

Reviewer Recommendation and Comments for Manuscript JPOP808 titled “An efficient protocol for direct somatic embryogenesis from *in vitro* leaf of *Rhododendron fortunei* L.”.

Line 2: ‘L.’ should be ‘Lindl.’.

Line 12: ‘L.’ should be ‘Lindl.’.

Line 13: This species is referred to have ‘highly appreciative and useful value’. What useful value does it have and how is it highly appreciative? What is this in perspective to?

Line 14: ‘has’ should be ‘have’.

Line 15: ‘microproagation’ is misspelled.

Line 16: ‘*Rhododendron fortunei* Lindl.’ Should be referred to as ‘*R. fortunei*’ from line 12 on.

Line 17: What is the pretreatment?

Line 18: ‘embryogenesis’ is misspelled.

Line 20: ‘For the pre...’ is formatted in a smaller font size than the rest of the document.

Line 23: ‘was much better’, is this significant and how was it compared?

Line 24: Conditioning medium has not been defined. There is mention of pretreatment and then somatic embryo regeneration coefficient and then conditioning medium with no details. This is very confusing.

Line 25: You must spell out abbreviations for the first time; TDZ and ZT are not acceptable unless first spelled out.

Line 27: When comparing two different PGRs you need to report in molarity (M) not concentration (mg/L).

Line 28: Leaves were not ‘sterilized’, they were ‘surface disinfested’.

Line 28: What were the leaves inoculated with in the test tubes?

Line 28: ‘After 1 week of transfer, the superficial of leaves give rise to bulging.’ This sentence doesn’t make any sense. Was meristematic differentiation occurring at this time interval?

Line 30(1): ‘After another week of transfer’ is not correctly stated. I cannot believe that it took an entire week to transfer the cultures.

Line 30: Define ‘They’.

Line 31: ‘And the...’. Do not start sentences with ‘And’.

Line 32: I do not understand the statement of ‘removed integrated easily’. This doesn’t make sense.

Line 32: Where were the somatic embryos located on the leaf tissue?

Line 36: Where the leaves transferred or introduced into culture? It is stated that somatic embryos developed after 4 weeks of transfer. There is no mention of transferring after they were introduced into culture.

Line 41: Keywords are not appropriate. 'R. fortune and direct somatic embryogenesis' are redundant because they are found in the title. The key word phrase of 'pre-treatment duration' is so vague that it would not be pertinent as a keyword. Suggest new keywords being used.

Line 47: Plant species are not 'popular' with respect to growing range. Is there a citation for the native range?

Line 51: 'ellipse' leaves should be 'elliptic leaves'.

Line 52: value is assumed to be of ornamental only.

Line 54: Is this micropropagation statement for *R. fortunei* only? I do not agree that it is a more economical method in the pure form. For some species it is if they are difficult to propagation by cuttings or grafting.

Line 56: 'several rhododendron species' are stated by not cited. Please cite the different species and how they relate to *R. fortunei*.

Line 57: What type of 'regeneration' has been achieved? Please define. Has somatic embryogenesis been successfully achieved prior to this study? On Line 15 of abstract it was mentioned that 'This study reported an improved and efficient protocol...' with no mention on previous published work on somatic embryogenesis. So how can this be improved?

Line 59: Deleted space before '2005'.

Line 60: First use of IAA, it must be spelled out first and then abbreviation can be used for the remainder of the article.

Line 61: 'It showed...' This statement is made that type of cytokinin and explant origin is important but no specifics are given.

Line 64: This is a very poor transition with respect to other types of clonal propagation and setting the stage why tissue culture is so important for this species.

Line 70: Seeds and cutting produce two completely different propagules, one sexual and one clonal. This relationship is not mentioned with respect to propagation and outcome of propagation.

Line 71(2): Authors state that because other means of propagation are not effective that it is so important to develop protocols for rapid propagation. This has already been accomplished by ChunYan et al. in 2006 with paper titled : In Vitro Rapid Micropropagation of Rhododendron Fortunei. The authors report an effective method of clonal propagation utilizing axillary shoots instead of somatic embryos for rapid propagation. This is a more desired method of propagation for conservation as compared to somatic embryogenesis because of the reduction of the potential for somaclonal variation.

Line 96: You have introduced a significant amount of error into your study by utilizing leaf tissue from seedlings. How do you account for sexual differences from leaf tissue among treatments? Axillary shoot culture should have been developed and then in vitro leaves utilized from a clonal population to ensure that there were no sexual differences being expressed with the explant tissue.

Line 103: See comment for line 18.

Line 104: 'leaces' is misspelled.

Line 111: This sentence is poorly written.

Line 112: BAP is mentioned and again must be spelled out for the first use.

Line 112: Why ½ WPM? Is this from another study?

Line 120: Why is week abbreviated here and nowhere else?

Line 128: 'An' is used incorrectly. What type of random experimental design was used? Was it CRD?

Line 133: Was there any unequal replication present as a result of contamination or explant death? If so then ANOVA would not be appropriate for analysis.

Line 139: Heading is different from M&Ms.

Line 140: Were the same explants evaluated across weeks 1, 2, 3 and 4? If so these are repeated measures and your analysis does not account for that and significant errors have been introduced into the experimental design. Is this the case?

Line 159: See comment for line 18.

Line 179: Again, leaves were not sterilized. By definition, if leaves were sterilized then all tissue was killed.

Line 180: What were the leaves inoculated with? This is very confusing.

Line 181: Again, were the explants transferred to fresh medium after 1 week and weekly up to week 4? This is how it is written but I do not believe this is how it was performed.

Line 185: I do not understand what it means to have embryos removed integrated easily.

Line 188: Mention of '2 weeks of transfer' is used incorrectly.

Line 189: '3 weeks of transfer' is misleading as well.

Line 191: Same comment for '4 weeks of transfer'.

Line 196: 'followed by a decrease', include germination following 'decrease'.

Line 200: Same comment as line 196.

Line 201: Delete extra space follow L-1

Line 201: Comparison cannot be made with different PGRs when using concentration (mg/L), they must be expressed in molarity (M).

Line 204: Figure 4 is cut off and not viewable.

Line 208: This sentence is not grammatically correct. 'Effectiveness' is used incorrectly.

Line 211: How has progress for somatic embryogenesis systems been limited? Again, improvement on somatic embryogenesis is mentioned but there is no mention of how it was improved on a previous system.

Line 219(222): Pecan and hickory are mentioned for somatic embryogenesis but no direct relation to this study is made. This relationship is not relevant as currently used.

Line 224: How is this similar to mango, Quassia and Coffea? This sentence is formatted incorrectly. All plants should be mentioned using common names with botanic names in parenthesis. There is no clear mention of direct vs. indirect somatic embryogenesis of *R. fortunei* in this manuscript and how it compares to other species.

Line 228(234): relationships between hickory and oak are not conclusive. Why are there no comparisons within the *Rhodendron* genus or within the Ericaceae family? This should be done and would be more applicable.

Line 240: Is pegeonpea supposed to be Pigeon pea (*Cajanus cajan*)? Again, improper use of plant naming.

Line 244: Delete space before 'Zhang'.

Line 247: Again, use common name with botanic name for plants.

Line 247: 'The' should be lowercase.

Line 251: 'used to rapid propagation' is not grammatically correct.

Line 252: This is the first mention of use of this system for transformation. This should have been mention much earlier in the manuscript as a primary reason for this research.

Line 259: All references are formatted incorrectly for JPOP. Numerous pertinent citations are missing from this manuscript.

Figures. Lower error bars are missing from all figures. Figure titles are not complete. Many of the figure titles have more than one font size present. Two of the figures (4 & 6) were not entirely viewable with the misplacement on the manuscript page.