Scientific Research Publishing

American Journal of Plant Sciences, 2020, 11, 262-275
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

A Versatile Liquid Culture Method to Control the *in Vitro* Development of Shoot and Root Apical Meristems of Bamboo Plants

Most Tanziman Ara[1], Taiji Nomura[2], Yasuo Kato[2], Shinjiro Ogita[1*]

[1]Graduate School of Comprehensive Scientific Research, Prefectural University of Hiroshima, Nanatsukacho, Shobara, Hiroshima, Japan
[2]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Kurokawa, Imizu, Toyama Japan
Email: *ogita@pu-hiroshima.ac.jp

How to cite this paper: Ara, M.T., Nomura, T., Kato, Y. and Ogita, S. (2020) A Versatile Liquid Culture Method to Control the *in Vitro* Development of Shoot and Root Apical Meristems of Bamboo Plants. *American Journal of Plant Sciences*, **11**, 262-275.
https://doi.org/10.4236/ajps.2020.112020

Received: January 10, 2020
Accepted: February 25, 2020
Published: February 28, 2020

Abstract

We focus on controlling morphological and histochemical responses of the shoot apical meristem (SAM) and root apical meristem (RAM) of bamboo node by using a simple and versatile liquid culture system. First, nodes of 11 different bamboo species that belong to seven major bamboo genera (*Bambusa*, *Dendrocalamus*, *Phyllostachys*, *Tetragonocalamus*, *Chimonobambusa*, *Pleioblastus*, and *Sasa*) were cultured using 2 mL per well of a liquid medium in a 6-well microplate to form a small-scale liquid culture environment (SLCE). The dormant lateral buds of all bamboo nodes resumed expanding and elongating within 7 days in the SLCE. The dormant and active lateral buds were sectioned longitudinally and stained with Sytox green (SG) to monitor mitotic activity and counterstained with safranin (SF) to detect the inward region of the SAM region. Further, mitotic activity was calculated using a digital imaging analysis, which showed an increase of up to 1.2- to 3.8-fold in terms of the SG/SF ratio after 7 days in the culture. Moreover, we used *in vitro* node cultures of two typical bamboo species, the sympodial clump-forming type (*Bambusa multiplex* Raeush, Bm) and the monopodial single culm-forming type (*Phyllostachys meyeri* McClure, Pm), and noted the following: 1) since gradual white-to-green tinge shoots were observed, we investigated the relation between color variation in the outer regions of culm and node tissues and their suitability as explants. By checking the autofluorescence property of whole shoots under LED 365 nm illumination with an RGB (red, green, and blue) digital imaging analysis using ImageJ software, we specified the color variation of explants as the relative intensity of the blue value. 2) Since the obtained shoots of a 1-month-old culture box showed

growth variation, we distinguished shoot types based on plant height, *i.e.*, short (less than 5 cm), medium (ca. 5 - 10 cm), and tall (more than 10 cm). Tall shoots that have ca. 5 nodes on average were suitable for explant. 3) Three types of node portions—the first node (the base node near a rhizome tissue), middle nodes (upper nodes near the 1st node), and the top meristem—were independently cultured in the SLCE, and it was found that the first node showed the best growth performance. 4) By culturing the first node in the SLCE system, we performed a quick survey during the 3 weeks in the culture and found that a combination of 10 μM benzyl adenine and 3 μM thidiazuron was effective for *in vitro* SAM development, while the addition of 2, 4-D was effective for promoting *in vitro* RAM development. 5) The detailed autofluorescence properties of the outer regions of culm and node tissues were also identified using an inverted fluorescent microscope under B- and U-excitation lights with RGB and HSB (hue, saturation, and brightness) digital imaging analysis.

Keywords

Bamboo, Digital Imaging, Node Culture, RAM, SAM, SLCE

1. Introduction

Bamboo plants that belong to the Poaceae family, which contains more than 1500 species, have been exploited for a range of uses, such as food, medicine, charcoal products, and housing materials, especially in Asia [1] [2]. It is well known that bamboo presents unique biological properties in its vegetative growth. A rhizome system is a key to maintain the lateral growth and form new culms with abundant nodes for longitudinal growth without secondary growth. The system is mainly classified into two types of growth feature: one is the sympodial and the clump-forming type (e.g., *Bambusa* sp.), which is distributed in tropical regions, and the other is the monopodial and single culm-forming type (e.g., *Phyllostachys* sp.), which grows mainly in temperate and subtropical regions [3].

A tissue culture protocol of bamboo (*Phyllostachys meyeri* McClure, Pm) was successfully achieved by Ogita *et al.* (2008) specifically regarding *in vitro* germination of the caryopses and plant regeneration from nodal segments of both the germinated seedlings and tissue-cultured clone plants [4]. *In vitro* node culture stocks of (*Bambusa multiplex* Raeush, Bm) have also been established using the method for Pm, as previously reported. Hence, several clonal small plantlets of two major bamboo species that have a high ability to form multiple shoots were obtained in a regular period (ca. every 1 - 2 months), as shown in **Figure 1**. The node portions of these culture stocks with apical and intercalary meristems were considered as excellent models for the morphological and histochemical response control of the shoot apical meristem (SAM) and root apical meristem

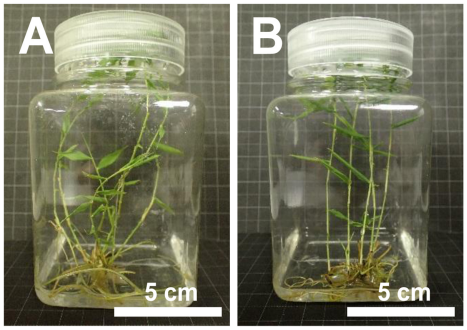

Figure 1. The features of *in vitro* node culture stocks of (A): *Bambusa multiplex* Raeush, Bm; and (B): *Phyllostachys meyeri* McClure, Pm.

(RAM) of bamboo. General tissue culture techniques for *in vitro* micropropagation of bamboos through enhanced axillary branching using node explants have been well reviewed, e.g. by Singh *et al*. (2013) [5]. Factors affecting success in micropropagation of bamboos such as medium, plant growth regulators, medium pH, carbon source, propagule size, and culture duration were summarized in this report. However, there is no detailed description of a versatile methodology to regulate *in vitro* growth of bamboo nodes. The aim of this study is to review a simple but versatile node culture system in a liquid culture environment to better understand unique biological properties of its vegetative growth and to, thus, reveal the relation between color variation in the outward regions of culm and node tissues and their suitability as explants.

The concept of the Plan-Do-Check-Act (PDCA) cycle in a plant tissue culture [6] is central for the present study. Generally, researchers should realistically consider the following requirements: explant selection, medium preparation, and culture environment setting in the P and D cycles. Based on a careful monitoring of size, shape, and color variation of the target cells and tissues by macro- and micro-scopic observations, they can recognize the specific pattern of growth promotion and/or inhibition during the culture of the target explant. These inputs are considered highly useful and interesting for the optimization of a protocol, especially regarding the C and A cycles.

A main topic of the present study is the culture environment setting. Sood *et al*. (2002) tested the effects of agar-solidified medium and liquid medium on shoot multiplication, whereby root formation of *Dendrocalamus hamiltonii* was investigated; it was found that a liquid culture condition is more suitable for the tissue culture of this bamboo species [7]. Using node culture system, we checked the effects of solid and liquid media on the growth of bamboo node cultures of Bm and Pm, and found that better growth performance was observed in the liquid medium condition (**Table 1**). Based on this preliminary result, a 6-well microplate that contains 2 mL per well of a liquid medium, which provides a small-scale liquid culture environment (SLCE) for optimizing the culture protocol of the bamboo within a short period was focused in the present study.

Table 1. Effect of solid and liquid medium conditions on growth performance of bamboo node cultures.

Species/ medium condition	Growth performance				
	Plant height (cm)	No of shoots/ explant	No. of roots/ explant	Length of root (cm)	Weight incensement (mg)
Bm/Solid	4.22 ± 0.95	1.2 ± 0.12	1.4 ± 0.47	5.84 ± 1.11	96 ± 0.03
Bm/Liquid	9.9 ± 1.16	1.2 ± 0.15	4.6 ± 0.59	8.26 ± 1.39	338 ± 0.04
p-value	0.00139**	0.5	0.011866**	0.165642	0.003522**
Pm/Solid	4.95 ± 1.17	1 ± 0.21	2.1 ± 0.45	2.1 ± 1.19	44 ± 0.42
Pm/Liquid	7.6 ± 3.00	1.6 ± 0.40	2.4 ± 0.75	8 ± 1.27	191 ± 0.38
p-value	0.216171	0.086002	0.85559	0.044966**	0.011815**

Growth performance was summarized after 30 days in culture. Data represent the average values ± SD from 5 explants. Data for each column were analyzed by a Student's t-test. **Significant at 0.05.

Another main topic of the present study is the autofluorescence measurement technique. Autofluorescence (primary fluorescence) is the fluorescence of naturally occurring substances, such as chlorophyll, collagen, and fluorite. Most plant and animal tissues show some autofluorescence when excited with ultraviolet light (e.g., light with a wavelength of approximately 365 nm) [8]. In the previous studies, we focused on evaluating the autofluorescence intensity of target plant cells, which reflects the histochemical features of the plant cell wall [9], and accumulation of specific secondary metabolites [10]. We also used digital imaging analysis to measure the growth features in a protoplast co-culture assay [11]. Currently, in the field of life sciences, the concept and application of autofluorescence measurement have developed with the requirement of non-destructive detection of a target cell and tissue [12] [13] [14]. In the present study, the color variation of explants as the relative fluorescent intensity by assessing the autofluorescence property of the whole shoots under LED 365 nm illumination with RGB (red, green, and blue) digital imaging analysis using ImageJ software was focused. The detailed autofluorescence properties of the outward regions of culm and node tissues were also identified using an inverted fluorescent microscope under B- and U-excitation lights with RGB and HSB (hue, saturation, and brightness) digital imaging analysis.

2. Materials and Methods

2.1. Plant Materials

Nodes of 11 different bamboo species that belong to seven major bamboo genera were cultivated using planter boxes in a greenhouse of Toyama Prefectural University, Japan: *Bambusa—B. multiplex* Raeush (Bm), *B. glaucescens* f. Horaiko-machi (Bg), *B. oldhamii* Munro (Bo); *Dendrocalamus—D. giganteus* Munro (Dg); *Phyllostachys—P. bambusoides* Sied. Et Zucc (Pb), *P. nigra* Munro var. Henonis (Pn), *P. pubescens* Mazel ex J. Houz (Pp); *Tetragonocalamus—T.*

angulatus (Munro) Nakai (Ta); *Chimonobambusa—C. marmorea* (Mitford) Makino (Cm); *Pleioblastus—P. simonii* (Carriere) Nakai (Ps); and *Sasa—S. kurilensis* Makino et Shibata (Sk). Fresh nodes were collected from the branches of these bamboo plants and cultured *in vitro* according to a method previously reported [4]. *In vitro* node culture stocks of Bm and Pm maintaining at Prefectural University of Hiroshima, Japan were also used in the following experiments to understand the sequential developmental processes of SAM and RAM of node explants. Briefly, a half-strength MS (Murashige and Skoog) [15] liquid medium that contains 30 g/L sucrose was prepared as a standard medium, unless otherwise specified. The pH of the medium was adjusted to 5.7 before autoclaving. All the cultures were maintained at 25˚C, with a 16 h photoperiod under fluorescent illumination (65 μmol m^{-2} s^{-1}).

2.2. Node Culture Protocol and Its Applications

A versatile node culture protocol using 2 mL per well of a liquid half-strength MS medium with a 6-well microplate (#353046, Corning), which provided a small-scale liquid culture environment (SLCE) was demonstrated. One node segment (1.5 - 2.0 cm in length) of each bamboo plant was placed in a well of the SLCE. Mature dormant nodes from greenhouse grown bamboo branches and the first node (the base node near a rhizome tissue) from the *in vitro* node cultures of Pm and Bm were used in the experiments, unless otherwise specified.

Three types of node portions—the first node, middle nodes (upper nodes next to the 1st node), and the top meristem—were independently cultured in the SLCE to define a bent for the explants. Effects of plant growth regulators, such as benzyl adenine (BA, Wako Pure Chemical Corp.) and thidiazuron (TDZ, Wako Pure Chemical Corp.), on induction of *in vitro* SAM development, and effects of 2,4-dichlorophenoxy acetic acid (2,4-D, Wako Pure Chemical Corp.) on the promotion of *in vitro* RAM development were also investigated after a short period in the SLCE.

2.3. Observations and Digital Imaging Analysis

Dormant and active lateral buds of 11 bamboo species were sectioned longitudinally and stained with Sytox green (SG, Thermo Fisher Scientific) to monitor mitotic activity and counterstained with Safranin O (SF, Wako Pure Chemical Corp.) to detect the inward region of the SAM region based on a previous method [16] with a minor modification.

In order to evaluate growth performance of explants, whole images of *in vitro* cultured shoots were captured with a digital camera system (UV CUBE, LC science Co., Ltd.) under a bright-field and LED 365 nm illumination without staining. The autofluorescence property of the obtained images was evaluated by RGB (red, green, and blue) digital imaging analysis using ImageJ software. A stereo microscope (SZ40, Olympus) was also used to monitor the morphological characteristics of the target tissue. The detailed autofluorescence properties of

the outward regions of culm and node tissues were observed using an inverted cell culture microscope (CKX53, Olympus) under B- and U-excitation lights [B; Band pass (BP) filter, 460 - 495; Barrier (BA) filter, 510IF; Dichroic mirror (DM), 505, U-FUW; BP filter, 340 - 390; BA filter, 420IF; DM, 410] with RGB and HSB (hue, saturation, and brightness) digital imaging analysis. The HSB color space in ImageJ software is regarded as equivalent to the HSV (hue, saturation, and value) color space.

3. Results

3.1. Histochemical Analysis of Mitotic Activity in Node Portions of 11 Bamboo Species

As shown in Figure 2, the dormant lateral buds of all bamboo nodes resumed expansion and elongation within the first 7 days in the SLCE. The mitotic activity was calculated by a digital imaging analysis and found an increase of up to 1.2- to 3.8-fold in terms of the SG/SF ratio after 7 days in the culture (Table 2).

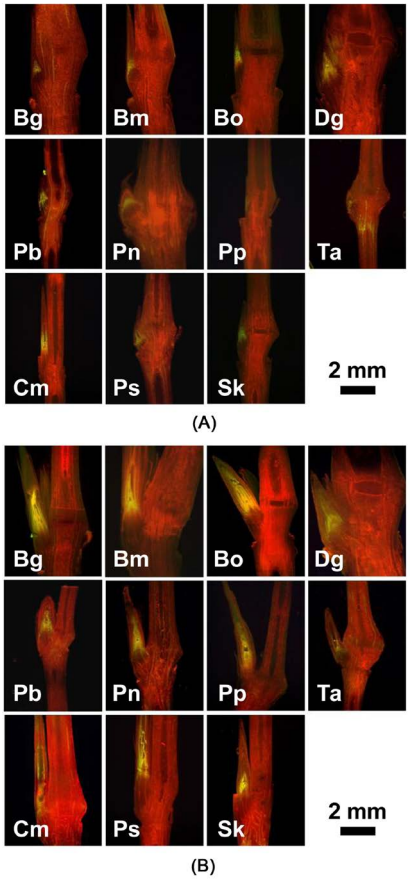

Figure 2. Histochemical analysis of mitotic activity in node portions of 11 bamboo species. A; dormant stage (0 day), B; active stage (7 days). Longitudinal sections of the lateral buds were stained with Sytox green (SG, yellow) and counter stained with Safranin (SF, red). Relative activity was calculated as the ratio of stained area (SG/SF) in Table 2.

Table 2. The mitotic activity in the node portion of 11 bamboo species.

Bamboo species	Relative activity (SG/Saf)*		
	Dormant stage	Active stage	Fold
Bg	0.069	0.084	1.22
Bm	0.058	0.083	1.45
Bo	0.079	0.133	1.68
Dg	0.040	0.062	1.57
Pb	0.037	0.069	1.86
Pn	0.036	0.059	1.63
Pp	0.050	0.109	2.17
Ta	0.007	0.025	3.82
Cm	0.040	0.064	1.59
Ps	0.068	0.098	1.44
Sk	0.032	0.056	1.75

*Relative activity was calculated as the ratio of stained area (SG/Saf) from **Figure 2** by a digital imaging analysis.

3.2. Morphological Features of *in Vitro* Bamboo Shoots

Systematically maintained *in vitro* node culture stocks of Bm and Pm, as shown in **Figure 1**, were used in this experiment. Since gradual white-to-green tinge shoots were observed, we investigated the relation between color variation in the outward regions of culm and node tissues and their suitability as explants (**Figure 3**). Color variation from pearl to light green under a white light could be observed, which is especially dependent on the position of the internode in the young shoot of Pn. On the other hand, there were no distinctive visible characteristics of the outward regions of the *in vitro* shoot of Pm and Bm under a white light. By checking the autofluorescence property of whole shoots under LED 365 nm illumination with RGB digital imaging analysis using ImageJ software, the color variation of explants as the relative intensity of the blue value was specified. To refer to a color scale, positional differences of the relative autofluorescence intensity were also identified.

3.3. Growth Performance of *in Vitro* Bamboo Shoots

As shown in **Figure 4**, we could distinguish types of shoots according to the plant height, *i.e.*, short (less than 5 cm), medium (ca. 5 - 10 cm), and tall (more than 10 cm)—specifically from a culture box of *in vitro* node culture stock. The number of nodes per shoot varied depending on the size of the shoots. Tall shoots that have ca. 5 nodes on average are suitable as explants.

Three types of node portions—the first node, middle nodes, and the top meristem—were collected from tall shoots that were more than ca. 10 cm in height and independently cultured in the SLCE to define a bent for the explants. The first node showed better growth performance in terms of weight increment (**Figure 5**). Further growth features of each node were also monitored (data not

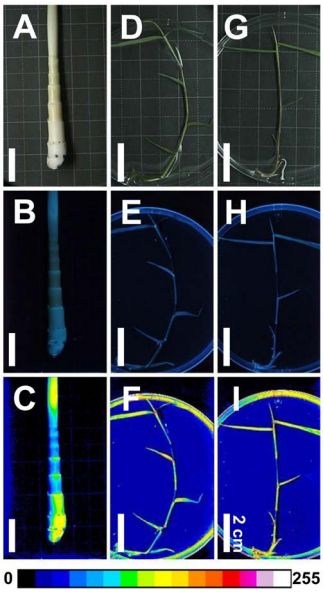

Figure 3. Autofluorescence of whole shoots under LED 365 nm illumination with an RGB (red, green, and blue) digital imaging analysis. Young shoot of Pn (A)-(C), *in vitro* shoot of Pm (D)-(F), *in vitro* shoot of Bm (G)-(I). Images in (A), (D), and (G) were captured under a white light. (B), (E), and (H) are autofluorescence images. (C), (F), and (I) are examples of digital imaging analyses in blue mode indicating histochemical differences in the outward regions of the tissues.

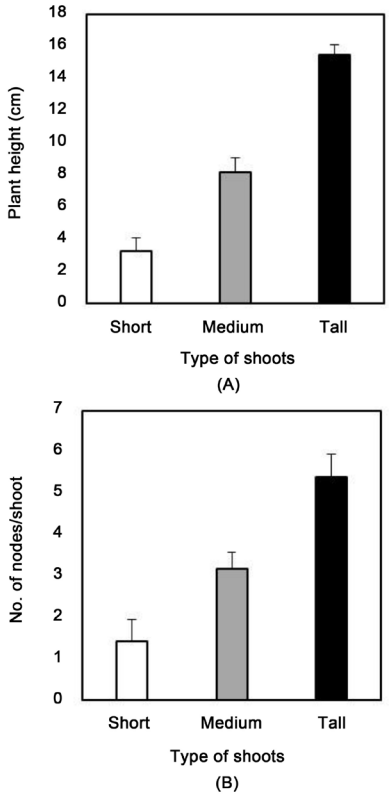

Figure 4. Categorization of the *in vitro* Pm shoot types according to (A) plant height, and (B) number of nodes per shoot. Values were summarized after being cultured for 30 days. Data represent the average values ± SD from 5 independent stock cultures.

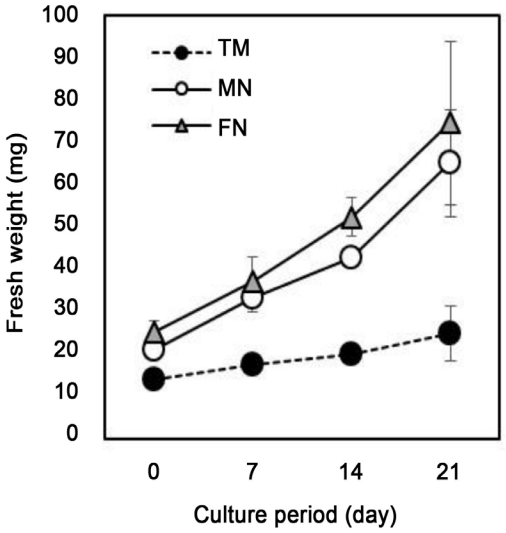

Figure 5. Growth performance of first node (FN), medium node (MN), and top meristem (TM). Data represent the average values ± SD from 3 explants.

shown) and it was concluded that the first nodes have superior bent for explants of the bamboo node culture. By selecting the first nodes as suitable explants, a quick survey to set a highly efficient culture condition became theoretically possible.

Without selecting the best node tissues, *i.e.*, first nodes, the resulting responses of a culture tend to vary widely. We effectively reconfirmed the superior bent of the first node when a test survey that set a highly efficient culture condition was performed as follows. Different concentrations and a combination of cytokinins, *i.e.*, 3 μM of TDZ, and 3 and 10 μM of BA, were used for *in vitro* SAM development, and different concentrations of auxin, *i.e.*, 0.1, 3, and 10 μM of 2,4-D, were also evaluated for whether *in vitro* RAM development was promoted. As expected, clear growth promotion and/or inhibition could be seen in the SLCE, especially in terms of the day to bud break values (Table 3).

3.4. Autofluorescence Properties of the Outward Regions of Culm and Node Tissues

Given the relation between color variation in the outward regions of culms and nodes and their suitability as explants (see Figure 3), we attempted to identify the detailed autofluorescence properties of the target tissues using an inverted fluorescent microscope under B- and U-excitation lights with an RGB and HSB digital imaging analysis. As shown in Figure 6, the mature node region, especially near the first node, showed various strong fluorescence intensities. When B-excitation light was adapted, the images from the R and G modes of the RGB analysis and the hue and brightness modes of the HSB analysis were estimable. When U-excitation light was used, the images from the G and B modes of the RGB analysis and the brightness mode of the HSB analysis were visible.

Table 3. Effect of plant growth regulators on shoot/root/callus development from first node explants.

Plant growth regulator (μM)	Growth performance		
	Days to bud breaking (d)	Length of shoot (cm)	Shoot/root/callus development
0	15.8 ± 2.34	4.1 ± 0.31	Single shoot elongation (control)
TDZ 3	8.00 ± 0.84	2.9 ± 0.32	Single shoot elongation with moderate inhibition
BA 3	5.5 ± 0.55	6.7 ± 0.22	Single shoot elongation with active promotion
BA 10	4.3 ± 0.55	7.1 ± 4.33	Single shoot elongation with active promotion
BA 10 + TDZ 3	3.00 ± 0.45	2.8 ± 0.26	Multiple shoots elongation with moderate inhibition
2,4-D 0.1	7.8 ± 0.82	4.4 ± 0.16	Single shoot elongation same as control with single root elongation
2,4-D 3	11.17 ± 0.89	3.8 ± 0.08	Single shoot elongation and slight callusing at the base of the node tissue
2,4-D 10	1.8 ± 3.03	0.15 ± 0.25	Moderate callusing at joint region of node tissue

Growth performances were summarized after cultured for 21 days. Data represent the average value ± SD from 5 explants.

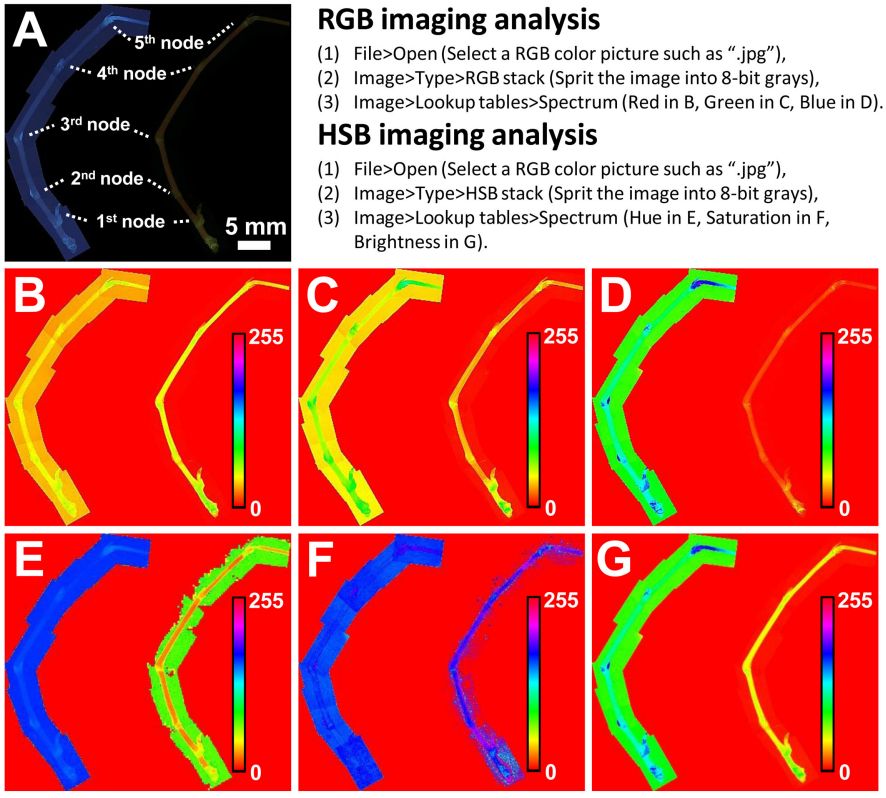

Figure 6. Autofluorescence of the outward regions of the tissues of culms and nodes under B- and U-excitation lights with an RGB and HSB (hue, saturation, and brightness) digital imaging analysis.

4. Discussion

A liquid medium condition has previously been suggested to be suitable for *in vitro* bamboo tissue cultures, especially in three major bamboo genera (*Bambusa*, *Dendrocalamus*, *Phyllostachys*) [4] [7] [17] [18]. In the present study, we investigated the effects of solid and liquid media on the growth of bamboo node cultures of Bm and Pm, and found that better growth performance could be observed in the liquid medium condition (see **Table 1**). Then, we focused on using a 6-well microplate that contains 2 mL per well of a liquid medium, which provides a small-scale liquid culture environment (SLCE). The underlying concepts of the SLCE are as follows: 1) to establish a simple and versatile liquid assay system to control morphological and histochemical responses of SAM and RAM of the bamboo node within a short period, and 2) to reveal the relation between color variation in the outward regions of culm and node tissues and their suitability as explants using techniques for autofluorescence measurement with a digital imaging analysis. For the first concept, we evaluated growth performance of nodes from 11 different bamboo species that belong to seven major bamboo genera (*Bambusa*, *Dendrocalamus*, *Phyllostachys*, *Tetragonocalamus*, *Chimonobambusa*, *Pleioblastus,* and *Sasa*), and concluded that the first node is the best explant for the bamboo culture system. As a test assay, we investigated the effects of plant growth regulators—both auxin and cytokinin—since it is well known that these chemicals interact in a complex manner to control many aspects of growth and differentiation [19]. The auxin-cytokinin interaction in the regulation of plant meristem development is overviewed in terms of biosynthesis, transport, and signaling control [20]. As shown in **Table 3**, we recognized that the combination of 10 μM of BA and 3 μM of TDZ was effective for *in vitro* SAM development during 3 weeks of culture. The addition of TDZ served as a trigger to induce multiple shoots. There are reports that TDZ is effective for enhancing micropropagation in cereal and grass plants [21] [22]. Moreover, the addition of 2,4-D effectively promoted *in vitro* RAM development. Interestingly, it was capable to monitor different bud breaking patterns (2 - 10 days after culture) with various RAM developments through the test assay. The low concentration of 0.1 μM was effective for shoot and root development, while the high concentration of 10 μM induced early bud breaking with callusing.

For the second concept, using macro- and microscopic fluorescence observation techniques, as described in the text, we estimated autofluorescence properties in the outward regions of bamboo node tissues by performing an RGB and HSB digital imaging analysis. The color of an object can be described by several color coordinate systems, referred to as color spaces. One of the most important decisions for imaging analysis is to select a color space, of which the most popular is RGB, often used in video monitors. HSV (HSB in ImageJ) is an alternative representation of the RGB color model designed to be more closely aligned to human vision and perception of color-making attributes [23]. As shown in **Figure 6**, we compared all the images obtained, and decided a suitable combination

of B- and U-excitation lights with an RGB and HSB digital imaging analysis.

5. Conclusion

To the best knowledge, this is the first report that suggests a versatile node culture method in the SLCE to control morphological and histochemical responses of SAM and RAM in *in vitro* bamboo plants. Clear growth promotion and/or inhibition could be seen in the SLCE within a short period. For future studies, we are constructing detailed quantitative imaging analyses on the variation of the autofluorescence property by adapting a method [24] with our new culture system.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research (C) (Grant No. 22580387 and 25450519 to S.O.) from the Japan Society for the Promotion of Science (JSPS). The author (M.T.A.) thanks to the Rotary Yoneyama Memorial Foundation, Japan Student Services Organization (JASSO), Hiroshima International Centre (HIC) and Daikyo Nishikawa Company Ltd., JAPAN for providing the scholarships to finalize this research work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bystriakova, N., Kapos, V., Stapleton, C. and Lysenko, I. (2003) Bamboo Biodiversity: Information for Planning Conservation and Management in the Asia-Pacific Region. UNEP-WCMC/INBAR 1, Cambridge, 1-72. https://archive.org/details/bamboobiodiversi03byst

[2] Clark, L.G., Londono, X. and Ruiz-Sanchez, E. (2015) Bamboo Taxonomy and Habitat. In: Liese, W. and Köhl, M., Eds., *Bamboo*: *The Plant and Its Uses*, Springer, Berlin, 1-30. https://doi.org/10.1007/978-3-319-14133-6

[3] Ohrnberger, D. (1999) The Bamboos of the World: Annotated Nomenclature and Literature of the Species and the Higher and Lower Taxa. Elsevier, Amsterdam, 596. https://doi.org/10.1016/B978-0-444-50020-5.X5000-X

[4] Ogita, S., Kashiwagi, H. and Kato, Y. (2008) *In Vitro* Node Culture of Seedlings in Bamboo Plant, *Phyllostachys meyeri* McClure. *Plant Biotechnology*, **25**, 381-385. https://doi.org/10.5511/plantbiotechnology.25.381

[5] Singh, S.R., Singh, R., Kalia, S., Dalal, S., Dhawan, A.K. and Kalia, R.K. (2013) Limitations, Progress and Prospects of Application of Biotechnological Tools in Improvement of Bamboo—A Plant with Extraordinary Qualities. *Physiology and Molecular Biology of Plants*, **19**, 21-41. https://doi.org/10.1007/s12298-012-0147-1

[6] Ogita, S. (2015) Plant Cell, Tissue and Organ Culture: The Most Flexible Foundations for Plant Metabolic Engineering Applications. *Natural Product Communications*, **10**, 815-820. https://doi.org/10.1177/1934578X1501000527

[7] Sood, A., Ahuja, P.S., Sharma, O.P. and Godbole, S. (2002) *In Vitro* Protocols and

Field Performance of Elites of an Important Bamboo *Dendrocalmus hamiltonii* Nees et Arn. Ex Munro. *Plant Cell Tissue and Organ Culture*, **71**, 55-63. https://doi.org/10.1023/A:1016582732531

[8] Rost, F. (1999) Fluorescence Microscopy, Applications. In: Lindon, J.C., Ed., *Encyclopedia of Spectroscopy and Spectrometry*, Academic Press, Cambridge, 565-570. https://doi.org/10.1006/rwsp.2000.0090

[9] Ogita, S., Kikuchi, N., Nomura, T. and Kato, Y. (2011) A Practical Protocol for Particle Bombardment-Mediated Transformation of *Phyllostachys* Bamboo Suspension Cells. *Plant Biotechnology*, **28**, 43-50. https://doi.org/10.5511/plantbiotechnology.10.1101a

[10] Ogita, S., Lee, J.B., Kurosaki, F. and Kato, Y. (2015) The Biosynthetic Activities of Primary and Secondary Metabolites in Suspension Cultures of *Aquilaria microcarpa*. *Natural Product Communications*, **10**, 779-782. https://doi.org/10.1177/1934578X1501000520

[11] Ogita, S. and Sasamoto, H. (2017) *In Vitro* Bioassay of Allelopathy in Four Bamboo Species; *Bambusa multiplex*, *Phyllostachys bambusoides*, *P. nigra*, *Sasa kurilensis*, Using Sandwich Method and Protoplast Co-Culture Method with Digital Image Analysis. *American Journal of Plant Sciences*, **8**, 1699-1710. https://doi.org/10.4236/ajps.2017.87117

[12] Croce, A.C. and Bottiroli, G. (2014) Autofluorescence Spectroscopy and Imaging: A Tool for Biomedical Research and Diagnosis. *European Journal of Histochemistry*, **58**, 2461. https://doi.org/10.4081/ejh.2014.2461

[13] Zheng, X., Xiong, H., Li, Y., Han, B. and Sun, J. (2016) RGB and HSV Quantitative Analysis of Autofluorescence Bronchoscopy Used for Characterization and Identification of Bronchopulmonary Cancer. *Cancer Medicine*, **5**, 3023-3030. https://doi.org/10.1002/cam4.831

[14] Wena, Z., Raffaelloa, T., Zenga, Z., Pavicica, M. and Asiegbua, F.O. (2019) Chlorophyll Fluorescence Imaging for Monitoring Effects of *Heterobasidion parviporum* Small Secreted Protein Induced Cell Death and in Planta Defense Gene Expression. *Fungal Genetics and Biology*, **126**, 37-49. https://doi.org/10.1016/j.fgb.2019.02.003

[15] Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiologia Plantarum*, **15**, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[16] Ogita, S., Nomura, T., Kishimoto, T. and Kato, Y. (2012) A Novel Xylogenic Suspension Culture Model for Exploring Lignification in *Phyllostachys* Bamboo. *Plant Methods*, **8**, Article No. 40. https://doi.org/10.1186/1746-4811-8-40

[17] Das, M. and Pal, A. (2005) *In Vitro* Regeneration of *Bambusa balcooa* Roxb.: Factors Affecting Changes of Morphogenetic Competence in the Axillary Buds. *Plant Cell Tissue and Organ Culture*, **81**, 109-112. https://doi.org/10.1007/s11240-004-3017-x

[18] Negi, D. and Saxena, S. (2011) Micropropagation of *Bambusa balcooa* Roxb. through Axillary Shoot Proliferation. *In Vitro Cellular and Developmental Biology Plant*, **47**, 604-610. https://doi.org/10.1007/s11627-011-9403-2

[19] Coenen, C. and Lomax, T.L. (1997) Auxin-Cytokinin Interactions in Higher Plants: Old Problems and New Tools. *Trends in Plant Science*, **2**, 351-356. https://doi.org/10.1016/S1360-1385(97)84623-7

[20] Su, Y.H., Liu, Y.B. and Zhang, X.S. (2011) Auxin-Cytokinin Interaction Regulates Meristem Development. *Molecular Plant*, **4**, 616-625. https://doi.org/10.1093/mp/ssr007

[21] Kumari, K., Lal, M. and Saxena, S. (2017) Enhanced Micropropagation and Tiller Formation in Sugarcane through Pretreatment of Explants with Thidiazuron (TDZ). 3 *Biotech*, **7**, 282. https://doi.org/10.1007/s13205-017-0910-7

[22] Schulze, J. (2007) Improvements in Cereal Tissue Culture by Thidiazuron: A Review. *Fruit, Vegetable and Cereal Science and Biotechnology*, **1**, 64-79. http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/FVCSB_1(2)/FVCSB_1(2)64-79o.pdf

[23] Lazaro, A., Boada, M., Villarino, R. and Girbau, D. (2019) Color Measurement and Analysis of Fruit with a Battery-Less NFC Sensor. *Sensors*, **19**, 1741. https://doi.org/10.3390/s19071741

[24] Kasajima, I. (2019) Measuring Plant Colors. *Plant Biotechnology*, **36**, 63-75. https://doi.org/10.5511/plantbiotechnology.19.0322a