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STUDY ON *in vitro* INDUCTION OF ROOTING AND CHANGES IN ENDOGENOUS HORMONE CONTENT OF *Lagerstroemia indica* 'ZIJINGLING'

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ABSTRACT

Different media had different effects on the induction of rooting of *Lagerstroemia indica* 'Zijingling'. The aims of this study were to identify the best rooting medium, determine the changes in endogenous hormone content in entire aseptic seedlings of 'Zijingling', and then analyze its role in the rooting process, to improve the rooting efficiency. Using a test tube seedling of 'Zijingling' as the experimental material, the tissue cells were observed via paraffin sectioning, and the changes in endogenous hormone content during the rooting process were determined using high-performance liquid chromatography. The results showed that half-strength Murashig and Skoog (1/2 MS) medium had the most significant effect on rooting in the basic medium. The promoting effects of different auxins on rooting decreased in magnitude in the order indole-3-butyric acid (IBA) > naphthalene acetic acid (NAA) > indole-3-aceticacid (IAA). The optimal rooting medium was 1/2 MS + 0.6 mg L^{-1} IBA+ 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon , and the highest induction rate of adventitious roots was 92.5%. The rooting of 'Zijingling' is classified as a primordial type of induced rooting. Exogenous IBA content promoted an increase in endogenous IAA and gibberellic acid (GA_3) contents. High contents of IAA, GA_3, and abscisic acid (ABA) and low content of zeatin riboside (ZR) promoted the growth of adventitious roots, whereas high contents of IAA and ZR, and low contents of GA_3 and ABA were required for the induction of root primordia. High levels of IAA/ZR and low levels of IAA/ABA promoted the differentiation of root primordia. However, low levels of IAA/ZR and high levels of IAA/ABA promoted adventitious root elongation.

Key words: culture medium, high-performance liquid chromatography, paraffin section, rooting efficiency, tissue culture

INTRODUCTION

Lagerstroemia indica L. belongs to the genus *Lagerstroemia* of the family Lythraceae. *Lagerstroemia indica* 'Zijingling' is a valuable new variety of *L. indica*. Compared with other *L. indica* varieties, it has unique and rare purple and blue flowers, which have remarkable ornamental value and provide more choices for creating a flower sea. *Lagerstroemia indica* has strong resistance to environmental pollution and a long flowering period, which enriches the plant landscape and is favored in landscape greening and gardening [Zhang et al. 2010].

Tissue culture is an ideal method for the mass propagation of 'Zijingling', which reproduces by asexual

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propagation. 'Zijingling' seedlings cultivated through plant tissue culture can maintain their original characteristics and genetic traits. Moreover, the cultivation process is easy to manage and the growth cycle is relatively short, which significantly improves the economic benefits [Teixeira da Silva et al. 2019, Corredoira and Costa 2021]. Haberlandt put forward the concept of "cell totipotency", which laid the theoretical foundation for plant tissue culture [Haberlandt 1902]. Skoog and Tsui found that the balance between adenine and auxin content determines the growth of buds and roots [Skoog and Tsui 1948]. Skoog and Miller found that a low cytokinin/auxin ratio could promote root formation; in contrast, a high ratio could promote bud formation [Skoog and Miller 1957]. Huang used the axillary buds of *L. indica* as explants for breeding experiments, which were among the earliest tissue culture studies on *L. indica* in China [Huang 1984]. Eymar's experiment showed that high doses of BA produced an inhibition of rooting in the absence of AC in themedium, but addition of activated charcoal to the mediacounteracted this trend, presumably by adsorbing the growth regulator [Eymar et al. 2000]. Niranjan and Sudarshana studied the culture of somatic embryos of *L. indica* [Niranjan and Sudarshana 2005]. Jiang and Song used Murashig and Skoog medium (MS) to induce the root formation of fascicular buds of *L. indica* [Murashige and Skoog 1962, Jiang et al. 2004]. In tissue culture, the auxins, IBA and NAA, are generally used to induce adventitious root formation [Stuepp et al. 2017].

Despite the low content of endogenous hormones in plants, they play an indispensable role in growth and development [Yan et al. 2017]. They promote or inhibit each other and regulate the growth and development of plants through induction or feedback regulation. For example, Maintenance of the root meristem size is balanced by antagonistic activities of CK and auxin [Müller and Sheen 2008, Benková and Hejátko 2009]. High-performance liquid chromatography (HPLC) is a technique not only simple in operation and has high in sensitivity and accuracy for detection of endogenous hormones, but can also simultaneously detect a variety of hormones with very little content [Das et al. 2020]. This makes the method suitable for the determination of endogenous hormones of 'Zijingling'.

Induction of adventitious roots of 'Zijingling' is critical research topic in the development and morphogenesis of *L. indica* tissue culture, because it can promote large-scale cultivation of the species. And the formation of adventitious roots is very important for the growth of woody plants [Negishi et al. 2014, Díaz-Sala 2021]. In tissue culture, the selection of a medium has become particularly important for optimal growth of plants [Phillips and Garda 2019]. In this study, we mainly investigated the influence of different media on the induction of adventitious roots. We observed morphology and determined endogenous hormone contents to analyze the in vitro rooting mode and effects of endogenous hormone changes on rooting, to provide a formidable theoretical reference for the induction of adventitious roots of *L. indica*.

MATERIAL AND METHODS

Selection of plant materials. Aseptic seedlings of 'Zijingling' were obtained from professor Wang Xiaoming of the Hunan Key Laboratory of Tree Clonal Breeding, Hunan Academy of Forestry, and used for in vitro rooting induction experiments, endogenous hormone determination, and preparation and observation of paraffin sections.

In vitro induction of 'Zijingling' rooting. Healthy aseptic seedlings of 'Zijingling', that were 2 cm in height, were selected and inoculated on different rooting media to induce rooting. Each treatment was applied to 44 aseptic seedlings and repeated thrice. The conditions used were a temperature of 25°C, illumination intensity of 1500–2000 lux, and illumination duration of 12 h per day. After 30 days of culture, the rooting efficiency, number of roots, root length, and plant growth efficiency were determined.

In vitro rooting induction by different basic media. Aseptic seedlings were inoculated on three basic media (half-strength Murashig and Skoog (1/2 MS) medium, half-strength Driver Kuniyuki Walnut (1/2 DKW) medium [Driver and Kuniyuki 1984], and half-strength Woody Plant medium (1/2 WPM) [Lloyd and McCown 1980]), which were supplemented with IBA (0.6 mg L^{-1}), sucrose (15.0 g L^{-1}), agar (5.0 g L^{-1}), and activated carbon (200 mg L^{-1}).

In vitro induction of rooting by different auxins and concentrations. Aseptic seedlings were inoculated

on 1/2 MS rooting medium containing IBA (0, 0.1, 0.6, or 1.2 mg L^{-1}), NAA (0.6 mg L^{-1}), or IAA (0.6 mg L^{-1}). Sucrose (15.0 g L^{-1}), agar (5.0 g L^{-1}), and activated carbon (200 mg L^{-1}) were added to the medium.

In vitro induction of rooting by different concentrations of activated carbon. Aseptic seedlings of 'Zijingling' were inoculated on 1/2 MS rooting medium containing different concentrations of activated carbon (0, 100, 200, and 300 mg L^{-1}). IBA (0.6 mg L^{-1}), sucrose (15.0 g L^{-1}), and agar (5.0 g L^{-1}) were added to the medium.

Histological and cytological observations. Paraffin sections were made for observations. We refered to Liu's method and improve it [Liu et al. 2020]; a 1 cm segment at the bottom of the stem was cut, and the sample was fixed with formalin–acetic acid–alcohol (FAA) fixative for more than 24 h. After pumping, dehydrating (10 min), inducing transparency (30 min), impregnating, penetrating, embedding, slicing, dewaxing, and staining (safranin-fixed green staining), the slices were sealed with neutral gum, to make them permanent, and observed under a microscope (Olympus-BX51, by Olympus Corporation, Japan). The model number of the microtome was RM2235 (LEICA), and the thickness of the section was 9 μm. Root samples of tissue culture seedlings of 'Zijingling' were collected on the 0, 3, 6, 12, 20 and 25 days of rooting.

Methods for determination of endogenous hormones. HPLC was performed to detect hormones. The experimental materials was the entire tissue culture seedlings cultured for 30 days. Indole-3-acetic acid (IAA), abscisic acid (ABA), zeatin riboside (ZR), and gibberellic acid (GA3) were the endogenous hormones to be determined. Endogenous hormone standards IAA, ABA, and ZR with a purity of 98% were provided by SolarBio (Beijing, China). GA_3 with a purity of 96% was supplied by Macklin (Shanghai, China). Tissue culture seedlings were cultured in the optimal rooting medium identified by rooting experiments, and tissue culture seedlings cultured in the medium without auxins were selected as the control (C). The samples were taken once before inoculation and once every 5 days after inoculation for a total of seven times.

Samples were treated before detection using the method by Rao with some modifications [Rao 2020]. The samples were weighed with an accuracy of 1 g. After the samples were fully ground to powder, precooled methanol and sodium diethyldithiocarbamatre were added. After storing in a refrigerator for 15 h, the supernatant was centrifuged and evaporated. After three extractions with petroleum ether and ethyl acetate, 2 mL of chromatography-grade methanol was added and dissolved at a constant volume. The sample endogenous hormone extract was obtained by filtration with a 0.45 μm microporous membrane. Each treatment was repeated thrice. Chromatographic conditions were based on the method by Rao [Rao 2020]. The peak area was quantified using the external standard method. The HPLC model was LC-20AT (Shimadzu, Japan). The content of each hormone in the sample was calculated using the following equation.

$A = (B \times C \times D) / E$, where A (μg g^{-1}) is the content of each hormone in the sample, B (μg mL^{-1}) is the sample hormone concentration, C (mL) is the volume coefficient, D (%) is the purity of the endogenous hormone standard, and E (g) is the mass coefficient.

Statistical analysis of data

All experimentation was repeated thrice, with 40 explants pertreatment. Statistical analysis of endogenous hormone content changes and rooting efficiency during root. The experimental results were subjected to analysis of variance (ANOVA), and the significant differences were selected by Duncan's multiple range test ($P < 0.05$) with SPSS 25.0 (IBM Inc., New York, NY, USA) and Origin 2018 software.

RESULTS

In vitro rooting induction experiment of 'Zijingling': effects of different basic media. The results of rooting induced by three basic media are shown in Table 1. Under the same auxin type, concentration, and other conditions, the rooting induction results of the three basic media were different, indicating that the media had a significant influence on the rooting induction of 'Zijingling'. Compared with the other two basic media, 1/2 MS medium had a better rooting effect; the rooting efficiency was 92.5%, and the average number of roots and average root length were the highest, which were significantly different from those of 1/2 WPM and 1/2 DKW. The rooting efficiency of 1/2 DKW was higher than that of 1/2 WPM, but there

was no significant difference in rooting coefficient and root length between them.

Effects of different auxin types and concentrations on in vitro rooting induction of 'Zijingling'. Rooting culture results of the three auxins and different concentrations of IBA are shown in Table 2. The data showed that different auxins had different effects on rooting induction. The rooting efficiencies and average number of rooting strips induced by the three auxins were significantly different. The effect of IBA was higher than that of NAA and IAA in terms of the rooting efficiency, average number of roots, and average root length, indicating that the addition of a certain amount of IBA had the most significantt promoting effect on the induction of in vitro rooting. With an increase in IBA concentration, the rooting efficiency, mean number of roots, and mean root length all increased initially and then decreased in all groups. When the concentration was of IBA 0.6 mg L^{-1}, the rooting promotion was most conspicuous, the rooting efficiency was the highest, and the growth state of the inoculated seedlings was the best.

Effects of different activated carbon concentrations on in vitro rooting induction experiment of 'Zijingling'. Activated carbon is an effective additive to promote the rooting of *L. indica*. To determine the best concentration suitable for the induction of rooting, we studied the promoting effects of different concentrations of activated carbon on rooting. As indicated by the experimental data in Table 3, there were no significant differences in rooting efficiency at day 30 between the four concentrations of activated carbon. In terms of average root number and average root length, the promotion effect of 200 mg L^{-1} activated carbon was significantly different from that of the other three concentrations, but there were no differences among the other three concentrations. With an increase in the concentration of activated carbon, the average number of roots, and average root length increased slowly at first and then decreased. Therefore, 200 mg L^{-1} activated carbon had the best promoting effect and was most suitable for addition to the rooting medium.

Transplant tissue culture seedlings. The roots of each treated tissue culture seedling were cleaned and planted into a disinfected matrix of peat soil and perlite in a ratio of 3 : 1, sprayed with water, and then placed in a clean and well-drained plastic greenhouse. After 30 days, the survival rate of transplanted seedlings under different treatments was more than 90%.

Histological and cytological observations. The optimal rooting medium was used to culture aseptic seedlings of 'Zijingling', which were then processed as paraffin section material to observe the cell structure. Simultaneously, a stereomicroscope (Olympus) was used to record the external morphological changes of roots during the rooting process.

External morphology of 'Zijingling' during rooting. Based on observations under a microscope, it was found that 'Zijingling' began to grow roots on the 6th day after culture. The incision at the stem base of 'Zijingling' showed a tendency of contractive healing on the 3rd day (Fig. 1B), compared with day 0, but no callus appeared. On the 6th day of culture (Fig. 1C), the bottom of the stem began to swell, the epidermis was swollen and cracked, thereby exposing the root cap, and no callus was found at this time. After 12 days (Fig. 1D), the adventitious roots elongated slowly and formed a complete root structure. The adventitious roots of 'Zijingling' continued to grow, and formed a complete root system (Fig. 1E and 1F), and the induced growth of adventitious roots was completed.

Anatomical structure of 'Zijingling' during rooting. The results of paraffin sections of 'Zijingling' are shown in Fig. 2. The cross-section consists of the epidermis, cortex, phloem, vascular cambium, primary xylem, and pith, arranged concentrically from the outside to the inside (Fig. 2A). The epidermal cells were oval and tightly arranged in a circle. The cortex was composed of multiple layers of collenchyma and parenchyma, which were loosely and irregularly arranged. Phloem was mainly composed of phloem fibers and phloem parenchyma cells in a loose arrangement. The cambium cells had the ability to divide. The central medulla consisted of round parenchymal cells, mainly used for nutrient storage.

The paraffin sections showed that no latent root primordia were present in the cortex, phloem, xylem, and pith ray before rooting induction, which indicated that the adventitious roots of 'Zijingling' were induced and differentiated.

On the 3rd day of culture (Fig. 2B), cells in the vascular cambium region regained their ability to divide, and formed a nearly round meristem mass with compact arrangement, small volume, and dense cytoplasm.

Table 1. Effects of different basal media on induction of rooting of *Lagerstroemia indica* 'Zijingling'

Basal medium	Concentration of IBA (mg L^{-1})	Average root number	Average root length (cm)	Rooting efficiency at day 30 (%)
1/2MS	0.6	6.9 ±0.5a	2.3 ±0.1a	92.5 ±2.5a
1/2DKW	0.6	4.9 ±0.2b	1.0 ±0.1b	59.2 ±1.4c
1/2WPM	0.6	5.4 ±0.1b	1.0 ±0.2b	75.8 ±1.4b

* Mean value [±SE and n = 40] in same column with different letter (s) indicate significant differences (P < 0.05) based on Duncan's multiple range test

Table 2. Effects of different auxins and concentrations on induction of rooting of *Lagerstroemia indica* 'Zijingling'

Basal medium	Auxin type	Auxin concentration (mg L^{-1})	Average root number	Average root length (cm)	Rooting efficiency at day 30 (%)
1/2MS	IAA	0.6	5.86 ±0.2b	1.1 ±0.1bc	71.7 ±2.9d
1/2MS	NAA	0.6	5.9 ±0.2b	1.2 ±0.1b	83.3 ±1.4b
1/2MS	IBA	0.1	4.55 ±0.2c	1.15 ±0.01b	71.7 ±1.4d
		0.6	6.94 ±0.5a	2.29 ±0.05a	92.5 ±2.5a
		1.2	4.74 ±0.2c	1.08 ±0.05bc	77.5 ±0c
1/2MS	No auxin	0	3.43 ±0.2d	1.0 ±0.06c	46.7 ±3.8e

* Mean value [±SE and n = 40] in same column with different letter (s) indicate significant differences (P < 0.05) based on Duncan's multiple range test

Table 3. Effects of different concentrations of activated carbon on induction of rooting of *Lagerstroemia indica* 'Zijingling'

Activated carbon concentration (mg L^{-1})	Average root number	Average root length (cm)	Rooting efficiency at day 30 (%)
0	5.8 ±0.2b	1.3 ±0.1b	89.2 ±1.4a
100	6.3 ±0.3b	1.5 ±0.2b	91.7 ±1.4a
200	6.9 ±0.5a	2.3 ±0.1a	92.5 ±2.5a
300	6.1 ±0.3b	1.4 ±0.1b	90.8 ±2.9a

* Mean value [±SE and n = 40] in same column with different letter (s) indicate significant differences (P < 0.05) based on Duncan's multiple range test

This mass expanded outward to form the secondary phloem, which was stained much darker than the surrounding parenchyma cells that formed the original root primordium. After the formation of root primordia, their division ability was continuously enhanced, and the division and expansion continued, resulting in the formation of protrusions in the phloem and cortex, that was, the growth point of root primordia (Fig. 2C). On the 9th day of induction (Fig. 2D), root primordia continued to grow and elongate outward, reaching the epidermal region, and some root tips broke through the epidermis and extended to form adventitious roots. With an increase in induction time, the root primordia finally broke through the epidermis and formed a complete root structure with root cap, meristem zone, and elongation zone (Fig. 2E). Adventitious roots continued to grow, forming an independent and complete root system (Fig. 2F–2H).

Changes in endogenous hormone content in 'Zijingling' during rooting. The mother liquor of ZR, GA_3, IAA and ABA standards was mixed and diluted to prepare standard samples with five concentration gradients for HPLC detection and analysis. The standard curves of the hormones were plotted taking the peak area as the ordinate (y) and the mass concentration of hormone (μg mL^{-1}) as the abscissa (x). The linear regression equation and correlation coefficient of the four endogenous hormones were derived. The correlation coefficients of the four endogenous hormone standards were all greater than 0.9999, indicating a good linear relationship between the concentration and peak area.

Change in ZR content. As shown in Fig. 3A, during the induction of rooting of 'Zijingling', there were two peaks for the overall change in ZR content in the entire tissue culture seedlings of the treatment group and the control group added with IBA. In the treatment group, the maximum value was 33.5 μg g^{-1} (fresh weight – fw) on the 25th day, and in the control group, the maximum value was 35.25 μg g^{-1} (fw) on the 20th day. The root primordium of 'Zijingling' differentiated from day 0 to 10th day. The ZR content in the control group initially decreased and then increased rapidly, whereas the ZR content in the treatment group increased slowly, and this increase was less than that in the control group. When the root primordia differentiated to form and grow root organs (10th to 20th day), the ZR content in the control group was always higher than that in the treatment group, and the differencewas a maximum of 8.8 μg g^{-1} (fw) on the 20th day, and subsequently both began to decline.

Change in GA_3 content. As shown in Fig. 3B, when the root primordia of 'Zijingling' differentiated between day 0 and 10th day, the GA_3 contents in both the treatment and control groups increased rapidly, and reached their maximum values on the 10th day, which were 526.77 and 457.25 μg g^{-1} (fw), respectively; the maximum difference between them was 69.52 μg g^{-1} (fw). Thereafter, the changes were the same between the 10th and 25th day, with a trend of rapid decrease followed by a small increase and subsequently, a decrease. During the increasing period, the GA_3 content in the treatment group was always higher than that in the control group.

Change in IAA content. As shown in Fig. 3C, the IAA content in the treatment and control groups showed the same trend during rooting of 'Zijingling' within 30 days of initially increasing, followed by a decrease, increase and a decrease. The IAA content in the treatment group was always higher than that in the control group. The two peak values of the treatment and control groups were exhibited on the 10th and 20th days, among which, the highest value of IAA content in the treatment group was 150.05 μg g^{-1} (fw) on the 20th day, whereas the highest IAA content in the control group was 101.38 μg g^{-1} (fw) on the 10th day, and the maximum difference was 58.15 μg g^{-1} (fw) on the 20th day.

Change in ABA content. The change trend of ABA in Fig. 3D shows that it was not synchronous in the treatment and control groups. There were two peaks in the treatment group, but only one in the control group. The specific change trend of the treatment group was a sharp increase in the content between the day 0 and 5th day, a decrease between the 5th and 20th day, followed by a rise again, and a decline between the 25th and 30th day. In the control group, the trend of change was a slow increase from the day 0 to 10th day and a downward trend from the 10th to 30th day. The maximum value of the treatment group was 39.52 μg g^{-1} (fw) on the 5th day, whereas that of the control group was 41.67 μg g^{-1} (fw) on the 10th day, and the maximum difference between the two was 15.3 μg g^{-1} (fw) on the 20th day.

Fig. 1. External morphological characteristics of *Lagerstroemia indica* 'Zijingling' during rooting. 0 day of culture (A), 3rd day (B), 6th day (C), 12th day (D), 20th day (E), 25th day (F)

* Rooting medium: 1/2 MS + 0.6 mg L^{-1} IBA+ 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon. By stereomicroscope (Olympus-SZX2, by Olympus Corporation, Japan).

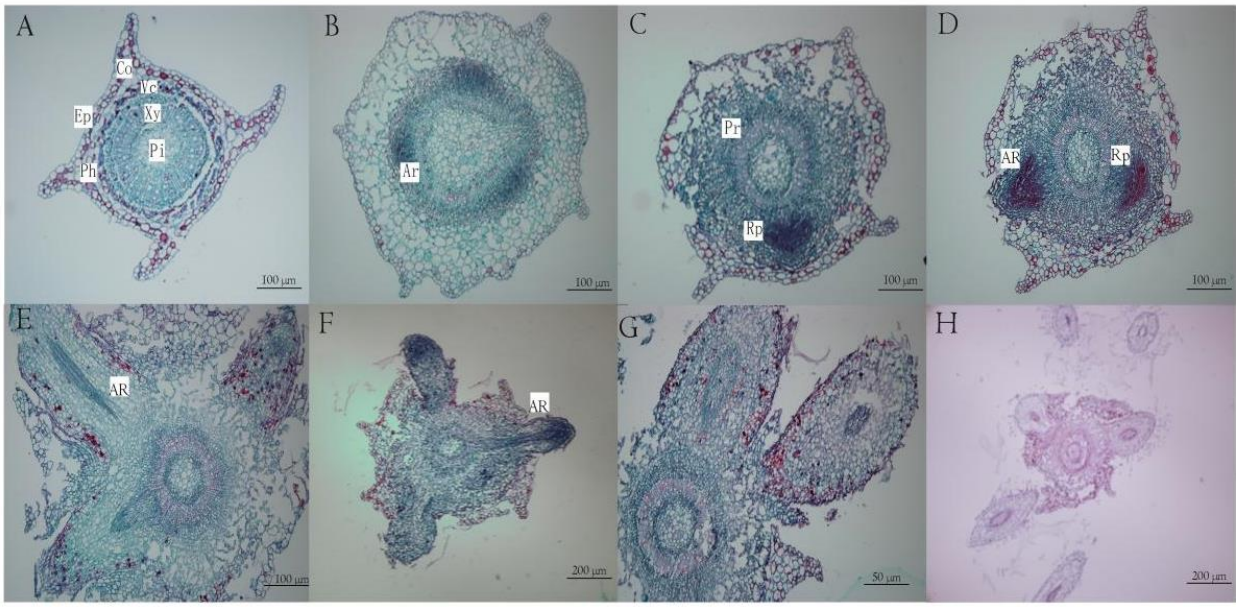

Fig. 2. Process of adventitious root formation in Lagerstroemia indica 'Zijingling'

Ep – epidermis, Co – cortex, Ph – phloem, Vc – vascular cambium, Xy – xylem, Pi – pith, Ar – adventitious root primordial body, Pr – pith ray, Rp – root primordium, AR – adventitious root.
Day 0 (A); 3rd day (B); 6th day (C); 9th day (D); 12th day (E); 15th day (F); 18th day (G); 21st day (H).
* Rooting medium: 1/2 MS + 0.6 mg L^{-1} IBA+ 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon. By microscope (Olympus-BX51, by Olympus Corporation, Japan).

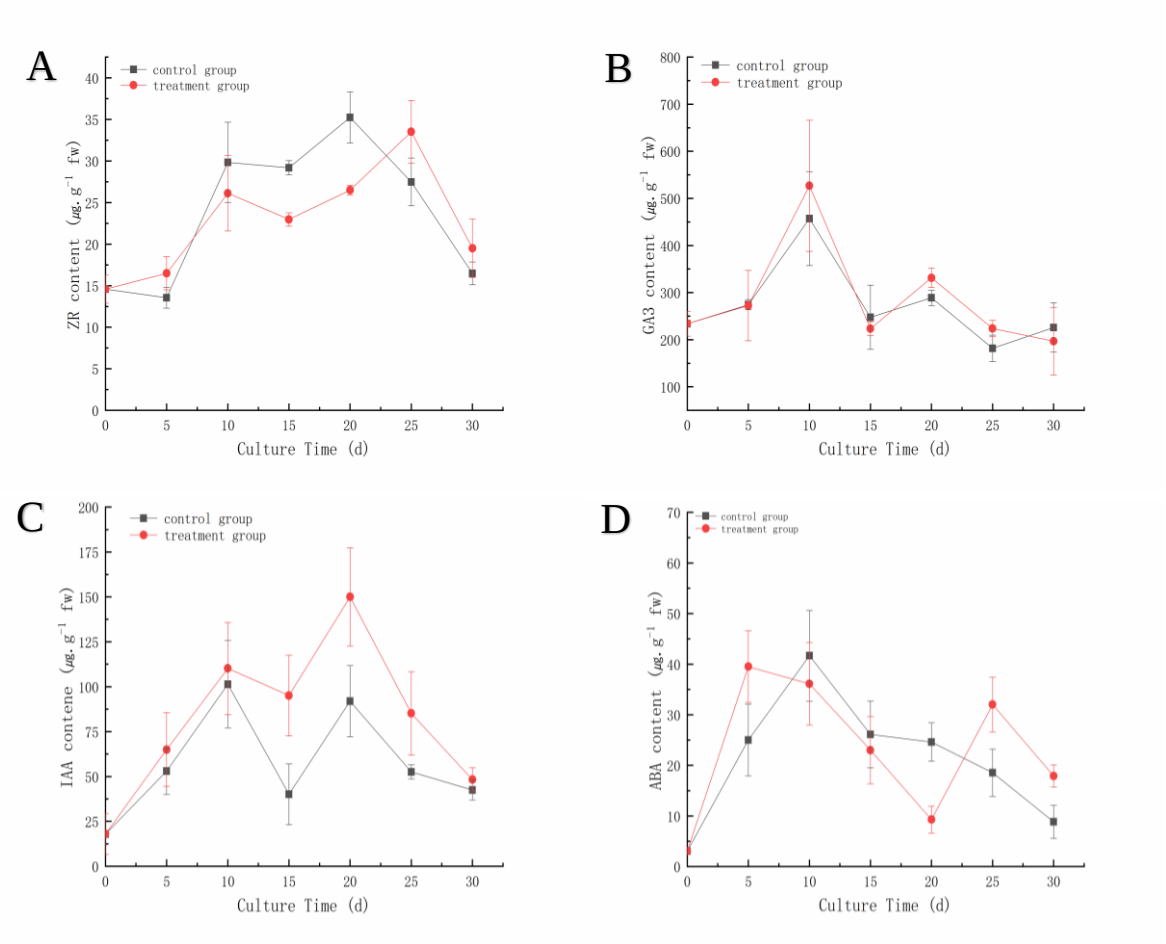

Fig. 3. Changes in ZR, GA_3, IAA, and ABA contents in the entire tissue culture seedlings during rooting of *Lagerstroemia indica* 'Zijingling'

* Treatment group: 1/2 MS + 0.6 mg L^{-1} IBA+ 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon;
Control group: 1/2 MS + 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon;
By HPLC (LC-20AT, Shimadzu, Japan)

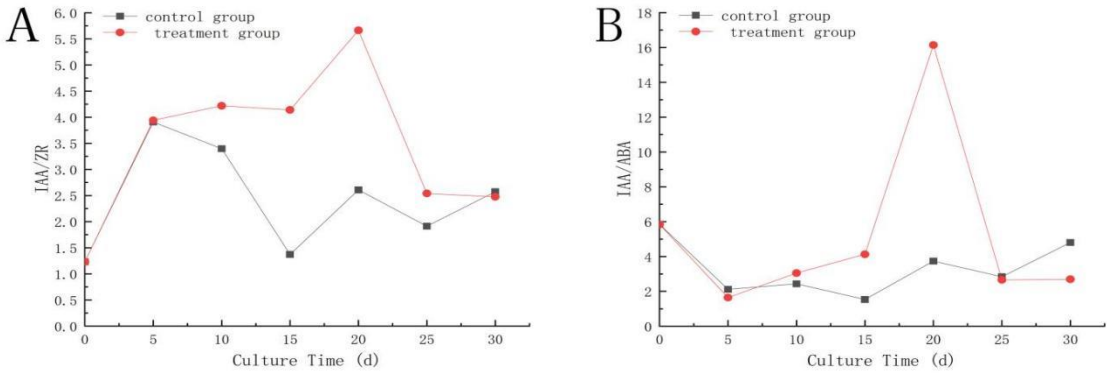

Fig. 4. Changes in IAA/ZR and IAA/ABA ratios in the entire tissue culture seedlings during rooting of *Lagerstroemia indica* 'Zijingling'.

* Treatment group: 1/2 MS + 0.6 mg L^{-1} IBA+ 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon;
Control group: 1/2 MS + 15 g L^{-1} sucrose + 5 g L^{-1} agar + 200 mg L^{-1} activated carbon;
By HPLC (LC-20AT, Shimadzu, Japan).

Changes in endogenous hormone ratio. The IAA/ZR ratio is an important indicator of adventitious root growth in tissue culture. A high ratio promotes adventitious root differentiation and growth, whereas a low ratio promotes bud differentiation. The IAA/ZR ratio increased rapidly during the induction of the rooting of 'Zijingling' to promote the differentiation and growth of the root primordium. In the control group, the ratio decreased after the adventitious roots broke through the epidermis, increased at the beginning of root growth, and subsequently decreased again. In the treatment group, it began to decline at the later stage of root growth (Fig. 4A).

The IAA/ABA values of the treatment and control groups showed a decreasing trend during root primordium induction. In the early stage of root primordium formation and adventitious root growth, the ratio decreased in the control group decreased and subsequently increased, whereas the ratio in the treatment group increased continually. Between the 15th and 20th day, the increase rate and ratio were highest, with a maximum value of 16.14; the ratio was 4.31 times that of the control group (Fig. 4B).

DISCUSSION

Effect of basal medium, growth regulators, and activated carbon on rooting induction

Induction and growth of adventitious roots is an essential step in tissue culture [Díaz-Sala 2021]. In this study we focused on selecting the most suitable medium for in vitro rooting of 'Zijingling' and improving the rooting efficiency. A comparison of three basic media, 1/2 MS, 1/2 WPM, and 1/2 DKW, indicated that 1/2 MS was the most suitable medium for the induction of rooting of *L. indica* 'Zijingling'. This is consistent with the rooting medium selected by Faisal [Faisal et al. 2017]. Chen selected ZW (modified WPM) as the basic medium for the rooting of *L. indica* [Chen et al. 2015]. Whereas Cai et al. selected 1/2 DKW as the best rooting medium for a superior variety of *Lagerstroemia indica* 'Xiaoming 1', which indicates that different species or varieties of *L. indica* have different requirements of mineral elements during rooting induction [Cai et al. 2016].

In terms of the effects of auxin type and concentration, an addition of 0.6 mg L^{-1} IBA had the most conspicuous promoting effect on the rooting induction of 'Zijingling'. This also proves that IBA is the most commonly used and effective auxin in plant rooting because it is very stable [Sousa Costa et al. 2018]. Duan et al. chose 0.5 mg L^{-1} IBA as the optimal concentration of auxin for root induction of *L. indica* [Duan et al. 2013]. A stuty showed that different concentrations of IBA had different results on the rooting efficiency of blueberry cultivars [Guo et al. 2019]. Zeng et al. reported that regenerated shoots transferred to the medium containing 0.5 mg L^{-1} IBA achieved the highest frequency of rooting (100%) [Zeng et al. 2019].

Our experiment showed that adding 200 mg L^{-1} activated carbon could effectively improve the root length of 'Zijingling'. Li et al. proposed that the function of activated carbon might be to adsorb and remove toxic substances produced by the culture, and provide a suitable dark growth environment for roots [Li et al. 2020]. Therefore, appropriate addition of activated carbon can promote the induction of rooting of 'Zijingling'.

Pathway of adventitious root formation in in vitro culture of 'Zijingling'

At present, adventitious roots mainly occur in direct, indirect, and mixed rooting modes. Direct occurrence refers to the direct development of adventitious roots from explants. Indirect occurrence is the main rooting type of the callus, which implies that adventitious roots can be formed first through the callus [Strzelecka 2007]. Sun et al. found that adventitious roots were formed by the differentiation of callus cells, which exhibited callus rooting [Sun et al. 2008]. In a study on the rooting of *Vaccinium ashei* Reade, adventitious roots grew not only from the skin, but also from the callus, and hence exhibited mixed rooting [Song et al. 2014]. Through stereomicroscopy and sectioning, it was observed that there was no callus in the process of adventitious root formation of 'Zijingling', and the adventitious roots were directly produced from the skin. Therefore, the rooting mode of 'Zijingling' was direct.

The generation of adventitious roots first involves the generation of root primordia, and the cell division and differentiation of the root primordia; subsequently, the root primordia further grow and extend through the epidermis and pericarp to form adventitious roots

[Joshi and Ginzberg 2021]. Since adventitious roots of different plants develop at different times, root primordia can be categorized into latent and inducible root primordia [Hartmann et al. 1990]. Xiang et al. found that *Albizia julibrissin* Durazz exhibited the primordial type of induced rooting based on their study on the rooting of hardwood cuttings [Zhou et al. 2016]. Latent root primordium was not found in the sections of 'Zijingling' before induction, indicating that the root primordium was induced after the culture and exhibited induced rooting. Moreover, the root primordia were mainly produced between the vascular cambium and the medullary rays, and root primordia were not found in other parts, indicating that the root primordia of 'Zijingling' developed at a specific point.

Effects of changes in endogenous hormone content on rooting

Adventitious roots are produced by the combined action of many internal and external factors. The fact that auxin is involved in the process of adventitious root initiation is well established [Blakesley et al. 1991]. The change in IAA content during the rooting process of 'Zijingling' shows that with the differentiation and growth of the root primordium (0–10 days), the IAA content in the treatment and control groups increased gradually, indicating that more IAA content was needed to promote the division and growth of the root primordium. Benková found that as lateral root primordia form, strong auxin response can be observed in the initial cells [Benková et al. 2003]. Moreover, the IAA content in the treatment group was always higher than that in the control group, indicating that added exogenous IBA can be transferred to the base tissues of the stem to convert it into endogenous IAA, and increase its content, and promote the growth of adventitious roots [Van der Krieken et al. 1993]. Jawahir and Zolman conclude that, LACS4 and LACS6 catalyze the addition of CoA onto IBA, the first step in IBA metabolism and a necessary step in generating IBA-derived IAA [Jawahir and Zolman 2021]. Fattorini also showed that the IBA alone induces adventitious root formation in thin cell layers of Arabidopsis thaliana by conversion of IBA into IAA [Fattorini et al. 2017]. After the completion of root primordia differentiation, the IAA content reached a peak when adventitious roots continued to divide and elongate, indicating that a large amount and high endogenous levels of IAA was also needed for subsequent adventitious root growth. Bai demonstrated that exogenous IBA is necessary for the formation of adventitious root primordia in apple [Bai et al. 2020].

The results of this study showed that the GA_3 content increased slightly during the induction and differentiation stages of the root primordium of 'Zijingling'. The effect of auxins, such as indole-3-acetic acid (IAA], is at least in part mediated by its effect on GA metabolism, since auxin upregulates biosynthesis genes and downregulates GA catabolism genes leading to elevated levels of bioactive GA_1 [Reid et al. 2011]. Further, the GA_3 content increased significantly when the root primordium was formed in the late stage and the root tip broke through the epidermis, and reached the maximum value. The value decreased at the later stage of adventitious root growth, indicating that a higher GA_3 content could promote the growth of root primordia and the formation of adventitious roots at the early stage. Fu and Harberd [2003] found that auxin can promote the growth of roots by enhancing the GA-induced destabilization of RGA, and perhaps of other DELLA proteins. However, most experiments indicate that GA_3 can inhibit the formation and growth of adventitious roots, which is likely due to the different mechanisms of endogenous hormones in different plants during adventitious root formation. Therefore, further studies are needed on this topic [Li and Pan 1993].

A study by Chen et al. [2011] have shown that ZR mainly inhibits the differentiation and formation of root primordia during rooting [Qi et al. 2009]. In this study, during the induction of differentiation and formation of root primordia, the ZR content increased slowly, and the content in the treatment group was less than that in the control group, indicating that low concentrations of ZR promoted the induction of differentiation of root primordia. Lohar et al. found that cytokinin response can be seen in the base of the lateral root primordium [Lohar et al. 2004]. During the division and elongation of adventitious roots, the content of ZR gradually increased, which may have been because of the promotion of the synthesis of ZR by exogenous IBA for active cell division during adventitious root growth [Chen et al. 2011]. This is consistent with the results of Qiao regarding the change in ZR content

during the cutting process of sterile *Lagerstroemia indica* 'Xiangyun' [Qiao et al. 2015].

In the treatment group, the content of ABA increased rapidly to the maximum value on the 5th day after culture, which indicated that a higher concentration of ABA was needed for the induction of root primordium growth. Bouza et al. also found that during root induction of Paeonia suffruticosa, when exogenous IBA was quickly absorbed by the explants and was transformed into IAA, the content of ABA was also signifcantly increased [Bouza et al. 1992]. Fu et al. reported that the expression of auxin response factor ARF2 was signifcantly up-regulated as the IAA content increased after rooting, and this may have promoted ABI transcription to activate ABA signaling pathway, this may be the reason for the increase of ABA content after rooting [Fu et al. 2021]. Further, the content of ABA decreased gradually after adventitious root formation, which indicated that low concentrations of ABA were conducive to adventitious root growth. Jiang and Guan also reported that high levels of ABA induced root primordia growth [Jiang and Guan 2000]. The change of ABA content during Rooting also showed a bimodal pattern, which was the same as that of Zhou [Zhou and Zhang 2010]. ABA could promote adventitious root formation by inhibiting the growth of terminal buds and facilitating the transport and accumulation of more nutrients to the base of branches. Gubler and Jacobsen's results confirmed that the TAACAAA box played a central role in both GA and ABA regulation of α-amylase gene expression, and functional analysis of α-amylase promoter sequences revealed that the TAACAAA box was also the likely site of ABA action in repressing GA promotion of gene expression [Gubler and Jacobsen 1992]. And whether the increase of ABA content in the final stage is related to the decrease of GA_3 content remains to be further studied.

Several experimental studies have indicated that during the induction of adventitious root growth, the effect produced by a relatively balanced ratio of multiple endogenous hormones is not produced by a single endogenous hormone that regulates the formation of adventitious roots through synergistic or antagonistic action. Cytokinins are negative regulators of root growth and lateral root formation, and auxins are positive regulator [Werner, et al. 2003]. Auxin and cytokinin are antagonistic during root meristem transition zone [Růžička et al. 2009, Su et al. 2011]. In the process of in vitro rooting, a high IAA/ZR ratio promoted the differentiation and growth of root primordia and the elongation of adventitious roots, and the ratio in the treatment group was higher than that in the control group, indicating that the addition of exogenous IBA helped increase the IAA/ZR ratio. Low levels of IAA/ABA benefited root primordia differentiation, whereas high levels of IAA/ABA benefited adventitious root elongation. Zheng et al. studied the rooting efficiencys of several woody plants and found that an increase in endogenous IAA/ABA ratio during the rooting process also contributed to the growth of adventitious roots [Zheng et al. 1991].

CONCLUSIONS

In this study, through experiments on in vitro rooting induction of 'Zijingling' variety of *L. indica*, it was found that the most suitable basal medium for in vitro rooting was 1/2 MS, and the auxin that best promoted the rooting effect was IBA, at a concentration of 0.6 mg L^{-1}, combined with activated carbon. 1/2 MS + 0.6 mg L^{-1} IBA + 15.0 g L^{-1} sucrose + 5.0 g L^{-1} agar + 200 mg L^{-1} activated carbon was selected as the most suitable medium for the rooting of *L. indica* 'Zijingling'. The results showed that adventitious roots were of the primordial type of induced roots that take root in a relatively, short time, and mainly grew between the vascular cambium and pith ray. We determined the changes in endogenous hormone content in entire aseptic seedlings of 'Zijingling'. Exogenous IBA may also promote the accumulation of endogenous GA_3 and ABA, and enhance the differentiation of root primordia at the early stage of induction. High levels of IAA/ZR were beneficial to the induction of root primordia and adventitious root growth, low levels of IAA/ABA were beneficial to the differentiation of root primordia, and high levels of IAA/ABA promoted root elongation.

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CONFLICTS OF INTEREST

The authors state that they have no conflict of interest.

REFERENCES

Bai, T., Dong, Z., Zheng, X., Song, S., Jiao, J., Wang, M., Song, C. (2020). Auxin and its interaction with ethylene control adventitious root formation and development in apple rootstock. Front. Plant Sci., 11, 574881. https://doi.org/10.3389/fpls.2020.574881

Benková, E., Hejátko, J. (2009). Hormone interactions at the root apical meristem. Plant Mol. Biol., 69, 383. https://doi.org/10.1007/s11103-008-9393-6

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G., Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell, 115(5), 591–602. https://doi.org/10.1016/s0092-8674(03)00924-3

Blakesley, D., Weston, G.D., Hall, J.F. (1991). The role of endogenous auxin in root initiation. Plant Growth Regul., 10(4), 341–353. https://doi.org/10.1007/bf00024593

Bouza, L., Sotta, B., Bonnet, M., Jacques, M., Arnaud, Y. (1992). Hormone content and meristematic activity of *Paeonia sufruticosa* Andr. cv. 'Madame de Vatry' vitroplants during in vitro rooting. Acta Hortic., 320(29), 213–216. https://doi.org/10.17660/ActaHortic.1992.320.29

Cai, N., Wang, X.M., Li, Y.X., Zeng, H.J., Qiao, Z.Q. (2016). Establishment of tissue culture and rapid propagation system of *Lagerstroemia indica* 'Xiaoming 1'. Chinese Agric. Sci. Bull., 1, 22–27 [in Chinese]. https://doi.org/10.11924/j.issn.1000-6850.casb15100075

Chen, L.Y., Zheng, Y., Chen, L.G., Rong, J.D., Zheng, Y.S. (2011). Study on rooting culture and changes of endogenous hormone content of *Rhododendron hybridum*. J. For Environ, 31, 131–135 [in Chinese]. https://doi.org/10.13324/j.cnki.jfcf.2011.02.011

Chen, Y.J., Cui, Y.Y., Zhang, X.M., Deng, X.M. (2015). Tissue culture and rapid propagation of *Lagerstroemia indica* 'pink Velour'. Plant Physiol. J., 51, 882–886 [in Chinese]. https://doi.org/10.13592/j.cnki.ppj.2015.0160

Corredoira, E., Costa, R.L. (2021). Application of tissue culture in plant reproduction. Forests, 12, 342. https://doi.org/10.3390/f12030342

Das, S., Sultana, K.W., Chandra, I. (2020). In vitro micropropagation of *Basilicum polystachyon* (L.) Moench and identification of endogenous auxin through HPLC. Plant Cell Tissue Organ Cult., 141, 633–641. https://doi.org/10.1007/s11240-020-01824-3

Díaz-Sala, C. (2021). Adventitious root formation in tree species. Plants, 10, 486. https://doi.org/10.3390/plants10030486

Driver, J.A., Kuniyuki, A.H. (1984). In vitro propagation of Paradox walnut rootstock. HortSci., 19, 507–509.

Duan, L.J., Li, G.R., Tong, J., Ye, Y.M. (2013). Study on rapid propagation system of *Lagerstroemia indica* stem segments in vitro. Acta Agric. Univ. Jiangxiensis, 35, 709–714. https://doi.org/10.13836/j.jjau.2013125

Eymar, E., Alegre, J., Toribio, M., López-Vela, D. (2000). Effect of activated charcoal and 6-benzyladenine on in vitro nitrogen uptake by *Lagerstroemia indica*. Plant Cell, Tissue Organ Cult., 63, 57–65. https://doi.org/10.1023/A:1006471519609

Faisal, M., Naseem, A., Mohammad, A., Abdulrahman, A.A., Ahmad, A.Q. (2017). Auxin-cytokinin synergism in vitro for producing genetically stable plants of Ruta graveolens using shoot tip meristems. Saudi J. Biol. Sci., 25(2), 273–277. https://doi.org/10.1016/j.sjbs.2017.09.009

Fattorini, L., Veloccia, A., Della Rovere, F., D'Angeli, S., Falasca, G., Altamura, M.M. (2017). Indole-3-butyric acid promotes adventitious rooting in Arabidopsis thaliana thin cell layers by conversion into indole-3-acetic acid and stimulation of anthranilate synthase activity. BMC Plant Biol., 17(1), 121. https://doi.org/10.1186/s12870-017-1071-x

Fu, X., Harberd, N. (2003). Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature, 421, 740–743. https://doi.org/10.1038/nature01387

Fu, Z., Xu, M., Wang, H., Wang, E., Li, Y., Wang, L., Gao, J., Zhang, J., Yuan, Xin, Zhang, H. (2021). Analysis of the transcriptome and related physiological indicators of tree peony (*Paeonia suffruticosa* Andr.) plantlets before and after rooting in vitro. Plant Cell Tissue Organ Cult., 147, 529–543. https://doi.org/10.1007/s11240-021-02145-9

Gubler, F., Jacobsen, J.V. (1992). Gibberellin-responsive elements in the promoter of a barley high-pI alpha-amylase gene. Plant Cell, 4(11), 1435–1441. https://doi.org/10.1105/tpc.4.11.1435

Guo, Y.X., Zhao, Y.Y., Zhang, M., Zhang, L.Y. (2019). Development of a novel in vitro rooting culture system for the micropropagation of highbush blueberry (*Vaccinium corymbosum*) seedlings. Plant Cell Tissue Organ Cult., 139, 615–620. https://doi.org/10.1007/s11240-019-01702-7

Haberlandt, G. (1902). Culturversuche mit isolierten Pflanzenzellen. In: Plant tissue culture, Laimer, M., Rücker, W. (eds.). Springer, Vienna. https://doi.org/10.1007/978-3-7091-6040-4_1

Hartmann, H.T., Kester, D.E., Davies, F.T. (1990). Anatomical and physiological basis of propagation by cuttings. In: Plant propagation, principles and practices. Inter Edit, London, 199–225.

Huang, Q.C. (1984). Axillary bud culture of *Lagerstroemia indica*. Plant Physiol. J., 3, 44 [in Chinese]. https://doi.org/10.13592/j.cnki.ppj.1984.03.013

Jawahir, V., Zolman, B.K. (2021). Long chain acyl CoA synthetase 4 catalyzes the first step in peroxisomal indole-3-butyric acid to IAA conversion, Plant Physiol., 185(1), 120–136. https://doi.org/10.1093/plphys/kiaa002

Jiang, L., Guan, X.C. (2000). Plant hormones and adventitious root formation. Bull. Biol., 11, 17–19 [in Chinese]. https://doi.org/CNKI:SUN:SWXT.0.2000-11-009

Jiang, X.H., Song, G., Zhang, H., Cao, R., Guan, B., Liu, G. (2004). Tissue culture and rapid propagation of Lagerstroemia indica. Plant Physiol. J., 6, 707 [in Chinese]. https://doi.org/10.13592/j.cnki.ppj.2004.06.016

Joshi, M., Ginzberg, I. (2021). Adventitious root formation in crops – Potato as an example. Physiol. Plant., 172, 124–133. https://doi.org/10.1111/ppl.13305

Liu, G.B., Zhao, J.Z., Zhang, Y.P., Liao, T., Guo, L.Q., Cao, J. (2020). Study on adventitious root formation mode of *Platycladus orientalis* cutting [J]. Acta Bot. Boreali-Occident Sin 40: 987–996 [in Chinese]. https://doi.org/10.11929/j.swfu.201806007

Li, H.H., Pan, R.C. (1993). Hormone control of adventitious rooting in mung bean stem cuttings. XV International Botanical Congress, Japan Pacifico Yokohama. 6183.

Li, X., Li, G.Z., Yang, L., Zhao, P., Ding, Y. (2020). Progress in tissue culture of the genus *Vaccinium*. Plant Physiol. J. 56, 921–930 [in Chinese]. https://doi.org/10.13592/j.cnki.ppj.2019.0613

Lloyd, G., McCown, B.H. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot tip culture. Int. Plant Prop. Soc. Comb. Proc., 30, 421–427. Available: https://ipps.org/search-advanced/?q=Commercially feasible+micropropagation+of+mountain+laurel%2C+Kalmia+latifolia%2C+by+use+of+shoot+tip+culture&filterby=All&search=Search&submit=submit

Lohar, D.P., Schaff, J.E., Laskey, J.G., Kieber, J.J., Bilyeu, K.D., Bird, D.M. (2004). Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. Plant J., 38, 203–214. https://doi.org/10.1111/j.1365-313X.2004.02038.x

Müller, B., Sheen, J. (2008). Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. Nature, 453, 1094–1097. https://doi.org/10.1038/nature06943

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Negishi, N., Nakahama, K., Urata. N., Kojima, M., Sakakibara, H., Kawaoka, A. (2014). Hormone level analysis on adventitious root formation in *Eucalyptus globulus*. New Forest, 45, 577–587. https://doi.org/10.1007/s11056-014-9420-1

Niranjan, M.H., Sudarshana, M.S. (2005). In vitro response of encapsulated somatic embryos of *Largerstroemia indica* L.. Indian J. Exp. Biol., 43, 552–554.

Phillips, G.C., Garda, M. (2019). Plant tissue culture media and practices: an overview. In vitro Cell. Dev. Biol., Plant 55, 242–257. https://doi.org/10.1007/s11627-019-09983-5

Qi, Y.S., Zhang, Z.H., Wang, T.K., Du, G.Q., Zhang, J.Z. (2009). Changes of endogenous hormones in adventitious root formation in soft-wood cuttings of autotetraploid Vitis vinifera L.. Acta Hortic. Sin., 36, 565–570. https://doi.org/10.16420/j.issn.0513-353x.2009.04.014

Qiao, Z.Q., Wang, X.M., Zeng, H.J., Li, Y.X., Cai, N., Wang, X.Y. (2015). Changes of endogenous hormone content in cutting of sterile *Lagerstroemia indica* 'Xiangyun'. Hunan Sci. Tech., 42, 49–53. https://doi.org/10.3969/j.issn.1003-5710.2015.01.012

Rao, D.D. (2020). Tissue culture and changes of endogenous hormone content of *Lagerstroemia indica* New Variety 'Ziyu'. Cent. South Univ. For. Technol., 2 [in Chinese]. https://doi.org/10.27662/d.cnki.gznlc.2020.000478

Reid, J.B., Davidson, S.E., Ross, J.J. (2011). Auxin acts independently of DELLA proteins in regulating gibberellin levels, Plant Signal. Behav., 6(3), 406–408. https://doi.org/10.4161/psb.6.3.14352

Růžička, K., Šimášková, M., Duclercq, J., Petrášek, J., Zažímalová, E., Simon, S., Friml, J., Montagu, M.E.V., Benková, E. (2009). Cytokinin regulates root meristem activity via modulation of the polar auxin transport. Proc. Natl Acad. Sci. USA, 106(11), 4284–4289. https://doi.org/10.1073/pnas.0900060106

Skoog, F., Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol., 11, 118–130.

Skoog, F., Tsui, C. (1948). Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. Am. J. Bot., 35, 782–787. https://doi.org/10.1002/j.1537-2197.1948.tb08148.x

Song, P.F., Chen, H.J., Jiang, Y.Q., Hong, Y., Qi, X.J. (2014). Studies on the morphology and anatomy of Softwood Cutting Rooting of *Vaccinium ashei Reade*. J. Zhejiang Agr. Sci. 4, 505–508. https://doi.org/10.16178/j.issn.0528-9017.2014.04.029

Sousa Costa, E. Jr, Melo Barbosa, M.S. de, Silva, C.M.A. da, Silva, R.C.S. da, Kiill, L.H.P., Beckmann-Cavalcan-

te, M.Z. (2018). Vegetative propagation of Rhaphiodon echinus Schauer (*Lamiaceae*): effects of the period of cutting in rooting, cuttings arrangement and IBA concentrations for seedlings production. Ornam. Hortic. 24(3), 238–247. https://doi.org/10.14295/oh.v24i3.1232

Strzelecka, K. (2007). Anatomical structure and adventitious root formation in *Rhododendron ponticum* L. cuttings. Acta Sci. Pol. Hortorum Cultus, 6(2), 15–22. Available: https://czasopisma.up.lublin.pl/index.php/asphc/article/view/4208

Stuepp, C.A., Wendling, I., Trueman, S.J., Koehler, H.S., Zuffellato-Ribas, K.C. (2017). The use of auxin quantification for understanding clonal tree propagation. Forests, 8, 27. https://doi.org/10.3390/f8010027

Su, Y.H., Liu, Y.B., Zhang, X.S. (2011). Auxin–cytokinin interaction regulates meristem development. Mol. Plant, 4(4), 616–625. https://doi.org/https://doi.org/10.1093/mp/ssr007

Sun, T.T., Hu, B.Z., Chen, Y.X., Fu, H.B. (2008). Anatomical study on rooting process of softwood cutting of *Rosa xanthina Lindl*. J. Northeast Agric. Univ., 39, 53–56. https://doi.org/10.19720/j.cnki.issn.1005-9369.2008.07.013

Teixeira da Silva, J.A., Zeng, S., Godoy-Hernández, G., Rivera-Madrid, R., Dobránszki, J. (2019). *Bixa orellana* L. (achiote) tissue culture: a review. In Vitro Cell. Dev. Biol. – Plant 55, 231–241. https://doi.org/10.1007/s11627-019-09969-3

Van der Krieken, W.M., Breteler, H., Visser, M., Mavridou, D. (1993). The role of the conversion of IBA into IAA on root regeneration in apple: Introduction of test system. Plant Cell Rep., 12, 203–206. https://doi.org/10.1007/BF00237054

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., Schmülling, T. (2003). Cytokinin-deficient transgenic arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell, 15(11), 2532–2550. https://doi.org/10.1105/tpc.014928

Yan, X., Tian, M., Liu, F., Wang, C., Zhang, Y. (2017). Hormonal and morphological changes during seed development of *Cypripedium japonicum*. Protoplasma, 254, 2315–2322. https://doi.org/10.1007/s00709-017-1128-6

Zeng, Q.Q., Han, Z.Q., Kang, X.Y. (2019). Adventitious shoot regeneration from leaf, petiole and root explants in triploid (*Populus alba* × *P. glandulosa*) × P. tomentosa. Plant Cell Tissue Organ Cult., 138, 121–130. https://doi.org/10.1007/s11240-019-01608-4

Zhang, Q., Luo, F., Liu, L., Guo, F.C. (2010). In vitro induction of tetraploids in crape myrtle (*Lagerstroemia indica L.*). Plant Cell Tissue Organ Cult., 101, 41–47. https://doi.org/10.1007/s11240-009-9660-5

Zheng, J.B., Liu, Y.J., Pei, B.H., Qiang, X.N. (1991). The relationship between rooting and rooting of several woody plants and endogenous IAA and ABA. Physiol. Mol. Biol. Plants, 3, 313–316.

Zhou, J., Zhang, L.J. (2010). Changes of endogenous hormone content in tissue culture seedlings of *Hippophae rhamnoides* L. J. Anhui Agric. Sci., 38(17). https://doi.org/10.13989/j.cnki.0517-6611.2010.17.118

Zhou, X.M., Liu, Y.T., Zhao, X.Z., Song, Z.W., Wang, S. (2016). Rooting anatomy of hardwood cutting for Albizia julibrissin Duraxx. and activity change of related enzymes during rooting process. Bull. Bot. Res., 36, 58–61. https://doi.org/10.7525/j.issn.1673-5102.2016.01.008