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

**Title of the manuscript:** **REGENERATION OF SOMATIC EMBRYOS FROM *IN VITRO* ISOLATED LIGULATE FLORETS OF CHRYSANTHEMUM**

**Author (s):** Tymoszuk A., Zalewska M., Lema-Rumińska J................................................

# No of the manuscript: JPOP741..........................

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

□ Adequate

□ Low

The quality is adequate, but the amount of relevant new data is low.

### C. Scientific quality

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

□ Yes

□ No

Comments: There are publications available that have reported on somatic embryogenesis from floral parts in chrysanthemum, so the overall information is not novel. New data are found in the genotype used and the combinations of plant growth regulators..…………………………………………………………………………

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

□ Yes

□ No. Comments: In general, the manuscript is well organized, but the English language should be corrected by a native speaker..........................................................

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

x Yes

□ No. Suggestions: The abstract could contain more hard data (e.g. regeneration rate with the best PGR combination)..........................................................

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

x Yes

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

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

□Adequate

xImprovement needed. Suggestions: see comments below, the study is well designed, and materials and methods are o.k., but it is just one experiment, performed only once.

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

***6. Results and Discussion***

xProperly drawn with regard to methods and data

□ Should be adjusted – Suggestions: ………………………………………………….

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

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

x Yes

□ Improvement needed. Suggestions:……………………………………………………

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

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

x Adequate

□ Improvement needed. Comments:……………………………………………………

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

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

x Yes

□ No. Suggestions: ....................................................................

**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

□ Rejected

To be decided by the editor in chief, to my opinion, there is not enough data justifying a publication.

#### 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.

This study describes the regeneration of somatic embryos from ligulate flowers of one chrysanthemum cultivar depending on the plant growth regulator combinations in the culture medium. It is well organized, but contains just the results of one single experiment without independent replication of the whole experiment, and thus has the extent of a Bachelors thesis. To my opinion, this is not enough to justify a publication as a full research paper in Journal Propagation of Ornamental Plants.

Essential aspects, as

1. the further development of the somatic embryos, i.e. their germination
2. the proposed single cell origin of the embryos resulting in segregation of chimera genotypes is not proven! This was the hypothesis behind the whole approach, and it is not clear and even not at all discussed, why the authors did not work with genotype that is a chimera (periclinal).
3. The tissue from which the embryos develop remains unclear, is it ovary tissue mainly? Photos, or even better histological analyses would be important to show this (see also ii).

However, if the editor in chief decides to accept it for publication, I would strongly recommend to pay attention to the following points:

* Callus tissue is an impossible term!!! Callus = undifferentiated cells, tissue = cells of certain functions = differentiated cells. Replace by callus!
* The classical model in somatic embryogenesis involves different phases (induction of embryogenic cells, proliferation, realization, maturation, germination) and only in the first two, 2,4-D normally is used. The authors need to discuss, why they did not go for a two-step protocol, that could improve the efficiency.
* The stability of regenerants is crucial, so at least in the discussion the authors should deal with this aspect.
* Can this protocol just be applied to this one (model) genotype?
* The regeneration of adventitious roots is not aimed at neither is it relevant for the readers of the journal, so this part (Table 3) could be omitted.
* Line 32/33: please cite a reference for this statement!
* Line 49/50 restitution from single explant tissue? What is meant here?
* Line 55: Broertjes 1976 missing in reference list
* Line 69/70: with which explants did Lema-Ruminska work? Was this protocol already published there?
* Line 73-76: What is the scientific basis for this statement? I do not believe that this holds true.
* What was the temperature regime in the greenhouse during explants donor plant cultivation?
* Which type of detergent was used?
* If 20 explants/replicates were used, how can % of 53.33 be explained??? If there were losses due to contaminations they should be indicated!
* When were the embryos observed?
* Lines 163ff. It is not clear to me, why a study working with Frittilaria is cited here, since there are reports available on chrysanthemum and other Asteraceae