA application (2000 mg L^{-1}) on blade+rachis cuttings in PM+PL substrate. These findings offer a practical protocol for efficient nursery production and large-scale propagation of ZZ plants.
Keywords: Leaf cutting, NAA, plantlet growth, rhizome, rooting medium.

Resumo
Este estudo teve como objetivo otimizar a propagação por estacas foliares da planta ZZ (*Zamioculcas zamiifolia*), que normalmente apresenta crescimento lento após o enraizamento. No primeiro experimento, quatro tipos de estacas foliares (lâmina+raquis, lâmina, basal e apical) foram testados em quatro substratos: fibra de coco (FC), fibra de coco+perlita (FC+PL), turfa+perlita (T+PL) e vermicomposto+vermiculita (VC+VM). O sucesso no enraizamento foi elevado (>88%) em todos os tratamentos, mas o crescimento pós-enraizamento variou significativamente. As estacas do tipo lâmina e lâmina+raquis nos substratos FC e T+PL apresentaram melhor desenvolvimento de rizoma, maior área foliar e biomassa, provavelmente devido às maiores reservas iniciais de carboidratos e nitrogênio. Com base nesses resultados, um segundo experimento avaliou os efeitos do ácido naftalenoacético (ANA) nas concentrações de 0, 1000 e 2000 mg L^{-1} nas estacas com melhor desempenho nos substratos FC e T+PL. O substrato T+PL mostrou-se ideal para o crescimento de raízes e rizomas, graças ao seu equilíbrio entre retenção de água e aeração. Os maiores valores de volume radicular (6,05 cm^3), área foliar (51,5 cm^2), teor de clorofila nas folhas (5,80 mg g^{-1}) e massa fresca da planta (15,93 g) foram obtidos com a aplicação de ANA (2000 mg L^{-1}) em estacas do tipo lâmina+raquis no substrato T+PL. Esses achados oferecem um protocolo prático para a produção eficiente em viveiros e propagação em larga escala da planta ZZ.
Palavras-chave: ANA, crescimento de plântulas, estaca foliar, rizoma, substrato de enraizamento.

Introduction

Zamioculcas zamiifolia (commonly referred to as ZZ plant) is a popular indoor ornamental species valued for its glossy, dark green foliage and tolerance to low-light conditions (Ashouri et al., 2025). It thrives under low light (Chen et al., 2003) and drought stress (Le Moullec et al., 2015), and has been shown to enhance indoor air quality (Ullah et al., 2020). While rhizome division is the conventional propagation method (Seneviratne et al., 2013), it requires mature donor plants and is often impractical for large-scale production (Kharrazi et al., 2023). Although in vitro techniques have been optimized, their adoption in standard nurseries is limited by the need for specialized equipment and high initial costs (Ashouri et al., 2025). Leaf cutting offers a cost-effective, scalable alternative that minimizes damage to the parent plant, though it typically results in slower post-propagation growth compared to rhizome division (Le Moullec et al., 2015).

Research indicates that the type and position of leaf cuttings significantly influence propagation success and subsequent plantlet vigor. Badizadegan et al. (2023) found that apical leaflet segments (from the tip of the rachis) produced 250% more rhizomes than basal segments, suggesting superior regenerative capacity at the distal end of the leaf. However, Thongkham and Phavaphutanon (2018) reported no significant differences in rooting or rhizome formation among basal, mid, and apical leaflet sections. Further studies confirm that the specific portion of the leaflet used, apical, mid, or basal, affects both rhizome number and growth (Cutter, 1962; Badizadegan et al., 2023). Full leaflets (entire leaflet blades) have been recommended as optimal for rapid propagation (Cutter, 1962; Seneviratne et al., 2013), although Thongkham and Phavaphutanon (2018) noted that full, apical, and basal segments exhibit similar rooting and rhizome-forming abilities, typically yielding only small rhizomes.

The choice of rooting medium and the use of rooting promoters are critical factors influencing the success and post-rooting growth of *Zamioculcas zamiifolia* leaf cuttings. An ideal substrate balances moisture retention with sufficient aeration and drainage. Although various media have been tested in plant propagation, only one study to date has specifically focused on identifying suitable rooting substrates for *Zamiifolia*. Early work used peat alone as the rooting medium (Cutter, 1962). More recently, a 1:1 mixture of perlite and cocopeat treated with chitosan (250-500 mg L^{-1}) enhanced rhizome number, rhizome diameter, and root count (Badizadegan et al., 2023). In coarse sand, coconut water acted as an effective natural rooting stimulant, accelerating rhizome initiation (46.5 days) and root formation (50.7 days), while also improving root number (8.73), main root length (4.93 cm), and rhizome diameter at 150 days after planting (Malla et al., 2023). Water has also been used successfully for rooting *Zamiifolia* cuttings (Gangani et al., 2023); however, it poses risks of microbial contamination by pathogenic bacteria and fungi, which can lead to cutting decay and inhibit rooting (Nion et al., 2015). Moreover, water lacks essential nutrients necessary for robust plantlet development (Górnik et al., 2008).

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Plant growth regulators, particularly auxins, further enhance rooting potential. In *Zamiifolia*, auxin application has consistently improved leaf-cutting propagation outcomes (Swandari et al., 2023). Cutter (1962) tested several auxins, IAA, IBA, NAA, and 2,4-D, and found that 2,4-D was phytotoxic, whereas NAA and IBA applied as pastes to the cutting base promoted rooting. Similarly, Gangani et al. (2023) demonstrated that 750 mg L^{-1} IBA significantly increased root number, root length, root diameter, rooting percentage, fresh and dry root weight, callus sprout formation, and survival rate at 75 days after rooting.

Building on this body of work, the present study aimed to optimize *Zamiifolia* propagation by evaluating the most effective combination of leaf cutting type, rooting substrate, and NAA concentration to enhance both rooting and post-rooting growth of daughter plants. Two sequential experiments were conducted, and morphological and biochemical parameters were analyzed to establish the most favorable conditions for efficient leaf-cutting propagation of *Z. zamiifolia*.

Materials and Methods

Experiment 1: Choosing the suitable rooting substrate and type of leaf cutting

This experiment was conducted as a factorial arrangement in a completely randomized design. The experimental factors included type of leaf cutting and type of rooting substrate. Four types of leaf cuttings were evaluated for propagation: A) Blade+rachis: whole leaflet + leaf axil; B) Blade: whole leaflet; C) Basal segment: Basal half of the leaflet; D) Apical segment: Upper half of the leaflet (Fig. 1 A-D). Cuttings were prepared using sharp, sterilized blades and immediately placed into the respective rooting substrates. Four different substrates were tested: Cocopeat (CP), Cocopeat + perlite (2:1 ratio, CP+PL), Peat moss + perlite (2:1 ratio, PM+PL), Vermicompost + vermiculite (1:1 ratio, VC+VM). The cuttings were maintained at a temperature of 31±2°C under a plastic tunnel in a greenhouse. After six months, both morphological and biochemical traits were measured. Each treatment consisted of five replications, with ten cuttings per replication.

Experiment 2: Evaluating NAA concentrations to increase the rooting efficiency

This experiment was arranged as a factorial experiment in a completely randomized design, involving three factors: Cutting type, rooting substrate, and NAA concentration. Each treatment had five replications, with ten cuttings per replication. Based on the results of Experiment 1, two effective cutting types and rooting substrates were selected for further investigation. This experiment aimed to obtain higher performance with NAA application.

Two preferable types of leaf cuttings identified in the first experiment treated with three concentrations of naphthalene acetic acid (NAA): 0 (control), 1000 and 2000 mg L^{-1}. Following treatment, the cuttings were planted in two selected rooting substrates. Plants were placed in the same environmental conditions as in the first experiment. After six months, morphological and biochemical traits were evaluated similar to the first experiment.

Measured Parameters

Fresh weight of roots, stems, leaves, and tubers were determined using an analytical balance. Rhizome diameter was measured using a digital caliper. Leaf area, root length, and shoot height were assessed by image analysis using ImageJ software after capturing photographs. Root volume was estimated by water displacement method. Briefly, freshly harvested root systems were washed and then carefully submerged in a graduated cylinder partially filled with a known volume of distilled water (V_1). The new water level (V_2) was recorded, and root volume was calculated as the difference between the final and initial volumes (V_2-V_1) (Karimi et al., 2022).

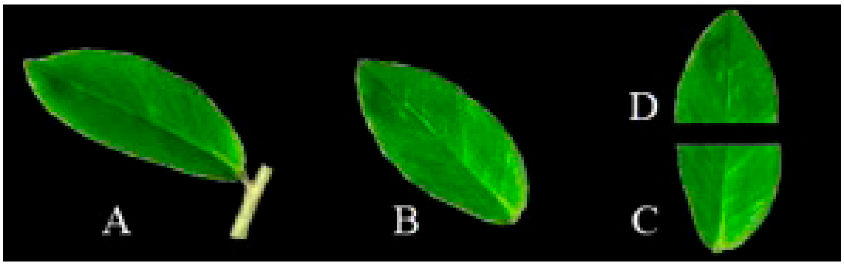

Fig. 1. Types of leaf cuttings, A) leaf blade+rachis, B) leaf blade, C) basal segment, and D) apical segment of leaflet.

Young mature leaves were collected in liquid nitrogen and used for biochemical analyses. Chlorophyll and carotenoid contents were quantified according to the method of Arnon (1967). Briefly, 0.25 g of leaf tissue was extracted with 80% acetone in darkness at 4 °C. The homogenate was centrifuged and absorbance of the supernatant was measured at 663, 645, and 480 nm using a UV-Vis spectrophotometer. Pigment concentrations were calculated using the equations of Arnon (1967). The concentration of soluble carbohydrates was determined using the anthrone reagent method (Yemm and Willis, 1954). Briefly, 0.50 g of leaf tissue was extracted twice with 80% ethanol at 80 °C for 30 min. After centrifugation, the supernatant was mixed with 3 mL of freshly prepared anthrone reagent (0.15% anthrone in 72% H_2SO_4) and heated in a boiling water for 10 min. After cooling, absorbance was read at 625 nm. Glucose was used as the standard.

Statistical Analysis

The experimental unit was defined as a single replication consisting of 10 leaf cuttings, with five replications per treatment. Prior to analysis of variance (ANOVA), data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. A factorial ANOVA was performed, and significant differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5% probability level. Correlations between variables were assessed using Pearson's two-tailed correlation test. All statistical analyses were conducted using SPSS version 21. Heat maps and regression graphs were generated in Microsoft Excel 2013; for heat map visualization, data values were normalized to a range of 0 to 1.

Results

The physicochemical properties of the four rooting substrates evaluated in this study are presented in Table 1. Significant variations were observed in pH, electrical conductivity (EC), nutrient content, cation exchange capacity (CEC), and water-holding characteristics among the substrates.

Table 1. The physicochemical properties of the rooting substrates evaluated in this study: Cocopeat (CP), Cocopeat + perlite (2:1 ratio, CP+PL), Peat moss + perlite (2:1 ratio, PM+PL), Vermicompost + vermiculite (1:1 ratio, VC+VM).

Parameter	CP	CP+PL	PM+PL	VC+VM
Acidity (pH)	5.9	6.3	4.9	6.7
EC (dS m^{-1})	0.61	0.49	0.26	1.5
N (mg kg^{-1})	56.2	31.5	53.8	1531.3
P (mg kg^{-1})	22.8	15.6	10.4	390.8
K (mg kg^{-1})	150.4	100.3	60.8	832.0
CEC (meq/100g)	48.5	40.2	102.8	84.3
SP (V%)	86.7	75.6	71.9	80.1
FC (V%)	65.7	54.8	60.1	55.4
PWP (V%)	28.3	23.0	24.2	26.6
AW (V%)	37.4	31.8	35.9	28.8

EC) electrical conductivity; N) nitrogen content; P) available phosphorus; K) available potassium; CEC) cation exchange capacity; SP) saturation percentage; FC) field capacity; PWP) permanent wilting point; AW) available water.

Experiment 1: Choosing the suitable rooting substrate and type of leaf cutting

High rooting percentages were observed in 86%-91% of the leaf cuttings across all treatments, with no significant differences found among the different combinations of substrates and cutting types. However, both the type of rooting substrate and the type of leaf cutting significantly influenced the growth indices and physiological performance of the rooted plantlets. The results of the measured growth and biochemical parameters are summarized in Fig. 2, presented as a heatmap using a color gradient from blue (low values) to red (high values). Prior to visualization, data were normalized between 0 and 1 to allow for comparative analysis.

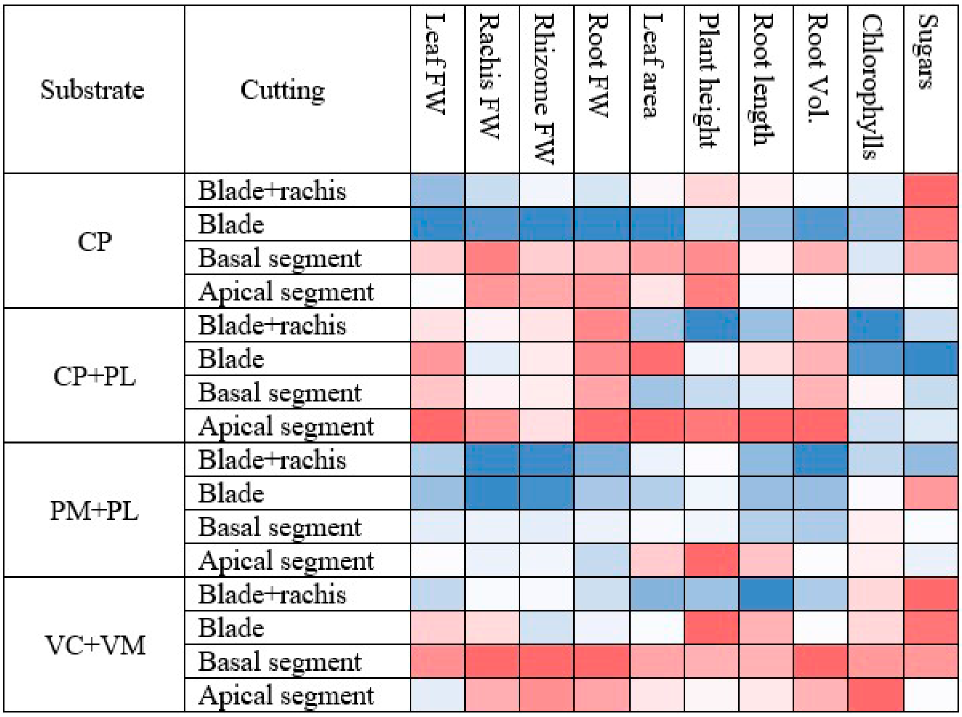

Fig. 2. Heatmap of growth and biochemical indices of daughter plants in response to rooting substrates (CP: Cocopeat; CP+PL: cocopeat + perlite; PM+PL: Peat moss + perlite; VC+VM: vermicompost + vermiculite) and types of *Zamiifolia* leaf cuttings. Data values were normalized to a range of 0 to 1 before visualization. In the heatmap, blue represents values closer to 1 (highest), white indicates values around 0.5 (intermediate), and red corresponds to values closer to 0 (lowest). This color scheme highlights the variation in data intensity across the dataset. CP: Cocopeat; CP+PL: cocopeat+perlite; PM+PL: Peat moss+perlite; VC+VM: vermicompost+vermiculite.

The highest leaf weight was recorded in plants grown in cocopeat (CP) and peat moss + perlite (PM+PL) substrates, while the lowest leaf weight was observed in those grown in cocopeat + perlite (CP+PL) (Table 2). Blade+rachis and blade cuttings produced the greatest leaf biomass without significant effects, whereas the lowest leaf weight was obtained from apical segment cuttings. A similar pattern was observed for fresh weight accumulation in the rachis, rhizome, and root of the daughter plants. The highest biomass in these organs was recorded in plants grown in PM+PL, while the lowest values were found in those cultivated in VC+VM and CP+PL substrates. In terms of cutting type, blade cuttings showed the highest biomass accumulation, while basal segment and apical segment resulted in the lowest accumulation. Leaf area was not significantly affected by the type of rooting substrate; however, cuttings derived from blade+rachis, blade, and basal segment produced larger leaf areas compared to those obtained from the apical segment of the leaves.

Table 2. Effects of rooting substrate (CP: Cocopeat; CP+PL: cocopeat + perlite; PM+PL: Peat moss + perlite; VC+VM: vermicompost + vermiculite) and *Zamiifolia* leaf cuttings on fresh weigh of different organs and leaf surface of daughter plants.

	Leaf	**Rachis**	**Rhizome**	**Root**	**Leaf area**
Substrate	FW (g plant^{-1})				cm^2
CP	0.442^a	0.145^b	2.727^b	0.765^b	24.0^a
CP+PL	0.202^c	0.090^{bc}	1.535^c	0.085^d	21.7^a
PM+PL	0.407^a	0.235^a	4.275^a	1.012^a	22.8^a
VC+VM	0.297^b	0.067^c	1.520^c	0.352^c	22.6^a
Cutting					
Blade+rachis	0.422^a	0.177^b	2.962^b	0.777^b	26.9^a
Blade	0.412^a	0.221^a	4.297^a	0.942^a	25.8^a
Basal segment	0.247^b	0.073^c	1.455^c	0.210^c	21.0^{ab}
Apical segment	0.265^b	0.075^c	1.342^c	0.285^c	17.4^b

Means in the same column with the same letter are not significantly different *(DMRT, $p \leq 0.05$).*

Plants grown in CP+PL and VC+VM substrates without significant difference with each other, exhibited greater plant height compared to those grown in CP and PM+PL (Table 3). The longest root length was observed in plants grown in PM+PL, which was not statistically different from those grown in CP (Table 3). The shortest roots were recorded in CP+PL and VC+VM substrates. The largest root volume was achieved in PM+PL, while the smallest was observed in CP+PL, which did not differ significantly from VC+VM. No significant effect of rooting substrate was detected on chlorophyll content in the leaves of daughter plants.

The highest values for plant height, root volume, leaf chlorophyll content, and soluble carbohydrates were recorded in plantlets derived from blade+rachis and blade cuttings (Table 3). The largest root volume was obtained from blade+rachis cuttings. Conversely, the lowest values for plant height, root volume, and soluble carbohydrates were observed in plantlets derived from basal segment and apical segment cuttings. Additionally, the shortest root length were recorded in plantlets obtained from apical segment cuttings. The type of cutting influenced leaf chlorophyll levels, with the largest and lowest content of chlorophyll recorded in plantlets of blade+rachis and apical segment cutting respectivily.

Table 3. Effects of rooting substrate (CP: Cocopeat; CP+PL: cocopeat + perlite; PM+PL: Peat moss + perlite; VC+VM: vermicompost + vermiculite) and *Zamiifolia* leaf cuttings on growth indices and accumulation of chlorophyll and soluble carbohydrates in leaves of daughter plants.

	Plant height	**Root length**	**Root volume**	**Chlorophylls**	**Sugars**
Substrate	cm		cm^3	(mg g^{-1} FW)	
CP	1.84^b	3.60^{ab}	2.24^b	4.81^a	385.7^a
CP+PL	2.74^a	3.28^b	1.24^c	4.34^a	430.6^a
PM+PL	1.88^b	4.12^a	3.16^a	4.57^a	372.5^a
VC+VM	2.04^{ab}	3.47^b	1.74^{bc}	4.52^a	200.0^b
Cutting					
Blade+rachis	2.98^a	4.61^a	2.66^a	5.38^a	385.5^a
Blade	2.09^{ab}	3.73^b	2.66^a	5.17^a	427.1^a
Basal segment	1.98^b	3.42^b	1.66^b	4.20^b	290.4^b
Apical segment	1.45^b	2.69^c	1.41^b	3.49^c	285.8^b

Means in the same column with the same letter are not significantly different *(DMRT, $p \leq 0.05$).*

Correlation analysis revealed that most of the growth indices, excluding plant height and root weight, were positively correlated with each other. However, these growth-related parameters did not show significant correlations with either leaf chlorophyll or soluble carbohydrates content (Table 4). A positive correlation was observed between soluble carbohydrates content and chlorophyll concentration in the leaves.

Table 4. Correlations between the measured indices in the first experiment.

	Leaflet FW	Rachis FW	Rhyzome FW	Root FW	Leaf area	Plant height	Root length	Root volume	Chlorophylls
Rachis FW	0.750**								
Rhyzome FW	0.742**	0.907**							
Root FW	0.788**	0.876**	0.922**						
Leaf area	0.667**	0.680**	0.742**	0.716**					
Plant height	0.343*	0.358*	0.353*	0.284	0.669**				
Root length	0.486**	0.372**	0.436**	0.428**	0.643**	0.590**			
Root volume	0.564**	0.626**	0.706**	0.640**	0.655**	0.324*	0.492**		
Chlorophylls	-0.162	-0.190	-0.148	-0.143	-0.167	-0.058	-0.178	-0.086	
Sugars	-0.095	-0.106	-0.091	-0.101	-0.145	-0.143	-0.081	-0.017	0.459**

** and *: Correlations are significant at the 0.01 and 0.05 levels (2-tailed), respectively.

A close and positive relationship was observed between rhizome fresh weight and leaf area with the total biomass of the daughter plants (Fig. 3). This indicates that conditions promoting greater rhizome development are closely associated with the production of more vigorous and robust plants. Based on the overall evaluation of both growth and biochemical parameters, the PM+PL substrate, in combination with blade or blade+rachis cuttings, is recommended as the most suitable method for the propagation of *Zamiifolia*.

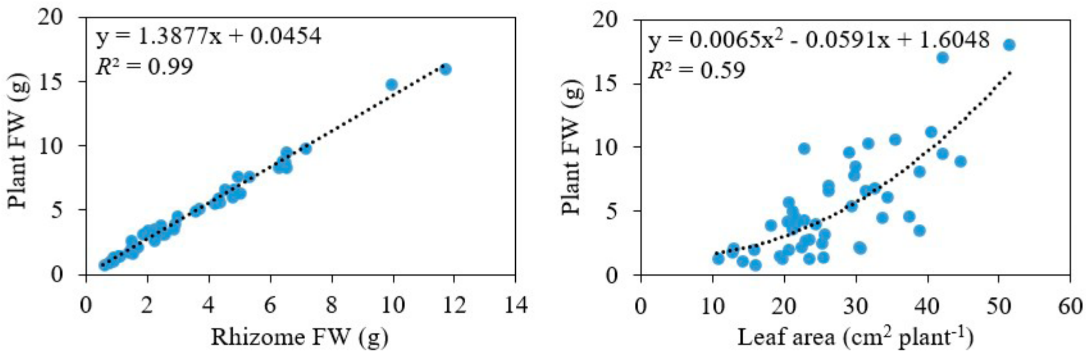

Fig. 3. Relationship between rhizome weight and total weight of daughter plants.

Experiment 2: Evaluating NAA concentrations to increase the rooting efficiency

The highest leaf fresh weight was observed in plantlets derived from blade+rachis cuttings grown in the PM+PL substrate and treated with 1000 or 2000 mg L^{-1} NAA (Table 5). In contrast, the lowest leaf fresh weight was recorded in plants obtained from blade+rachis cuttings in the CP substrate treated with 2000 mg L^{-1} NAA, as well as in those derived from blade cuttings in the PM+PL substrate treated with either 0 or 1000 mg L^{-1} NAA (Table 5).

The maximum rachis weight was achieved in plantlets treated with 2000 mg L^{-1} NAA and derived from blade+rachis cuttings in the CP substrate. On the other hand, the lowest rachis weight was found in plantlets from blade cuttings in the CP substrate treated with 1000 mg L^{-1} NAA, and in those from blade cuttings in the PM+PL substrate treated with 2000 mg L^{-1} NAA (Table 5). Rhizome weight was highest in plantlets derived from blade+rachis cuttings grown in the PM+PL substrate and treated with either 1000 or 2000 mg L^{-1} NAA. The lowest rhizome weight was observed in plantlets from blade+rachis cuttings grown in the CP substrate without any NAA application (Table 5). Root weight was greatest in plantlets treated with 1000 mg L^{-1} NAA, derived from blade+rachis cuttings, and grown in the PM+PL substrate. The lowest root weight was recorded in plantlets from blade+rachis cuttings without NAA application and in those from Blade cuttings grown in the CP substrate and treated with 1000 mg L^{-1} NAA (Table 5).

Table 5. Interactive effects of rooting substrate (CP: Cocopeat; PM+PL: Peat moss + perlite), leaf cutting type, and naphthaleneacetic acid (NAA) on fresh weight of *Zamiifolia* daughter plants.

Substrate	Cutting	NAA (mg L^{-1})	Leaf	Rachis	Rhizome	Root
			FW (g $Plant^{-1}$)			
CP	Blade+rachis	0	0.53^{c}	0.17^{g}	2.03^{g}	0.70^{g}
	Blade+Rachis	1000	0.72^{b}	0.31^{de}	5.29^{e}	1.34^{de}
	Blade+Rachis	2000	0.47^{d}	0.32^{de}	6.39^{cd}	1.70^{cd}
	Leaf blade	0	0.71^{b}	0.32^{de}	6.50^{cd}	2.07^{bc}
	Leaf Blade	1000	0.64^{bc}	0.23^{f}	3.62^{cd}	0.69^{g}
	Leaf Blade	2000	0.73^{b}	0.45^{c}	7.16^{bc}	1.49^{d}
PM+PL	Blade+rachis	0	0.47^{d}	0.35^{d}	6.46^{cd}	1.54^{d}
	Blade+Rachis	1000	1.12^{a}	0.76^{a}	9.94^{ab}	3.08^{a}
	Blade+Rachis	2000	0.96^{a}	0.69^{b}	11.7^{a}	2.58^{b}
	Leaf blade	0	0.52^{cd}	0.35^{d}	6.27^{d}	1.15^{efg}
	Leaf Blade	1000	0.48^{d}	0.47^{c}	4.82^{ef}	0.94^{fg}
	Leaf Blade	2000	0.71^{b}	0.26^{ef}	4.51^{f}	1.22^{ef}

Means in the same column with the same letter are not significantly different *(DMRT, $p \leq 0.05$).*

The highest values of total plant fresh weight were observed in plantlets treated with 1000 or 2000 mg L^{-1} NAA (without significant difference), derived from blade+rachis cuttings, and grown in the PM+PL substrate (Fig. 4). In contrast, the lowest fresh weights were recorded in plantlets from blade cuttings grown in the CP substrate, either without NAA application or with 1000 mg L^{-1} NAA (Fig. 4).

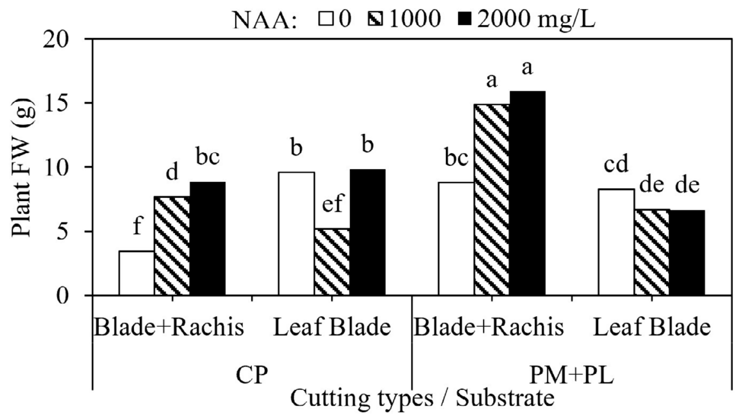

Fig. 4. Interactive effects of rooting substrate (CP: Cocopeat; PM+PL: Peat moss + perlite), leaf cutting type, and naphthaleneacetic acid (NAA) on total fresh weight (FW) of *Zamiifolia* daughter plants. Means with the same letter are not significantly different (DMRT, $p \leq 0.05$).

The highest plant height was observed in plantlets treated with 1000 or 2000 mg L^{-1} NAA, derived from blade+rachis cuttings, and grown in the PM+PL substrate (Table 6). In contrast, the lowest plant height was recorded in plantlets obtained from blade cuttings in the CP substrate treated with 2000 mg L^{-1} NAA, as well as in those derived from blade+rachis cuttings without NAA application in the PM+PL substrate (Table 6).

Root volume was significantly greater in plantlets treated with 2000 mg L^{-1} NAA and grown in the PM+PL substrate, regardless of whether they were derived from blade or blade+rachis cuttings, compared to other treatments (Table 6). The largest leaf area was measured in plantlets treated with 2000 mg L^{-1} NAA, derived from blade+rachis cuttings, and grown in the PM+PL substrate (Table 6). The highest leaf chlorophyll content was also observed in plantlets treated with either 1000 or 2000 mg L^{-1} NAA, derived from blade+rachis cuttings, and grown in the PM+PL substrate (Table 6). Conversely, the lowest chlorophyll content was recorded in plantlets derived from blade+rachis cuttings grown in the CP substrate without any NAA application (Table 6).

Table 6. Interactive effects of rooting substrate (CP: Cocopeat; PM+PL: Peat moss + perlite), leaf cutting type, and naphthaleneacetic acid (NAA) on growth indices and chlorophyll concentration in *Zamiifolia* daughter plant.

Substrate	Cutting	NAA (mg L^{-1})	Plant height (cm)	Root vol. (cm^{3})	Leaf area (cm^{2})	Chlorophylls (mg g^{-1} FW)
CP	Blade+rachis	0	1.78^{f}	1.66^{d}	20.6^{e}	2.80^{f}
	Blade+Rachis	1000	2.53^{cd}	1.66^{d}	30.0^{cd}	3.30^{de}
	Blade+Rachis	2000	2.34^{d}	4.33^{b}	31.8^{cd}	4.60^{c}
	Leaf blade	0	2.84^{b}	4.33^{b}	40.5^{bc}	3.80^{d}
	Leaf Blade	1000	1.79^{f}	3.33^{c}	20.8^{e}	3.10^{def}
	Leaf Blade	2000	2.31^{d}	4.33^{b}	35.4^{c}	4.30^{c}
PM+PL	Blade+rachis	0	2.03^{ef}	4.66^{b}	22.7^{e}	4.30^{c}
	Blade+Rachis	1000	3.80^{a}	4.80^{b}	42.2^{b}	6.01^{a}
	Blade+Rachis	2000	3.02^{ab}	6.05^{a}	51.5^{a}	5.80^{a}
	Leaf blade	0	2.16^{e}	3.33^{c}	28.9^{de}	4.20^{cd}
	Leaf Blade	1000	3.08^{ab}	3.33^{c}	29.7^{cd}	4.10^{b}
	Leaf Blade	2000	2.65^{bc}	6.66^{a}	38.8^{c}	4.30^{b}

Means in the same column with the same letter are not significantly different *(DMRT, $p \leq 0.05$).*

The interactive effects of rooting substrate, cutting type, and NAA concentration on the chlorophyll-to-carotenoid ratio in leaves were found to be statistically significant. The lowest values of this ratio were primarily observed in plantlets derived from blade+rachis cuttings treated with 0 or 1000 mg L^{-1} NAA, as well as in those derived from blade cuttings treated with 1000 mg L^{-1} NAA and grown in the CP substrate (Fig. 5A). Furthermore, the interaction of rooting substrate, cutting type, and NAA application had a significant effect on the soluble carbohydrates content in leaves. The highest levels of soluble carbohydrates were measured in plantlets treated with 2000 mg L^{-1} NAA, derived from blade+rachis cuttings, and grown in the PM+PL substrate. On the other hand, the lowest soluble carbohydrates content was observed in plantlets derived from both blade and blade+rachis cuttings grown in the CP substrate without any NAA application (Fig. 5B).

Visual assessment of plantlets confirmed the quantitative data, with blade+rachis and blade cuttings in PM+PL substrate exhibiting the most vigorous rhizome and shoot development (Fig. 6).

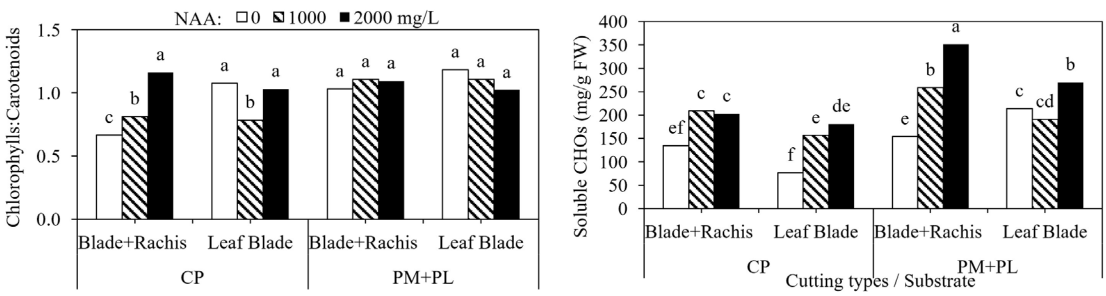

Fig. 5. Interactive effects of rooting substrate (CP: Cocopeat; PM+PL: Peat moss + perlite), leaf cutting type, and naphthaleneacetic acid (NAA) on the A) chlorophylls to carotenoids ratio and B) concentration on soluble carbohydrates content (CHOs) in leaves of *Zamiifolia* daughter plants. Means with the same letter are not significantly different (DMRT, $p \leq 0.05$).

Discussion

The first experiment revealed that *Zamioculcas zamiifolia* leaf cuttings exhibit high rooting success (exceeding 88%) even in the absence of exogenous growth regulators. This aligns with earlier findings: Gangani et al. (2023) reported 69.1% rooting, which rose above 93.3% with IBA and an optimized medium; Thongkham and Phavaphutanon (2018) observed 90%–100% rooting; and Malla et al. (2023) recorded ~80% success across cultivars without special treatments. Chen and Henny (2003) attributed this to the species' inherent high rooting capacity, indicating that routine auxin application may not be necessary for root initiation. However, despite near-universal rooting, a critical limitation emerged: the slow post-rooting growth of daughter plants, which hinders commercial scalability. Notably, while rooting percentage did not differ significantly among cutting types, substrates, or (in this phase) auxin treatments, the study found that cutting type and substrate choice profoundly influenced subsequent plantlet vigor.

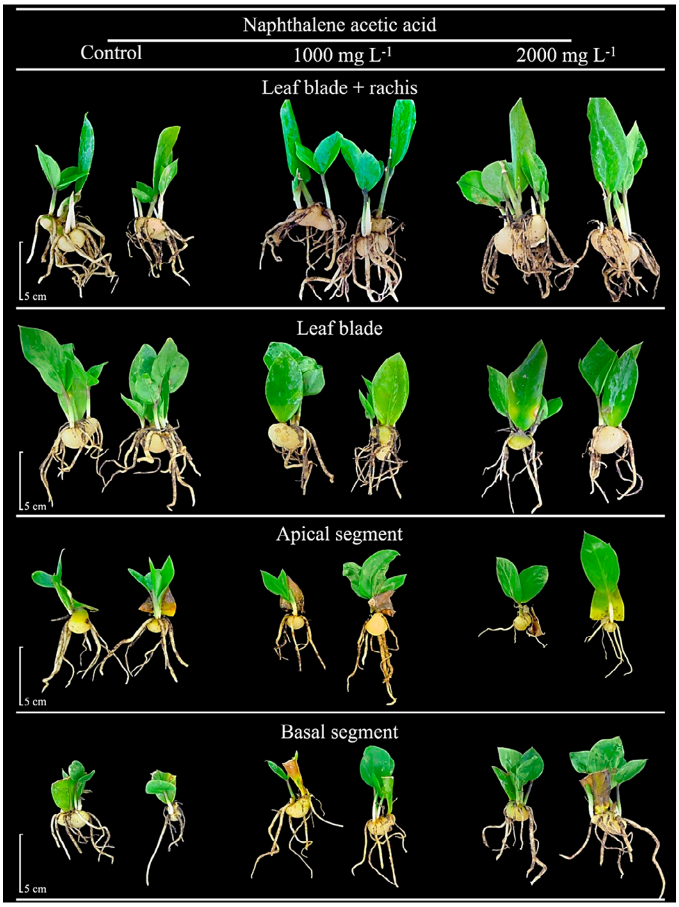

Fig. 6. Morphological development of *Zamioculcas zamiifolia* daughter plants derived from different leaf cutting types (blade+rachis, blade, apical segment, and basal segment) grown in peat moss + perlite (PM+PL) substrate. The images illustrate rhizome formation, root system development, and overall plantlet vigor.

Among the evaluated substrates, CP+PL (cocopeat + perlite) and VC+VM (vermicompost + vermiculite) underperformed relative to cocopeat (CP) and PM+PL (peat moss + perlite), a divergence primarily attributable to differences in mineral nutrition and water availability (Table 1). The pH of the substrates ranged from 4.9 (PM+PL) to 6.7 (VC+VM), with CP and CP+PL exhibiting near-neutral values (6.2-6.5); the slightly acidic conditions of CP and PM+PL likely enhance micronutrient, phosphorus and potassium availability, whereas the higher pH of VC+VM may alter nutrient solubility and uptake dynamics (Sonneveld and Voogt, 2009). Nutrient analysis revealed substantially higher concentrations of primary macronutrients in VC+VM; however, PM+PL demonstrated the highest cation exchange capacity (CEC; 102.8 meq 100 g^{-1}), indicating a superior ability to retain and gradually supply nutrients to developing roots. Moreover, electrical conductivity (EC) was highest in VC+VM (1.5 dS m^{-1}), suggesting that the elevated salinity in VC+VM, derived from its vermicompost component, may impose osmotic stress on the rudimentary root systems of newly established cuttings, where even minor water or ionic imbalances can severely restrict growth (Górnik et al., 2008).

Water retention characteristics further differentiated substrate performance: although CP exhibited the highest saturation percentage (86.7%), field capacity (65.7%), and available water content (37.4%), its coarse, fibrous structure naturally balanced moisture retention with adequate aeration. The addition of perlite to CP increased porosity but excessively reduced water availability, thereby impairing growth in CP+PL. Similarly, despite vermiculite's high CEC, its combination with vermicompost created a coarser matrix that limited water accessibility and negatively impacted plant performance, consistent with Gangani et al. (2023), who reported that vermiculite mixes with cocopeat or sand reduce rooting efficiency and delay post-rooting growth in *Zamiifolia*. In contrast, PM+PL proved highly effective; while peat moss alone offers a fine texture and excellent water-holding capacity (Carlile et al., 2015), it risks waterlogging and poor aeration (Cutter, 1962).

Blending it with perlite optimized the water-air ratio, creating ideal conditions for root and shoot development, while the inherent low salinity and natural fungistatic compounds of peat moss further suppressed pathogens and minimized abiotic stress (Carlile et al., 2015). Consequently, the superior performance of CP and PM+PL can be attributed to their balanced pH, low salinity, high CEC, and optimal moisture–aeration equilibrium, whereas the limitations of VC+VM and CP+PL stem from elevated osmotic pressure, reduced available water, and suboptimal pore structure, despite their high nutrient potential.

Daughter plants from blade and blade+rachis cuttings exhibited significantly better growth than those from apical or basal segments. This advantage is likely due to their greater initial carbohydrate reserves (Tombesi et al., 2015). Thongkham and Phavaphutanon (2018) similarly found that whole leaf cuttings produced more roots, higher root quality, and larger rhizomes compared to partial segments. Multiple studies confirm a strong positive correlation between cutting size/weight and resulting rhizome dimensions (Thongkham and Phavaphutanon, 2018).

Carbohydrates are essential during early rhizogenesis, providing energy and carbon skeletons for cell division and callus differentiation (Agulló-Antón et al., 2011). Since newly rooted cuttings have minimal capacity for external nutrient uptake, they rely heavily on internal reserves. Thus, cuttings with greater biomass (e.g. blade and blade+rachis types) support faster and more efficient rhizome initiation and subsequent plantlet development. Furthermore, rhizome formation emerged as a key determinant of long-term plantlet vigor. Acting as the primary growth center, the rhizome gives rise to new shoots and roots (Le Moullec et al., 2015), enabling both horizontal and vertical expansion and increasing overall biomass. It also plays a central role in resource acquisition and allocation (Ning et al., 2014). Structurally, the *Zamiifolia* rhizome functions as a "living battery", a dynamic storage organ rich in carbohydrates and water (Lubbe et al., 2023). This reservoir sustains physiological activity during drought or nutrient stress, enhancing resilience. Consequently, cuttings that generate larger, more robust rhizomes are better equipped to withstand the variable conditions of propagation environments, leading to improved survival and growth. These findings underscore the importance of selecting high-biomass cutting types, for establishing vigorous, stress-tolerant plantlets from the outset.

Once daughter plants developed leaves and established photosynthetic capacity, they transitioned to autotrophic carbohydrate production (Ainsworth and Bush, 2011). A strong positive correlation between leaf area and total plant biomass highlighted that early leaf expansion and the development of functional source tissues are critical for overall growth vigor. The superior performance of plantlets from blade and blade+rachis cuttings thus stems not only from higher initial carbohydrate reserves but also from their enhanced ability to rapidly expand leaf area. This early photosynthetic advantage enables a stronger source-sink relationship, allowing more efficient allocation of energy toward root and rhizome development (Rapaka et al., 2005). Consequently, the greater inherent resource availability in these larger cuttings supports both immediate metabolic needs and sustained long-term growth. Although root size, not leaf chlorophyll concentration, was the primary driver of overall growth performance, a positive correlation between chlorophyll content and soluble carbohydrates underscores the importance of a healthy photosynthetic apparatus in supplying carbon skeletons during early development. Chlorophyll synthesis and leaf health were significantly influenced by cutting type, but not by rooting substrate. Given that no external nutrients were provided during propagation, this effect likely reflects differences in initial nitrogen reserves within the cuttings. Larger cuttings (blade and blade+rachis) appear to contain sufficient nitrogen to support chlorophyll biosynthesis and the production of associated protective proteins (Monder et al., 2014). This, in turn, enhances photosynthetic output and the translocation of soluble carbohydrates, further boosting growth.

In summary, the combination of greater biomass, higher nutrient reserves, and superior photosynthetic capacity in blade and blade+rachis cuttings creates a synergistic advantage for plantlet development. Based on their consistently strong performance in both cocopeat (CP) and peat moss + perlite (PM+PL) substrates, these cutting–substrate combinations were selected for the second experiment to evaluate the efficacy of NAA in further enhancing rooting and early growth. The application of NAA significantly enhanced rhizome formation and leaf area expansion, which in turn drove more vigorous shoot and root development. Auxin-mediated rooting is a multifaceted process involving stimulation of cell division (particularly longitudinal), tissue differentiation, regulation of organogenesis-related genes, and redirection of resources toward developing sinks (Di Mambro et al., 2017). These mechanisms collectively accelerate and improve the efficiency of root initiation. However, rooting success depends on several interacting factors, including auxin type, concentration, timing of application, hormonal crosstalk, and environmental conditions (Gangani et al., 2023).

In this study, 1000 and 2000 mg L^{-1} NAA applied to blade+rachis cuttings in PM+PL substrate produced the most pronounced improvements in rhizome development and leaf expansion. These treatments also yielded the highest total biomass, reflecting enhanced overall plant vigor. NAA further promoted vegetative sink development, as evidenced by increased plant height and root volume, likely a consequence of greater photosynthetic source capacity from expanded leaf area. Additionally, robust root systems not only improved water and nutrient uptake but also served as active sites for synthesizing growth-promoting compounds, amplifying other growth parameters. Beyond vegetative growth, NAA boosted photosynthetic capacity by increasing leaf chlorophyll content. Since chlorophyll levels and photosynthetic integrity are critical for carbon assimilation and source strength, this effect likely sustained long-term growth (Fatichi et al., 2014). Further physiological insight came from the chlorophyll-to-carotenoid ratio, a recognized indicator of photosynthetic health (Song and Wang, 2022). Chlorophyll is more stress-sensitive than carotenoids and degrades under suboptimal conditions; thus, a lower ratio suggests stress. The lowest ratio was observed in CP (cocopeat), implying that this substrate imposed physiological stress, possibly due to inadequate water or nutrient availability. Critically, the optimal treatment, 2000 mg L^{-1} NAA on blade+rachis cuttings in PM+PL, produced the highest overall plantlet growth, confirming the synergistic benefits of combining an effective cutting type, a well-balanced substrate, and an appropriate auxin concentration.

Conclusion

This study aimed to optimize the vegetative propagation of *Z. zamiifolia* by evaluating cutting type, rooting substrate, and exogenous auxin application. While leaf cuttings exhibited a high natural rooting capacity, post-rooting growth was significantly limited. Optimization of these three factors successfully overcame this constraint. Blade and blade+rachis cuttings consistently produced the most vigorous plantlets, outperforming apical and basal segments. Among tested media, peat moss + perlite (PM+PL) provided the most favorable environment for establishment. Application of NAA (1000-2000 mg L^{-1}) further enhanced growth parameters, with the combination of blade+rachis cuttings, PM+PL substrate, and 2000 mg L^{-1} NAA yielding the highest overall plantlet vigor and biomass. Consequently, this integrated protocol is recommended as an efficient, reproducible strategy for commercial *Zamiifolia* propagation. Future studies should investigate the effects of rooting temperature, assess oxidative stress biomarkers during clonal propagation, and evaluate long-term acclimatization to fully validate commercial scalability.

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Author Contribution

NA: Investigation, Formal Analysis, Writing – Original Draft. **SDD**: Conceptualization, Methodology, Review and Editing, Supervision. **SK**: Validation, Writing – Review and Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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