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Dear Editor,

Thank you very much for reconsidering our manuscript entitled “The genetic basis for panicle traits variation in switchgrass (*Panicum virgatum*)” for publication in Theoretical and Applied Genetics.

We appreciate the thoughtful comments and suggestions provided by both of the anonymous reviewers. We have addresses each of the comments in the following attached document.

Thank you for your time and reconsideration.

Sincerely,

 

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**Responses to reviewer 1:**

1. The comments about aiding breeding and cultivar development (line 27) are really not appropriate for this type of analysis. These types of comments have been made many times over the years, but the reality is that single-marker QTL studies, such as this one, have NEVER led to the incorporation of a single QTL into an improved cultivar. This conclusion was developed by a very extensive literature survey of all agronomic crops by none other than Rex Bernardo. Furthermore, with the recent advancements in sequencing technologies and cost reductions, breeders are now conducting whole-genome sequencing, just as was done in this study, and are using whole-genome prediction methods to place selection pressure on hundreds or thousands of genes throughout the genome. The days of marker-assisted selection based on one or a few QTL, as in the original Meuweissen et al. proposal, are long gone. Thirdly, the actual cost to develop a small number of QTL into high-throughput screenable markers for breeding and selection on thousands of plants is astronomically high. This is another reason why these types of QTL have never been used to develop new cultivars - it's a great theory, but it's completely impractical. In summary, these types of statements would reflect poorly on the authors' knowledge of how plant breeding and cultivar development are actually conducted. Please just stick to your excellent efforts to advance the science of genetics and genomics for this species - really, it's enough, you don't need to justify your efforts in terms of having impacts on breeding and cultivar development. The worst of these statements is on line 381, where the authors claim that incorporation of these QTL into breeding programs is easy. This is an extremely naïve statement that drives home my point that the authors should stick to fundamental genetics/genomics and not try to make any claims about plant breeding.

One more point about this. It did not require very much effort to dig into the literature on this species and discover that plant breeders located at different latitudes are using different germplasm, really no different than maize or soybean breeders across the gradient from Texas to South Dakota. So, my point is if those switchgrass breeders out there already know what germplasm is adapted to their (broadly) local environmental conditions, why do they need to know about one or two QTL that may or may not be partly responsible for these GxE interaction. What would be the benefit to these breeders to go to all the work to design primers and screen germplasm for one tiny chromosome segment that probably only accounts for a small percentage of the variability for these panicle traits? The authors do not give any indication of this percentage, but I doubt it can be more than about 5% or so, based on other published studies of this type.

*This is fair point. We have taken out or reworded the statements where we claimed that this type of QTL analyses would help with plant breeding, and focused on justifying that the goal of this study was to understand the genetics of panicle traits in switchgrass.*

2. I cannot help but wonder how much of the GxE or QTLxE interaction was due to differential survival at the various locations. It is impossible for me to imagine that there was no bias in which genotypes survived in Texas vs. South Dakota at the extremes. We know there is tremendous genetic variation for heat and cold tolerance. Yes, I can imagine that the heat tolerance of the lowland grandparents could combine with the cold tolerance of the upland grandparents, so that many of the F2 progeny could have both traits to be able to survive the entire latitudinal gradient. But, the converse will also be true - not all F2 genotypes will have both traits. Differential mortality of non-cold tolerant genotypes in South Dakota and non-heat tolerant genotypes in Texas would undoubtedly cause differential QTL effect across locations, i.e. a QTLxE interaction. This seems rather artificial to me. This really needs to be addressed in the discussion, maybe even with some aspect of the analysis that could point to which of the putative QTLxE effects might have been caused by this phenomenon. Ignoring this phenomenon is not the answer. I would like some statement of assurance that the putative QTLxE interactions claimed in this paper are not simply due to differential mortality of some plants in Texas and different plants in South Dakota.

*We thank the reviewer’s consideration of mortality or survivorship of plants in the northern sites and in the southern sites. All the plants were transported to each field site in 2015, and well-watered and maintained for establishment. There was not much mortality of plants at each site when we collected the panicle phenotypes in 2016. There are some NA values in the dataset at some sites especially in Pickle, TX (i.e., PKLE) due to data lost but not mortality. To visualize this, we made a histogram of the number of overlapping plants among sites (see below), showing most of the plants occurred at most of the sites (84% of plants survived at least at seven field sites). We added this into discussion as well (Line 317-322).*

3. The "across sites" correlations are really quite meaningless, given the variation and magnitude of the correlations for the individual sites. The most glaring example is the genetic correlation between PL and PBN of 0.03, whereas the range of the individual site values was 0.35 to 0.68. This value of 0.03 clearly has no meaning or value, so the conclusion that PL and PBN are independent of each other is clearly wrong. I do not know how readers are expected to believe this conclusion, given the data in Table 3. I certainly do not believe it. I do not know why the "across sites" value is so low, given the other 10 values, but it gives me no confidence in that value at all.

*We agree with the reviewer. The across sites correlations did not add much to the context. We have taken it out from the manuscript. But to address the reviewer’s concerns, the reason that the correlation became very low after pooling together all the 10 field site data was due to the non-convergence data from Stillwater, OK which did not converge when evaluating by site (Table 3). When pooling together, the estimation of genetic correlation converged but yielded very poor result.*

4. I am having a lot of trouble accepting that Figure 6 depicts "low to moderate prediction accuracy". In my assessment, the prediction accuracy is what I would call either extremely low or near zero. For panels (a) and (b), these are just two shotgun scatters of points, almost as round as can be, with each one only accounting for ~12% of the variability. For panel (c), the high correlation is highly misleading, because it reflects only an ability to predict the mean of the three locations - that linear regression is nothing more than a connection between the means of the three locations. Within each location, there are just three more round (or horizontally ovoid) shotgun scatters of points.

*We agreed with the reviewer that the prediction accuracy is low after we split the prediction panel for each site (data not shown). To address this, we further performed a genomic prediction based on the 4-way kinship matrix using a R package ‘BMTME’, which is a Bayesian multi-trait and multi-environment approach for analyzing breeding data, and allows parameter estimation and evaluates the genomic predication performance. This analysis also reflected the reviewer’s comment (i.e., comment 1) that breeders are now using whole-genome prediction methods to place selection pressure on hundreds or thousands of genes throughout the genome, and we agree with this comment.We have added this into the main text (M&M: line 177-194; Results:255-264; Discussion: 343-358)*

5. I am not very convinced about the value and usefulness of the environmental regression analysis in Table 5. Simply using BIC as the model selection criterion and telling readers which terms were included in the model is not very useful or informative, especially insofar as helping readers to understand whether or not to put much credence into these results. Readers need to be able to assess the results using statistics that are familiar to most of them. At an absolute minimum, this table needs to include the coefficient estimates (sign and magnitude), some sort of SE value for each estimate to give an assessment of how much faith to put into the value, and an overall assessment of the proportion of variance explained (Rsq). With the lack of data in this table, I feel like I'm being told to just accept this as fact and not ask any questions. I also come back to my comment above, with the strong confounding of temperature and daylength across the latitudinal gradient and the role that those two factors undoubtedly played in creating differential survivorship along the north-south gradient, how do we know that these effects are real and not simply an artifact of that non-heat-tolerant genotypes died out in Texas and non-cold-tolerant genotypes died out in South Dakota?

*We agree with the reviewer. As analyzed and discussed in previous version of the manuscript, there were other environmental factors, and the confounding/interactions between factors we are not taking into consideration, resulting in weak analyses. We have taken this section out from our manuscript as it did not add much information to the context. Future work including all possible environmental factors and their interaction may help identify the drivers underlying the QTL-by-environmental interaction for the traits of interest in switchgrass.*

*Additionally, we thank the GxE expert who was not reviewing the manuscript but giving suggestion of using a recently published approach for analyses (Li. Xin, T. Guo, Q. Mu, Xianran Li\*, and J. Yu\*. 2018. Genomic and environmental determinants and their interplay underlying phenotypic plasticity. PNAS 115:6679-6684). As we mentioned, we took out the section of environmental driver exploration underlying QTL-by-environment interaction, so we will not be able to implement this approach in this study. We would like to try this approach in future work.*

**Response to reviewer 2:**

This is a well-constructed paper, featuring a large data set and very good analysis. The findings will be of interest to both switchgrass breeders and to comparative genomic investigations in the grasses. I think the Key Message at the beginning is somewhat misleading, as it implies that GxE is the major finding, when the results clearly indicate a large proportion of the variation is stably inherited between environments. The extra step of validating the QTL mapping via the three extra populations is unusual in such a paper and of great value, although please provide more detail on constructing the model included in the main text. Other than that, I can find little in the analysis to improve. However, I have rated the literature review as poor, even though they do cover much of the available literature very well. It is somewhat embarrassing to say so, but the one they missed was an early publication of mine from 2005 on foxtail and green millet (Doust, A.N., K.M. Devos, M. Gadberry, M.D. Gale, and E.A. Kellogg. 2005. The genetic basis for inflorescence variation between foxtail and green millet (Poaceae). Genetics 169: 1659-1672). Why this is important is because it measures one of the same traits that they do in a QTL analysis of the genetic basis of inflorescence variation in Setaria, and includes investigations and discussions on pleiotropy, epistasis and candidate genes that are relevant to the switchgrass manuscript. Setaria is closer to Panicum than the examples of rice and sorghum that are (briefly) discussed. I would encourage the authors to look at the Genetics paper and see what value it might have to their findings.

*We agree with the reviewer’s comments. First, we have retitled our manuscript into ‘The genetic basis of panicle traits variation in switchgrass (Panicum virgatum)” to reflect our study. Second, we have deleted the QTL model evaluation and replaced with a genomic prediction for the extra genotypes grown at the three field sites. Finally, we have read the paper the reviewer suggested and added it into our references in several places, also in the candidate gene section (Line 276-280). This reference adds great value to the manuscript as it performed similar analyses on inflorescence between foxtail and green millet (Poaceae), which belong to Setaria family and are closer to Panicum. However, we would like to address a little bit more about why we used rice more as example when references are needed. We agree with the reviewer that Setaris is closer to Panicum, but the panicle architecture of switchgrass is more similar to rice as stated ‘The rice inflorescence or panicle, together with those of Sorghum, Panicum, and Avena, is categorized as a raceme, in which the spikelets are not attached directly to the main axis but are formed on the lateral branches” (Taguchi-Shiobara et al., 2011; Itoh et al., 2005).*