Journal Name:	**PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY**
Manuscript Number:	**Ms_PCBMB_12280**
Title of the Manuscript:	**Effect of different Agrobacterium rhizogenes strains on in-vitro hairy root induction for cucurbitacin E production in Citrullus colocynthis**
Type of the Article	**Original Research Article**

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
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PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write few sentences regarding the importance this manuscript for scientific community. Why do you like (or dislike) this manuscript? Minimum 3-4 sentences may be required for this part.	This manuscript is significant for the scientific community because it demonstrates the potential of hairy root cultures induced by different strains of Agrobacterium rhizogenes for enhanced production of bioactive compounds, particularly cucurbitacin E from Citrullus colocynthis. The study highlights the effectiveness of the R1000 strain in inducing hairy roots, which can facilitate mass production of valuable medicinal compounds. The findings also contribute to the understanding of the role of explant type and co-cultivation conditions in optimizing hairy root induction and production. The manuscript's thorough methodology and detailed results provide a valuable reference for future research in plant biotechnology and the production of bioactive compounds from medicinal plants.	
Is the title of the article suitable? (If not please suggest an alternative title)	The title is concise, informative and accurately represents the content of the manuscript. No alternative title is necessary.	

Is the abstract of the article comprehensive? Do you suggest addition (or deletion) of some points in this section? Please write your suggestions here.	The abstract of the article is comprehensive and covers the key aspects of the study. It provides a concise overview of the research objectives, methodology, and main findings.	
Are subsections and structure of the manuscript appropriate?	The subsections and structure of the manuscript are appropriate and follow a logical flow.	
Please write few sentences regarding the scientific correctness of this manuscript. Why do think that this manuscript is scientifically robust and technically sound? Minimum 3-4 sentences may be required for this part.	The study employs well-established techniques in plant biotechnology, such as Agrobacterium rhizogenes-mediated hairy root induction and HPLC analysis for quantifying the bioactive compound cucurbitacin E. The authors have used four different strains of A. rhizogenes (A4, Ar532, ARqua1, and R1000) to infect various explants (leaf, cotyledon, stem, and callus) of Citrullus colocynthis and optimized the transformation protocol by testing different parameters like acetosyringone concentration, infection time, and co-cultivation duration.The results are supported by quantitative data, including the frequency of transformation, number of hairy roots induced, and the concentration of cucurbitacin E in hairy root cultures compared to normal in vitro root cultures. The authors have also performed GUS assay and PCR analysis to confirm the integration of T-DNA from the Ri plasmid into the host plant genome.The discussion section compares the findings with previous studies on hairy root induction in other plant species, demonstrating the authors' thorough understanding of the existing literature and the significance of their results in the broader context of plant biotechnology. The manuscript follows a clear and logical structure, with well-defined sections and appropriate use of tables and figures to present the data.	

Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form. **-**	**yes**	
Minor REVISION comments **Is language/English quality of the article suitable for scholarly communications?**	The language and English quality of the article are suitable for scholarly communications. The writing is clear, concise, and follows standard scientific conventions. The manuscript is well-structured, with appropriate use of technical terminology, and the overall presentation is professional.Some minor suggestions to further improve the language and readability: 1. Occasionally, the sentence structure could be slightly simplified or rephrased to enhance clarity, for example: "The study highlights the effectiveness of the R1000 strain in inducing hairy roots, which can facilitate mass production of valuable medicinal compounds." 2. Some minor grammatical or typographical errors could be corrected, such as: • "Ar532" should be "Ar 532" for consistency with other strain names. • "Arqua1" should be "ARqua1" for consistency. 3. Occasionally, the use of passive voice could be converted to active voice to improve flow, for example: "Hairy root cultures accumulated approximately two-fold higher levels of the anticancer compound cucurbitacin E compared to normal in vitro root cultures."	
Optional/General comments		

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

Name:	**Anamika Rana**
Department, University & Country	**India**