Dear Dr. Abd El-Moneim,

I have reviewed the manuscript and recommend its acceptance for publication after minor revisions. The study makes significant contributions, and I believe that with a few adjustments, it can be further refined and enhanced. Below, I provide both positive feedback on the strengths of the work and constructive suggestions for improvement, which I hope will help in finalizing the manuscript.

Best regards,
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Strengths of the Study

1. **Clear Research Focus and Relevance:**

The study is well-focused on identifying and characterizing four novel SUMO genes in wheat (TaSUMO4-7), which is a crucial area of research in plant biology. SUMOylation plays an essential role in various cellular processes, and this paper adds to the understanding of its molecular mechanisms in wheat, an important crop.

2. **Comprehensive Bioinformatics Analysis:**

The paper provides a thorough bioinformatics analysis using multiple software tools, offering valuable insights into the TaSUMO4-7 genes. The results highlight the close evolutionary relationship between these genes and their counterparts in other monocot species, supporting the idea of the conservation and functional importance of the SUMO family. This multi-tool approach strengthens the study by offering a detailed computational perspective on the TaSUMO genes, contributing to a deeper understanding of their potential roles across plant species.

3. **Subcellular Localization Experiments:**

The paper presents well-executed subcellular localization experiments using both DsRFP-tagged and GFP-tagged TaSUMO4-7 proteins in onion cells. These experiments effectively illustrate the distribution of the proteins in both the nucleus and cytoplasm. Additionally, the use of organelle-specific markers to confirm the localization further strengthens the validity of the findings. The use of two distinct fluorescent markers and organelle localization tools enhances the confidence in the observed patterns. This data serves as a solid foundation for future studies that could explore the functional roles of these proteins in the SUMOylation process, providing a starting point for further functional validation.

4. **Contribution to Wheat Research:**

The identification of the TaSUMO4-7 genes, their expression patterns, and the localization studies fill a significant gap in our knowledge of the SUMO system in wheat. This has practical implications for understanding stress responses and other cellular functions in wheat, which could be valuable for agricultural applications.

5. **Well-supported Conclusion**

The conclusions drawn are well-supported by the data presented in the study. The connection between the SUMOylation process and the localization of TaSUMO proteins in different cellular compartments is effectively highlighted. The potential involvement of these genes in abiotic and biotic stress responses is thoughtfully discussed, although further studies could explore these aspects in more detail.

Recommendations for Strengthening the Work (Minor revisions needed)

1. Clarity of Experimental Design:
The subcellular localization experiments using DsRFP-tagged TaSUMO proteins are mentioned, but the methods for these experiments (e.g., concentrations, time points, number of replications, and controls) lack sufficient detail. A more thorough description of these methodologies would improve reproducibility and help the reader understand the experimental setup more clearly.

2. Tissue-Specific Expression Data:
The paper mentions that TaSUMO4-7 genes are constitutively expressed across wheat tissues. However, it would be more impactful to include quantitative expression data to support this claim. While the study is not focused on differential gene expression, providing some form of expression data (e.g., relative expression levels in various tissues like leaf, root, stem) would offer additional clarity and confirm the extent of gene expression across tissues. Presenting numerical values or statistical analyses (such as fold changes or comparisons between tissues) would strengthen the findings.

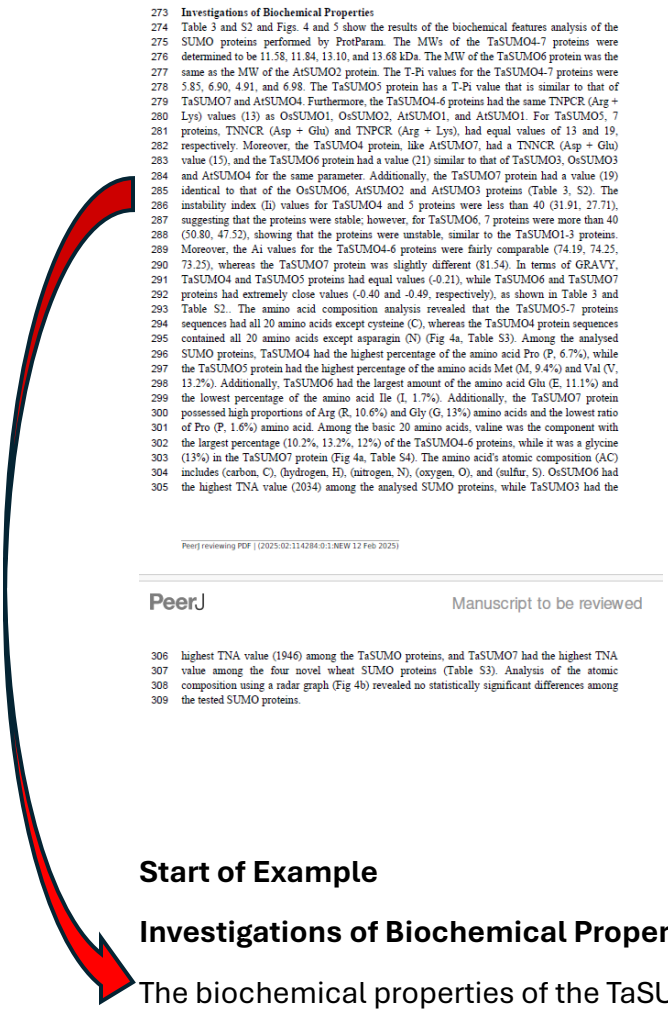
3. Language and Structure: Clarity, Grammar, and Readability

For language and structure, I have two comments: A. Paragraph Length and B. Grammar and Flow.

A. Paragraph Length:

The manuscript includes several long paragraphs, some extending to an entire page, which could benefit from being broken into smaller, more manageable sections. Shorter paragraphs would enhance readability and make the content easier to follow, especially when conveying complex ideas or data. Consider dividing lengthy paragraphs into focused ones that address a single concept or related ideas. This will help maintain the reader's attention and improve the overall clarity of the manuscript.

While shorter paragraphs are often preferred for clarity and readability, it is also common in research papers to use longer paragraphs, especially when discussing complex ideas or technical data. The key is to ensure that each paragraph maintains unity—meaning all sentences in the paragraph should support one central idea or concept. If multiple ideas are introduced, it's usually a sign that a new paragraph is needed. However, combining related points into a single paragraph is acceptable as long as the paragraph remains focused and coherent. I have provided an example below of lines 273 - 309 broken down into paragraphs. However, feel free to adjust the paragraph length or combine sections as you see fit, especially if the example I provided doesn't align with your preferred structure. The primary goal is that each paragraph should maintain unity and effectively convey the intended ideas.



273 **Investigations of Biochemical Properties**
274 Table 3 and S2 and Figs. 4 and 5 show the results of the biochemical features analysis of the
275 SUMO proteins performed by ProtParam. The MWs of the TaSUMO4-7 proteins were
276 determined to be 11.58, 11.84, 13.10, and 13.68 kDa. The MW of the TaSUMO6 protein was the
277 same as the MW of the AtSUMO2 protein. The T-Pi values for the TaSUMO4-7 proteins were
278 5.85, 6.90, 4.91, and 6.98. The TaSUMO5 protein has a T-Pi value that is similar to that of
279 TaSUMO7 and AtSUMO4. Furthermore, the TaSUMO4-6 proteins had the same TNPCR (Arg +
280 Lys) values (13) as OsSUMO1, OsSUMO2, AtSUMO1, and AtSUMO1. For TaSUMO5, 7
281 proteins, TNNCR (Asp + Glu) and TNPCR (Arg + Lys), had equal values of 13 and 19,
282 respectively. Moreover, the TaSUMO4 protein, like AtSUMO7, had a TNNCR (Asp + Glu)
283 value (15), and the TaSUMO6 protein had a value (21) similar to that of TaSUMO3, OsSUMO3
284 and AtSUMO4 for the same parameter. Additionally, the TaSUMO7 protein had a value (19)
285 identical to that of the OsSUMO6, AtSUMO2 and AtSUMO3 proteins (Table 3, S2). The
286 instability index (Ii) values for TaSUMO4 and 5 proteins were less than 40 (31.91, 27.71),
287 suggesting that the proteins were stable; however, for TaSUMO6, 7 proteins were more than 40
288 (50.80, 47.52), showing that the proteins were unstable, similar to the TaSUMO1-3 proteins.
289 Moreover, the Ai values for the TaSUMO4-6 proteins were fairly comparable (74.19, 74.25,
290 73.25), whereas the TaSUMO7 protein was slightly different (81.54). In terms of GRAVY,
291 TaSUMO4 and TaSUMO5 proteins had equal values (-0.21), while TaSUMO6 and TaSUMO7
292 proteins had extremely close values (-0.40 and -0.49, respectively), as shown in Table 3 and
293 Table S2.. The amino acid composition analysis revealed that the TaSUMO5-7 proteins
294 sequences had all 20 amino acids except cysteine (C), whereas the TaSUMO4 protein sequences
295 contained all 20 amino acids except asparagin (N) (Fig 4a, Table S3). Among the analysed
296 SUMO proteins, TaSUMO4 had the highest percentage of the amino acid Pro (P, 6.7%), while
297 the TaSUMO5 protein had the highest percentage of the amino acids Met (M, 9.4%) and Val (V,
298 13.2%). Additionally, TaSUMO6 had the largest amount of the amino acid Glu (E, 11.1%) and
299 the lowest percentage of the amino acid Ile (I, 1.7%). Additionally, the TaSUMO7 protein
300 possessed high proportions of Arg (R, 10.6%) and Gly (G, 13%) amino acids and the lowest ratio
301 of Pro (P, 1.6%) amino acid. Among the basic 20 amino acids, valine was the component with
302 the largest percentage (10.2%, 13.2%, 12%) of the TaSUMO4-6 proteins, while it was a glycine
303 (13%) in the TaSUMO7 protein (Fig 4a, Table S4). The amino acid's atomic composition (AC)
304 includes (carbon, C), (hydrogen, H), (nitrogen, N), (oxygen, O), and (sulfur, S). OsSUMO6 had
305 the highest TNA value (2034) among the analysed SUMO proteins, while TaSUMO3 had the

306 highest TNA value (1946) among the TaSUMO proteins, and TaSUMO7 had the highest TNA
307 value among the four novel wheat SUMO proteins (Table S3). Analysis of the atomic
308 composition using a radar graph (Fig 4b) revealed no statistically significant differences among
309 the tested SUMO proteins.

Start of Example

Investigations of Biochemical Properties

The biochemical properties of the TaSUMO proteins were analyzed using ProtParam, with the molecular weights (MW) of the TaSUMO4-7 proteins ranging from 11.58 kDa for TaSUMO4 to 13.68 kDa for TaSUMO7. Notably, the MW of TaSUMO6 was identical to that of AtSUMO2. The theoretical isoelectric points (T-Pi) for the proteins varied, with values of 5.85, 6.90, 4.91, and 6.98 for TaSUMO4-7, respectively, and TaSUMO5 exhibited a T-Pi value similar to that of TaSUMO7 and AtSUMO4.

The analysis revealed significant similarities in the TNPCR and TNNCR values across the TaSUMO proteins. The TNPCR (Arg + Lys) values for TaSUMO4-6 were consistent (13), matching those of OsSUMO1, OsSUMO2, AtSUMO1, and AtSUMO2. For TaSUMO5 and TaSUMO7, TNNCR (Asp + Glu) and TNPCR (Arg + Lys) had identical values of 13 and 19, respectively. Furthermore, TaSUMO4 had a TNNCR (Asp + Glu) value of 15, similar to AtSUMO7, while TaSUMO6 showed a value of 21, akin to TaSUMO3, OsSUMO3, and AtSUMO4. The TNNCR value of TaSUMO7 was 19, aligning with OsSUMO6, AtSUMO2, and AtSUMO3.

Stability analysis of the TaSUMO proteins, assessed using the instability index (Ii), indicated that TaSUMO4 and TaSUMO5 were stable with values of 31.91 and 27.71, respectively, both under 40. In contrast, TaSUMO6 and TaSUMO7 were unstable, with Ii values exceeding 40 (50.80 and 47.52), similar to TaSUMO1-3. Additionally, the aliphatic index (Ai) for the

TaSUMO4-6 proteins was comparable (74.19, 74.25, 73.25), whereas TaSUMO7 had a slightly higher value of 81.54.

The Grand Average of Hydropathy (GRAVY) values showed a trend in hydrophobicity among the TaSUMO proteins, with TaSUMO4 and TaSUMO5 having identical values of -0.21, while TaSUMO6 and TaSUMO7 exhibited closely related values of -0.40 and -0.49. The amino acid composition revealed that TaSUMO5-7 proteins contained all 20 amino acids except cysteine (C), while TaSUMO4 lacked asparagine (N). TaSUMO4 had the highest proportion of proline (P, 6.7%), TaSUMO5 had the highest percentages of methionine (M, 9.4%) and valine (V, 13.2%), and TaSUMO6 had the highest amount of glutamic acid (E, 11.1%) but the lowest amount of isoleucine (I, 1.7%).

Amino acid composition differences were also notable across the proteins. TaSUMO7 had high proportions of arginine (R, 10.6%) and glycine (G, 13%) and the lowest amount of proline (P, 1.6%). Among the basic 20 amino acids, valine was the most abundant in TaSUMO4-6 (10.2%, 13.2%, 12%), whereas glycine was most prevalent in TaSUMO7 (13%).

Analysis of the atomic composition showed no significant differences among the tested SUMO proteins. The total nucleotide abundance (TNA) values for the SUMO proteins varied, with OsSUMO6 having the highest TNA value (2034), followed by TaSUMO3 (1946), and TaSUMO7, which had the highest TNA value among the four novel TaSUMO proteins. A radar graph analysis of the atomic composition confirmed the lack of statistically significant differences between the proteins.

End of Example

A. Grammar and Flow:

The manuscript is generally well-written, but there are a few areas where clarity could be improved by refining the language and grammar. Some sentences are lengthy and could benefit from rephrasing for better readability. Additionally, minor grammatical errors were noticed, such as inconsistencies in tense usage and subject-verb agreement. A thorough proofreading would help streamline the writing and enhance the overall flow of the paper. It may be helpful to have a native English speaker review the manuscript to ensure grammatical accuracy and clarity. Here are a few things I noticed:

Line 35: "the wheat's SUMO genes"

The phrase "the wheat's SUMO genes" should be "The SUMO genes in wheat"

- **Explanation:** This revision removes the possessive form, making the sentence clearer and more grammatically correct.

Line 48: replace "record" with "most consumed"

Line 56: "like drought" Problem:

The use of "like" in formal writing should be avoided as it is too casual.

Explanation: "Such as" is a more formal and appropriate choice for listing examples in scientific writing.

Line 58:

The phrase "employed via plants" is unclear and could be replaced by "Utilized by plants"

Line 66

The term "dissimilar ubiquitylation" is and unclear and can be replaced with "In contrast to ubiquitination"

Line 73: "Finally, SUMOylation refers to the modification of a substrate using a small-ubiquitin-like modifier (SUMO)."

- **Problem:** The phrase "Finally, SUMOylation refers to..." feels somewhat redundant, as the paper has already defined SUMOylation earlier.
- **Fix:** "SUMOylation refers to the modification of a substrate by a small-ubiquitin-like modifier (SUMO)."
- **Explanation:** Removing "Finally" improves the sentence's flow and removes unnecessary repetition.

Line 79: "Ubiquitous across eukaryotic cells, the SUMO protein has known as a crucial controlling mechanism that regulate various cellular pathways through targeted protein modification..."

- **Problem:** The phrase "has known as a crucial controlling mechanism" is grammatically incorrect and unclear.
- **Fix:** "Ubiquitous across eukaryotic cells, the SUMO protein is known as a crucial controlling mechanism that regulates various cellular pathways through targeted protein modification..."

- **Explanation:** "Has known" should be changed to "is known as," and "regulate" should be corrected to "regulates" to maintain subject-verb agreement.

Line 81:

SUMO “cross talk” should be “crosstalk”

Line 91: "The engagement of SUMO to a certain protein (substrate) isn’t permanent but rather a dynamic process controlled by SUMO proteases."

- **Problem:** The phrase "engagement of SUMO to a certain protein" sounds and unclear.
- **Fix:** "The attachment of SUMO to a protein (substrate) is not permanent but rather a dynamic process controlled by SUMO proteases."
- **Explanation:** "Attachment" is a clearer and more accurate term than "engagement" in this context, and "is not" is a more formal construction.

Line 120:
Original: "SUMO sequences in Arabidopsis thaliana (AtSUMOs) **got** from TAIR"
Correction: "SUMO sequences in *Arabidopsis thaliana* (AtSUMOs) **were obtained** from TAIR"

Line 133

Remove the hyphen in "controlled-environment" to make it "controlled environment"

Line 159:

Replace “via” with “using”

Line 209:

(Change "a vectors" to "vectors" and "was" to "were" to agree with plural subject "vectors")

Line 211*:*
***“its** role” should be “their role”*