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

**Title of the manuscript:** **SEED PROPAGATION OF *COLCHICUM CAPENSE* SUBS. *CILIOLATUM***

**Author (s):** **Seiichi Fukai1\*, Yasuhiro Monden1, Takako Narumi1 and Eiichi Kodaira2**

# No of the manuscript: JPOP705

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

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

**- the answer to point A.1 is NO**

**- the answer to point A.2 is YES**

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

**A. Relevance of the paper.**

***1. Is the subject of the manuscript within scope of the journal?***

**x** Yes

**□** No

***2. Previous publication of the material***

**X** No

**□** Yes. What and where…It is not clear for me what data will be published in the Acta Horticulturae manuscript (Fukai et al. in press).

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

**□** High

**x** Adequate

**□** Low – See comments

### C. Scientific quality

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

**x** Yes

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

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

**x** Yes

**□** No. Comments: Minor information is missing (see below) and in some passages the language should be corrected by a native speaker.…………………………………

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

**□** Yes

**x** No. Suggestions: It would be very important to add Germination and dormancy as key words…………………………………………………………………………

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

**x** Yes

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

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

**x** Adequate

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

**□** Inadequate. Comments:…………………………………………………………

***6. Results and Discussion***

**x** Properly drawn with regard to methods and data

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

…………………………………………………………………………………………..

**□** Insufficiently supported – Comments: ……………………………………………..

………………………………………………………………………………………….

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

**□** Yes

**x** Improvement needed. Suggestions: Title of Figure 7 is missing ……………

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

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

**□** Adequate

**x** Improvement needed. Comments: The authors announce statistical evaluation of the data in materials and methods, but do not show the statistics in the figures, moreover, they describe the Tukey test to be used for comparison of means, but in Table 1 used the t-test. Therefore, a very careful revision regarding this point is necessary. (Data distribution, ANOVA??)

**□** Inadequate. Comments:………………………………………………………………

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

**x** Yes

**□** No. Suggestions:…………………………………………………………………….

**E. Recommendations (after corrections)**

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

x 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

#### If adjustments or revision is recommended

□ The writer is allowed to contact me (sign both copies of this checklist)

X I want to be anonymous (sign only one copy of this checklist)

□ I am not willing to review this paper again (revision)

Please add further comments.

This manuscript gives new and relevant information on dormancy and germination of a plant species, *Colchicum capense*, based on carefully designed and conducted experiments. The authors see a possible use of this species in floriculture, which unfortunately cannot be judged by the reviewers, since no photos of flowering plants are provided. There are few, but very important data missing, that are urgently needed before the manuscript might be accepted for publication:

1. The genetic background of the starting plant material is crucial. If the plants used for pollination experiments were clones, self-pollination would be the same as crossing two plants!!! It is essential to clarify this!
2. The data would point to the possibility that the dormancy type is rather morphophysiological than physiological, as also assumed for *Colchicum autumnale*. The authors need to comment on this, best by showing transversal cuts (maybe even hand-cuts) of seeds at the time of harvest and seeds after after-ripening. I would assume that the embryo will be small and underdeveloped first and mature/ripen and increase in size during storage.
3. The conditions in the unheated greenhouses in Japan need to be specified. What average temperatures can be observed, and what was the daylength (to answer the latter question the latitude would be sufficient).
4. It would be interesting to know, if all seeds with radicle emergence developed into normal seedlings.
5. Experiment V: Were the seeds incubated under moist conditions (on filter paper soaked with water) in this experiment? Otherwise the data would contradict the results of Exp. II.
6. For discussion of the uniconzole effect shown in Fig. 6 the following source might be very interesting: It was shown that uniconazole not only inhibits GA synthesis, but also ABA degradation! This would be very important to discuss.

[Biosci Biotechnol Biochem.](http://www.ncbi.nlm.nih.gov/pubmed/16819156) 2006 Jul;70(7):1731-9. Epub 2006 Jul 1.

# A plant growth retardant, uniconazole, is a potent inhibitor of ABA catabolism in Arabidopsis.

[Saito S](http://www.ncbi.nlm.nih.gov/pubmed?term=Saito%20S%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Okamoto M](http://www.ncbi.nlm.nih.gov/pubmed?term=Okamoto%20M%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Shinoda S](http://www.ncbi.nlm.nih.gov/pubmed?term=Shinoda%20S%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Kushiro T](http://www.ncbi.nlm.nih.gov/pubmed?term=Kushiro%20T%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Koshiba T](http://www.ncbi.nlm.nih.gov/pubmed?term=Koshiba%20T%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Kamiya Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Kamiya%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Hirai N](http://www.ncbi.nlm.nih.gov/pubmed?term=Hirai%20N%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Todoroki Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Todoroki%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Sakata K](http://www.ncbi.nlm.nih.gov/pubmed?term=Sakata%20K%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Nambara E](http://www.ncbi.nlm.nih.gov/pubmed?term=Nambara%20E%5BAuthor%5D&cauthor=true&cauthor_uid=16819156), [Mizutani M](http://www.ncbi.nlm.nih.gov/pubmed?term=Mizutani%20M%5BAuthor%5D&cauthor=true&cauthor_uid=16819156).

### Source

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### Abstract

Plant growth retardants (PGRs) reduce the shoot growth of plants by inhibiting gibberellin biosynthesis. In this study, we performed detailed analyses of the inhibitory effects of PGRs on Arabidopsis abscisic acid (ABA) 8'-hydroxylase, a major ABA catabolic enzyme, recently identified as CYP707As. In an in vitro assay with CYP707A3 microsomes expressed in insect cells, uniconazole-P inhibited CYP707A3 activity more effectively than paclobutrazol or tetcyclacis, whereas the other PGRs tested did not inhibit it significantly. Uniconazole-P was found to be a strong competitive inhibitor (K(i)=8.0 nM) of ABA 8'-hydroxylase. Uniconazole-P-treated Arabidopsis plants showed enhanced drought tolerance. In uniconazole-P-treated plants, endogenous ABA levels increased 2-fold as compared with the control, and co-application of GA(4) did not suppress the effects, indicating that the effects were not due to gibberellin deficiency. Thus uniconazole-P effectively inhibits ABA catabolism in Arabidopsis plants. We also discuss the structure-activity relationship of the azole-type compounds on ABA 8'-hydroxylase inhibitory activity.

Further minor comments:

* Title and following: what do you mean by subs.? Subspecies would be subsp. or ssp.
* Line 31, please give more details on the type of DNA analyses: ITS sequences?
* Please indicate raphe and caruncula in Fig. 1A by arrows
* What are pre pods? (line 100)
* Line 165: co-existence is not a suitable term here, application?
* Ellington et al. not cited in manuscript
* Finch\_Savage not cited in manuscript
* Han not cited in manuscript
* Variants 25 °C and GA concentrations not given in Fig. 4
* What does cont. stand for in Fig. 3