

Draft template for Applications in Plant Sciences - Protocol Note

Short Title for Running Head: Surname of the first author followed, as appropriate, with the surname a sole co-author or et al. (if there are three or more authors) - 2 to 4 descriptive words [e.g., Smith et al. - Root staining in Fabaceae]

An innovative method for identifying mycorrhizal connections in root tissue¹

[no more than 125 characters; after a species name, include family name in parentheses]

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Number of words: YYYY *[3000 or fewer, consisting of Introduction, Methods and Results, and Conclusions sections]*.

¹ Manuscript received _____; revision accepted _____.

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Acknowledgments should be limited to no more than 75 words.

ABSTRACT

The abstract should capture the interest of the general botanical community as well as specialists within the area. The abstract must be 150 words or less, written in the following structured format:

- Premise of the study (why the work was done)
- Methods and Results (how the protocol was developed and tested)
- Conclusions (how the protocol is applicable)

Avoid references; if essential, cite parenthetically with journal name, volume number, pages, and year.

Here is a sample abstract:

- *Premise of the study:* A novel preparation and staining technique was developed to examine the arbuscular mycorrhiza symbiosis within root tissue of the Fabaceae, in order to facilitate future ecological studies.
- *Methods and Results:* Living root tissue was obtained from three species (*Amorpha crenulata*, *Medicago sativa*, and *Trifolium pratense*), cleared, and subjected to a series of fluorescent stains. When examined with epifluorescence microscopy, the new method resulted in brighter staining of mycorrhizal filaments with a greater number of identified connections to root tissue than other standard staining techniques.
- *Conclusions:* Compared with existing techniques, the protocol described here has the potential to identify more reliably and with greater accuracy arbuscular mycorrhizal connections within complex root tissue in legumes.

Key words: arbuscular mycorrhizae; Fabaceae; root staining.

[List 3 to 6 key words here in alphabetical order, separated by semicolons.]

INTRODUCTION

It is recommended that this section consist of no more than three paragraphs outlining the reasons behind the development of the new protocol, a brief explanation of its importance, and any information regarding the protocol that would be of interest to the general botanical community. In this section, authors must also justify the need for the new protocol given current technology, and be able to clearly explain why it is a novel technique and not just a minor modification of an existing protocol (using appropriate citations). This section should also briefly mention how the new protocol will be compared to existing techniques.

METHODS AND RESULTS

The combined **Methods and Results** section will consist of no more than six paragraphs. In the first one to four paragraphs of this section, the methods used to develop and conduct the protocol should be adequately described. These should be in enough detail to allow readers to understand the overall process, as well as reasons behind each of the individual steps. As necessary and appropriate, a figure or multimedia content (e.g., an animated video) can be used to clarify difficult concepts. Authors are encouraged to present the finer details of the protocol in a separate printable document suitable for the lab bench; this document should be included as an appendix (see below), with appropriate references to it within the text of this section. Any important troubleshooting notes or cautionary comments should also be presented here. Suppliers and/or manufacturers of chemicals or supplies should be provided, including the location of the company in parentheses (e.g., Li-Cor, Lincoln, Nebraska, USA).

In the final one to two paragraphs of this section, the authors must clearly demonstrate the feasibility and utility of the protocol by testing it against existing protocols available today. This comparison must involve a reasonable number of individuals to adequately demonstrate the advantage(s) of the new method, including advances in utility, cost, and/or time. If plant samples were used in the development or testing of the protocol, the number and geographic origin of specimens analyzed (using GPS decimal degrees or to the nearest second) must also be included here; any voucher specimens must also be given here or in a table footnote, or in an appendix (in the case of multiple voucher specimens).

CONCLUSIONS

In this section, the author(s) should clearly state in one to two paragraphs the main conclusions that have been reached, focusing on the effectiveness and applicability of the protocol in comparison to other existing methods. In addition, the authors may address potential advances that would be gained through the application of this protocol, as well as any drawbacks that may exist.

LITERATURE CITED *[no more than 25]*

- GOUDET, J. 1995. FSTAT: A computer program to calculate *F* statistics, version 1.2. *Journal of Heredity* 86: 485–486.
- STEBBINS, G. L. 1974. Flowering plants: Evolution above the species level. Belknap Press,

Cambridge, Massachusetts, USA.

STEVENS, P. F. 2001 onward. Angiosperm phylogeny website, version 8, June 2007 [more or less continuously updated]. Website <http://www.mobot.org/MOBOT/research/APweb/> [accessed 00 Month Year].

TURNER, B. L., AND R. M. KING. 1977. Chromosome numbers in the Compositae. VIII. Mexican and Central American species. *Southwestern Naturalist* 9: 27–39.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: A guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

Tables

Tables are optional but up to two tables can be included if necessary in protocol papers. They must follow the general format of all APPS manuscripts in which each table has a brief legend, with footnotes explaining any abbreviations within the table itself.

Figure and Legend

[limited to two optional figures, which should be uploaded as separate files; the legend should be included in the text file]

Appendix

[appendices should be numbered numerically in the order they are cited in the text]

This section should include an appendix consisting of a highly detailed protocol document that can be printed out for use at the bench. This document should be broken down into individual steps of the protocol, including the specific quantities of chemicals used at each step, as well as source information for chemicals and supplies (including the location of the supplier, and for chemicals, the product number). Enough information must be provided so that a reader can independently replicate the process in a different laboratory. Difficult steps can be clarified through the use of separate figures or multimedia content (e.g., a video showing a close-up view of a particular step).

If there are multiple voucher specimens, they should be listed in a separate appendix, in one of the following formats:

Appendix 2. Species, population voucher, municipality/state, country, and GPS coordinates of all samples used in this study. Abbreviations: AM = Amazonas; BA = Bahia; MG = Minas Gerais; MS = Mato Grosso do Sul; MT = Mato Grosso; PR = Paraná; SC = Santa Catarina; SP = São Paulo.

Utricularia gibba—UG3: Mogi das Cruzes/SP, Brazil (−23.532917, −46.143972); UG4: Mogi das Cruzes/SP, Brazil (−23.557844, −46.137386); UG5: Itararé/SP, Brazil (−24.084417, −49.201639); UG6: Guaratuba/PR, Brazil (−26.023625, −48.770411); UG7: São Bento do Sul/SC, Brazil (−26.361697, −49.388964); UG8: Corumbá/MS, Brazil (−19.008889, −57.652778); UG9: Presidente Figueiredo/AM, Brazil (−60.020556, −2.052489).

Utricularia neottioides—UN1: Chapada dos Guimarães/MT, Brazil (15.383333, −55.833333); UN2: Piatã/BA, Brazil (−13.151356, −41.758842); UN3: Santa Bárbara/MG, Brazil (−19.958889, −43.415000); UN4: Raudal Caldero/Amazonas, Venezuela (4.766667, −66.683333).

Utricularia reniformis—UR1: Salesópolis/SP, Brazil, (−23.649222, −45.677833); UR2: Biritiba-Mirim/SP, Brazil (−23.658306, −46.034556); UR3: Bananal/SP, Brazil (−22.798722, −44.377917); UR5: Itararé/SP, Brazil (−24.115472, −49.363611); UR6: Mogi das Cruzes/SP, Brazil (−23.751353, −46.126506); UR7: Campina Grande do Sul/PR, Brazil (−25.245278, −48.834167); UR8: Corupá/SC, Brazil (−26.393211, −49.354878); UR9: Morretes/PR, Brazil (−25.127778, −48.820278).

Utricularia subulata—US1: Salesópolis/SP, Brazil (−23.556856, −46.137842); US2: Mogi das Cruzes/SP, Brazil (−23.534294, −46.144850); US3: Jaguariaíva/PR, Brazil (−24.250833, −49.705833).

Appendix 2. Voucher information for *Armeria* species used in this study.

Species	Voucher specimen accession no. ^a	Collection locality ^b	Geographic coordinates	No. of individuals
<i>A. caespitosa</i>	Acp-003-AG	Cabeza de Hierro, Madrid	40°47'57.14"N, 3°57'3.21"W	20
<i>A. caespitosa</i>	Acp-015-AG	Pico del Lobo, Guadalajara	41°11'0.23"N, 3°27'58.91"W	20
<i>A. bigerrensis</i>	Abg-002-AG	Morezón, Ávila	40°14'56.13"N, 5°16'11.33"W	5
<i>A. cantabrica</i>	Act-001-AG	Torrecedredo, Asturias	43°12'3.26"N, 4°50'53.19"W	5
<i>A. maritima</i>	Amt-001-AG	Cabo Mayor, Santander	43°29'26.94"N, 3°47'26.27"W	5

Note: AG = Alfredo García, collector.

^a Vouchers deposited at Universidad Rey Juan Carlos, Departamento de Biología y Geología, Germplasm bank.

^b Locality and Spanish province.