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Article

# Micropropagation of *Philodendron* 'White Knight' Through Adventitious Shoot Regeneration

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**Abstract:** *Philodendron* 'White Knight' is a popular climbing evergreen plant typically propagated through stem cuttings. However, this method is slow and inefficient, making it challenging to meet the rising market demand. In vitro culture could enhance the multiplication rate of this cultivar. However, research on its in vitro propagation is limited. Therefore, the objectives of the present study were to investigate the impact of silver nanoparticles (Ag NPs) on the surface sterilization of *Philodendron* 'White Knight' petioles, to study the influence of plant growth regulators on adventitious shoot regeneration, shoot multiplication, and root induction. Aseptic petiole explants (100%) were obtained after treatment with 40 mg L<sup>-1</sup> Ag NPs for 60 minutes. The highest rate of adventitious shoot induction (52.6%) and the maximum shoot number (13.9 shoots per petiole) were achieved on Murashige and Skoog shoot multiplication B (MS-B) medium with 20 μM of 2-isopentenyl adenine (2-IP) and 5.0 μM of naphthalene acetic acid (NAA). The highest number of shoots (34 shoots per shoot tip) with a length of 5.1 cm was obtained on MS-B medium with 5.0 μM 2-IP and 2.5 μM NAA. The highest root induction (100%), the maximum root number (8.2 roots per shoot), and the greatest plantlet height (9.1 cm) were achieved on half-strength Murashige and Skoog medium containing indole-3-butyric acid (10.0 μM). The rooted plantlets of *Philodendron* 'White Knight' were transplanted into a substrate composed of 10% peat moss, 50% orchid stone, and 40% coconut husk chips, and acclimatized in a greenhouse environment, achieving a survival rate of 100%. This micropropagation protocol can be used for the commercial propagation of *Philodendron* 'White Knight' and related species.

**Keywords:** auxin; cytokinin; in vitro propagation; petiole; silver nanoparticles

## 1. Introduction

The genus *Philodendron* Schott, belonging to the Araceae family, comprises 625 species native to South America, Central America, the Caribbean, and parts of Colombia, French Guiana, and Guyana [1]. Many *Philodendron* species are popular indoor plants due to their attractive foliage and low maintenance requirements, with several cultivars (cv.) emerging in recent decades [2]. *Philodendron* cultivars with orange, red, and yellow foliage, either variegated or non-variegated, tend to attract more attention and command higher market value, especially the variegated varieties (Chen et al. 2002, Oboni and Hossain 2025). Although they are typically propagated through stem cuttings and seeds, these methods do not meet market demand due to the lack of healthy plant materials and the lengthy propagation process [2–4]. In contrast, in vitro propagation techniques offer excellent alternatives for mass propagating *Philodendron* species and cultivars, using less plant material and allowing for year-round production with reduced space and a shorter time to satisfy market demand [2].

Micropropagation is a powerful in vitro plant culture technique widely used for the commercial propagation of various ornamental plants [5], including *Philodendron billietiae* Croat [6], *Philodendron*

*bipinnatifidum* Schott ex Endl. [7], *Philodendron erubescens* cv. Pink Princess [4], *Philodendron pertusum* Kunth & C.D.Bouché [8], *Philodendron selloum* Koch [9], *Philodendron tuxtlanum* G.S.Bunting [10], *Philodendron* cv. Birkin [11], Imperial Green, Imperial Red, and Imperial Rainbow [2], as well as Blue Mystic, Painted Lady, Pink Prince, Pluto, Royal Queen, and Green Emperor [3]. The authors have utilized various explants, including axillary buds, axillary shoots, leaf laminae, nodes, petioles, shoot tips, and stem nodal segments, cultured on media containing a range of plant growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2-isopentenyl adenine (2IP), benzyladenine (BA)/6-benzylaminopurine (BAP), indole-3-butyric acid (IBA), kinetin (KN), naphthalene acetic acid (NAA), and thidiazuron (TDZ), either alone or in combination, to achieve adventitious shoot regeneration or axillary shoot multiplication. However, shoot induction depends on explant type, culture media composition, genotype, and plant growth regulators [2]. Therefore, the development of a micropropagation protocol for the mass production of a new cultivar of *Philodendron* is necessary.

Micropropagation involves multiple stages, including the establishment of aseptic culture, shoot multiplication, shoot elongation, rooting, and acclimatization [5]. Although all stages are equally important, the initial establishment of in vitro plant culture is a critical stage, and contamination poses a significant challenge. Success depends on effectively disinfecting explant surfaces while preserving their viability [12]. *Philodendron* explants were often surface disinfected with 70-95% ethyl alcohol (EtOH), 0.16-5.0% sodium hypochlorite (NaOCl), and 0.05-0.1% mercuric chloride (HgCl<sub>2</sub>) [3,5,8,11,13,14]. Nanoparticles (NPs) effectively combat various bacteria, fungi, and viruses that can contaminate plant explants [15]. Their small size allows them to penetrate microbial cell walls easily, making them more efficient than traditional disinfectants. NPs can be used in lower concentrations, reducing toxicity risks to plant tissues while remaining effective. Furthermore, they are generally more environmentally friendly than HgCl<sub>2</sub>, making them a promising alternative for disinfecting plant tissue culture explants [16,17]. Silver (Ag) NPs are environmentally friendly chemicals that have been used to eliminate microorganisms in plant tissue culture [14] because of their powerful antimicrobial properties. Recently, Lara-Ascencio et al. [17] demonstrated that the Ag NPs treatment reduces the presence of contaminants in the *Philodendron xanadu* Croat explants. *Philodendron* 'White Knight' is a climbing evergreen plant native to South America. It is one of the popular *Philodendron* cultivars due to its variegated foliage and is used as both an indoor and garden plant. It is commonly multiplied through stem cutting; however, vegetative propagation is slow and requires effective alternative methods for multiplying this important cultivar to meet market demand. So far, no reports have documented the in vitro propagation of *Philodendron* 'White Knight'. Thus, this study aimed to develop an efficient micropropagation protocol for the mass production of *Philodendron* 'White Knight' through adventitious shoot regeneration using petiole explants. The objectives of the present study were to evaluate the effect of Ag NPs on the surface disinfection of petiole explants and to investigate the effect of plant growth regulators (PGRs) on adventitious shoot regeneration, shoot multiplication, and rooting of *Philodendron* 'White Knight'.

## 2. Materials and Methods

### 2.1. Plant Materials and Surface Disinfection

Petioles were collected from fully developed leaves of three-year-old *Philodendron* 'White Knight' shoots cultivated in a polyhouse. The cut ends of the petioles were sealed, thoroughly cleaned under running tap water for 20 minutes, and then washed with distilled water (DH<sub>2</sub>O) containing 0.15% (w/v) polyvinylpyrrolidone (PVP). Petioles were surface disinfected in laminar airflow by treating them with 70% (v/v) EtOH for 30 seconds; rinsed three times in sterile DH<sub>2</sub>O containing 0.15% PVP (SDH<sub>2</sub>O-PVP) and 1.0% (v/v) NaOCl solution for 12 minutes; and washed five times with SDH<sub>2</sub>O-PVP. To study the effect of Ag NPs (< 100 nm particle size, 99.5% purity, Sigma-Aldrich) on surface disinfection, the NaOCl-treated petioles were immersed in 10, 20, 40, or 80 mg L<sup>-1</sup> Ag NPs for 30, 60, or 90 minutes and then washed five times with SDH<sub>2</sub>O-PVP. The sealed ends were discarded, and the petioles were cut transversely into 0.4-0.6 mm segments and then cultured on Murashige and

Skoog shoot multiplication B (MS-B) medium (Duchefa Biochemie, The Netherlands) containing 8.0  $\mu\text{M}$  BA. All media contained 0.6 g L<sup>-1</sup> activated charcoal (AC), 30 g L<sup>-1</sup> sucrose, 0.8 g L<sup>-1</sup> agar, and the pH of the media was adjusted to 5.70-5.80 before autoclaving at 123 °C for 23 minutes. For each treatment, 15 petiole explants with three replicates were used, and the experiment was repeated twice. The cultures were kept at 23 ± 1°C under a 12-hour photoperiod with a photosynthetic photon flux density (PPFD) of 18-23  $\mu\text{Mol s}^{-1} \text{m}^{-2}$ . The rates of contamination and survival of *Philodendron* 'White Knight' explants were recorded after 21 days of incubation. Explants that turned black were deemed dead, while those that remained purple or increased in size were considered alive.

### 2.2. Effect of PGRs on Adventitious Shoot Regeneration

To study the impact of PGRs on adventitious shoot induction, the explants were prepared from petioles treated with 40 mg L<sup>-1</sup> Ag NPs for 60 minutes and cultured on MS-B medium containing 5, 10, 20, or 30  $\mu\text{M}$  of BA or 2-IP combined with 2.5 or 5.0  $\mu\text{M}$  of NAA or IBA. The medium MS-B, devoid of PGRs, served as a control. For each treatment, 20 petiole explants with three replicates were used, and the experiment was repeated twice. The cultures were kept at 23 ± 1°C under a 12-hour photoperiod with a PPFD of 35-40  $\mu\text{Mol s}^{-1} \text{m}^{-2}$ . The rate of shoot formation and the number of shoots per *Philodendron* 'White Knight' petiole were recorded after 13 weeks of incubation.

### 2.3. Effect of Combinations of 2-IP and NAA on Shoot Multiplication and Shoot Elongation

The adventitious shoot buds were transferred to PGR-free MS-B medium and subcultured at four-week intervals. After three subcultures, the in vitro developed shoots were utilized to obtain shoot tips explants. For shoot multiplication, shoot tips were cultured on the MS-B medium containing 2.5, 5.0, or 10.0  $\mu\text{M}$  2-IP combined with 2.5  $\mu\text{M}$  NAA. The medium MS-B, devoid of 2-IP and NAA, served as a control. For each treatment, 30 shoot buds with three replicates were used, and the experiment was repeated twice. The cultures were kept at 23 ± 1°C under a 14-hour photoperiod with a PPFD of 45-50  $\mu\text{Mol s}^{-1} \text{m}^{-2}$ . The number of shoots per *Philodendron* 'White Knight' shoot bud, and shoot length (mm) were recorded after 13 weeks of incubation.

### 2.4. Effect of IBA and NAA on Rooting

Well-developed shoots separated from the shoot clusters were transferred to half-strength Murashige and Skoog (MS) [18] medium containing 0, 2.5, 5.0, 10.0, or 20.0  $\mu\text{M}$  NAA or IBA to induce roots. For each treatment, 30 shoots with three replicates were used, and the experiment was repeated twice. The cultures were kept at 23 ± 1°C under a 14-hour photoperiod with a PPFD of 60-65  $\mu\text{Mol s}^{-1} \text{m}^{-2}$ . The rate of root induction and the number of roots per *Philodendron* 'White Knight' shoot, were recorded after 8 weeks of incubation.

### 2.5. Acclimatization

The well-developed 300 *Philodendron* 'White Knight' plantlets were separated from the rooting medium, thoroughly washed to remove any traces of it, and then transplanted into trays containing a mixture of peat moss (10%), orchid stone (50%), and coconut husk chips (40%). They were subsequently acclimatized in a greenhouse at a temperature of 20-25 °C and a relative humidity of 95-100% for 2 weeks, which was gradually reduced to 60%. The plants were fertigated with ¼ MS nutrients, and the survival of the plantlets was recorded after 6 weeks of cultivation.

### 2.6. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) tests, and means were distinguished by Duncan's multiple range test (DMRT) at  $p \leq 0.05$ . The statistical analyses were performed using SAS Release 9.4.

### 3. Results and Discussion

#### 3.1. Impact of Ag NPs on the Surface Disinfection of *Philodendron* 'White Knight' Petiole Explants

The petiole explants of *Philodendron* 'White Knight' treated with EtOH and NaOCl (control) yielded 25.9% aseptic explants, while 74.1% of the petioles were contaminated with bacteria and fungi; however, the survival rate of *Philodendron* 'White Knight' explants remained unaffected. The percentage of aseptic cultures increased when the petioles were treated with Ag NPs compared to the control (Table 1). The positive effect of Ag NPs on the surface disinfection of explants has also been documented in *Ardisia mamillata* [12], *Begonia tuberosus* [19], *Limonium sinuatum* 'White' [20], *Monstera deliciosa* 'Thai Constellation' [21], *Ocimum basilicum* 'Italian Large Leaf', *Salvia farinacea*, and *Thymus vulgaris* [22]. Increasing the concentration of Ag NPs and soaking period reduced explant contamination; however, higher concentrations and a longer soaking period also resulted in a decrease in the explant survival rate (Table 1). Similar results have been observed in *A. mamillata* [12], *Kaempferia parviflora* [23], *M. deliciosa* 'Thai Constellation' [21], *O. basilicum* 'Italian Large Leaf', *S. farinacea*, and *T. vulgaris* [22]. In this study, soaking *Philodendron* 'White Knight' explants in 10 mg L<sup>-1</sup> silver nanoparticles for 30, 60, and 90 minutes resulted in 29.3%, 40.1%, and 51.4% of aseptic explants, respectively. Additionally, the survival rate (100%) of explants remained unaffected. Among the various Ag NPs treatments, 40 mg L<sup>-1</sup> Ag NPs for 60 minutes was found to be the most effective, resulting in 100% aseptic explants with a 100% survival rate. In contrast, the survival rate of explants decreased when treated with 40 mg L<sup>-1</sup> Ag NPs for 90 minutes (Table 1).

**Table 1.** Effects of Ag NPs concentration and soaking period on the surface disinfection of *Philodendron* 'White Knight' Petiole Explants.

Ag NPs (mg L <sup>-1</sup> )	Soaking Period (min)	Aseptic Explant (%)	Survival (%)
Control	0	25.9 ± 1.6 h	100 ± 0.0 a
10	30	29.3 ± 1.2 h	100 ± 0.0 a
20	30	34.8 ± 1.2 g	100 ± 0.0 a
40	30	47.8 ± 1.3 e	100 ± 0.0 a
80	30	54.7 ± 1.7 d	100 ± 0.0 a
10	60	40.1 ± 1.4 f	100 ± 0.0 a
20	60	72.1 ± 2.1 c	100 ± 0.0 a
40	60	100 ± 0.0 a	100 ± 0.0 a
80	60	100 ± 0.0 a	77.0 ± 3.0 d
10	90	51.4 ± 1.5 d	100 ± 0.0 a
20	90	81.2 ± 1.9 b	92.7 ± 1.5 b
40	90	100 ± 0.0 a	84.9 ± 2.4 c
80	90	100 ± 0.0 a	68.2 ± 2.3 e

Means ± standard errors in a column that share the same letter(s) are not significantly different, as determined by DMRT at  $p \leq 0.05$ .

#### 3.2. Influence of PGRs on Adventitious Shoot Regeneration from Petiole Explants of *Philodendron* 'White Knight'

##### 3.2.1. Combination of 2-IP and Auxins (NAA and IBA)

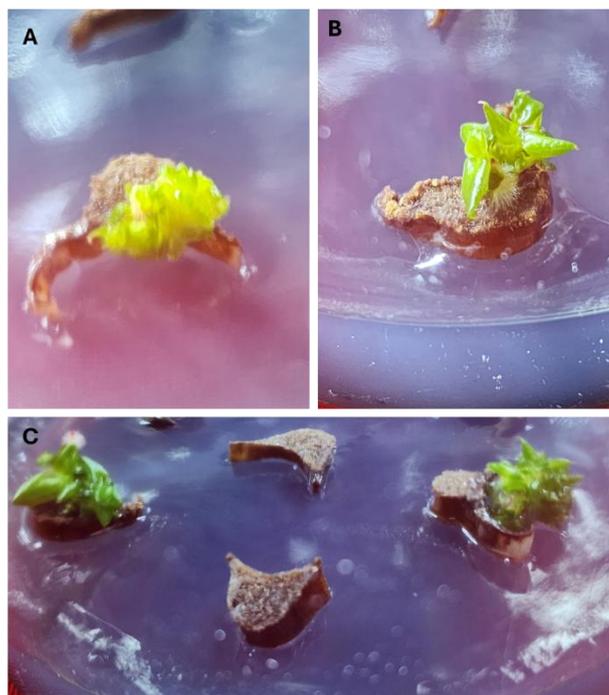
Direct adventitious shoot regeneration is an effective technique that enhances efficiency and sustainability in commercial plant propagation [24] and is essential for *Agrobacterium*-mediated genetic transformation [25]. It has been reported that the petiole segments of *Philodendron scandens* [26] and *Philodendron* 'Imperial Green' [2] do not produce shoots. In this study, the petiole explants of *Philodendron* 'White Knight' did not yield adventitious shoots on the MS-B medium lacking PGRs or containing 5.0 μM 2-IP plus auxins, and they died after 13 weeks of culture. However, adventitious shoot buds developed from the explants within 8 weeks of culture when the MS-B medium was fortified with higher concentrations of 2-IP and auxins (Figure 1A and B). It has been revealed that supplementing both auxin and cytokinin can induce adventitious shoot regeneration in several

members of the Araceae family, including *Aglonema* 'Lady Valentine' [27], *Anthurium andraeanum* [28], *Epipremnum aureum* [29], *Spathiphyllum wallisii* 'Domino' [30], and *Zamioculcas zamiifolia* [31]. When the MS-B medium was fortified with 10-30  $\mu\text{M}$  of 2-IP and 2.5  $\mu\text{M}$  of NAA, 15-38.9% of *Philodendron* 'White Knight' petioles developed 2.8-7.0 adventitious shoots per explant (Figure). The highest adventitious shoot induction (52.6%) and the maximum shoot production (13.9 shoots per explant) were achieved on MS-B medium with 20  $\mu\text{M}$  of 2-IP and 5.0  $\mu\text{M}$  of NAA (Table 2). This result surpassed previous findings, in which only 2.8% of petioles from *Philodendron* Imperial Red and 11.1% from Imperial Rainbow produced shoots [2]. When 2-IP was combined with IBA, 14.5-34.9% of *Philodendron* 'White Knight' petioles developed 1.9-6.3 adventitious shoots per explant (Table 2). The optimal shoot production (34.9% explants induced 6.3 shoots per petiole) was obtained on MS-B medium containing 20  $\mu\text{M}$  of 2-IP and 5.0  $\mu\text{M}$  of IBA.

**Table 2.** Effect of different concentrations of 2-IP combined with NAA and IBA on adventitious shoot regeneration from petiole explants of *Philodendron* 'White Knight'.

2-IP	PGRs ( $\mu\text{M}$ )		Shoot Induction (%)	Number of Shoots/Explant
	NAA	IBA		
0	0.0	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 h
5	2.5	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 h
10	2.5	0.0	15.0 $\pm$ 1.9 h	2.8 $\pm$ 0.4 fg
20	2.5	0.0	38.9 $\pm$ 1.7 bc	7.0 $\pm$ 0.7 bc
30	2.5	0.0	33.0 $\pm$ 1.4 de	4.7 $\pm$ 0.3 de
5	5.0	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 h
10	5.0	0.0	30.0 $\pm$ 2.2 ef	4.2 $\pm$ 0.7 def
20	5.0	0.0	52.6 $\pm$ 2.0 a	13.9 $\pm$ 1.0 a
30	5.0	0.0	42.2 $\pm$ 2.5 b	8.4 $\pm$ 1.0 b
5	0.0	2.5	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 h
10	0.0	2.5	14.5 $\pm$ 1.3 h	1.9 $\pm$ 0.4 g
20	0.0	2.5	21.2 $\pm$ 1.9 g	5.4 $\pm$ 0.7 cd
30	0.0	2.5	17.8 $\pm$ 1.8 gh	3.5 $\pm$ 0.6 efg
5	0.0	5.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 h
10	0.0	5.0	20.6 $\pm$ 1.2 g	2.2 $\pm$ 0.4 g
20	0.0	5.0	34.9 $\pm$ 2.0 cd	6.3 $\pm$ 0.6 c
30	0.0	5.0	28.2 $\pm$ 2.1 f	3.4 $\pm$ 0.5 efg

Means  $\pm$  standard errors in a column that share the same letter(s) are not significantly different, as determined by DMRT at  $p \leq 0.05$ .



**Figure 1.** Adventitious shoots developed from *Philodendron* 'White Knight' petioles cultured on MS-B medium with 2-IP and NAA (A), 2-IP and IBA (B), BA and IBA (C).

### 3.2.2. Combination of BA and Auxins (NAA and IBA)

When BA (10-30  $\mu\text{M}$ ) was combined with NAA (2.5 or 5.0  $\mu\text{M}$ ), 8.3-38.8% of petioles produced 1.2-4.8 shoots per explant. The maximum adventitious shoot induction (38.8%) and the highest number of shoots (4.8 shoots per petiole) were achieved on MS-B medium containing 20  $\mu\text{M}$  BA and 2.5  $\mu\text{M}$  NAA. However, the shoot production potential of *Philodendron* 'White Knight' petioles decreased when the medium MS-B was fortified with BA and 5.0  $\mu\text{M}$  NAA (Table 3). The presence of BA and IBA in the MS-B medium enhanced shoot production, with the best adventitious shoot induction at 46.1% and the maximum number of shoots at 7.1 shoots per petiole achieved on MS-B medium with 20  $\mu\text{M}$  BA and 5.0  $\mu\text{M}$  IBA (Table 3, Figure 1C). The results (Tables 2 and 3) indicated that adventitious shoot production from petiole explants of *Philodendron* 'White Knight' was influenced by the type of cytokinin and auxin. The petioles of *Philodendron* 'White Knight' showed the best response to MS-B medium containing 2-IP and NAA. The presence of 2-IP and NAA in the culture medium has been shown to effectively promote optimal shoot formation from petioles of *Bixa orellana* [32].

**Table 3.** Effect of different concentrations of BA combined with NAA and IBA on adventitious shoot regeneration from petiole explants of *Philodendron* 'White Knight'.

BA	PGRs ( $\mu\text{M}$ )		Shoot Induction (%)	Number of Shoots/Explant
	NAA	IBA		
0	0.0	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 g
5	2.5	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 g
10	2.5	0.0	22.8 $\pm$ 1.9 d	2.2 $\pm$ 0.3 de
20	2.5	0.0	38.8 $\pm$ 2.0 b	4.8 $\pm$ 0.4 b
30	2.5	0.0	30.9 $\pm$ 1.2 c	2.9 $\pm$ 0.4 cd
5	5.0	0.0	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 g
10	5.0	0.0	10.8 $\pm$ 1.3 gh	1.6 $\pm$ 0.2 ef
20	5.0	0.0	16.7 $\pm$ 1.7 ef	3.0 $\pm$ 0.4 cd
30	5.0	0.0	8.3 $\pm$ 1.2 h	1.2 $\pm$ 0.2 f
5	0.0	2.5	0.0 $\pm$ 0.0 i	0.0 $\pm$ 0.0 g

10	0.0	2.5	18.9 ± 1.4 e	1.8 ± 0.2 ef
20	0.0	2.5	24.8 ± 1.6 d	2.7 ± 0.2 cd
30	0.0	2.5	13.9 ± 1.4 fg	1.4 ± 0.2 ef
5	0.0	5.0	0.0 ± 0.0 i	0.0 ± 0.0 g
10	0.0	5.0	32.2 ± 2.2 c	3.3 ± 0.4 c
20	0.0	5.0	46.1 ± 1.8 a	7.1 ± 0.6 a
30	0.0	5.0	39.3 ± 1.5 b	4.6 ± 0.4 b

Means ± standard errors in a column that share the same letter(s) are not significantly different, as determined by DMRT at  $p \leq 0.05$ .

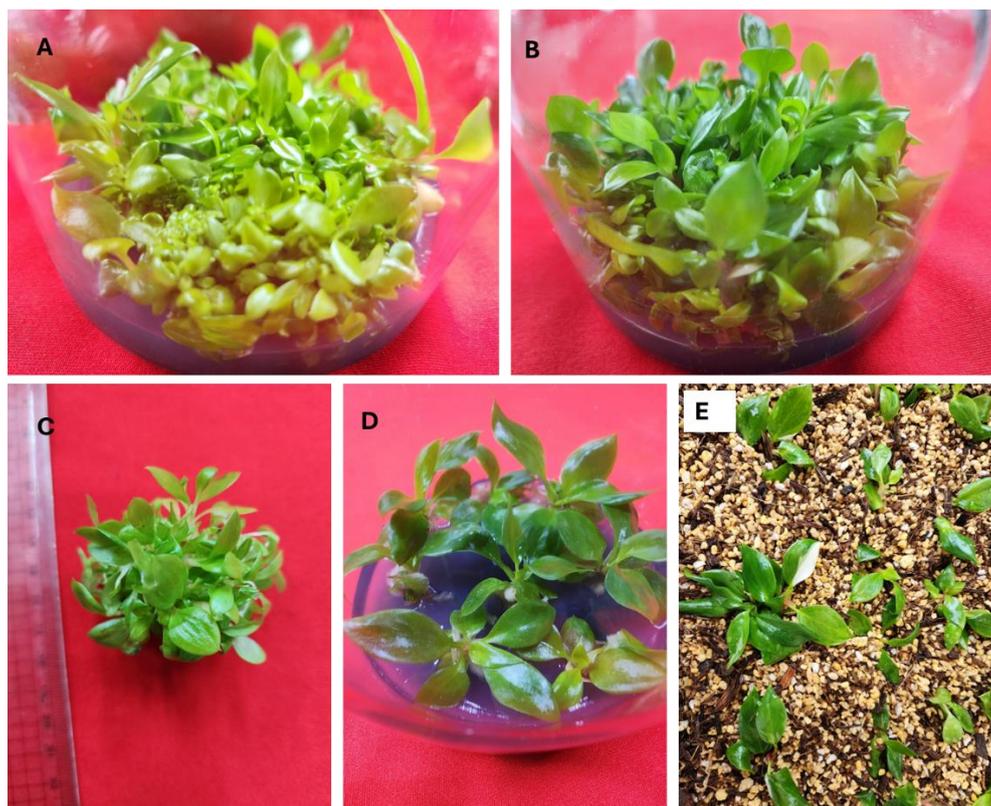
### 3.3. Combination of 2-IP and NAA on *Philodendron* 'White Knight' Shoot Multiplication

Chen et al. [2] utilized in vitro regenerated shoots of *Philodendron* cultivars as the explant (node) source to examine the impact of cytokinins (BA, KN, and TDZ) on shoot multiplication. Similarly, the shoot multiplication of *Philodendron* cultivars or species has been achieved using in vitro derived nodes, protocorm-like bodies, and shoot tips on culture media containing BA alone or in combination with auxins [4,6,7,33]. These studies have demonstrated that BA/BAP, whether used alone or in combination with auxins, is more effective for shoot multiplication than other cytokinin treatments. In this study, the results indicated that a combination of 2-IP and NAA produced a higher number of shoots. Therefore, the impact of various concentrations of 2-IP combined with 2.5  $\mu\text{M}$  NAA was examined on multiple shoot induction from in vitro derived shoot tips of *Philodendron* 'White Knight'. The shoot tips of *Philodendron* 'White Knight' cultured on MS-B medium devoid of 2-IP and NAA produced multiple shoots (4.9 shoots per shoot tip) with a length of 6.4 cm (Table 4). In contrast, Alawaadh et al. [7] reported that shoot tip explants of *Philodendron bipinnatifidum* developed only a single shoot on PGR-free media. On the other hand, *P. erubescens* 'Pink Princess' protocorm-like bodies developed 4.4 shoots on PGR-free medium [4]. The addition of 2-IP and NAA to MS-B medium significantly increased the number of shoots per *Philodendron* 'White Knight' shoot tip compared to the control, while the length of the shoot significantly decreased in a dose-dependent manner. The highest number of shoots (34 shoots per shoot tip) with a length of 5.1 cm was obtained on MS-B medium containing 5.0  $\mu\text{M}$  2-IP and 2.5  $\mu\text{M}$  NAA (Table 4, Figure 2A-C). Likewise, shoot multiplication has been achieved in the presence of 2-IP and NAA in *Allium sativum* [34], *Philodendron bipinnatifidum* [7], and *Philodendron goeldii* [35].

**Table 4.** Effect of concentrations of 2-IP along with NAA on shoot multiplication.

2-IP ( $\mu\text{M}$ )	NAA ( $\mu\text{M}$ )	Number of Shoots/Explant	Shoot Length (cm)
0.0	0.0	4.9 ± 0.7 d	6.4 ± 0.7 a
2.5	2.5	11.2 ± 1.0 c	5.1 ± 0.7 ab
5.0	2.5	34.0 ± 2.4 a	3.7 ± 0.6 bc
10.0	2.5	21.4 ± 1.5 b	2.9 ± 0.5 c

Means ± standard errors in a column that share the same letter(s) are not significantly different, as determined by DMRT at  $p \leq 0.05$ .



**Figure 2.** Shoot multiplication, rooting and acclimatization. Shoot tips of *Philodendron* 'White Knight' developed multiple shoots on MS-B medium containing 5.0  $\mu\text{M}$  2-IP and 2.5  $\mu\text{M}$  NAA after 6 weeks (A), and 13 weeks (B) of cultivation; C) Multiple shoot cluster; D) Regenerated shoots of *Philodendron* 'White Knight' developed roots on  $\frac{1}{2}$  MS medium with 10.0  $\mu\text{M}$  IBA; D) Acclimatized *Philodendron* 'White Knight' plantlets. .

#### 3.4. Effect of IBA and NAA on Rooting

Rooting is a crucial stage of micropropagation influenced by the composition of the shoot induction medium, the rooting medium composition, the type and concentration of auxins, as well as the genotype and plant species [5]. In vitro rooting of *Philodendron* cultivars and species has been achieved on basal medium without [3,9] or with auxins [2,4,6–8,13]. Previous studies have shown that in vitro rooting of *Philodendron* cultivars and species is best achieved on a medium containing IBA or NAA [2,4,6–8,13]. Thus, this study investigated the influence of IBA and NAA on the rooting of *Philodendron* 'White Knight' shoots. In the control medium (auxin-free  $\frac{1}{2}$  MS), 18.9% of shoots successfully developed an average of 2.2 roots, with an average plantlet height of 3.9 cm (Table 5). The inclusion of IBA or NAA at concentrations ranging from 2.5 to 20.0  $\mu\text{M}$  to the  $\frac{1}{2}$  MS medium significantly increased the rooting percentage (52.2–100%), the average number of roots (2.7–8.2), and the plantlet height (4.3–5.8) compared to the control. However, it is noteworthy that elevated concentrations of NAA, particularly at 20.0  $\mu\text{M}$ , resulted in a marked decrease in plantlet height, which measured only 3.1 cm compared to the control. Of the various concentrations (2.5–20.0  $\mu\text{M}$ ) of auxins tested, 10.0  $\mu\text{M}$  IBA resulted in the highest root induction (100%), the maximum root number (8.2 roots per shoot), and the greatest plantlet height (9.1 cm) (Table 5). Among the two auxins tested, IBA was found to be the best for in vitro rooting of *Philodendron* 'White Knight' shoots (Figure 2D). The beneficial effects of IBA on the rooting of Araceae members have been shown in *Aglaonema* 'Lady Valentine' [36], *M. deliciosa* 'Thai Constellation' [21], *Philodendron* cultivars [2], *Philodendron* 'Birkin' [11], *P. erubescens* 'Pink Princess' [4], *P. pertusum* [8], *Philodendron xanadu* [17], and *Spathiphyllum wallisii* 'Domino' [30]. In this study, most shoots that regenerated in vitro during the rooting phase displayed normal green leaves; however, a subset of shoots exhibited variegated leaves (Figure 3).

**Table 5.** Effect of Auxins on Rooting of *Philodendron* 'White Knight' Shoots.

IBA	Auxin ( $\mu\text{M}$ )		Rooting (%)	Number of Roots/Explant	Plantlet Height (cm)
	NAA	IBA			
0.0	0.0	0.0	18.9 $\pm$ 2.0 f	2.2 $\pm$ 0.1 d	3.9 $\pm$ 0.3 ef
2.5	0.0	0.0	52.2 $\pm$ 2.1 e	3.6 $\pm$ 0.4 cd	5.8 $\pm$ 0.2 cd
5.0	0.0	0.0	75.1 $\pm$ 3.1 c	4.7 $\pm$ 0.4 bc	7.3 $\pm$ 0.4 b
10.0	0.0	0.0	100 $\pm$ 0.0 a	8.2 $\pm$ 0.7 a	9.1 $\pm$ 0.3 a
20.0	0.0	0.0	87.2 $\pm$ 2.6 b	3.1 $\pm$ 0.3 d	6.4 $\pm$ 0.3 c
0.0	2.5	0.0	55.8 $\pm$ 3.2 e	2.7 $\pm$ 0.4 d	5.3 $\pm$ 0.5 d
0.0	5.0	0.0	91.7 $\pm$ 1.7 b	5.2 $\pm$ 0.3 b	4.3 $\pm$ 0.2 e
0.0	10.0	0.0	74.4 $\pm$ 1.8 c	3.2 $\pm$ 0.5 d	3.6 $\pm$ 0.2 ef
0.0	20.0	0.0	65.9 $\pm$ 2.1 d	2.9 $\pm$ 0.4 d	3.1 $\pm$ 0.2 f

Means  $\pm$  standard errors (SE) in a column that share the same letter(s) are not significantly different, as determined by DMRT at  $p \leq 0.05$ .



**Figure 3.** In vitro regenerated normal and variegated plantlets of *Philodendron* 'White Knight'.

### 3.5. Acclimatization

Acclimatization is the final stage of micropropagation, influenced mainly by the growth substrate. *Philodendron* cultivars and species have been successfully acclimatized using a mixture of various substrates such as peat moss and perlite [2,7], river sand, farmyard manure, and soil [3], perlite, vermiculite, and peat moss cocopeat [4], sand, soil, and farmyard manure [8], and coir and *Luffa* sponge [13]. In this study, both the normal and variegated plantlets of *Philodendron* 'White Knight' were successfully transplanted into a substrate composed of 10% peat moss, 50% orchid stone, and 40% coconut husk chips and acclimatized (Figure 2E) in a greenhouse environment, achieving a survival rate of 100%.

## 4. Conclusions

For the first time, we have established an efficient micropropagation protocol for the commercial propagation of *Philodendron* 'White Knight'. Our findings demonstrate that a combination of cytokinin and auxin is essential for inducing adventitious shoot induction and maximizing shoot multiplication. The in vitro grown shoots of *Philodendron* 'White Knight' exhibit optimal rooting when treated with IBA. In vitro regenerated normal and variegated plantlets can be utilized in

breeding programs aimed at enhancing this cultivar. To ensure the genetic integrity of the acclimatized plantlets, molecular analysis will be conducted.

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## Abbreviations

The following abbreviations are used in this manuscript:

Ag NP	silver nanoparticles
MS-B	Murashige and Skoog shoot multiplication B
2-IP	2-isopentenyl adenine
IBA	indole-3-butyric acid
NAA	naphthalene acetic acid
BA	benzyladenine
PPFD	photosynthetic photon flux density

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