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Effect of Different Types and Concentration of Rooting Hormones on High Yielding Genotype of *Strobilanthes crispus* Stem Cuttings

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ABSTRACT

Strobilanthes crispus is a medicinal shrub recognised for its rich composition of bioactive compounds, particularly phenolics and flavonoids, which are responsible for its antioxidant, anti-inflammatory, and antidiabetic properties. The demand for consistent, high-quality planting materials necessitates the development of an efficient propagation method, particularly through stem cuttings. The propagation efficiency of high yielding *S. crispus* genotypes through stem cuttings was evaluated using three auxin types namely Indole -3-Acetic Acid (IAA), Indole -3- Butyric Acid (IBA), and 1-Naphthalene Acetic Acid (NAA) at concentrations of 0, 0.5, and 2.0 mg/L each. A significant interaction between hormone type and concentration was observed for the number of primary roots, indicating that these traits are highly responsive to specific hormone-concentration combinations. Indole-3-butyric acid (IBA) at 0.5 mg/L resulted in the greatest root number and length, confirming its effectiveness in promoting adventitious root formation. No significant interaction effects were found for survival rate, mean root length, sprouting percentage and number of shoots, suggesting these parameters were influenced more by individual hormone or concentration effects. The results emphasize the importance of selecting appropriate auxins and concentrations based on propagation objectives. This hormone-specific strategy offers a practical and accessible approach to improving vegetative

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propagation protocols for *S. crispu*s, with implications for both conservation and commercial cultivation.

Keywords: Auxin, medicinal plant, plant propagation, rooting performance, stem cuttings

INTRODUCTION

Strobilanthes crispus is a perennial shrub belonging the Acanthaceae family that has long played an important role in traditional medicine across Malaysia and nearby regions. In Malaysia, *S. crispus* is locally known as 'pecah beling' or 'pecah kaca' in Malay, and 'Hei Mian Jiang Jun', translated as "black-faced general" in Chinese dialects, reflecting its recognition across different cultural traditions. This plant naturally grows along riverbanks and in abandoned fields, reaching heights of up to 1.5 metres. It's easy to identify with its dark green, slightly rough leaves and bright yellow flowers that form in dense, spike-like clusters. Originally found in Madagascar and the Malay Archipelago (Burkill & Birtwistle, 2002), *S. crispus* continues to attract attention for its medicinal uses, especially as both local communities and global industries turn back to nature for remedies and wellness solutions.

Traditionally, people consume *S. crispus* as a tea or water decoction, believing it to help with kidney stones (Ernawati et al., 2018), diabetes, and general health maintenance (Permatasari et al., 2025). These traditional beliefs are increasingly supported by modern science. Over the years, researchers have discovered a wealth of phytochemicals in the plant that explain its healing potential. Scientific studies have shown it to possess powerful antioxidant (Ismail et al., 2000; Qader et al., 2011), antimicrobial (Hanafiah et al., 2023; Mahyantika et al., 2025), antidiabetic (Fadzelly et al., 2006; Norfarizan et al., 2009; Sukendi et al., 2025), anti-ulcer (Al-Henhena et al., 2011), and even wound-healing effects (Norfarizan et al., 2009). One of the most promising discoveries about *S. crispus* is its potential role in cancer therapy. A review by Ng et al. (2021) highlighted that extracts from this plant demonstrated significant anticancer effects in both laboratory experiments and animal models, particularly showing strong activity against breast, liver, and colon cancer cells (Hakimi et al., 2025).

Given its remarkable medicinal potential, *S. crispus* has become increasingly sought after, not only within traditional medicine but also in the herbal and pharmaceutical industries. However, the majority of raw material currently available is still sourced through wild collection or imported from unverified origins. This reliance on inconsistent sources raises major concerns regarding product quality, genetic uniformity, and long-term sustainability (Zurinawati, 2004). To address these challenges and ensure a stable, high-quality supply chain, there is a demanding need to establish a reliable system for producing high quality planting material. In line with this, the Forest Research Institute Malaysia (FRIM) has initiated a structured breeding programme focused on identifying, selecting,

and propagating *S. crispus* superior genotypes were collected from diverse populations across the Peninsular Malaysia.

One of the most efficient ways to multiply selected plants while keeping their valuable traits intact is through stem cuttings. However, success with this method can vary depending on the plant's genetic makeup and whether or not rooting hormones are used. Compounds like IBA, NAA, and IAA are known to stimulate root formation and improve survival rates of cuttings (Saini & Anmol, 2024), but their effectiveness often depends on both dosage and the plant genotype. The present study aims to evaluate the effects of different types and concentrations of auxins on the propagation efficiency of high yielding *S. crispus* genotypes through stem cuttings.

MATERIALS AND METHODS

Study Site and Materials

The experiment of stem cutting conducted at the nursery of the Tree and Herb Improvement Branch, Forest Research Institute Malaysia (FRIM). The propagation area was maintained under a high-humidity environment (70–80% RH) and 50% shade, with an intermittent misting system operating for 10 minutes, daily three times at 8.00 am, 12.00 pm and 4.00 pm. A high-yielding *S. crispus* genotype, BPB23, known for its high verbascoside content, was used as the planting material. Stem cuttings approximately 10 cm long, each containing two nodes, were prepared. Leaves were removed to minimize transpiration and prevent desiccation prior to root initiation. The cuttings were then immersed in hormone solution for one minute and immediately planted in 100% coarse river sand as the rooting medium.

Experimental Design

A Randomised Complete Block Design (RCBD) with three replications was used to carry out the experiment. Treatments consisted of three auxin types, i) IAA, ii) IBA and iii) NAA, each applied at three concentrations (0, 0.5, and 2 mg/L) on stem cuttings of the high yielding *S. crispus* genotype. The concentrations of auxins selected for this study were chosen based on effective ranges reported in previous studies on vegetative propagation of medicinal and woody plants. The 0 mg/L treatment served as a control to assess the baseline rooting capacity without hormonal influence.

Data Collection

Data were collected after eight weeks to ensure adequate root development and evaluate the influence of various treatments on the rooting performance, following the procedure described by Ghimire et al. (2022). Parameters recorded included survival rate (%), number of primary roots, mean root length (cm), sprouting percentage, and number of shoots. To

analyse the data two-way ANOVA (analysis of variance) was used, and DMRT (Duncan's Multiple Range Test) was used to evaluate the statistically significant (at $p < 0.05$) among the treatments using the SPSS 25.0 software.

RESULTS AND DISCUSSION

A significant interaction was observed between the type of plant growth regulator (IAA, IBA, and NAA) and their respective concentrations (0, 0.5, and 2.0 mg/L) on the number of primary roots in stem cuttings of the high-yielding *S. crispus* genotype ($p \leq 0.05$; Table 1). In contrast, no significant interaction effects were observed between auxin type and the concentration for survival rate, mean root length, sprouting percentage and number of shoots (Table 1). This suggests that these parameters were more likely influenced by the main effects of either hormone or concentration alone, rather than a combination of the two. Therefore, the discussion will focus primarily on the significant interaction effects observed for the number of roots as this variable is more responsive to the combined influence of hormone treatment and concentration in high yielding *S. crispus* genotype.

Table 1
The main and interaction effects of different hormone types and concentrations on the survival rate, number of roots, means root length, sprouting percentage and number of shoots of high yielding genotype Strobilanthes cripsus *stem cuttings*

Factor	Survival rate (%)	Number of primary roots	Means root length (cm)	Sprouting percentage (%)	Number of shoots
Hormone (H)					
IAA	86.53a	7.23b	5.16b	86.15a	9.96a
IBA	86.53a	8.52a	5.79a	86.53a	9.68a
NAA	88.07a	7.18b	5.27b	88.07a	9.98a
Concentration (mg/L) (C)					
0	83.84a	8.08a	5.21a	83.07b	8.92c
0.5	86.15a	7.43b	5.24a	85.90ab	9.86b
2.0	87.95a	7.61ab	5.57a	87.95a	10.59a
Significance					
Hormone (H)	ns	*	*	ns	ns
Concentration (C)	ns	*	ns	*	*
H x C	ns	*	ns	ns	ns

*significant at $p \leq 0.05$

The effect of auxin concentration on root induction varied significantly depending on the hormone type used, as demonstrated by the hormone × concentration interaction (Figure 1). Among all treatment combinations, 0.5 mg/L IBA produced the highest number

of primary roots, significantly outperforming other hormone *x* concentration combinations. Notably, IBA consistently induced superior rooting performance at all tested concentrations, with 0.5 mg/L yielding the highest root number, followed closely by 2.0 mg/L. This trend is consistent with previous findings by Nabilah et al. (2023), who stated that generic *S. cripus*s genotype stem cuttings produced the most roots when treated with 0.5 mg/L IBA.

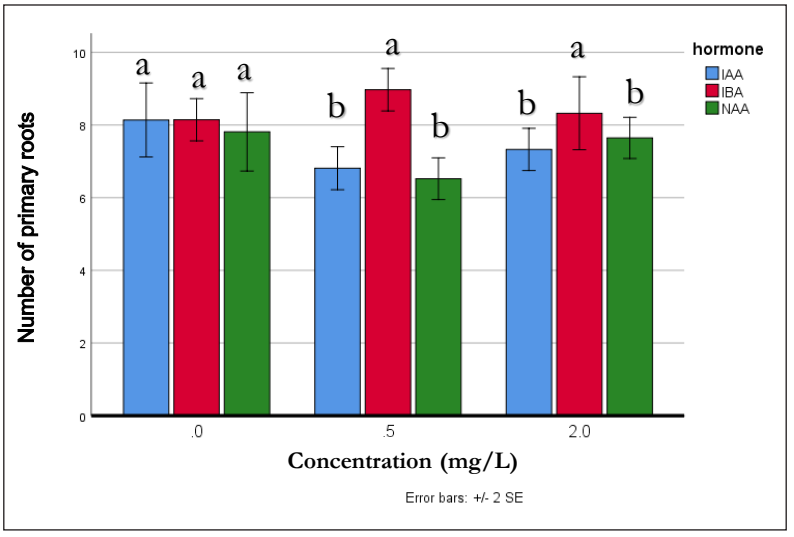

Figure 1. Interaction effect of hormone type (IAA, IBA, and NAA) and concentration (0, 0.5, and 2.0 mg/L) on the number of primary roots in high-yielding Strobilanthes crispus stem cuttings. Bars with different letters indicate statistical significant

Indole-3-butyric Acid (IBA) is one of the most important effective auxins type plant growth regulators which can stimulate adventitious root development from the stem cuttings of various plants, including *Piper betle* (Muttaleb et al., 2017*), Solanum procumbens* (Tien et al., 2020), and *Andrographis paniculata* (Hossain et al., 2021). Its superior efficacy is often associated with its higher chemical stability and lower susceptibility to enzymatic breakdown, which allows it to remain active for a longer duration at the application site (Klerk et al., 1999; Păcurar et al., 2014). These properties likely explain the improved rooting response observed in *S. crispus* stem cuttings treated with IBA, particularly at 0.5 and 2.0 mg/L concentrations in this study.

In contrast, IAA and NAA produced more moderate and inconsistent rooting responses across the tested concentrations. As reported by Hartmann et al. (2011) and Pacurar et al. (2014), IAA is known to degrade rapidly within plant tissues, reducing its effectiveness over time, whereas NAA, though more chemically stable, may exhibit limited uptake and translocation efficiency in stem. Additionally, it is possible that *S. crispus* cuttings possess sufficient endogenous auxin to initiate rooting without the need for exogenous application.

The introduction of external auxins such as IAA and NAA at 0.5 or 2.0 mg/L may have disrupted the internal hormonal equilibrium, leading to suboptimal rooting or physiological stress (Hartmann et al., 2011). These factors may explain the observed variability and overall lower performance of IAA and NAA treatments compared to the more consistently effective IBA formulations in this study.

While this study offers valued understandings into the interactive effects of auxin type and concentration on root growth and development in. *crispus* stem cuttings, several areas need further exploration. The investigation was limited to a single high yielding genotype; therefore, future studies should include multiple genotypes to evaluate genotypic variability in hormonal responsiveness, especially since auxin sensitivity can differ among genetic backgrounds. Moreover, exploring a broader concentration gradient (e.g., 0.25, 1.0, 1.5 mg/L) could help identify more precise threshold levels for optimal growth responses and minimize the risk of hormonal overdose or under treatment. Future studies may also benefit from incorporating endogenous hormone profiling or gene expression analysis of root and shoot related genes to understand the underlying molecular mechanisms driving differential responses to exogenous auxins.

CONCLUSION

This study highlights the importance of hormone type and concentration in optimizing root induction of high yielding *S. crispus* stem cuttings, with IBA at 0.5 mg/L producing the highest number of primary roots. The significant hormone × concentration interaction indicates that rooting response is highly dependent on the specific auxin treatment used, with IBA demonstrating greater effectiveness compared to IAA and NAA. These findings provide a reliable protocol for vegetative propagation, supporting the large scale production of uniform and genetically stable planting materials for commercial cultivation.

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