

The present study "Molecular characterization and expression profiling of cystatin family genes reveal their high evolutionary conservation and functional divergence in sorghum (*Sorghum bicolor* L.)" attempt to underpin the evolutionary conservation and functional divergence of cystatin family genes in *Sorghum bicolor*. It is an interesting study and relevant to current focus. This study does highlight cystatin gene characterization evolutionary relatedness and their expression profiles. However, considering the interest of the global readers of this journal, authors must improve the manuscript further. Overall, English grammar is poor and sentence formation needs specific improvement.

Q. There is a lot of repetition in manuscript creating a confusion, should be written precisely grammatical errors need to be checked.

Q. Abstract is elaboratively written. It can be written in a Precise manner. Delete the following sentence:

“Cystatin is a class of proteins known to reversibly inhibit the activities of cysteine proteases. Although cystatin family genes have been well characterized in many plant species, little is known about this family and their roles in sorghum (*Sorghum bicolor* L.)”.

Q. Paraphrase 58 and 59 lines.

Q. Line 72 and 73 Read as “unfortunately, the genome-wide studies of cystatins family genes in sorghum (*Sorghum bicolor* L.) are quite limited”. When there is no data available about genome wide studies of Cystatins in Sorghum, mention as “unfortunately, the genome-wide studies of cystatins family genes in sorghum (*Sorghum bicolor* L.) are not available.

Q. Line 92 Replace “Predicted” with “predicted”.

Q. Mention *Arabidopsis* in italics.

Q. Line 138 25 °C

Q. Line 144 -80 °C

Q. Line 173: Replace “this consisted” with “corroborate”

Q. Line 234: 1.5 kb

Q. Why authors have performed only native expression analysis for different tissues, but not with stress conditions

Q. Why there is a variation in number of genes in expression profiling experiment? Why it is not performed for all 18 Cystatin genes?

- Q. Figure 1: Description is missing below the figure
- Q. Figure 2: Mention the software name used for gene structure analysis and range of exons and introns present in them.
- Q. Figure 3: Distribution of SbCys conserved motifs and amino acid alignment
- Q. Figure 6: delete organ and mention it as tissue. Mention about figure description clearly like tissue types, color blocks, and range of expression.
- Q. At many places sorghum is written instead of Sorghum, author needs to check them.
- Q. Line 268: change the word from depressed to suppressed
- Q. Line 272 : deleted during aphid infection, it is already mentioned in line 269
- Q. Line 274: delete not and replace with no and write rest of genes instead of rest genes
- Q. Line 280: replace “excepted” with “except”
- Q. Line 290: delete in different stresses
- Q. Line 292: delete excepting and replace with except
- Q. Line 371: delete profiles and mention as profile
- Q. 371-373: Paraphrase the sentence to: The expression profiling of *SbCys* genes of Sorghum tissues reveals the role of regulation activity like storage seed reserves mobilization.
- Q. Focus more on discussion and add few more references of the recent ones.
- Q. Conclusion can be written focusing mainly on the present work carried out instead of generalized findings. Mention the group of genes fall into different localizations, exon-intron characterization and real time expression analysis, duplication events and phylogeny analysis.
- Q. Does the author tried to find the interacting partners of these genes, it could add novelty to the work.
- Q. Cite the references in chronological order althrough the text.

The MS can be recommended if the author address all the above issues.