Response to comments from Review editor

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.  
We have corrected some small errors that are not described in this letter, and have also highlighted these changes in green.

• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.  
1) E.g. Line 66 should be a note.

L. 68, We have added ‘Note: ’ before ipecac (*Carapichea ipecacuanha*).

In addition, L. 82-83, We have revised the second sentence to imperative tense.

L. 131, We have deleted ‘Further’.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more details to the following protocol steps.**There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:  
1) Line 72: cute using scissors?

L. 76, 82, We have added ‘using a surgical scalpel with a blade No. 22 on a sterile acryl plate’.   
2) Line 74: What is the culture medium/substrate?  
L. 79, We have added ‘on phytohormone-free B5 medium’.

In addition, we have added some details in the text. See green highlight.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.  
We have changed the numbering of protocol section as you proposed. In addition, we have inserted a one-line space between each step.

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.  
We have revised the protocol accordingly, but the total length was within 3 pages. Thus, we have not highlighted in yellow in the text.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.  
1), 3), 5) We compared comprehensive analysis of all phytohormones and described on critical steps in our protocol (L. 207-218).

2) We discussed the detection limit of phytohormone analysis (L. 219-224).

4) We added one sentence to the end of Discussion as the application of our method, ‘In the future, this method would be applied to investigate dynamics of the endogenous auxin and cytokinin during organogenesis in other plant species.’ (L. 231-233).

• **Figure/Table Legends:**:  
1) Fig 3: Define the insets

We have defined the insets in Fig.3.  
2) Tables 1-3: Add legends.

L. 198-203, Table legends have been added after figure legends.  
  
• **References:**  
1) Please abbreviate all journal titles.

We abbreviated the journal titles in references.

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Leica Microsystems, TissueLyser II (Qiagen, Hilden, DHS1000, Oasis HLB, Oasis MCX, 3200 QTRAP; Sciex, HPLC, Prominence, MultiQuant v. 2.0.2 software (Sciex), etc.  
1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.  
We have replaced commercial names with generic names in the text. And we have added the commercial names in a material file.

• Please define all abbreviations at first use.  
We defined all abbreviations at first use.

• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.  
We used abbreviations for SI units and non-SI units with a single space between the numerical value and unit.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

We have obtained the permission to re-use the figures and tables from the Springer. Text for the reprint permission was submitted as a supplemental file. In figure legends of Figs. 2 and 3, we added ‘This figure has been modified from [Koike et al. 2017]9.’ In Tables 2 and 3, we added ‘This table has been modified from [Koike et al. 2017]9.’

Response to comments from Reviewer 1

1. I would like to know the hormone levels in other plant species that adventitious shoots can be induced on phytohormone free culture media.

Thank you for your comments. The JoVE is a scientific method journal. We hope that someone who read our paper would try to analyze the hormone levels in other plant species using our protocol.

1. I would like to know why ipecac does not need exogenously applied phytohormones for adventitious shoot formation.

This is an important question. However, nobody probably knows the mechanism at this moment. In the future, we will try to answer the question.

1. Authors described in abstract, 'the optimum concentrations and combinations differ by species' and `The results will allow us to estimate the most efficient timing of auxin and cytokinin treatment to induce efficiently adventitious shoots in other plant species. I felt these two sentences are conflict a little bit.

We have revised the words 'the optimum concentrations and combinations differ by species' to be more clearly. And we have deleted a sentence, 'The results will allow us to estimate the most efficient timing of auxin and cytokinin treatment to induce efficiently adventitious shoots in other plant species'. We have added the advantages, limitations, and applications in our methods in stead of the sentence.

Response to comments from Reviewer 2

1. JoVE is a methods journal, I am not sure the manuscript is also a methods article. If it is, then I have a question. What are the advantages of the method you used? To clarify this, some comparison should be included in the results.

We have added the advantages of our method in Abstract (L. 29-31). Our method is simple compared with all hormone analysis established previously because we are focusing on two hormones, auxin and cytokinin. We have discussed it in Discussion (L. 216-227).

1. Why did you choose these two hormones (auxin and CKs) in your experiments, but not others such as cytokinins and gibberellins?

Because auxin and cytokinin mainly regulate adventitious shoot formation, we focused on quantification of these two phytohormones. To clarify the point, we have changed the title to ‘Quantification of endogenous auxin and cytokinin during internode culture of ipecac’.

1. Long abstract: The section should be re-written. In the "Long Abstract", the authors focused on the background, material and method, but not the results.

The instruction for authors requires that Abstract should be written on the method rather than the results of a specific method, and that we should state about the purpose of the method, advantages, limitations, and applications. Thus, we have revised the long abstract and we have added the advantages, limitations, and applications in our methods.