Response to Reviewer 1 Comments

Reviewer #1

Point 1: This paper explores the application of ISSR molecular marker technology to assess the genetic similarity between interspecies Brassicaceae F2 hybrids and their parental forms. The study employs a specific combination of materials that may not have been thoroughly reported in previous research, suggesting a degree of originality. However, the paper presents certain limitations regarding the depth and breadth of the research. Firstly, the study is confined to particular species and their hybrids, which may restrict the generalizability of the findings. Secondly, the research relies solely on specific molecular marker technology, omitting other potential technologies such as SNP markers, which could enhance the dimension and accuracy of genetic similarity analysis. Additionally, while the article addresses the applicability of various measurement methods, it does not provide an in-depth discussion of the applicability and limitations of each method.

Response: We would like to thank you very much for appreciating our research and the results from it. The evaluation of genetic similarity between interspecific hybrids of F_2 *Brassicaceae* and their parental forms is very important to us, since we depend on the results obtained for further research on the material under consideration. Therefore, at this stage of the research we have limited ourselves to these specific genotypes. It seems to us that the results obtained can be generalized. We are aware that further research on other materials may expand knowledge and shed light on aspects that were only hinted at in our study. Our study is based solely on a specific molecular marker technology, but, as we discuss below, this does not detract from the significance of the results obtained.

Major comments:

Point 2: (1) Check whether the number and selection of ISSR molecular markers used sufficiently represent the diversity of genotypes of the Brassicaceae family across generations, and evaluate their possible impact on the reliability of genetic similarity results?

Response: Previous studies have shown that the type of markers did not affect the evaluation of genetic similarity coefficients of rapeseed genotypes. We added this information in the new version of the manuscript. The ISSR markers used sufficiently represent the diversity of genotypes of the *Brassicaceae* family and in this respect are a good tool for obtaining reliable results of genetic similarity and clustering of the genotypes studied. As for the number of markers, it is, of course, important in assessing genetic similarity. It must not be too small. "Small" is a relative term. The most important thing is that the markers should represent the entire genome as far as possible.

Point 3: (2) The selection of the six genetic similarity measures in the study seemed appropriate, taking into account the advantages of the different algorithms. However, the paper is not sufficiently detailed on the reasons for the choice of methods, comparisons between them, and their respective advantages and disadvantages.

Especially when the results of the Rogers method are significantly different from those of other methods, the reasons and their impact on the research results should be explained in detail.

Response: The choice of the six genetic similarity measures in our study was based on the fact that they are the most commonly used in practical research. Five of these methods (in addition to Rogers' method) are based on counting alleles present simultaneously in both genotypes being compared. The Rogers method takes into account the number of alleles present in only one of the compared genotypes, while the other methods are based on the number of alleles present simultaneously in both compared genotypes. This is explained in detail in the Discussion.

Point 4: (3) The article points out that the two genetic similarity measurement methods, Sokal and Michener, and Euclidean, yield the same results. This phenomenon is briefly discussed. The paper should provide more explanation and discussion of why the two results are consistent, and discuss whether this similarity reflects substantial biological information or is just a coincidence of computational methods. If the methods are similar in nature, it is necessary to discuss which method has more advantages in practical applications?

Response: Two methods of measuring genetic similarity, Sokal and Michener and Euclidean, give the same results. We discussed this phenomenon only briefly out of respect for the scientists who introduced the formula, which went down in history with the names Sokal and Michener. The similarity, or in fact identity, with Euclid's method is due to the fact that the aforementioned authors 'adopted' Euclid's method by using a bivalent variable in it: zero and one.

Point 5: (4) UPGMA is a commonly used method in population genetic analysis, but it assumes that the evolutionary rate of all species is constant, which may not always be reasonable. The authors are advised to elaborate on the rationale for choosing this method and discuss the impact that other clustering methods, such as neighborhood similarity or maximum likelihood methods, may have on the study results. If possible, conducting a comparative analysis between these methods may give more confidence to the results.

Response: We have previously performed clustering not only with the UPGMA method, but also with other methods such as maximum likelihood, neighbor-joining, minimum-evolution and maximum parsimony. The results we obtained were very similar, and some were identical. In the manuscript, we chose to present only one clustering method, UPGMA. We have added this information in the revised version of the manuscript.