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| Logo | **PROPAGATION OF ORNAMENTAL PLANTS**  Editorial Office, University of Forestry, 10 Kliment Ohridski blvd.,  Sofia 1756, Bulgaria, Fax: (++ 359 2) 862 28 30, e-mail: [ivilievltu@yahoo.com](mailto:ivilievltu@yahoo.com),  [www.journal-pop.org](http://www.journal-pop.org/) |

##### **CHECKLIST FOR REVIEWERS**

**Title of the manuscript:** **Cyclic Secondary Somatic Embryogenesis And Plant Regeneration *In Vitro* Leaf Culture of *Rosa multiflora* Thunb. var. *carnea* Thory**

**Author (s):** Yuxiao Shen, Meng Ding, Wen Xing, Manzhu Bao, Guogui Ning

# No of the manuscript: 813..........................

Deadline for the receiving of your review: 30 days after the receiving of the manuscript

**Please consider main point A and B. Please DO NOT CONTINUE TO REVIEW the manuscript if:**

**- the answer to point A.1 is YES**

**- the answer to point B is LOW.**

**A. Relevance of the paper.**

**1. *Previous publication of the material***

x No

□ Yes. What and where………………………………………………………………...

### B. Scientific and practical importance of the data

□ High

x Adequate, but not in the present form

□ Low

### C. Scientific quality

***1. Are the data in this manuscript new?***

x Yes

□ No. Comments:.…………………………………………………………………………

***2. Is the manuscript clearly written and well-organized?***

□ Yes

x No. Comments: The manuscript is really very hard to read and in many places hard to understand due to improper use of the English language. Moreover, the experimental design is not clear, due to discrepancies in the Table footnotes and materials and methods (see comments).

***3. Are the Abstract and the Key words adequate?***

□ Yes

x No. Suggestions: The abstract is really confusing and not understandable without having read the full manuscript. This is in part due to the language problems.

***4. Does the Introduction state the present knowledge and aim of the research?***

□ Yes

x No. Comments: There are more studies dealing with somatic embryogenesis in roses, that also followed the approach of secondary somatic embryogenesis. E.g. Dohm et al. (2001) being the first and more (see comments)

***5. Materials, methods, and study design***

□Adequate

xImprovement needed. Suggestions:…see comments…………………

□Inadequate. Comments: ...........................................!

***6. Results and Discussion***

□Properly drawn with regard to methods and data

x Should be adjusted – Suggestions: ……see comments…………………………….

□ Insufficiently supported – Comments: ................................................

***7. Are the tables , figures titles, and legends presented well and necessary?***

□ Yes

x Improvement needed. Suggestions:…………see comments……………………

□ No. Comments: ........................................................................

***8. Data and statistical treatment***

□ Adequate

x Improvement needed. Comments:…… see comments………………………………

□ Inadequate. Comments: .....................................................

***9. Have all relevant literature been cited***

□ Yes

x No. Suggestions: ..see 4. and comments..................................................................

**D. Recommendations (after corrections)**

□ The paper should be published as it is now, or with minor editorial changes

□ The paper could be published after minor revision, and need not be re-reviewed

□ The paper could be accepted after major revision according to the comments

x Rejected, maybe reconsidered after complete rewriting

#### E. If adjustments or revision is recommended

□ The writer is allowed to contact me

x I want to be anonymous

□ I am not willing to review this paper again

□ I agree to review the manuscript again after the revision

Please add further comments.

Somatic embryogenesis in members of the genus *Rosa* is difficult to achieve and often not efficient. Therefore, a new study investigating effects of different treatments to establish and optimize somatic embryogenesis in *R. multiflora* would be worth publishing. However, this study in its present form can not be accepted for publication due to severe language problems and also inconcise experimental designs (or at least their description) and unclear regeneration steps.

Most reports (see below) and also the Figure 1 of this manuscript show, that germination of somatic embryos in rose is not taking place, but instead adventitious shoots are formed on the cotyledons of somatic embryos. Thus, the process can not be termed germination.

Also shown in this publication:

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| 15. |  | Plant regeneration from the root-derived embryonic tissues of *Rosa hybrida* L. cv. Charming via a combined pathway of somatic embryogenesis and organogenesis.  Kim SukWeon Oh MyungJin Liu, J. R.  Plant Biotechnology Reports; 2009. 3(4):341-345.    This study describes culture conditions for a plant regeneration system via a combined pathway of somatic embryogenesis and organogenesis in root explant cultures of the commercial rose cultivar 'Charming'. Root explants formed white calluses at a frequency of 30% after 6 weeks of culture on Schenk and Hildebrandt (SH) medium supplemented with 11 mg l-1 2,4-dichlorophenoxyacetic acid. After 6 weeks of transfer to SH medium without growth regulators, initial white calluses gave rise to globular somatic embryos at a frequency of 2.8%, which were subsequently dedifferentiated to embryonic tissues. Somatic embryos or embryonic tissues initially derived from root explants did not undergo development beyond cotyledonary stage. To produce adventitious shoots, embryonic tissues were sliced and cultured on SH medium with 0.5 mg l-1 6-benzyladenine. After 4 weeks of culture, 28% of embryonic tissue explants formed adventitious shoots. Regenerated shoots were rooted on half strength SH medium with 0.1 mg l-1 alpha -naphthalaneacetic acid and subsequently grown to maturity. Root-derived embryonic tissues were proliferated by subculture, while retaining the capacity for shoot production for a few years. |

**Comments in detail:**

* Title should be: … plant regeneration from …
* Abstract: accuracy of induction frequencies are not justified by the data, by far too low number of explants to present two decimals!
* Abstract: last sentence: it is not possible to state that this protocol can be a reference model for woody plants!
* Introduction: I could not understand the first sentences. Please first refer to restrictions of conventional propagation methods of *R. multiflora* and then explain, why you would like to use in vitro culture techniques. For vegetative propagation cuttings would be a much easier and cheaper way. To me, the only use of the somatic embryogenesis system would be for genetic transformation.
* Line 70: there are other studies that have suggested secondary embryogenesis in roses:

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| 37. |  | Somatic embryogenesis, secondary somatic embryogenesis, and shoot organogenesis in *Rosa*.  Li, X. Q. Krasnyanski, S. F. Korban, S. S.  Journal of Plant Physiology; 2002. 159(3):313-319. 18 ref.  The influence of various 2,4-D concentrations (11.3-181 micro mol/litre) on the induction of callus from leaf tissues of *Rosa hybrida* cultivars Carefree Beauty and Grand Gala and *R. chinensis minima* cv. Red Sunblaze was evaluated. Following transfer of callus to a regeneration medium containing different concentrations of thidiazuron (TDZ) (0-90.8 micro mol/litre), 6-benzyladenine (BA; 0-44.4 micro mol/litre), or 2.9 micro mol/litre gibberellic acid (GA3), alone or in various combinations, the highest frequency of embryogenic (32%) and organogenic (55.3%) callus was induced on Carefree Beauty. Secondary somatic embryos were also induced on somatic embryos of Carefree Beauty. The effects of different concentrations of TDZ (2.3 micro mol/litre), BA (2.2 or 4.4 micro mol/litre), and abscisic acid (ABA; 3.8 or 7.6 micro mol/litre), alone or in combinations, on proliferation and germination of secondary somatic embryos were also evaluated. ABA was found to be the most effective in promoting proliferation and germination of somatic embryos. The growth rate of secondary embryogenic callus grown on ABA increased by 36-fold, while germination rate of somatic embryos was more than five times higher than those derived from embryogenic callus grown on BA and TDZ. For *R. chinensis minima* cv. Red Sunblaze, only somatic embryogenesis (6.6%) was observed; while, for *R. hybrida* cv. Grand Gala, only shoot organogenesis (3.3%) was observed. |
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| 40. |  | Transformation of roses with genes for antifungal proteins.  Dohm, A. Ludwig, C. Schilling, D. Debener, T.  Acta Horticulturae; 2001. (547):27-33. 16 ref.  In Northern Europe, the cultivation of garden roses is tremendously impaired by fungal diseases. In order to obtain partial resistance to the major diseases blackspot, powdery mildew, downy mildew and rust simultaneously, we followed the biotechnological approach to overexpress genes for different antifungal proteins. First, a protocol for transformation by *Agrobacterium*-mediated gene transfer and regeneration via somatic embryogenesis was established. The transformation frequency was up to 3%. According to this protocol, different combinations of antifungal resistance genes, encoding a Class II chitinase, a Class II beta -1,3-glucanase and a Type I ribosome-inhibiting protein from barley as well as the antibacterial T4-lysozyme gene were introduced into the garden rose (*Rosa hybrida*) cultivars Heckenzauber and Pariser Charme. About 80 true transgenic plants were analysed for expression of their transgenes, somaclonal variation and resistance to blackspot. Overall, in 80% of the putative transgenic plants, expression of the transgenes could be detected by Northern analysis. Compared to non-transgenic control plants, the susceptibility to blackspot did not decrease in the case of cytosolic expression of the antifungal proteins. The secretion of the ribosome-inhibiting protein into the extracellular space, however, reduced the susceptibility against blackspot to 60% in the mean compared to non-transgenic control plants. Several transgenic plants showed severe morphological deviations, mainly in leaf shape and flower morphology, which may be the result of both somaclonal variation and the transformation procedure. |

* Line 81: protocorms do only exist in orchids, the study of Tian et al. misused this term, but the authors of this manuscript should not follow them!
* Materials and methods (lines 116 ff) Here it is stated that the explants were incubated in the respective media for 2 months, but in Table 1 it is said, that the explants were cultivated for only 4 weeks on the respective media and then transferred to a medium containing 1 mg/l 2,4-D for further two months! What is right, what is wrong?
* The same problem occurs with lines 121 ff. and Table 2.
* Lines 131 ff. How where the somatic embryos cut/the explants prepared? Normally you would use clusters of embryos. In the paper of Bao et al. 2012 the proliferation coefficient is described as follows:

The proliferation coefficient of somatic embryos was calculated as the ratio of weight of somatic embryos after proliferation to the weight of somatic embryos before proliferation. This is cited in line 148, but should also be explained in this manuscript. What was the average fresh mass of the explants?

* Have the experiment been repeated as a whole? If not, the results are not very reliable due to high variations normally observed between repetitions of whole experiments.
* The number of explants per replicate (12-22) needs to be specified for all experiments, at least in the tables.
* Why are the data for 1 and 2 mg/l 2,4-D not included in Table 1?
* Lines 188: Please indicate the type of light used (lamps, intensity, colour)
* Lines 214 ff : contradiction to introduction
* Refer to respective tables in discussion part.
* Discussion: Please comment on the stability of the regenerants!
* References: Murashige and Skoog (1962) missing.
* Table 4: There is no clear effect of one of the additives, but really extreme variation in the data.
* Figure 1: please indicate the media on which the explants had been cultivated.

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