Review of the manuscript "Proanthocyanidins in seed coat's tegmen and endospermic cap inhibit seed germination in the bioenergy plant *Sapium sebiferum*"

The manuscript needs thorough reworking to improve all sections of the documents, developing the state of the art, better presenting the objectives, completing the material and methods, centering the result section on the results by moving additional methodological details to the previous section, and focusing the discussion on the interpretation by synthesizing the statement of the conclusions rather than repeating detailed results.

Therefore, I recommend to reject the manuscript for publication, with possibility to submit again following completion of the modifications. This seems feasible, as some of the necessary elements can be found dispersed in the text at the wrong place or in the figure legends. It seems that no additional experimental work nor analyses should be done, but a huge effort to correctly and fully explain the design, the methods, the results.

General comments

The **introduction** contains a short state of the art, a good presentation of the socio-economic context and the results of the current study. You should remove the results (line 65 to line 73) and rather develop the state of the art regarding the role of proanthocyanidins as germination inhibitor. Indeed you started with few elements (l 38-41) then you mentioned (l58-59) works by Debeaujon et al. (2000); Wada et al. (2011), as providing results that suggested you the main hypothesis of your research, but you provide no elements on the results or conclusions drawn by these authors. The same comment holds for works by Debeaujon & Koornneef 2000; 61 Debeaujon et al. 2000; Jia et al. 2013; Jia et al. 2012; Liguo et al. 2012) that are hardly presented (l60-61). Then the question "Whether PAs response to nitrates signalling or not? It is not clear yet." is not clear. Please rework this paragraph. Then conclude the introduction by clearly presenting the hypothesis you want to test, with the detailed questions and objectives that will help solve this hypothesis, rather than providing results.

The **material and method** section is well structured but lack of some information in several sections that need to be completed (see detailed remarks below for more information). Some elements are difficult to understand and need reworking: for instance l 90-97, we do not understand the purpose of what is described. How do you relate the medium on the petri dishes, to the PA that you bought or to the PA from the seed coats? What does "analyzed by the previous protocol as described by Xuan" means exactly? Without providing all details, you still need to explain what this protocol is and what it aims at doing; finally: how is it different from the content analysis protocol from Herald et al.?

The design presented in the "Pre-germination treatments and Germination conditions" section is not clear. You should indicate clearly how many seeds and pots were used per treatment, specifying how many modalities you used: in the introduction, it seemed like you were going to test ABA GA3 etc. but in this section, it seems that you only tested PA and SCE treatments. We don't know either the design corresponding to the application of seed coat abstract. We don't know if you applied the same treatment to uncoated seeds / coated scarified seeds / coated unscarified seeds, either. Please develop the description of your experiment fully and properly.

I am not skilled enough in molecular biology to judge of the adequacy of the “Primer designing, RNA extraction, cDNA synthesis, and RT–qPCR conditions” section.

Finally, the material and method section present no elements regarding statistical analyses although some test results are indicated on the manuscript figures (ex. Post-hoc tests, number of replicates).

The **results** section is a mixture of results and methodology. Some elements that should have been provided in the material and methods section are presenting here: for instance, among many other examples, the different concentrations of sulfuric acid used to test the best suitable dormancy breaking methodology; this element was never mentioned in the “Pre-germination treatments and Germination conditions” section where it should be. This remark is relevant for each paragraph of the results section. In a similar although more moderate manner, some sentences in the results section are either interpretation of the results or comparison to the literature; all these elements should be moved to the discussion section. You should reorganize totally the document to present separately the methods from the results, and avoid starting the discussion.

The **figures** are nicely presented to illustrate the effect of the treatments on the seeds and to sustain the results; the legends are clearly commented, even presenting some elements regarding statistical analyses.

The **discussion** section presents some interesting interpretation of the results of the current work, with comparison and use of appropriate references. However, the results are presented again several times in the discussion, with too many details, making it difficult to keep up the logic of the discussion (for instance l269 to 273, l 281 to l288). You should remove the detailed presentation of the results and rather state the conclusion drown from your experiment to confront it to the literature and interpret it.

The **conclusion** recalls the main results and aims at proposing some perspectives. However, the perspectives remain vague (for instance l 374-375 “Further studies of molecular levels are needed to find out the relationship between…”, l380 “it remained a debate for the future”, l382 “more investigations from other species seed are required”) and you should be more specific regarding which questions or elements need to be enquired and solved.

Overall the English is sound and easy to understand, but some grammatical errors are remaining that you should check and correct, all through the text. For instance, l38 “hard seed coat blocks water uptake by the seed” rather than “hard seed coat blocks the water uptake of seed”; l39 “gas exchange” not “the gasses exchange”, l41 remove THE in “the seed germination”, it is not clear what means the expression “Tallow layer of its fruits”; l76 remove THE before “the plants” etc. etc.

Detailed remarks and questions.

All the abbreviations used further in the manuscript are defined only one time in the abstract (ex. PA, NDGA, GA3, SCE etc.). Please use full name before using abbreviation the first time you are referring to each word in the content of the manuscript, apart from the abstract.

There is a typographic error all through the document: S. Sebiferum. Please remove the uppercase and replace Sebiferum by sebiferum.

L76: could you please add more information on seed harvesting. How many plants were used as seed source? When did you collect the seeds (year, season)? How long did you store the seeds before the experiment?

L80-81: please indicate the complete references and characteristics (ex. Concentration) of the products that you bought, and complete the address of the companies that sold them to you (same remark on line 93).

L82-83: please indicate the characteristics of the solutions: final concentration, volume etc.

L85-89: indicate the machines that you used in the experiment: weighting device, centrifuge machine etc.

L90-91: Reword this sentence “SCE and PAs were adjusted to 0.1%, 0.2%, and 0.3% in 0.5×MS containing 15mg/L sucrose and 8 g/L agar before sterilization”. We don’t understand what MS means, nor the meaning of “adjusted” and what you did with agar and sucrose together with SCE and PA. Did you mix them together? In which proportions?

L95-97: please give more information on the protocol by Xuan to explain what it is made for. Is it differing from the one of Herald et al? reword your sentence “Seed coat proanthocyanidins contents were analyzed conventional HCl–vanillin assay (Herald et al. 2014)” to “Seed coat proanthocyanidins contents were determined following the conventional HCl–vanillin assay (Herald et al. 2014)”.

L100: how long did you maintain the seeds in the sulfuric acid?

L104: “Uncoated seed kernels were sterilized”. Seed macrobiota was thus removed. Why did you sterilized the uncoated seeds? Why did you not sterilize the coated unscarified or scarified seeds? Macrobiota is known to play a role in seed germination (see for instance special issue in Plant and Soil, with Editorial special issue: the soil, the seed, the microbes and the plant, 2018, by Nelson Simoneau Barret Mitter and Compant). If you sterilized some seeds but not others, how can you separate the effect of the treatment uncoated / coated from the effect of sterilization?

L160: moderate your statement “These results suggested that sulfuric acid scarification promoted the seed germination by cracking the seed coat” since it depends on the duration of sulfuric acid application, as 60 mn is maybe cracking the seed coat but also depressing germination and growth. Moreover, all interpretation of the results should be moved to the discussion section.