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Studies on cuttings propagation of Ilex cassine 'angustifolia'

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Abstract

This study explored the effects of different rooting hormones on cutting rooting and physiological indices of Ilex cassine'angustifolia'. Cuttings were treated with KIBA, IBA, NAA, IAA (1000 mg/L each) and CK (water control) via 20-second quick-dip. Results showed KIBA 1000 mg/L as optimal, with 67.6% survival rate, 56.25% rooting rate, and significantly higher root activity, soluble protein, and soluble sugar content. IBA 1000 mg/L followed (54.4% survival rate), while CK and IAA had low rooting rates (12.50% and non-significant, respective-ly). Conclusion: KIBA 1000 mg/L is optimal for promoting rooting and growth of Ilex cassine'angustifolia' cuttings.

Keywords

Ilex cassine 'angustifolia'; cutting propagation; rooting hormones; physiolog-ical indicators; rooting rate

1. Introduction

Ilex cassine 'angustifolia' is an evergreen small tree with distinctive willow-like leaves, dense branching, a round-tower crown, and abundant red fruits, making it highly valuable for wetland greening, waterfront landscaping, and artistic shaping[1]. However, its propagation is severely constrained by biological and technical limitations: seeds exhibit deep dormancy leading to extremely low germination rates, while cutting rooting is notoriously difficult[2].

Notably, existing research on Ilex propagation is scarce, with studies focusing only on a few species, leaving critical gaps in knowledge for this cultivar[3]. To address these challenges, hormone treatment emerges as a promising strategy to enhance rooting efficiency.

This study specifically aims to identify the optimal hormone type and concentration, with the goal of improving cutting survival rate and rooting quality. The findings are expected to provide a theoretical basis for the rapid and efficient propagation of Ilex cassine ‘angustifolia’.

2. Materials and Methods

2.1. Plant Materials

Cuttings were collected from healthy, disease-free semi-lignified current-year shoots with 1-2 axillary buds from the experimental base of Yangtze University in September 2022.

Processing Specifications：Trimmed to 5.5-8 cm length, retaining 1/3 of leaves (each leaf cut to 1/2 area), with flat top cuts and oblique basal cuts.

2.2. Site

The experiment was conducted in Jingzhou City, Hubei Province (111°15′—114°05′ E, 29°26′—31°37′ N), characterized by a subtropical monsoon climate with annual temperature 15.9-16.6°C and annual rainfall 1100-1300 mm. Temperatures above 28°C accounted for 63.6% of the experimental period, characterizing the microclimate.

2.2.3. Cutting Treatments

The experimental design included 5 treatments (4 hormones + control) with 32 cuttings per group:

- CK： Water control, quick-dip for 20 s；
- IBA： 1000 mg/L, quick-dip for 20 s；
- IAA： 1000 mg/L, quick-dip for 20 s；
- NAA： 1000 mg/L, quick-dip for 20 s；
- KIBA： 1000 mg/L, quick-dip for 20 s.

After treatment, cuttings were inserted 3-5 cm deep into the substrate. Subsequent management included shading, plastic film covering, moderate watering, fertilization, weeding, and pest control (chemical spraying for moths or aphids).

2.2.4. Data Collection and Analysis

Measured parameters included **morphological indicators** (survival rate, rooting rate, average root length, root surface area, projection area, new shoot occurrence rate, new shoot number) and **physiological indicators** (root activity via TTC method[4], soluble protein content via Coomassie brilliant blue method, soluble sugar content via anthrone colorimetry). Data were analyzed using Microsoft Office Excel 2019, SPSS 27.0 (correlation analysis), and Origin 2021 (graphing) .

2.2.5. Experimental Design

A randomized complete block design was adopted with 5 treatments, 32 cuttings per group, and 3 replicates to ensure statistical robustness.

3. Results

3.1. Effects of Hormones on Root Morphology

Different hormone treatments significantly affected root morphology of *Ilex cassine* 'angustifolia' cuttings (Figure 1). **KIBA 1000 mg/L treatment exhibited the most developed root structure** with significantly higher root density than other treatments; IBA 1000 mg/L promoted root growth but with less development than KIBA. NAA 1000 mg/L resulted in sparse roots, while CK and IAA 1000 mg/L showed minimal or no rooting.

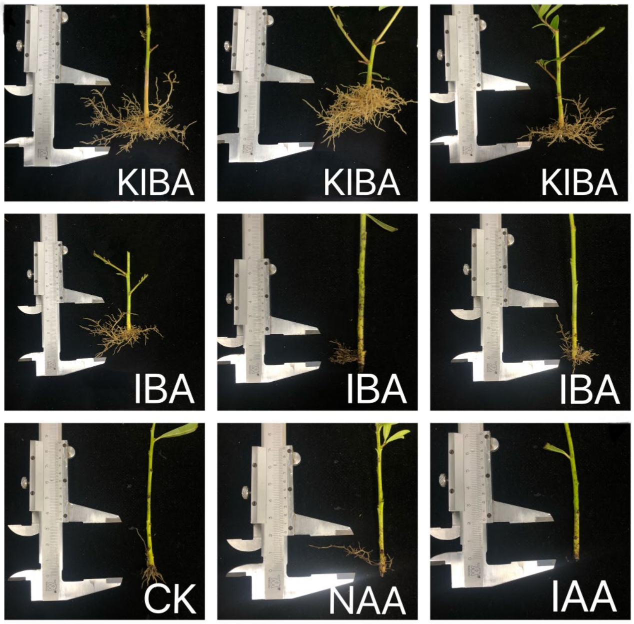

Figure 1. Root Growth Under Different Hormone Treatments

3.2. Rooting Indices Across Treatments

Table 1 presents the effects of different rooting hormones on rooting traits of *Ilex cassine* 'angustifolia' cuttings (ANOVA, P<0.05). KIBA 1000 mg/L performed optimally across all indicators: rooting rate 56.25%, significantly higher than other treatments (2.8 times that of NAA at 19.87%), with highest survival rate (67.6%), root length (83.47 mm), surface area (14.68 cm^2), and projection area (6.70 cm^2). IBA 1000 mg/L followed with 40.62% rooting rate; NAA and CK showed lower rates (19.87%, 12.50%), while IAA had no significant effect.

Table 1. Comparisons of rooting traits of Ilex cassine 'angustifolia' cuttings treated with different rooting hormones

Treatment/mg·L	Survival rate/%	Rooting rate/%	Root length/mm	Surface area/cm^2	Projection area/cm^2
IBA	54.40±2 ab	40.62±3.1 b	44.52±6.7 b	9.84±1.14 ab	4.16±0.32 b

NAA	55.00±3 ab	19.87±5.7 c	25.93±2.9 c	6.38±0.84 ab	1.52±0.61 c
KIBA	67.60±7 a	56.25±5.2 a	83.47±6.4 a	14.68±1.65 a	6.70±1.32 a
CK	52.00±1 b	12.50±1.9 c	12.63±3.7 d	4.17±0.90 b	0.61±0.17 c

Note: Different lowercase letters in the same column indicate significant differences (P<0.05). The same below.

3.3. New Shoot Growth

New shoot growth served as a secondary indicator of cutting vigor (Figure 2). KIBA 1000 mg/L treatment showed the highest new shoot rate (75.00%) and shoot number per plant (24), significantly higher than IBA (66.66%, 16) and control (9.37%, 3), presumably related to enhanced root system development (Table 2).

Table 2. The effect of different rooting hormone treatments on the number of new branches of Ilex cassine 'angustifolia' cuttings

Treatment/mg·L	New shoot rate/%	New shoot number/plant
KIBA	75.00	24
IBA	66.66	16
NAA	18.75	6
CK	9.37	3

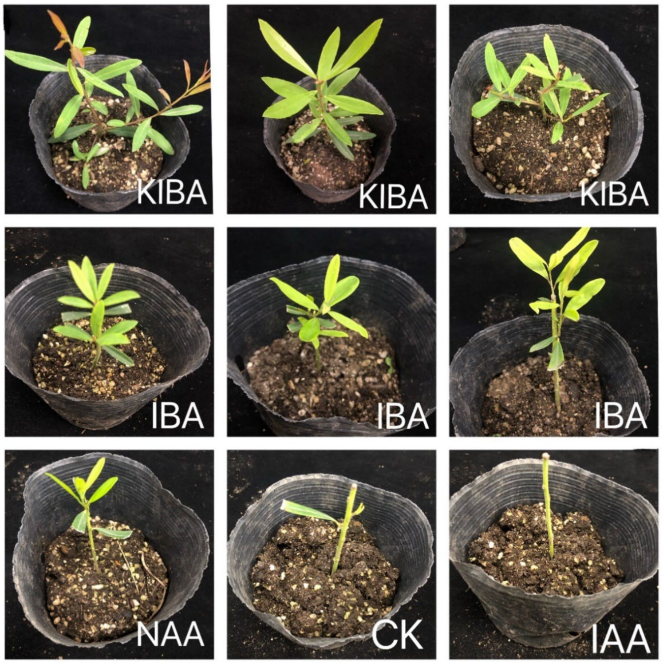

Figure 2. Comparison of new branches sprouting from Ilex cassine 'angustifolia' treated with different rooting hormones

3.4. Root Activity

Root activity is a core indicator of root physiological health (Figure 3).

Experimental data showed: KIBA 1000 mg/L treatment group had the highest activity (193.449 μg/g), significantly higher than IBA 1000 mg/L (173.575 μg/g), NAA (162.59 μg/g), and control (129.913 μg/g), with IAA group showing 0 μg/g.

Higher root activity indicates KIBA treatment enhances root physiological function and nutrient absorption capacity.

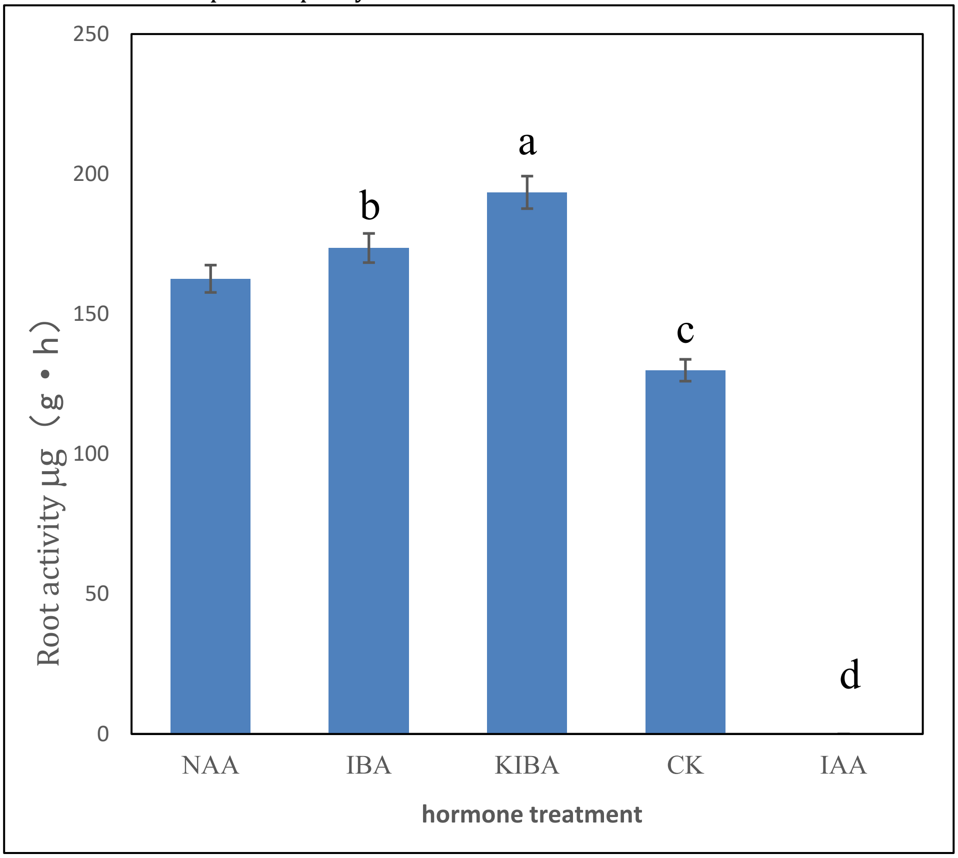

Figure 3. Root activity of Ilex cassine 'angustifolia' cuttings

3.5. Soluble Protein Content

Soluble protein content is closely related to plant stress tolerance and metabolic activity.

This study showed (Figure 4) that KIBA 1000 mg/L treatment group had the highest content (0.212 μg/g), significantly higher than IBA (0.1589 μg/g), NAA (0.145 μg/g), control (0.1299 μg/g), and IAA (0 μg/g) groups, indicating KIBA effectively enhances protein synthesis, potentially improving cutting stress resistance and metabolic levels.

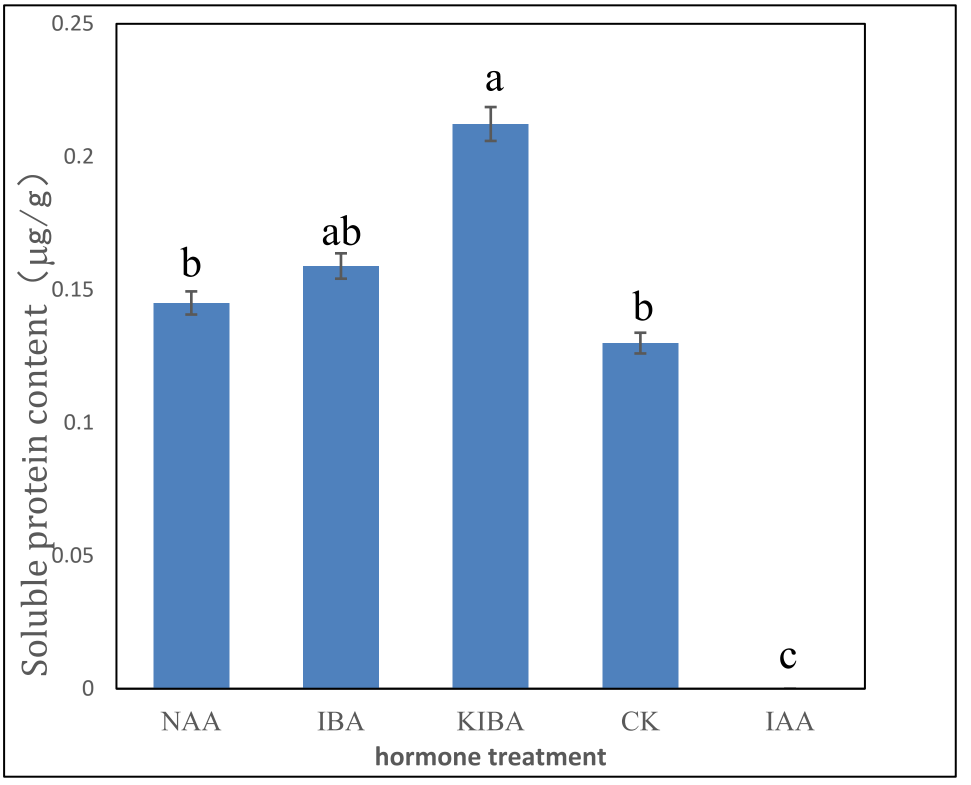

Figure 4. Soluble protein content of Ilex cassine 'angustifolia' cuttings

3.6. Soluble Sugar Content

As key energy reserves for rooting, soluble sugar content directly affects rooting potential (Figure 5). Soluble sugar content across treatments was: KIBA 1000 mg/L (0.196 μg/g) > IBA 1000 mg/L (0.1609 μg/g) > NAA (0.1405 μg/g) > CK (0.1289 μg/g) > IAA (0 μg/g). Higher content under KIBA may enhance rooting capacity.

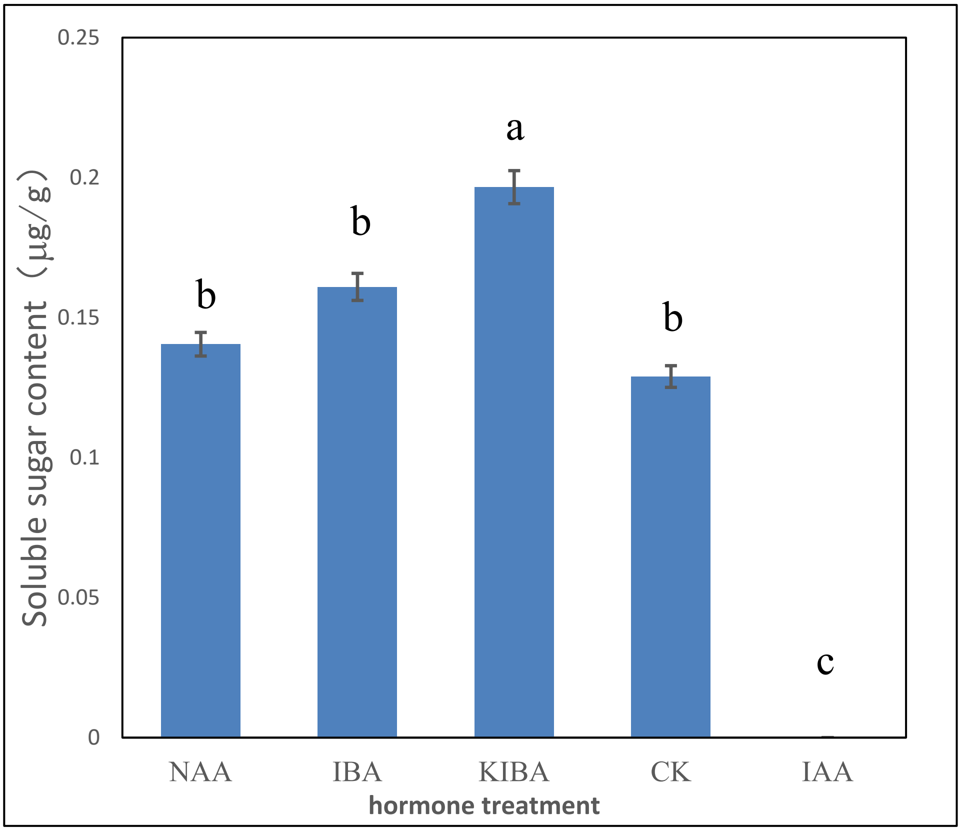

Figure 5. Soluble sugar content of Ilex cassine 'angustifolia' cuttings

4. Discussion

Results demonstrate that exogenous hormone treatments significantly promote rooting of Ilex cassine 'angustifolia' cuttings, consistent with previous studies showing IBA and NAA enhance rooting in Ilex species[5]. KIBA exhibited optimal rooting promotion, improving not only rooting rate but also comprehensively optimizing rooting capacity and growth potential through enhanced root physiological activity (promoting nutrient absorption), soluble protein accumulation (improving stress tolerance), and soluble sugar synthesis (providing energy supply)[6].

In practical application, findings provide critical technical reference for nursery-scale propagation of Ilex cassine 'angustifolia', confirming KIBA's value as the preferred growth regulator. However, optimization opportunities remain: testing KIBA concentrations below 1000 mg/L to reduce costs while maintaining efficacy[7]; and investigating molecular mechanisms (e.g., gene expression, signaling pathways) regulating rooting, which could be explored through transcriptomics or metabolomics[8].

5. Conclusion

This study systematically investigated the effects of exogenous rooting hormones on the cutting propagation of *Ilex cassine* 'angustifolia', revealing that such hormones exert significant regulatory effects on cutting survival and rooting. The core conclusion drawn is that the optimal treatment for promoting the cutting propagation of this species is a 20-second quick-dip in 1000 mg/L KIBA (keto-indolebutyric acid): this treatment not only achieved a survival rate of 67.6% and a rooting rate of 56.25% but also significantly improved root morphology and physiological activity. Overall, this technical protocol provides a scientific basis for the large-scale horticultural propagation of *Ilex cassine* 'angustifolia' and holds important practical significance for enhancing the propagation efficiency and seedling quality of rare Ilex varieties.4.2. Identify the Headings

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