Identification of candidate reference genes for qRT-PCR normalization studies in salinity stress and injury on *Onchidium struma*

Review

Generally speaking the manuscript requires comprehensive English editing. I have made several corrections/suggestions for the abstract and Introduction but stopped at some point as there are simply too many typos and/or grammatical errors. Please have a native English speaker edit the manuscript.

The poor English aside, I think that the abstract and introduction are well structured and present the study well. Some of the references should be updated as many of them are over a decade old and to my knowledge are not fundamental papers in this field.

There is one important (perhaps very important) “take home message” from this paper which is not highlighted sufficiently by the authors and is of potential wide-ranging implication (outside the Onchidium community…). That is, that one cannot assume that a particular “housekeeping gene” which was found to be good (i.e. stable) in one series of experiments on a particular organism will still be relevant for standardization in other experiments (and certainly not in other organisms). The notion that stress induced by extreme environmental conditions (such as salinity) may alter the expression pattern of “putative” housekeeping genes is not apparent and should be highlighted. I suspect this will make the paper more appealing to a broader audience. I am not sure but this finding may even warrant changing the title of the paper accordingly because at the moment the title speaks to a very very small niche group of interest.

Abstract

1. Until now, researches on stable housekeeping genes in *Onchidium struma* under salinity stress and injury are scare. – To date, studies on…..
2. housekeeping genes are selected as candidates – …were selected….
3. suitable reference genes to facilitate analysis mRNA transcript levels – analysis of mRNA…
4. The aim of the study – this study
5. under salinity stress even injury stress – under salinity and injury stress
6. Discoveries from this study will – The findings of this study…
7. under other stress researches. – under other stress conditions

Introduction

Line 3 – it has become one of the most….
Line 6 – References 1,2 and 3 are quite out of date. Is there a particular reason why they specifically are cited?
Line 9 – standardize
Line 11 – tissues and conditions
Line 19 – thus their expression…

Line 20 – have revealed
Line 25 – O. struma is a brackish water…
Line 27 – The species
Line 28 – I understand the potential importance of O. struma for food etc. However, its problematic to infer from that to it's breeding potential under an agricultural setting.

Materials and Methods

Line 38 – The animals were collected and maintained according to Shen's method (ref. 18). This paper has an English abstract, however the main text is in Chinese. Question to editor – Is it ok to leave this as is? Or since the reference is in Chinese, perhaps the actual method should be presented here in English?

Line 62 – the manuscript states that the NovoStart SYBR qPCR SuperMix was used to reverse transcribe…..However, if I understand correctly this is a kit for qPCR once the RNA has already been reverse transcribed with some other kit. Is so, which kit was used and under what conditions? Please clarify this and move this part down to section 5 which deals with the qPCR analysis.

Line 64 – states that the cDNA samples were diluted x5. How and why was this value chosen?

Line 81 – software

Line 96 – To evaluate the respective stabilities of the candidate reference genes….

Results

As with the previous section, the English is poor and requires editing.

In tables 1-9 the number of samples (N) is given. It is unclear to me to what this refers to. For example, table 9 presents weight differences, so does the N=5 refer to different weights? If so what are they?
Please clarify this for the further tables as well.

Figure S1 – Please add X and Y axis value titles (I assume that the Y axis is geNORM V values?).

Figure S2 – The letter D is partially hidden behind a table bar.

Lines 140-143 – This section needs to be rewritten and thoroughly clarified.

Line 144 – This and all the following titles – In terms of….Should be rephrased.

Discussion

In general, the discussion is short and not comprehensive enough. Only three other similar studies are quoted. Admittedly, I am not familiar enough with the cited literature and to what extent there are further such studies (Assessing the suitability of appropriate house-keeping genes for qPCR in general, and more specifically, under varying stress/physiological conditions), however I think this section should be more comprehensive.

Line 216-218 – This entire sentence needs to be rephrased and although the assumptions presented are obvious, perhaps a reference is also in need.

Line 323, 235 and 237 – Full stop instead of the current symbol used.

Line 238 – “current academic community” – Rephrase.

Line 241 – Rephrase.

Conclusions

Line 246 – among six tissue samples.

Line 247 – The results imply or the data imply but not both.