For research article

Response to Reviewer 1 Comments

1. Summary

Thank you very much for taking the time to review this manuscript. Please find the detailed responses below and the corresponding revisions/corrections highlighted/in track changes in the re-submitted files

2. Questions for General Evaluation	**Reviewer's Evaluation**	**Response and Revisions**
Does the introduction provide sufficient background and include all relevant references?	Yes/Can be improved/Must be improved/Not applicable	*The responses are addressed in the section of point-by-point response letter.*
Are all the cited references relevant to the research?	Yes/Can be improved/Must be improved/Not applicable	
Is the research design appropriate?	Yes/Can be improved/Must be improved/Not applicable	
Are the methods adequately described?	Yes/Can be improved/Must be improved/Not applicable	
Are the results clearly presented?	Yes/Can be improved/Must be improved/Not applicable	
Are the conclusions supported by the results?	Yes/Can be improved/Must be improved/Not applicable	

3. Point-by-point response to Comments and Suggestions for Authors

Comments 1: The paper reports that the C-CH morphotype produced the highest mucilage yield (800 mL Kg^{-1} FW) under optimum and suboptimum soil moisture conditions, while showing lower relative water content. How do the authors explain the basic chemical and biological reasons behind the higher mucilage production in C-CH compared to the other morphotypes under both good and low water conditions?

Response 1: The following text and references were added and marked in yellow into the Manuscript:

… under environmental stress conditions, by tolerating low water potential at the cellular level under conditions of water deficit in the soil and capable of recovering its water status reflected in the relative water content, under favorable soil moisture contents (Time et al. 2018; Feng et al. 2016), which corresponds to … **Pag. 11, Paragraph: 2, Lines: 401 – 404.**

Comments 2: 2. The authors classified the morphotypes based on physical traits and noted different responses to water stress. Could the use of gene expression tests or molecular markers help explain why the C-CH type retains more water and produces more mucilage

than C-NA and C-HE?

Response 2: Yes, I agree. The following text was added, and marked in yellow into the Manuscript:

This suggests molecular studies of genetic expression, as reported by Tomkowiak et al. (2024) to identify the different responses to water deficit and performance among different genetic materials as the nopal morphotypes evaluated in this study. **Pag. 12, Paragraph: 3, Lines: 484 – 486.**

Comments 3. The study observed a strong positive correlation between chlorophyll content and mucilage yield, suggesting that higher pigment levels are linked to better mucilage production. What simple photosynthetic or metabolic processes might explain this connection, and how might these processes differ among the morphotypes?

Response 3: For better explanation, the following text was added, and marked in yellow into the Manuscript:

Chlorophyll is a green pigment in plants that absorbs light energy and transforms it into chemical energy through photosynthesis, which is used to synthesize organic compounds (Martins et al. 2023) such as nopal mucilage. Since nopal morphotypes are different in the intensity of the green color of the cladode, being stronger in the case of the C-CH morphotype, this may explain the different responses among the nopal genetic materials evaluated in this study. **Pag. 13, Paragraph: 2, Lines: 513 – 518.**

Comments 4: The authors noted that relative water content (RWC) decreases with lower soil moisture, yet C-CH shows a good recovery in water status. What possible cell signaling pathways or control systems might work differently in each morphotype during water stress, and how do these differences relate to their mucilage production?

Response 4: The following text marked in yellow was added into the Manuscript:

According to Xiong et al (2002), the soil moisture content influences the C4 and CAM metabolism, stomatal closure, and changes in gene expression, including those encoding potentially protective proteins, key enzymes in the osmolyte synthesis pathway, antioxidant enzymes, and transcription factors, which regulate stress-induced gene expression through signaling pathways of the hormonal action of abscisic acid and that lead to plant tolerance to water stress, which could explain the differential behavior among the nopal morphotypes evaluated in this study**. Pag. 12, Paragraph: 2, Lines: 466 – 472.**

Comments 5. Although the quality of mucilage (pH, ash content, total solids) did not change with soil moisture, the paper suggests that genetic factors strongly control mucilage composition. What ideas do the authors have regarding the genetic regulation of mucilage quality, and might this involve simple water balance or other chemical pathways?

Response 5: The following text marked in yellow was added into the Manuscript:

This means that the cactus plant has a high tolerance to stress and the lowest level of soil moisture included in this study may not have been low enough to drive on the mucilage

quality in response to hydric stress. **Pag. 12, Paragraph: 4, Lines: 490 – 492.**

Comments 6. The study highlights that water availability directly affects mucilage yield, and that C-CH is more resilient under both good and poor water conditions. Could differences in water channel proteins, such as aquaporins, be a reason why C-CH handles drought better and produces more mucilage?

Response 6: The following text marked in yellow was added into the Manuscript:

In addition, Parent et al. (2009) indicate that ABA effect on plant hydraulic properties through aquaporin activity, which contributes to the maintenance of a favorable plant water status. **Pag. 13, Paragraph: 3, Lines: 525 – 528.**

Comments 7. The paper shows clear differences in water response and mucilage production among the Opuntia morphotypes, what do these findings imply for selecting and improving Opuntia types for farming in dry areas, particularly regarding water efficiency and the quality of animal feed?

Response 7: The following text marked in yellow was added.

The findings of this study suggest that the genetic materials evaluated in this study may be the basis for selecting, and improvement for tolerance to water stress in livestock regions in arid zones, which would allow for the availability of genetic materials with greater efficiency in the use of water and the quality of fresh matter for animal feed in dry areas. **Pag. 12, Paragraph: 3, Lines: 525 – 528.**

4. Response to Comments on the Quality of English Language

No comments.

5. Additional clarifications

Additional changes were made by the co-authors. The texts modifieds were marked in yellow color into the Manuscript.