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Optimization of plant growth regulators for *in vitro* mass propagation of *Philodendron* cv. Birkin through shoot tip culture

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ABSTRACT

In vitro cultures provide a promising tool for large-scale multiplication of valuable plant species with an important role in the global ornamentals industry. In the current investigation, a rapid and efficient protocol was described for *in vitro* mass propagation of attractive ornamental plant *Philodendron* cv. Birkin through shoot tip culture. In shoot proliferation stage, 11 treatments of plant growth regulators were evaluated. The highest shoot multiplication was achieved by culturing explants in MS medium containing 3 mg/l benzyladenine (BA) and 0.5 mg/lit indole-3-butyric acid (IBA), resulting in average of 16.65 shoots per explant over a four weeks period. To induce adventitious root formation, the regenerated shoots were subsequently transferred to MS media supplemented with various concentrations of IBA and naphthalene acetic acid (NAA) (0.5-2 mg/l). In this regard, the superior performance of IBA compared with NAA was observed with the best response achieved using 1 mg/l IBA (resulting in a 95% rooting rate) with an average of 6.13 roots per shoot and the root length of 2.59 cm. Finally, the obtained plantlets were successfully acclimatized in a greenhouse, with 100% *ex vitro* survival rate. This established protocol can serve as an effective alternative to classical propagation methods for mass multiplication of this valuable decorative plant.

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1. Introduction

Economically, the Araceae family holds significant importance as it encompasses numerous ornamental plants, including *Philodendron* Schott. In terms of leaf morphology and life form, the genus is considered as one of the most diverse genera of the family (Canal *et al*, 2018). The morphological variability of *Philodendron* plants, as the second largest genus of Araceae, besides their attractive foliage and tolerance to indoor conditions make them favorable for use as ornamental plants (Alawaadh *et al*., 2020; Lara-Ascencio *et al*., 2021). Among them, the *Philodendron* cv. Birkin is a beloved and widely used cultivar with a unique variegation pattern of plant leaves.

Generally, plant tissue cultures refer to the process of culturing plant materials on a synthetic media under aseptic and controlled conditions. This method has gained significant commercial importance as it allows for the large-scale propagation of desired plants, thus playing a crucial role in the global ornamental industry. In fact, *in vitro* cultures offer several advantages over traditional methods of plant propagation, including the relatively rapid mass production of plant species and a reduced cost of production (Nakano *et al*., 2004; Al-Aizari *et al*., 2020; Raju and Divya, 2020; Showkat Bhat *et al*., 2022).

The successful propagation of economically valuable plant species through *in vitro* culture methods heavily relies on the composition of culture media, particularly the inclusion of plant growth regulators (PGRs). In fact, optimized application of plant growth regulators is a prerequisite for efficient organogenesis and growth. Cytokinins and auxins, either individually or in combination, are generally responsible for regulating *in vitro* morphogenesis, and their effects have been extensively studied across various steps of micropropagation (Veraplakorn, 2016; Desai *et al*., 2018; Rafiq *et al*., 2021; Sethy and Kullu, 2022). Furthermore, it is important to note that micropropagation protocols are cultivar-specific and should be optimized for each specific case. Here, we have reported a rapid and efficient protocol for *in vitro* mass propagation of *Philodendron* cv. Birkin using shoot tip explant and the successful acclimatization of rooted plantlets.

2.Materials and Methods

2.1. Explant collection and sterilization

In this study, shoot tip explants were collected from greenhouse grown plants of *philodendron* cv. Birkin. Prior to surface sterilization, the healthy explants were thoroughly washed under tap water to clean adhering contaminants. The collected explants were then surface sterilized by immersing them in a 1% sodium hypochlorite solution for 15 min with continuous agitation. After disinfection, the explants were thoroughly washed with sterile distilled water in a laminar air flow. Afterwards, shoot tip explants were cultured in glass jars containing 40 ml MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 7 g/lit agar along with 1 mg/lit benzyladenine (BA) for initial shoot proliferation and sub-cultured in the same medium every about three to four weeks. The pH of culture medium was adjusted to 5.8 and autoclaving was performed at 121 ˚C for 15 min. All cultures were maintained at 25 ± 2 ˚C and 16 h photoperiod provided by cool white fluorescent lamps. Finally, proliferated shoots were transferred individually to PGR free MS medium for two successive subcultures during two months and used in further experiment.

2.2.Shoot multiplication

In this stage, the effects of plant growth regulators on shoot proliferation of shoot tip explants were evaluated. The shoot tips (approximately 1 cm in length) separated from *in vitro* shoot cultures were cultured in MS media supplemented with benzyladenine, kinetin and indole-3-butyric acid, alone or in combinations, to set up 11 treatments as presented in Table 1. After four weeks of culture, the number of regenerated shoots was measured.

2.3.Shoot rooting and acclimatization of plantlets

To induce root formation, the regenerated shoots (approximately 2-3 cm in length) were transferred to root inducing MS media supplemented with different concentrations of naphthalene acetic acid and indole-3-butyric acid (0.5-2 mg/ml). After four weeks, the rooting percentage, as well as the number and length of root were determined.

For hardening process, the *in vitro* raised plantlets were carefully rinsed with tap water to remove adhering gel. Subsequently, the plantlets were transferred to 8 cm diameter plastic pots filled with a mixture of cocopeat and perlite (2:1, v/v). Cultured pots were then kept in greenhouse at 18-25 ˚C under a relative humidity of about 70%.

3.Statistical analyses

All the experiments in the study were arranged in a completely randomized design (CRD) and treatments were replicated four times. Obtained data were analyzed by one-way ANOVA and mean values were compared by Duncan's multiple range test ($P \leq 0.05$) using computer program of MSTAT-C.

4. Results

4.1. Effects of PGRs on shoot proliferation

In this experiment, shoot tips of *philodendron* cv. Birkin were cultured for a duration of four weeks on MS media supplemented with various levels of PGRs including BA, Kin, NAA and IBA. According to the results, there were significant differences among the used PGR treatments during the shoot multiplication phase, as illustrated in Fig 1. In this respect, the highest shoot proliferation rate was observed on MS medium supplemented with 3 mg/l BA + 0.5 mg/lit IBA (16.65 shoots per explant), followed by 3 mg/l BA + 0.5 mg/l Kin and 3 mg/l BA treatments. Conversely, the lowest regenerated shoot number was observed on PGR free MS medium, and among the PGR-containing media, those fortified with 1 mg/l BA and 1 mg/l kin exhibited the lowest shoot proliferation rates (Fig. 1).

Table1. Treatments used for shoot proliferation of *Philodendron* cv. Birkin shoot tips

Treatment*	BA (mg/l)	Kin (mg/l)	IBA (mg/l)
T1	-	-	-
T2	1	-	-
T3	3	-	-
T4	5	-	-
T5	-	1	-
T6	-	3	-
T7	-	5	-
T8	2	1	-
T9	1	2	-
T10	3	-	0.5
T11	-	3	0.5

* BA, Kin and IBA are benzyladenine, kinetin and indole-3-butyric acid, respectively.

Table 2. Mean squares from analysis of variance of PGRs effects in shoot proliferation (top) and rooting (bottom) stages of *Philodendron* cv. Birkin micropropagation

Source of variations	df	Parameters
		Shoot number
Treatments	10	66.23**
Error	33	1.20

Source of variations	df	Rooting percentage	Root length	Root number
Treatments	6	4028.57**	2.83**	16.81**
Error	21	171.43	0.08	0.16

** Significant differences at $P<0.01$.

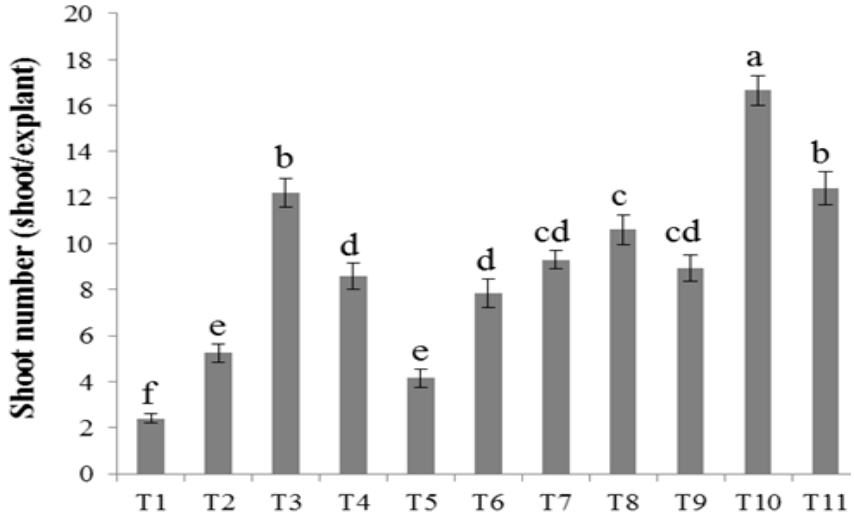

Fig 1. Effects of plant growth regulators on shoot multiplication of *Philodendron* cv. Birkin shoot tips. For abbreviations, see Table 1.

4.2. Rooting and acclimatization

To induce adventitious root formation on proliferated shoots of *philodendron* cv. Birkin, different concentrations of IBA and NAA (0.5-2 mg/l) were used in this study. As depicted in Figs. 2-4, *in vitro* rooting of *philodendron* cv. Birkin microshoots was significantly influenced by both the type and concentration of PGRs used, as evidenced by the rooting percentage, root length and root number. In total, while PGR-free MS medium failed to induce root formation, root development was observed in media supplemented with any of the auxins. In this regard, efficiency of IBA was generally found to be superior to NAA. The highest and lowest rooting rates were observed in 1 mg/l IBA and 0.5 mg/l NAA treatments, respectively. Furthermore, shoot cultured on MS media fortified with 1 mg/l (6.13 root/shoot) and 2 mg/l (5.74 root/shoot) IBA exhibited the maximum numbers of adventitious roots and the minimum root number was found in NAA 0.5 mg/l (3.15 root/shoot) treatment. Similarly, the highest and lowest root lengths were recorded in 1 mg/l IBA and 0.5 mg/l NAA treatments (2.59 and 1.64 cm, respectively). Finally, acclimatization of *Philodendron* cv. Birkin *in vitro* plantlets was completely successful (100% survival rate), following their transfer to greenhouse conditions.

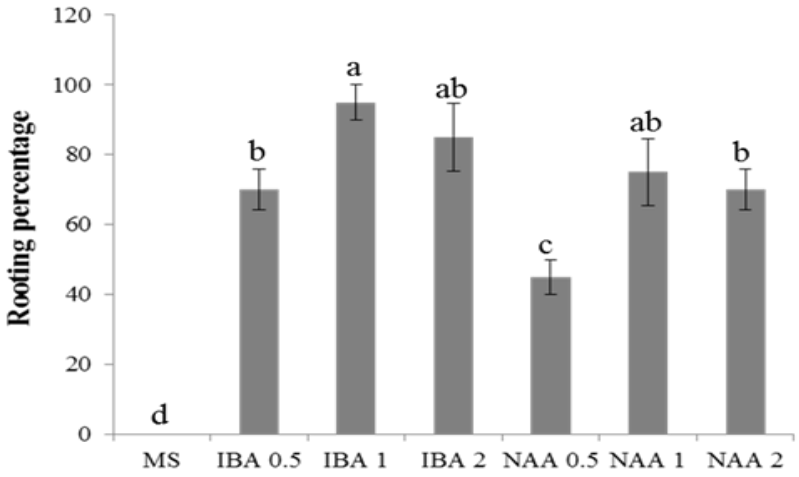

Fig 2. Effects of Auxins types (IBA and NAA) and concentrations (0.5-2 mg/l) on rooting percentage of *Philodendron* cv. Birkin proliferated shoots.

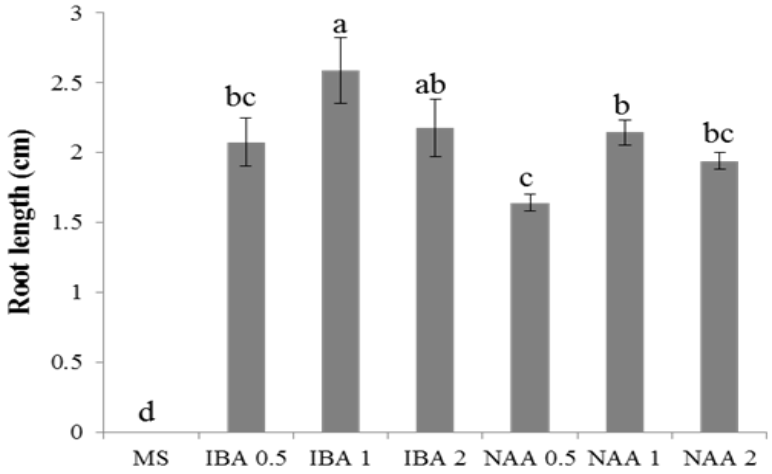

Fig 3. Effects of auxins types (IBA and NAA) and concentrations (0.5-2 mg/l) on root length of *Philodendron* cv. Birkin proliferated shoots.

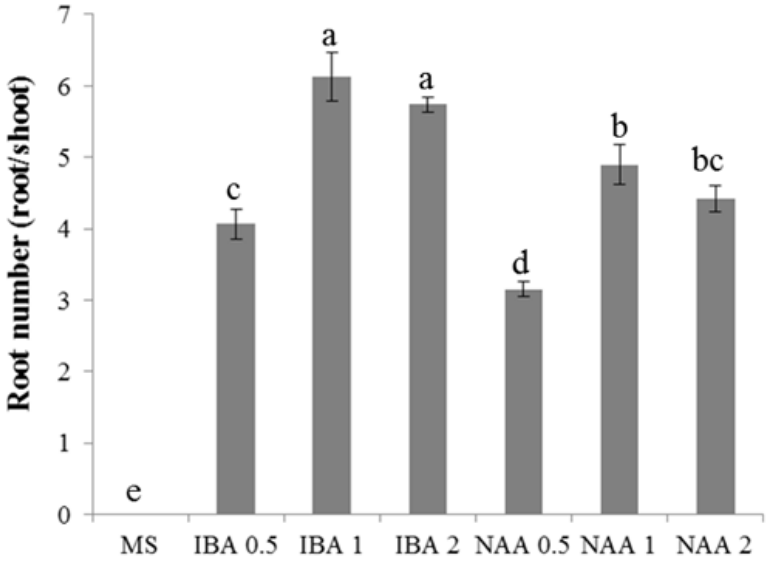

Fig 4. Effects of Auxins types (IBA and NAA) and concentrations (0.5-2 mg/l) on root number of *Philodendron* cv. Birkin proliferated shoots.

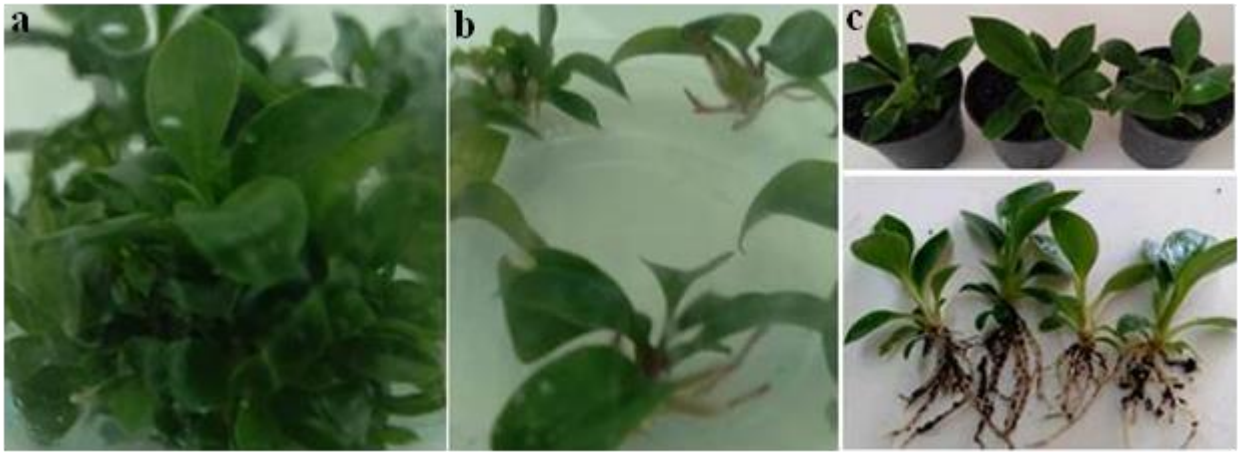

Fig 5. Stages of *Philodendron* cv. Birkin micropropagation: (a) shoot proliferation; (b) shoot rooting; (c) acclimatized plantlets.

5. Discussion

Cytokinins and auxins are two major groups of plant growth regulators commonly used in various types of *in vitro* plant cultures. Similar to results of our study, the principal role of cytokinins in *in vitro* organogenesis processes including shoot proliferation has been widely reported (Veraplakorn, 2016; Hosseinabadi *et al*., 2020; Kumar and Giridhar, 2020; Nithya and Kamalam, 2021; Farooq *et al*., 2022). In fact, cytokinins play a crucial role in stimulating cell division and synthesis of various macromolecules including RNA, proteins and enzymes and they are notably used in *in vitro* cultures to suppress apical dominance and promote axillary shoot proliferation (Gomes *et al*., 2010; Mohamad *et al*.., 2022; Farooq *et al*., 2021; Fawzia *et al*., 2018; Raju and Divya, 2020). However, optimal rate of shoot proliferation largely depends on the concentration of cytokinins used and it has been well established that different types of cytokinins have varying efficiencies in inducing shoot multiplication, and their effectiveness is dose-dependent (Jun-jie *et al*, 2017; Bayraktar *et al*., 2020; Jagiełło-Kubiec *et al*., 2021). Hence, in plant tissue culture studies aimed at achieving maximum shoot proliferation, various types of cytokinins are typically examined in proliferation stage. In this study, we evaluated the effectiveness of BA and kinetin, either alone or in combination, in inducing shoot proliferation of *Philodendron* cv. Birkin. As depicted in Fig. 1, BA cytokinin generally exhibited a higher rate of shoot multiplication compared to kinetin. Such differences in the activity of various cytokinins have also been reported in previous studies and can be attributed to factors such as tissue sensitivity, translocation rates, degradation and conjugation with physiologically inert compounds like sugars and amino acids. On the other hand, the addition of IBA auxin to the culture media resulted in a higher shoot regeneration frequency (Fig. 1). These results are in agreement with the findings of other investigations that have described the promotive effects of cytokinins in combination with auxins on shoot proliferation rate of other plant species (Kaliamoorthy *et al*, 2008; Alawaadh *et al*., 2020; Raju and Divya, 2020).

In the present study, the supplementation of MS medium with two tested auxins (IBA and NAA) successfully induced root formation on *Philodendron* cv. Birkin regenerated shoots and the effects were more pronounced in IBA treatments, as evidenced by higher rooting percentage, root length and root number per explant. Generally, *in vitro* rooting is a key step of plant micropropagation process as a prerequisite of successful *ex vitro* plant acclimatization. Regarding the unique ability in induction of adventitious roots, auxins are indispensable components of plant tissue culture media in rooting phase. Our findings regarding superior performance of IBA auxin compared to NAA in adventitious root

formation are consistent with earlier reports in other plant species (Cartabia *et al*., 2022; Farooq *et al*., 2021; Rafiq *et al*., 2021; Showkat Bhat *et al*., 2022). These differences in root induction abilities among auxins can be attributed to factors such as variations in auxin receptors involved in the rooting process and their stability (Mohamad *et al*., 2022; Cartabia *et al*., 2022).

6. Conclusion

Establishing a rapid and efficient micropropagation protocol is crucial for the economically viable mass propagation of any plant species *in vitro*. Cytokinins and auxins are essential components of plant tissue cultures, playing a significant role on growth and morphogenesis processes. Through the assessment of different cytokinins and auxins during the proliferation and rooting stages, our study has developed an efficient micropropagation method for large-scale multiplication of *Philodendron* cv. Birkin using shoot tip culture. In this regard, the best response in terms of shoot proliferation was achieved in MS medium supplemented with 3 mg/l BA and 0.5 mg/lit IBA (16.65 shoots/explant). Subsequently, the Regenerated shoots successfully formed adventitious roots when transferred to auxin-containing MS media, especially 1 mg/l IBA. The acclimatization process in the greenhouse was completely successful, with a 100% survival rate.

Conflict of interest

The authors have no conflicts of interest.

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