

Dear Editor,

Thank you for considering our manuscript for publication.

Reviewers have made some pertinent remarks. We modified the text with their help, convinced that this will improve the quality and also the comprehensibility of the text, the objectives of the study and the results. Below are the responses to the reviewers' comments and questions. We answered point by point. Our responses are organized in tables, on the left are the comments, and on the right, our responses.

We have is loaded in arXiv a revised version of the manuscript (<https://arxiv.org/submit/3568226>) taking account of a large number of your suggestions in the preprint. We prefer not to consider some propositions of English modifications. In fact, the manuscript has been edited before submission by a professional, native English-speaking editor with a PhD in a relevant discipline, working for Sees-editing Ltd., UK. We preferred also to keep some sentences which were added following specific requests from certain colleagues who revised the manuscript. We think that they help to understand the work for - less specialist - readers.

With My Best Regards,

Caroline Teyssier

## Editor

Dear authors

This is a very nice and clearly presented preprint dealing with a comparison of seed properties in two contrasting french populations of *Populus nigra* L, a riparian tree species submitted to severe threads due to mismanagement of riparian ecosystems and to genetic hybridization with other poplars. Important efforts are currently made for the conservation of this species present across Europe but restricted mostly to river banks. Your work concentrates on seed properties of two quite different french populations of black poplar, one along the Loire river, and another one along the Drome river in south eastern France.

Our knowledge about seed properties of this species needed be completed, and a comparison of intra and interpopulation variability in seed properties is really welcome. The preprint describes many properties of these small and recalcitrant seeds, with the ambition to relate those properties with propagation of black poplar under natural conditions. The manuscript was submitted to two experts about seed physiology, who found the study very interesting. One of the reviewers expressed nevertheless some concerns about some aspects of the preprint with which I fully concur. I did also a careful reading of the preprint myself, and made a number of suggestions and comments directly on the attached version of the preprint (see below).

I therefore reached the conclusion that the preprint could be recommended by the PCI Forest & Wood Sciences under the condition of a careful revision along the points raised by the external reviewers and by myself.

Here are the points you mention (right column) and our responses (left column) :

1- The manuscript is in general very well written and clearly presented. However, a number of small typos need be corrected, and some sentences require rewriting (see comments in the attached version).	We corrected the text in that way
2- The manuscript also refers to supplementary material. My recommendation would be either to integrate the most important material as an annex, or to add it to the data set available on the INRAE data portal.	All the annex files are already in the INRAE data portal.
3- The approach used remains rather descriptive (albeit very clear). I wonder whether it would not be possible to provide some more explicit research questions or even testable hypotheses. The structure of the discussion (which is very clear), defines some important focus points that should already be identified at the end of the introduction under the form of questions or hypotheses. This would be of real help to the reader and for structuring the presentation of the results.	The introduction has been modified to take into account this remark
4- The discussion refers in places to results (which should be as much as possible avoided) and	idem

<p>sometimes provides sentences that belong rather to the introduction (I identified such sentences in the attached copy of the preprint).</p>	
<p>5- Data display should be standardised to follow the general rule of showing three significant figures (implying a 1 permil accuracy).</p>	<p>This rule has been applied in the whole text.</p>
<p>Thank you very much for providing access to the data sets. I would recommend to cite the data set with its DOI in the reference list.</p>	<p>They have been added</p>
<p>I would also recommend to provide the DOIs of the papers cited in the reference list when they are available. This would enable readers to easily retrieve the cited material.</p>	<p>They have been added.</p>
<p>I found also that some references were missing.</p>	<p>They have been added.</p>
<p>In addition to these presentation details, I have a few more general points that deserve some attention:</p> <p>1. The main aim is to document the ability of black poplar to propagate by seeds in these particular environments where the establishment of seedlings depends so strongly of the level of water in the river. I wondered whether, similarly to other poplars (for instance aspen, or <i>P. euphratica</i>) black poplar is able to propagate by root suckers (vegetative propagation) or whether it depends solely on seed dissemination and germination.</p>	<p>Yes, you are right. Both regeneration (via seeds and root suckers) do exist in black poplar, but it is not the scope of this paper to compare both strategies. We focus on seed and its composition and its putative role in germination ability.</p>
<p>The second question would be related to the ability of deriving rather general conclusions about dissemination and stand regeneration from seeds, just by describing seed properties and germination under very peculiar conditions (in Petri dishes). I had the feeling of a gap between the two scales (seed properties and in situ regeneration). However, I found the discussion quite clear in this respect with no overinterpretation of the data. This feeling is probably due to the lack of explicit description of the experimental framework in the intro (see also above).</p>	<p>The <i>in vitro</i> germination test is required to compare seed germination in an unique environment. I do agree that the petri dish is far away from the gravelly sandy texture of the alluvial bar, but the idea is not to compare both tests. The idea is to have access to the potential of germination of seeds in comparable optimal conditions : thus Petri dish is the classical standardized test.</p>
<p>Two populations is rather a small number (albeit already a lot of work) to provide clear conclusions about the correlations between seed properties and climate. Are there other data published that could help better discuss this issue from a broader range of populations?</p>	<p>We do know that data from only two populations could not allow us to generalize data to all populations from the whole natural range of the species. I think we have been clear in that sens and we have never overinterpreted these data. However, these two populations belong to very contrasted environments, from the French range. These populations are also genetically distinct, characterized with a set of nearly 8000 SNP DNA markers (Faivre-Rampant et al. 2016). These were scientific reasons (and also excellent relationships with park managers, that help us to collect seeds at the adequate period) that allow us to choose these two populations. There is no other data published on seeds from other populations.</p>

I therefore warmly recommend to revise the preprint, taking into account the insightful comments of the referees. I believe this should be a rather easy task, and should ultimately lead to a very nice preprint I would be pleased to recommend. While submitting this revision, please prepare a point by point reply to our comments.

I am looking forward to the pleasure of considering the revised version for a recommendation.

With best regards and my apologies for the time it took to handle this preprint.

Erwin Dreyer.

**Additional requirements of the managing board:**

As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:

<p>-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.</p>	<p>This is done</p>
<p>-Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.</p>	<p>We did it in that way</p>
<p>-Details on experimental procedures are available to readers in the text or as appendices.</p>	<p>This is checked</p>
<p>-Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."</p>	<p>This is done</p>

**Reviews**

*Reviewed by anonymous reviewer, 2020-11-16 12:18*

Dear Editor, this manuscript addresses an important issue for understanding of the physiology, ecology and regeneration requirements of black poplar (*Populus nigra*). I agree with the Authors that our knowledge on the chemical constitution of seeds of tree species is scarce, and more information about it will allow us to improve our understanding of tree adaptation to changing environment. Additionally, such research should be done at the first place on species threatened with extinction such as black poplar or economically

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significant. Therefore, I think that this article could be interesting for broad scientific community, particularly scientists gathered on the topic on seeds biochemistry as well as plant ecology. The presented manuscript is consistent and well written. The statements are clear to me. The results are presented in a transparent and clear manner.

<p>The only minor drawback of the research is a sparse climatic data which is limited to average temperature only. It seems to me that work would gain a lot if this part was expanded.</p>	<p>We agree with the reviewer, but no other detailed climatic data were available. We have done our best to analyse our phenotypic and chemical data with the climatic data available.</p>
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Nevertheless, in my opinion the presented manuscript is very interesting could be published in PCI Forest Wood Sci.

Best regards, Beata Plitta-Michalak

*Reviewed by Tomasz A. Pawlowski, 2020-11-06 11:49*

Revision of the manuscript entitled “Variability in seeds’ physicochemical characteristics, germination and seedling growth within and between two French Populus nigra populations” for PCI Forest & Wood Sci.

Comments to the Authors

The authors report about analysis of the biochemical composition of Populus nigra seeds in two contrasting environments in France. Significant between population and individual trees differences were detected. General comment: the paper is well written and the topic has been studied in depth by the authors. The achieved data is important in the prediction of the regeneration requirements of black poplar.

<p>Remarks Materials and Methods Fresh weigh, dry weight, and water content. The methods of fw and dw determination were not clearly described.</p>	<p>We changed this section to consider all remarks about it.</p>
<p>Was determined for years 2017 – 2019 and two populations only dw, and for 2019 and Loire only fw? That should be explained.  Results Table 2. What I see from it is a lack of some sample fw and dw determination. What was a reason of that? Moreover, family and population effects were showed, but the time factor could be the interesting approach in these investigations. Why this was not investigated during the study despite seed collections during three years (as for dw determination)? These information could make this work more interesting.</p>	<p>The original idea was to collect seeds and measure dw, easy to do with a standardized way (even when we have to collect seeds present in the Drome population at 525 kms far away from our research lab). But the last year of the study, we investigated fw, in order to make comparison dw-fw and to have data (no published data available). Collected seeds in order to measure fw is complex, we have to be at the right period for collecting fresh seeds. Each mother tree has its own seed maturity period, we have to come back to check seed maturity in the field many times. So, we were able to do this, because the Populus nigra Loire population is close to our research lab.</p>

<p>Fig. 2. Show the arrows properly the investigated bands?</p>	<p>The arrows have been readjusted.</p>
<p>Discussion Structural and biochemical characterisation of the seeds. The Populus nigra seeds were classified as recalcitrant as well as intermediate, so that should be taken into account during discussion.</p>	<p>This Reviewer suggests that we would consider both the recalcitrant and the intermediate character of poplar seeds. The classification of poplar seed as an intermediate refers to its ability to be kept for a long period of storage under specific conditions (by freezing). Our publication does not deal with seed storage, and can then only discuss the recalcitrance of poplar seeds, namely their loss of germination potential by dehydration. We did this on page 12 with an analysis of the contribution of the biochemical content to the recalcitrance of these seeds.</p>
<p>the Populus nigra seeds we examined had far higher contents of storage proteins (ca. 60% of total proteins; Fig. 2), than of structural and enzymatic proteins. How was this proven because results did not show that? A ne regarder pas sur le gel, car saturation de la coloration, mais plus sur l'analyse en SM. J'analyse et rédige la réponse dans la lettre aux reviewers</p>	<p>It is always difficult to evaluate a proportion of proteins from an electrophoretic gel: it is necessary to take into account the saturation of the bands by the dye and the resolution of the protein bands. We agree with this Reviewer that on gel, storage proteins appear less abundant than 60% of total proteins, but could be close. However, legume and vicilin-like proteins are polymeric proteins with 2 types of subunits. On SDS-PAGE, these subunits are visible each time in two bands, and it is the sum of their respective intensity that must be taken into account to evaluate the abundances. The extract buffer is also an important parameter modulating the extraction yield of each type of protein. Our extraction protocol was effective for all types of proteins, even membrane proteins.</p>
<p>Loire seeds had higher dw than Drôme seeds, as the temperature is warmer) at the Drôme site than the Loire site. It means that colder temperature cause the dw increase. How you can explain that?</p>	<p>The discussion already contained the main hypothesis given in the literature to explain this increase of dw in cooler place.</p>
<p>Consequences of seed size for germination and growth of juvenile seedlings Did you observe the correlation between family (individual tree) seed size and seedlings growth?</p>	<p>As the poplar seed is very small (<math>0.8 \pm 0.15</math> mg), the weighing uncertainty did not technically allow individual correlations between mass and root elongation to be made.</p>
<p>Seedling emergence is sometimes better parameter of seed quality than germination rate, however for light seeds as are black poplar the influence of photosynthesis can be main factor of the differences in seedlings growth.</p>	<p>We have tried to measure a kinetics of germination, but with no significant demonstrative results. Thus, it why we prefer to use the more reliable 'classical' germination test, waiting for five days for standardized results. Moreover, measurement of photosynthesis is technically difficult on such tiny cotyledons with an area of less than <math>1\text{mm}^2</math>.</p>

*Reviewed by Eduardo Notivol, 2020-11-26 11:32*

Through biochemical composition of seeds the draft studies the variability of families (2x10) in two contrasting rivers (Drôme and Loire), looking for relationships between composition and germination and intra- and inter-population variability. It seems to be the first reference on biochemical composition of species' seeds, and this is worthy to mention.

Main findings are

Family variation (important for adaptation) In some traits population differentiation (lipids and s. sugars) but not in others (proteins). One population always bigger than other (3 years) No differences on germination. One population longer juvenile roots No critical factors in selection pressure

The paper is well written and presented. Methodology is well-defined and proper and the results are clearly exposed. Discussion is rich and raises interesting ideas about the relationships between the morphological traits, biochemical composition and germination and early steps of the seedlings.

I enjoyed reading the draft and only have very light comments for improvement:

Perhaps the introduction is a little too long even though it provides a comprehensive description and justification of the study.

In Mat and Met, for biochemical procedures there is no description about the family sampling, only number of biological replicates of seeds with weight is described but not about families' information, same for carbohydrates and lipids. It lacks some reference (like in the germination description they do) "per family".	We modified this paragraph; it is now more understandable.
In table 2 caption describe dw and and fw in the same way than WC.	We did not understand the request. The meaning of dw and fw are given in this legend.
In Fig 3 Drôme ellipse is too big and comprises 3 Loire trees, if you diminish the size of it the difference between populations will be better shown.	The size of the ellipse are representative of the confidence interval. So, we can not change it. This reviewer was right to propose us to mention this meaning in the legend.