

## **Reviewer 2**

In this study, the authors compared populations of Algarrobos (*Neltuma pallida*). They conducted morphological surveys of useful genetic resources and attempted to evaluate their value.

I commend the authors for conducting surveys of various populations from the perspective of diversity in the search for such useful genetic resources.

**However, there are several concerns.**

Dear Reviewer, thank you for your comments and feedback on how to improve the manuscript. Each suggestion will be addressed below. All changes in the manuscript are highlighted in red.

**Comments 01: The main one is the mistake of confusing the population-based analysis with the analysis for the concept of the heritability parameter.**

Heritability analysis involves treating genetic groups at progeny test sites (where the offspring of a mother tree is planted) or clonal test sites (where clone seedlings are planted) as random effects, with the aim of estimating genetic variance and heritability, and selecting families, clones, and superior individuals.

However, in this study, the authors did not consider family or clone (often planted within common experimental sites) as random effects, as is clear from the equation in lines 213-214.

The locality-based analysis referred to by the authors is primarily referred to as a provenance test. This analysis does not satisfy the strict formula for estimating heritability. The Vg and variance referred to by the authors here are merely variation between populations. Therefore, I recommend that the wording and theme of this entire paper be redefined, focusing on the concept of "Qst," which is a parameter of trait-based quantitative variation between populations, as opposed to Fst in population genetic analysis.

**Response 01:** We appreciate the comment. Although we initially considered estimating  $Q_{ST}$  to assess quantitative genetic differentiation, this approach requires estimating additive genetic variances through common garden or progeny tests, which are not feasible for long-lived tree species such as *Neltuma pallida*. As Brommer (2011) noted, “estimating the additive genetic variances needed for  $Q_{ST}$  generally requires rearing individuals from different populations in a common environment, which, for a variety of reasons, may not be feasible.” Therefore, we used the phenotypic analogue  $P_{ST}$  and compared it with the neutral genetic differentiation ( $F_{ST}$ ) as a practical and theoretically justified alternative for inferring adaptive population divergence (Blondeau Da Silva & Da Silva,

2018). This information was included in the manuscript at Material & Methods (lines 243-287), Results (lines 443-495) and Discussion (lines 618-646). Also this information was updated in the abstract and conclusions.

**References:**

Brommer, J.E. Whither  $P_{ST}$ ? The Approximation of  $Q_{ST}$  by  $P_{ST}$  in Evolutionary and Conservation Biology. *Journal of Evolutionary Biology* 2011, 24, 1160–1168, doi:10.1111/j.1420-9101.2011.02268.x. <https://doi.org/10.1111/j.1420-9101.2011.02268.x>

Blondeau Da Silva, S., & Da Silva, A. (2018). Pstat: An R package to assess population differentiation in phenotypic traits. *The R Journal*, 10(1), 447–456. <https://doi.org/10.32614/RJ-2018-010>

**Comments 02:** Once again, the term "heritability" is not normally used for Locality when using current analytical formulas. If this is the case, what variance components are used to calculate the clear genetic variance? It should be made clear what the heritability formula looks like.

**Response 02:** We appreciate the feedback. In this revised version, we no longer refer to "heritability" as a variance component because our analytical framework does not estimate additive genetic variance directly. Instead, we assess population differentiation in phenotypic traits ( $P_{ST}$ ), which quantifies phenotypic variance between and within populations without assuming specific values of  $h^2$ . Accordingly, the term *heritability* has been removed from the text, and the methodology now focuses on evaluating phenotypic differentiation among populations rather than estimating genetic parameters.

**Comments 03:** Also, looking at the formula on lines 213-214, are the categories indicated by the lowercase letters i, j, and k correct?

The trait "i" is used in a single formula. Does this mean that a multi-trait model has been adopted? Calculating traits with completely different unit systems using a single mixed model formula requires delicate consideration of the genetic variance-covariance structure. However, the authors do not explicitly state what  $\mu$  means, and the current formula does not make clear how the genetic covariance structure is estimated for the observed values corresponding to trait i (i.e., the random slope model).

Furthermore, looking at i, j, and k in the formula, the treatment of "individuals" within the population within Locality is not made clear.

**Response 03:** We thank the reviewer for this observation. The previous formula and related notation have been removed. The revised version no longer implies a multi-trait mixed model. These corrections and clarifications are now explicitly described in the section “Assess population differentiation in Phenotypic traits” within Materials and Methods (lines 243-287).

**Comments 04:** It is unclear how the 631 individuals mentioned in Line 138 were handled in this study.

Response 04: Thank you for the comment. We have clarified in the revised manuscript that a total of 631 *Neltuma pallida* trees were evaluated in this study. This information has been incorporated into the revised manuscript, specifically in Section 2.2 “Tree Sampling Methodology” (lines 142-152).