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The Induction of Adventitious Roots Regeneration before Transplanting Rootless *Ficus elastica* Heritage Tree

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Abstract: Heritage trees carry both botanical and historical value for a city's resilience and sustainability and hence are precious and unique. Their transplant is costly and very rare due to tremendous cost and 100% survival requirement by law. Rootless transplant is even more detrimental to the heritage tree due to removal of roots infected by brown root rot (BRR) before transplanting. This study examined the adventitious roots (AR) induction ability of the *Ficus elastica* Roxb. heritage tree infected with BRR. The experimental design considered three factors: root diameter (RD), wounding method (WM), and auxin solution on aerial roots under fractional factorial experiment in completely randomized design (CRD). There were four RD groups: RDI (RD < 2 cm), RDII (2 ≤ RD ≤ 4.3 cm), RDIII (4.3 < RD ≤ 22), and RDIV (RD > 22); three WMs: cutting off (CF), girdling (GD), and rectangular shape peeling (RP) of aerial roots; and three auxin solutions: 2000 mg·L⁻¹ IBA (Indole-3-butyric acid) (2B), 2000 mg·L⁻¹ IBA + 2000 mg·L⁻¹ NAA (1-Naphthaleneacetic acid) (2NB), and 4000 mg·L⁻¹ IBA (4B) plus water as control (C). The number of rooting wounds, number of roots, and the mean length of the three longest adventitious roots in each wound were recorded to evaluate the AR rooting performance. Twenty four treatment combinations including 328 wounds were tested. The results showed that rooting ability was significantly correlated with RD and WM. Smaller RDs had better rooting and declined with increased RDs. CF had the best rooting followed by GD and then RP. Auxin solution did not significantly affect the rooting ability. It may be due to the abundant endogenous auxin in the heritage tree, which mitigated the effect of exogenous auxin for AR induction. We conclude that cutting off small-diameter aerial roots is the best approach to induce ARs from rootless *F. elastica* heritage trees to enhance transplantation success.

Keywords: brown root rot; aerial roots; auxin; girdle; rooting

1. Introduction

Ficus spp. belongs to the Moraceae family and is one of the most popular heritage tree species in Taiwan and China [1]. Heritage trees are keystones of a landscape and possess cultural and ecological value. They carry both botanical and historical value for a municipality and contribute to a city's resilience and sustainability [2]. However, their numbers are declining due to urban development [3,4], so they have become a subject of conservation. Governments register and monitor all heritage trees and demand approval before tree works, including pruning and transplants, can be performed.

Transplanting a heritage tree is sometimes inevitable due to urban development and can endanger its survival. A guaranteed 100% success rate is required by Tree Code so meticulous planning and arboriculture skills are needed. A sufficient number of roots needs to be retained to endure the

transplant shock. Root pruning is usually used to stimulate the growth of new absorbing roots inside the root ball when transplanting large mature trees [5]. The root balls were usually very large and that meant tremendous cost involving heavy equipment and excessive manpower. The high cost and guaranteed survival limited the number of heritage tree transplant cases in a year [6].

It is not unusual to take shoots from the single source or the same tree to compare the rooting performance of stem cuttings under various treatments [7]. The *Ficus elastica* tree is known for its multiple prop roots, which are like many trunks, and we treated as duplicates [8].

The root pruning to grow new roots inside of the root ball is not possible if a heritage tree is infected with brown root rot (BRR). Due to poor drainage and soil conditions in urban areas, BRR has been one of the most prevalent diseases harming heritage trees. *Phellinus noxius* is a destructive, fast-growing fungus affecting woody plants in tropical and subtropical areas in Asia, Australia, Africa, and Oceania [9]. The fungus causes BRR in urban areas where the soil is disturbed. The leaves of the infected tree will change color to pale green or brown; then the tree starts a death spiral and declines or dies within six months to two years due to drought stress. That could cause tree failure, which can damage property or endanger human lives. Infected trees have to be removed to prevent tree failure. For heritage trees, people try to conserve them by surgery to remove the infected roots and transplant them to new sites or keep them at the site but fumigate the habitat soil [9,10].

Removing the infected roots makes the heritage tree rootless, but it continues to transpire, resulting in drought stress and transplant shock. It also takes time to grow sufficient new roots to absorb water and minerals. The root growth is affected by the transplanting season, soil, weather, and tree vigor [11]. The new root growth may not be fast enough to survive the transplantation.

F. elastica Roxb., originating from tropical Asia [12], belongs to the *Urostigma* subgroup of *Ficus* spp., which has many overhanging aerial roots to help with absorbing nutrition and moisture from the surroundings. “*Elastica*” comes from the high rubber content in the sap, which makes it milky or become latex. The aerial roots grow down to the ground from the stem or branches, then develop tension wood to mature and become prop roots. These are adventitious roots (ARs), which form from nonroot tissues, induced by stress [13].

AR formation is regulated by environmental stimuli, including light, temperature, nutrients, and endogenous hormone signals. [14] The ARs can be induced by stress such as wounding (through cutting, layering, and girdling), flooding, etiolation, and other propagation techniques [8,15].

Various studies suggest that the exogenous application of auxin can improve the rooting of stem cuttings [16], while IBA (Indole-3-butyric acid) and NAA (1-Naphthaleneacetic acid) are more commonly used [17]. IBA had been shown by Erdogen and Smith [18] to be more stable than IAA. The best IBA concentration for ARs varies by plant species and environmental conditions. It ranges between 50 mg L⁻¹ and 8000 mg L⁻¹, depending on the plant type, application method, and medium [19,20]. For quick dip or paint methods on stem cuttings, 2000 to 4000 mg L⁻¹ seemed to be the most commonly used concentration.

Wounding is a common approach for inducing de novo ARs. Wound-induced ARs are used in propagation. Stem cutting and girdling are often used for promoting ARs in horticulture practice [21,22]. After cutting or girdling the stem, the carbohydrates and auxin from the leaves accumulate at the wound to induce ARs or callus. [23–26]. Cutting off or girdling the ground roots are common practices before transplantation to induce ARs within the root balls. It is difficult to cut or girdle large trunks, hence creating a wound window on the surface of the trunk or prop roots is an option to generate ARs. These ARs can be kept in bags filled with a moisturized medium to provide water for transpiration needs [27]. In the case of *F. elastica*, the overhanging aerial roots grow from trunks or branches with no secondary cell wall. They extend to the ground and become large prop roots [8]. We thought that if the aerial roots or prop roots are wounded, enough ARs to complement the deficiency of water uptake from those severed infecting roots can be induced. Finding the most effective way to generate sufficient ARs should be able to help the rootless tree survive the transplantation.

Rooting ability was usually measured by rooting%, root number, and root length, as demonstrated by Al-Saqri and Alderson [28] and Alegre et al. [29]. This study aimed to identify the suitable size of root diameter (RD) of aerial roots, the most effective root wounding methods (WMs), and the optimal concentration of auxin spray to induce ARs by spraying on wounds for adventitious roots regeneration on the aerial roots or prop roots of *F. elastica*.

2. Materials and Methods

The experiment was conducted at a construction site in Hsinyi district, a central business area of Taipei City in Taiwan. One large *F. elastica* heritage tree with a height of 20 m and a canopy spread of 40 square m was supported by about 50 prop roots and 100 overhanging aerial roots and was estimated to be over 100 years old. It needed to be transplanted to Peitou, a suburban district of Taipei City to vacate the space for housing construction. The ground roots of the heritage tree were diagnosed by the certified arborist to have been infected with BRR. All the roots needed to be removed before transplanting the tree shoots to the new planting site.

The experiments were conducted in a completely randomized design (CRD) with treatment combinations included RD, WM, and auxin solution as the three factors. The four RD size groups included RD I (<2 cm), RD II ($2 \leq RD \leq 4.3$ cm), RD III ($4.3 < RD \leq 22$ cm), and RD IV (>22 cm). Each group had 82 ± 1 wounds in duplicates. The three WMs were cutting off (CF), girdling (GD), and rectangular shape peeling (RP): (a) CF, we cut off the aerial roots with a pruning shear or hand saw, (b) GD, we used a girdling knife to girdle the aerial roots deep into the xylem, and (c) RP, we used a carving knife (Godhandtool Co., Niigata, Japan 959) to incise a rectangular window on the surface of the prop roots 4×2 cm deep into the xylem, then peeled off the skin of the window (Figure 1).

Based on the 4 RD \times 3 WM \times 4 auxin treatments, the full factorial experiment would have been 48 treatment combinations. However, some treatments were not feasible and not practical: it was not feasible to conduct GD/RP on RDI (<2 cm) and RP on RDII due to small root diameters. CF and GD were not used on RD III because of the large prop root diameter, and we only conducted RP for RD IV as the root diameters were too large to do CF or GD. We adopted Fractional 3-Factorial Designs (Plackett–Burman design) to test 24 relevant treatment combinations for their main effects [30]. The detailed treatment combinations were listed in Table 1.

Table 1. Fractional 3-factorial experiment design.

WMs ^z	AUXIN ^y	RDI ^x	RDII ^x	RDIII ^x	RDIV ^x
CF	C	1	1		
CF	2B	1	1		
CF	2NB	1	1		
CF	4B	1	1		
GD	C		1	1	
GD	2B		1	1	
GD	2NB		1	1	
GD	4B		1	1	
RP	C			1	1
RP	2B			1	1
RP	2NB			1	1
RP	4B			1	1
Total treatment combinations		4	8	8	4

^z Wounding methods (WMs). Cutting off (CF): cut off the aerial roots; girdling (GD): girdle the aerial roots deep into the xylem, and rectangular shape peeling (RP): incise a rectangular window on the surface of the prop roots of 4×2 cm deep into the xylem, then peel off the skin of the window. ^y The three auxin solutions were $2000 \text{ mg}\cdot\text{L}^{-1}$ IBA (2B), $2000 \text{ mg}\cdot\text{L}^{-1}$ IBA + $2000 \text{ mg}\cdot\text{L}^{-1}$ NAA (2NB), and $4000 \text{ mg}\cdot\text{L}^{-1}$ IBA (4B) and water as the control (C). ^x The four root diameter (RD) size groups included RD I (<2 cm), RD II ($2 \leq RD \leq 4.3$ cm), RD III ($4.3 < RD \leq 22$ cm), and RD IV (>22 cm).



Figure 1. Wounding methods (WMs): (a) cutting off (CF), (b) girdling (GD), and (c) rectangular shape peeling (RP). (a) CF: cut off the aerial roots, (b) GD: girdle the aerial roots deep into the xylem, and (c) RP: incise a rectangular window on the surface of the prop roots of 4×2 cm deep into the xylem, then peel off the skin of the window.

There were 24 treatment combinations in the fractional 3-factorial experimental design. Some treatment combinations were eliminated due to practicality to test the main effect of the AR induction ability of these 3 factors.

The three auxin solutions were $2000 \text{ mg}\cdot\text{L}^{-1}$ IBA (2B), $2000 \text{ mg}\cdot\text{L}^{-1}$ IBA + $2000 \text{ mg}\cdot\text{L}^{-1}$ NAA (2NB), and $4000 \text{ mg}\cdot\text{L}^{-1}$ IBA (4B) and water as the control (C). The water or auxin solution was sprayed onto the wounds of aerial or prop roots until the solution or water fully covered the wound and flowed

out of the wound, then we filled the wounds with medium (moisturized peat/coconut fiber 50/50) and wrapped them with clear plastic sheets. The top and bottom of the plastic sheets were tied up and covered with a linen cloth to reduce moisture loss and shade it from the sunlight. (Figure 1).

A total of 328 wounds were created between 29 October 2019 and 3 November 2019. Rooting% (wounds with ARs/total wounds), root number in each wound, and the mean length of the three longest roots in each wound were recorded every month. Root numbers and root lengths were recorded in 5 classes: 0, 5, 10, 20, and 30 [31]. For non-rooting wounds, we recorded 0. For root number or root length in the wound to be between 1–5, we recorded 5, 10 for root number between 6–10, 20 for root number between 11–20, and 30 for root number >21. There were 328 sets of data including rooting rate, root number, and root length for statistical analysis.

A mist spray system circulating around the various wounds was installed and activated for 10 min at 6 a.m. and 12 p.m. every day to maintain the humidity of the environment. Black PVC sheet shading was used to cover the wounds to block direct sunlight from hitting the wounds.

According to the local weather bureau, the average temperature was between 13 and 24 °C and the humidity was between 63% and 99% at the test site during the experimental period. Taipei City is located in the subtropical area, and the elevation is 8 m above sea level.

On 27 March 2020, the plastic wrappings on wounds were opened up, and the records of rooting%, root numbers, and root lengths of all the 328 wounds were analyzed. The root lengths were recorded in centimeters (cm).

All the shoots and prop roots of the *F. elastica* heritage tree were cut off 1 m above ground on 7 April 2020. They were in six blocks, transported by six trucks to the new site, leaving all the ground roots behind. The induced ARs went along with the shoots and prop roots to the new site. The ground roots were dug out and the soil was fumigated. The six blocks of shoots were assembled into one tree with the ARs for recovery at the new planting site.

We used these 24 sets of relevant treatment combination values to make inference on the whole 48 values for the main effect of the 3 factors as a fractional factorial experiment [30,32]. Analysis of variance (ANOVA) was performed using R Studio version 1.2.1335 (R tools technology. Boston, MA, USA) to test the significance of the treatments. SigmaPlot version 10.0 (Systat software Inc. San Jose, CA, USA) was employed to create bar charts for reading convenience.

3. Results

The rooting%, root number, and root length data of the 24 treatment combinations are shown in Tables 2–4. The ANOVA results are in Table 5.

ANOVA revealed that RD sizes significantly influenced the rooting% of wounds, root number, and root length in the wounds. Different WM had significant effects on rooting%, root number, and root length in treatments. There was no significant interaction between RD and WM.

Table 2. The rooting percentage of wounding method (WM), root diameter (RD), and auxin solution treatment combinations on the *F. elastica* heritage tree.

Auxin Solutions ^y	WM ^z				CF ^z				GD ^z				RP ^z			
	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B
RD I ^x	92.5	100.0	100.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-
RD II	86.1	100.0	100.0	100.0	70.0	66.7	60.0	100.0	-	-	-	-	-	-	-	-
RD III	80.0	-	-	-	100.0	75.0	100.0	-	56.3	41.7	18.2	50.0	-	-	-	-
RD IV	-	-	-	-	-	-	-	-	34.9	16.7	50.0	66.7	-	-	-	-

^x RD sizes: RD I (RD < 2 cm), RD II (2 ≤ RD ≤ 4.3 cm), RD III (4.3 < RD ≤ 22 cm), and RD IV (>22 cm). ^y Auxin solutions and water: control group sprayed water onto to the wounds; 2NB: 2000 mg·L⁻¹ IBA + 2000 mg·L⁻¹ NAA; 2B: 2000 mg·L⁻¹ IBA; and 4B: 4000 mg·L⁻¹ IBA. ^z WM: CF, we cut off the aerial roots; GD, we used a knife to girdle the aerial roots deep into the xylem; and RP, we used a knife to incise a rectangular window on the surface of the prop roots of 4 × 2 cm deep into the xylem, then peeled off the skin of the window.

Table 3. The root numbers of wounding method (WM), root diameter (RD), and auxin solution treatment combinations on the *F. elastica* heritage tree.

Auxin Solutions ^y	WM ^z				CF ^z				GD ^z				RP ^z			
	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B
RD I ^x	14.6	19.2	21.7	15.9	-	-	-	-	-	-	-	-	-	-	-	-
RD II	17.4	20.0	17.5	20.0	8.5	6.7	5.0	6.7	-	-	-	-	-	-	-	-
RD III	19.0	-	-	-	7.0	7.5	10.0	-	3.8	2.9	0.9	5.8	-	-	-	-
RD IV	-	-	-	-	-	-	-	-	2.3	0.8	3.25	3.3	-	-	-	-

^x RD sizes: RD I (RD < 2 cm), RD II (2 ≤ RD ≤ 4.3 cm), RD III (4.3 < RD ≤ 22 cm), and RD IV (>22 cm). ^y Auxin solutions and water: control group sprayed water onto to the wounds; 2NB: 2000 mg·L⁻¹ IBA + 2000 mg·L⁻¹ NAA; 2B: 2000 mg·L⁻¹ IBA; and 4B: 4000 mg·L⁻¹ IBA. ^z WM: CF, we cut off the aerial roots; GD, we used a knife to girdle the aerial roots deep into the xylem; and RP, we used a knife to incise a rectangular window on the surface of the prop roots of 4 × 2 cm deep into the xylem, then peeled off the skin of the window.

Table 4. The root length of wounding method (WM), root diameter (RD), and auxin solution treatment combinations on *F. elastica* heritage tree.

Auxin Solutions ^y	WM ^z				CF ^z				GD ^z				RP ^z			
	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B	Control	2NB	2B	4B
RD I ^x	17.2	22.5	21.7	15.9	-	-	-	-	-	-	-	-	-	-	-	-
RD II	17.9	22.5	18.5	20.0	8.0	5.0	5.0	6.7	-	-	-	-	-	-	-	-
RD III	20.0	-	-	-	12.0	12.5	10.0	-	5.6	3.3	1.4	5.8	-	-	-	-
RD IV	-	-	-	-	-	-	-	-	2.8	1.3	4.0	5.0	-	-	-	-

^x RD sizes: RD I (RD < 2 cm), RD II (2 ≤ RD ≤ 4.3 cm), RD III (4.3 < RD ≤ 22 cm), and RD IV (>22 cm). ^y Auxin solutions and water: control group sprayed water onto to the wounds; 2NB: 2000 mg·L⁻¹ IBA + 2000 mg·L⁻¹ NAA; 2B: 2000 mg·L⁻¹ IBA; and 4B: 4000 mg·L⁻¹ IBA. ^z WM: CF, we cut off the aerial roots; GD, we used a knife to girdle the aerial roots deep into the xylem; and RP, we used a knife to incise a rectangular window on the surface of the prop roots in the size of 4 × 2 cm deep into the xylem, then peeled off the skin of the window. Root length unit in cm.

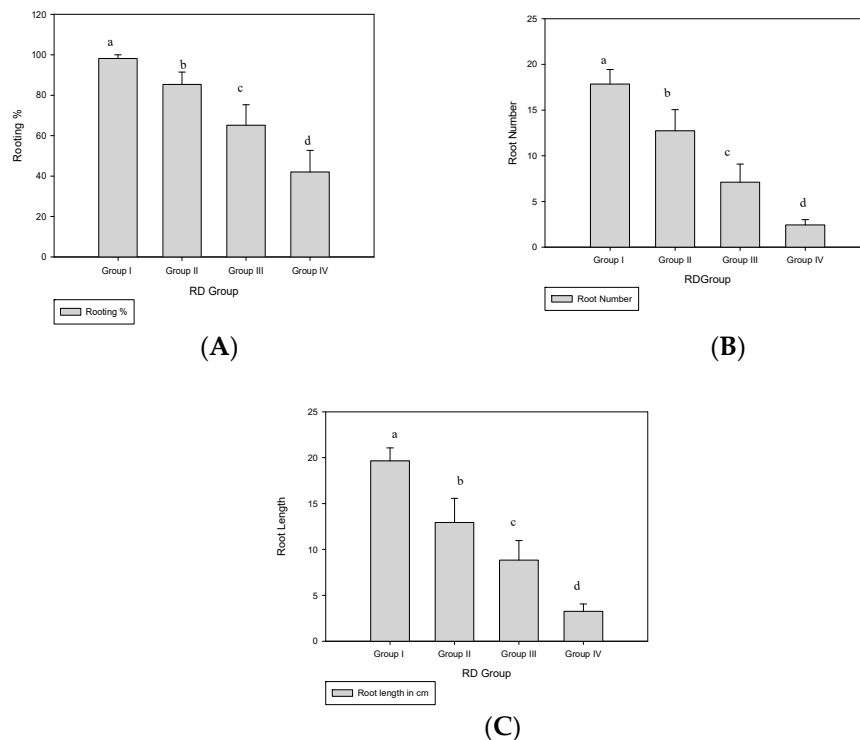
Table 5. ANOVA results of the main effects of wounding method (WM), root diameter (RD), and auxin solutions, and their interaction effect on the rooting, root length, and root number of *Ficus elastica* Roxb.

Source of Variation	Rooting%	Root Number	Root Length (cm)
Wounding method (WM)	***	***	***
Root diameter (RD)	***	***	***
Auxin solution	ns	ns	ns
WM × RD	ns	ns	ns

Significance code: ns: nonsignificant; *** $p < 0.001$.

3.1. Aerial Root Diameter Size (RD) Had Significant Effect on Adventitious Root Regeneration

ANOVA showed that RD groups had significant variance in terms of rooting%, root number, and root length. RD I showed the best rooting rate of 98.1%, followed by 85.3% for RD II, 65.1% for RD III, and 42.1% for RD IV. The smaller the RDs, the higher the rooting%. The rooting% of individual wounds within RD I were consistently higher than 92%. More variations in rooting% of individual wounds were found in RD II, III, and IV (Figure 2A).

**Figure 2.** Effect of root diameter on rooting (A), root number (B), and root length (C) of *Ficus elastica* Roxb. Means within a treatment followed by different letters significantly differ at $p < 0.05$ by the least significant difference (LSD) test.

Root numbers were 17.8, 12.7, 7.1, and 2.4 for RD I, II, III, and IV, respectively. RD I had the highest number of roots in the wounds. The root numbers in individual wounds within RD I were rather similar, while large in-group variations of wounds were found in RD II, III, and IV (Figure 2B).

Mean root lengths were 21 cm, 10.1 cm, 8.8 cm, and 3.3 cm for RD I, II, III, and IV, respectively. RD I had the longest roots in wounds. The mean root lengths in individual wounds within RD I were more consistent within the group, while large in-group variations of wounds were noticed in RD II, III, and IV (Figure 2C).

The rooting ability, including rooting%, root number in wounds, and mean root length in wounds, favored the smaller RD groups; RD I > RD II > RD III > RD IV.

3.2. Wounding Method (WM) Had Significant Effect on Adventitious Root Regeneration

The CF method had an average of 91.5% rooting percentage, followed by 82.9% for GD, and 41.8% for RP from the treatments. CF and GD had a similar rooting% and were better than RP. The CF rooting% was more than double that of RP (Figure 3A). However, there was no significant difference in rooting rate between CF and GD.

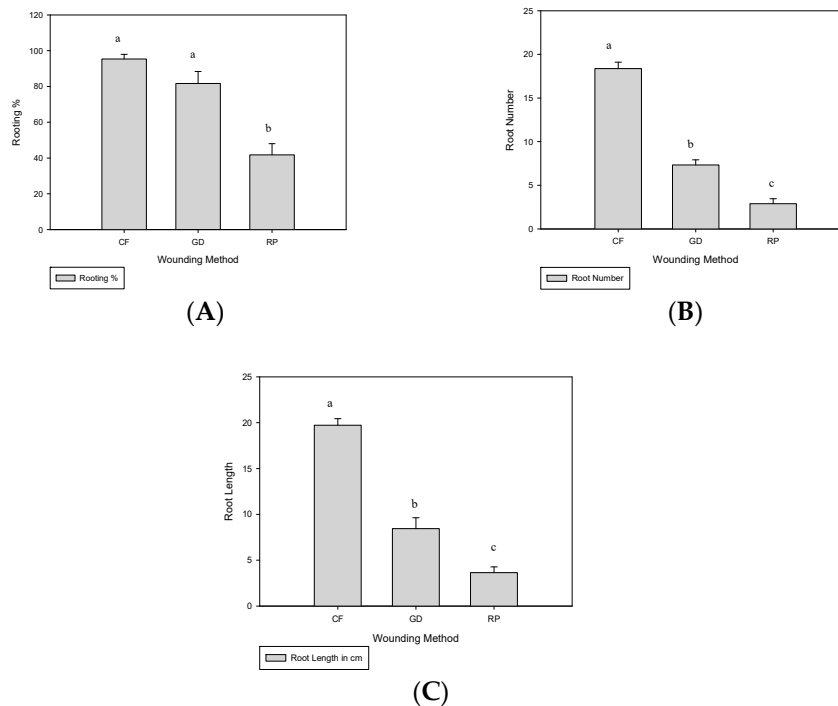


Figure 3. Effect of wounding method on rooting (A), root length (B), and root number (C) of *Ficus elastica* Roxb. Cutting off (CF): we cut off the aerial roots; Girdling (GD): we used a knife to girdle the aerial roots deep into the xylem; and rectangular shape peeling (RP): we used a knife to incise a rectangular window on the surface of the prop roots of 4 × 2 cm deep into the xylem, then peeled off the skin of the window.

The average root number was 18.5 roots for CF, trailed by 7.4 roots for GD, and 2.9 roots for RP. There was significant variation among the three wounding methods in terms of the root number. CF had far more roots in wounds than GD, and GD had more roots in wounds than RP (Figure 3B).

The mean of the 3 longest root length in wounds was 18.8 cm for CF, 8.2 cm for GD, and 3.6 cm for RP. There was significant variation among the three wounding methods. CF had longer roots in wounds than GD, and GD had longer roots than RP (Figure 3C).

The rooting performance of WM was ranked CF > GD > RP (Figure 3).

There was no significant interaction between RD and WM (Table 5).

3.3. Auxin Treatment Had Nonsignificant Effect on Adventitious Root Regeneration

The rooting% of different auxin solutions was 71% for water as control, 72.3% for 2B, 64.6% for 2 NB, and 79.2% for 4B. 4B seemed to have better results, but the difference was not significant (Figure 4A).

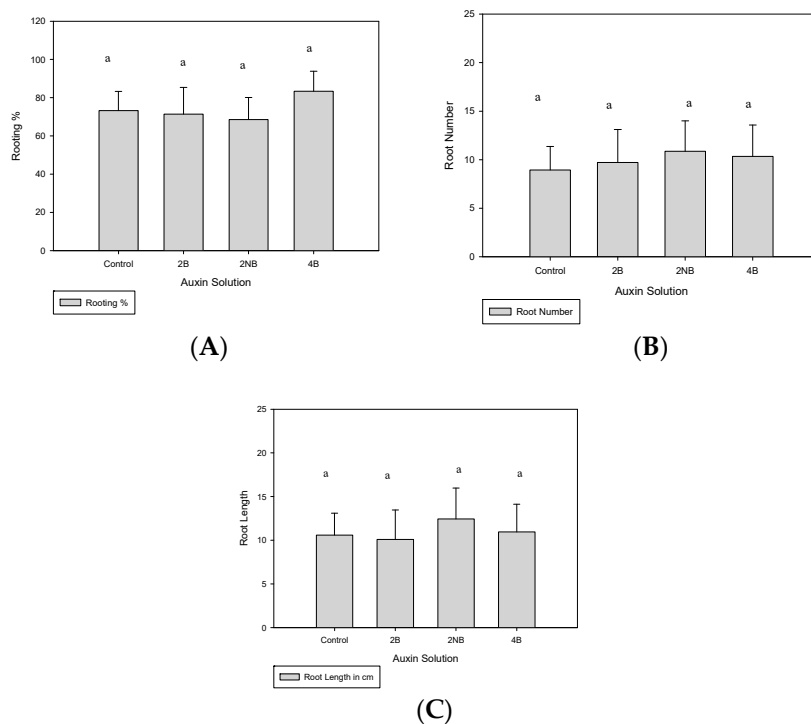


Figure 4. Effect of different auxin solutions and water on rooting (A), root length (B), and root number (C) of *Ficus elastica* Roxb. Control was water, 2B was 2000 mg·L⁻¹ IBA (Indole-3-butyric acid), 2NB was 2000 mg·L⁻¹ IBA + 2000 mg·L⁻¹ NAA (1-Naphthaleneacetic acid), and 4B was 4000 mg·L⁻¹ IBA. ^a There was no significant variation at $p \leq 0.05$ by the least significant difference (LSD) test among the auxin treatments.

The root numbers were 8.9 roots for the control, 9.7 roots for 2B, 10.9 roots for 2NB, and 10.3 roots for 4B. No significant variance among treatments of auxin solutions was found (Figure 4B).

The mean root lengths were 10.6 cm for the control, 10.1 cm for 2B, 10.3 cm for 2NB, and 11 cm for 4B. No significant variance among treatments of auxin solutions was found (Figure 4C).

4. Discussion

4.1. Root Diameter Groups (RD)

Overhanging aerial roots in their juvenile stage only have primary cell wall, protophloem, and protoxylem. When roots reach the ground, a secondary cell wall will develop. The juvenile characteristics of aerial roots allow them to generate ARs more easily than the mature prop roots that have established rings of phloem and xylem [33–38].

RD I and RD II contained overhanging aerial roots and developing prop roots that were juvenile. These were more vigorous than the mature larger prop roots in RD III and IV. That was why the smaller RD groups had a better rooting%, more root number, and longer root length in wounds than the larger RD groups.

4.2. Wounding Methods (WM)

Korvor and Testerink [39] reviewed stress-induced rooting and suggested that the stress level affected auxin activity. The rooting ability of different WMs comes from the severity of stress, accumulation of auxin, soluble sugar, and proteins. The CF method completely blocked the auxin and nutrition flux to accumulate at the upper side of the wound to induce ARs [23–26]. The higher level of accumulated soluble sugar at the wound can induce root primordia formation and root emergence,

accelerating the formation of callus and AR [40]. CF also blocked the upstream water and mineral supply from roots, creating drought stress. Drought stress prompts rooting [41].

GD blocked the downward phloem flow of auxin and nutrition. Auxin accumulated at the upper side of the wound due to polar transport and local biosynthesis to create a peak of IAA concentration at the wound, which induced ARs. However, the xylem flow of water was not cut off, so there was no drought stress like in CF, and the stress level was not that severe. The high endogenous IAA level and accumulation of soluble sugars facilitated adventitious rooting. Research found that the pericycle behind the cortex and endodermis layers initiated ARs. We suspect that some GD cuts were not deep enough to reach the endodermis and pericycle layer to change the cell fate and induce the formation of founder cells.

The RP method blocked only part of the nutrition and auxin flow, which resulted in the accumulation of auxin and nutrition at the wound under a lower stress level. The water and mineral supply were not creating drought stress, hence triggered fewer needs for ARs. Like in GD, the incised depth might not have reached the endodermis and pericycle layer for the parenchymal cells to change their fate to become founder cells. That was the probable reason for the inferior rooting results for the RP method compared to CF and GD.

The sap of *F. elastica* is a white, viscous latex that exudes upon wounding. It quickly coagulates when exposed to air and seals up wounds. Latex coagulation created a problem when coating auxin on the stem cuttings in Yates's experiments [42]. We saw the latex of *F. elastica* coagulated a few minutes after WM treatment. The wounds were healed quickly for RP because we only incised a window on the prop roots. CF and GD had less of a problem with sealing up wounds. That was the probable reason for the poor rooting performance of RPs.

4.3. Exogenous Auxin Solution

The rooting ability of exogenous auxin relies on the release of free auxins from the conjugates of endogenous auxin. Ludwig-Muller [43] found that the endogenous IAA within the plant itself was much more important for effecting the necessary auxin influence on the wound than the exogenous IBA applied.

Our experiment showed that the auxin solutions did not significantly increase the rooting ability compared with the control, which was consistent with the findings of Stefanci et al. [44] and Atefancic et al. [45]. They found that IBA treatments did not increase the percentage of rooting compared to the control for *Prunus avium* 'GiSelA 5' cuttings, although IBA treatment expanded the endogenous IAA pool in cuttings. They suggested that it was due to the higher level of free auxin in the IAA pool of *Prunus avium* 'GiSelA 5'. The level of the endogenous IAA pool was high enough to induce AR itself. Adding exogenous IBA reduced the callus% and increased the rooting quality but not the rooting%.

In our experiment, *F. elastica* heritage trees had an average rooting% of 72.7%, which means it could be considered an easy-to-root species. The easy-to-root species have abundant auxin conjugates to convert to IAA and slow metabolism of IAA to maintain a high level of IAA pool, which makes the impact of exogenous IAA relatively minor. It is the IAA pool (the total of the endogenous free IAA, IAA conjugates, and the exogenous IAA) induces the AR rooting process.

Most stem cuttings have only a limited number of buds or leaves to synthesize IAA; hence the endogenous auxin decays quickly after excision from shoots [46,47]. The exogenous auxin played an important role in raising the IAA level at the basal end of stem cuttings to trigger the AR process when the endogenous auxin decayed after leaving the mother shoots.

Therefore, with abundant endogenous free IAA and conjugated IAA, it is possible to induce AR without the exogenous IAA contribution to the pool. Ahkami et al. [48] reported several AR experiments with a high level of IAA-asp in the base of the stem cuttings, resulting in significant rooting without treating with auxin.

Auxin can be produced by developing and expanding leaves [49,50] in addition to the apical buds and young leaves. Our experimental heritage tree had a canopy spread of 40 square m, with many leaves and apical buds to produce endogenous IAAs. Such a large tree stored a large amount of IAA conjugates to sustain the high endogenous IAA level. The exogenous IBA/NAA applied in our experiment did not produce a significant result in the treatments.

The sensitivity to auxin is different between roots and shoots. Roots are more sensitive to auxin than shoots. We used auxin concentrations of 2000 mg·L⁻¹ ppm to 4000 mg·L⁻¹, which was reported to be optimal in many experiments for quick dip or spray applications for stem cuttings. However, this concentration for stem cuttings may be too high for the wounds on the aerial roots and prop roots. The high concentration probably inhibited the emergence and elongation of the ARs.

Another possibility is that the latex or sap of *F. elastica* could heal and seal up wounds quickly. It forms a barrier between the pericycle and the aerial root surface when spraying the auxin solutions onto the wound. It is likely that the auxin solutions failed to reach the pericycle and meristem layer.

Finally, some arborists believe that trees with latex such as *Ficus* spp. are easier to transplant. Cao and Lin [51] used PGRI (Plant Growth Regulators Immunoassay) and gas chromatography to test the latex of *Hevea brasiliensis* and reported abundant endogenous IAA. Because *F. elastica* has abundant latex, it is likely that it contains rich endogenous auxin and induced AR itself. It will be interesting to determine the level of endogenous IAA in *F. elastica* in future experiments.

5. Conclusions

In this study, we showed that cutting off or girdling young and vigorous aerial roots could induce and grow a sufficient number of ARs before transplanting. Our findings helped a rootless *F. elastica* heritage tree survive transplant shock and settle into a new habitat successfully. It will be useful to test if the practice works for the other *Ficus* heritage trees such as *F. microcarpa* and *F. benjamina* to rescue them from BRR. Furthermore, inducing adventitious roots before transplant can be applied to the large mature trees with root balls. Large root balls are required for balled and burlapped transplant especially transplanting in summer when desiccation due to transpiration is a major concern. That caused the root ball to be too heavy and expensive to protect its integrity during transplant. By inducing more adventitious roots before transplant, we can probably reduce the size of the root ball, enhance transplant success rate, and reduce costs.

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