### Article

**Genetic Diversity of Purple Passion Fruit, *Passiflora edulis* f. *edulis*, Based on Single-Nucleotide Polymorphism Markers Discovered through Genotyping by Sequencing**

Nohra Cecilia Rodríguez Castillo 1,2, Xingbo Wu 3, María Isabel Chacón 4, Luz Marina Melgarejo 1,2 and Matthew Wohlgemuth Blair 3,*

1 Laboratorio de Fisiología y Bioquímica Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, D.C. 111321, Colombia; nohra.rodriguez@uptc.edu.co (N.C.R.C.); lmmelgarejom@unal.edu.co (L.M.M.)
2 Escuela de Biología, Facultad de Ciencias, Grupo de Investigación GEBIMOL, Universidad Pedagógica y Tecnológica de Colombia, Tunja 153610, Boyacá, Colombia
3 Department of Agricultural and Environmental Sciences, College of Agriculture, Tennessee State University, Nashville, TN 37209-1561, USA; xwubbeans@gmail.com
4 Departamento de Agronomía, Facultad Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá, D.C. 111321, Colombia; michacons@unal.edu.co
* Correspondence: mblair@tnstate.edu

**Abstract:** Orphan crops, which include many of the tropical fruit species used in the juice industry, lack genomic resources and breeding efforts. Typical of this dilemma is the lack of commercial cultivars of purple passion fruit, *Passiflora edulis* f. *edulis*, and of information on the genetic resources of its substantial semiwild gene pool. In this study, we develop single-nucleotide polymorphism (SNP) markers for the species and show that the genetic diversity of this fruit crop has been reduced because of selection for cultivated genotypes compared to the semiwild landraces in its center of diversity. A specific objective of the present study was to determine the genetic diversity of cultivars, genebank accession, and landraces through genotyping by sequencing (GBS) and to conduct molecular evaluation of a broad collection for the species *P. edulis* from a source country, Colombia. We included control genotypes of yellow passion fruit, *P. edulis* f. *flavicarpa*. The goal was to evaluate differences between fruit types and compare landraces and genebank accessions from in situ accessions collected from farmers. In total, 3820 SNPs were identified as informative for this diversity study. However, the majority distinguished yellow and purple passion fruit, with 966 SNPs useful in purple passion fruits alone. In the population structure analysis, purple passion fruits were very distinct from the yellow ones. The results for purple passion fruits alone showed reduced diversity for the commercial cultivars while highlighting the higher diversity found among landraces from wild or semi-wild conditions. These landraces had higher heterozygosity, polymorphism, and overall genetic diversity. The implications for genetics and breeding as well as evolution and ecology of purple passion fruits based on the extant landrace diversity are discussed with consideration of manual or pollinator-assisted hybridization of this species.

**Keywords:** GBS technique; genetic diversity; SNP markers; ward-MLM; population structure

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### 1. Introduction

Passion fruits are a juice source that are highly sought after for exotic tropical drinks [1]. Belonging to a medium-sized family of plants, the Passifloraceae, passion fruits are native to the New World and reach their highest diversity in the Brazilian/Peruvian Amazon lowlands and into the eastern slopes of the Andes mountains of South America [2]. The most commercial species of passion fruit is *P. edulis*, which has two subforms: a purple-fruited type, *P. edulis* f. *edulis* (purple passion fruit, known as gulupa in Colombia), and a yellow-fruited type, *P. edulis* f. *flavicarpa* (yellow passion fruit, known as maracuja/maracuya...
in Brazilian Portuguese- and Spanish-speaking countries, respectively). These two subspecies make up the bulk of commercial trade and export production of passion fruits, both in Africa and Latin America where they are native. Despite this importance, no whole genome-based molecular marker studies have been carried out on the species.

Both the purple and yellow passion fruit subspecies of *P. edulis* are acidic and mostly used for juice extraction [2]. In contrast, other tropical passion fruit species are eaten directly as fresh fruit, including badea (*Passiflora quadrangularis*) and granadilla (*Passiflora ligularis*). The curuba (*Passiflora tripartita var. mollissima*) is another juice species from the Andes mountains. Additional semiwild *Passiflora* species of passion fruits in South America and the Caribbean include tumbos water lemon (*Passiflora laurifolia*), sweet calabash (*Passiflora maliformis*), and wild turmeric (*Passiflora turbinata*). Various *Passiflora* species are found in Venezuela, where they are known as taxo or parcha, as well as in Bolivia and Peru, where they are also called trompos or tintin [3]. None of these species have had the export market success of purple and yellow passion fruit because of their perishability, and these species are consumed in the local markets [2]. Most passion fruit species have beautiful flowers with colors ranging from red to blue and purple, making them desirable ornaments in tropical and subtropical gardens [3]. Many species of the family have spread around the world, often through botanical gardens and plant collectors but also through multinational companies interested in the fruit potential of the genus. Nevertheless, *P. edulis* remains the top cultigen.

The South American country of Colombia is one of the main centers of diversification of the *Passiflora* genus and particularly of the purple passion fruit species, *P. edulis f. edulis* [5]. The plant grows wild in many parts of the country and is especially prevalent as landraces at mid-elevation altitudes of the Andes Mountains [6,7]. Purple passion fruits are viny plants often found in forest margins or cacao and coffee plantations [8]. The fruit was first grown as a crop in monocultures starting around 1990 using trellises to support the vines [9]. Given that plants were selected from just a few wild populations and landraces and have multiplied through very few nurseries during that time, the purple passion fruit in Colombia has a narrow genetic base [10]. Studies on the genetic diversity of purple passion fruits, even with highly polymorphic simple sequence repeat (SSR) or microsatellite markers [11] and inter simple sequence repeat (ISSR) patterns [12], have all found low levels of variability in collections of commercial cultivars of the species *P. edulis* [10]. Most of the diversity for passion fruits is believed to be in the wild or in situ on farms, with only small germplasm collections of limited diversity held in the national germplasm seed banks of Andean countries or by commercial companies [13]. As a perennial vine that flowers sparsely and interruptedly in wild accessions and has slow-germinating recalcitrant seed, the purple passion fruit is hard to multiply by seed. Nonsynchronous flowering between plants and synchronous flowering along a vine encourages population inbreeding, although heterozygosity is common. Passion fruits are amenable to clonal propagation as their vines can root themselves with some species producing runners, but genotype x environment (GxE) interactions are common.

Single-nucleotide polymorphism (SNP) markers are the most abundant form of DNA polymorphism found in plants and other higher organisms [14]. Therefore, this marker type would be a useful method to evaluate diversity in passion fruits. Few genomic resources exist for passion fruits, and whole-genome sequencing (WGS) is incipient [15]. As a result, SNP markers are not widely available for purple passion fruits but would offer valuable information of high specificity and quality for crop improvement. Related to this, genotyping by sequencing (GBS) is considered an efficient method of SNP discovery used to identify inter- or intraspecific genetic diversity at multiple loci as well as for fingerprinting cultivars and varieties [14].

The GBS technique has not been undertaken for *Passiflora*. Although the method can be carried out with or without a reference genome, it usually results in fewer SNP markers
when undertaken without sequence alignment. The reliability of the loci discovered by GBS remains high in nonreference and reference genome-based GBS evaluations. Therefore, GBS as a modern sequencing method is useful, efficient, and rapid to develop DNA-based markers while simultaneously characterizing the population structure and diversity for any orphan or nonorphan crop [14].

Our overall objective in this work was to use GBS to evaluate and compare the diversity of purple passion fruit accessions from three sources: (a) commercial cultivars, (b) genebank accessions, and (c) landraces collected for this work. We generated the first SNPs based on GBS technology and then used these loci for diversity analysis. One of the main interests in having a wide genetic diversity of purple passion fruits is the possibility of generating new varieties (single plant selections or populations) that present suitable innate characteristics (fruit color and quality) or resistance to biotic factors (pests and diseases) [16] as well as good performance in a variety of environmental conditions. Loss of diversity evidently reduces the capacity for adaptive response to biotic and abiotic stresses. Another problem of purple passion fruits is slow seed multiplication, which results in only a few varieties being selected for nursery stock. This low diversity puts the crop at risk of a range of diseases and insects or climate fluctuations. Indeed, cultivation of *P. edulis f. edulis* has declined in Colombia and other tropical countries, especially due to root rot and wilt diseases, as farmers find monoculture of purple passion fruits to be uneconomical to maintain. Excessive use of pesticides to control important pathogens, such as *Fusarium* spp., is common. Therefore, new collections and their analysis with molecular tools, such as GBS performed in this study, are useful to expand the diversity within breeding programs for *P. edulis f. edulis*, the purple passion fruit, as well as related species. While the yellow passion fruit has been better studied, our molecular research is one of the first to apply GBS, or for that matter next-generation sequencing and modern genomics, to this minor or orphan crop species.

2. Materials and Methods

2.1. Plant Materials

A total of 97 genotypes of passion fruit (*P. edulis*) were used in this study, consisting of 92 purple passion fruits (*f. edulis*) and 5 yellow passion fruit controls (*f. flavicarpa*). Although all genotypes were used in DNA extraction, only 88 of the 92 purple passion fruits provided sufficiently high-quality nucleic acid for GBS study. The purple passion fruits were divided into (a) landraces, (b) commercial cultivars, and (c) genebank accessions from all over Colombia, while the yellow passion fruits were samples collected from farmers from the Putumayo department in southern Colombia (Supplemental Table S1). All yellow passion fruits produced good DNA. The collection of landraces collected in situ has been described in [17] and was based on single plants collected for germplasm conservation by the Department of Biology at the Universidad Nacional de Colombia, Bogota campus. Purple passion fruit landraces were from farmlands throughout the Colombian Andean region in the departments of Antioquia, Boyacá, Caldas, Cauca, Cundinamarca, Huila, Nariño, Putumayo, Quindío, Risaralda, Santander, Norte de Santander, and Tolima, providing a total of 56 accessions. The landraces were collected growing semiwild on roadsides and in longstanding farmer “orchards”, especially where purple passion fruits grow next to and often over other farm products, such as coffee and citrus. The landraces were compared to a total of 22 commercially cultivated purple-fruited cultivars from farms in Cundinamarca and Boyacá, and the entire group of collected genotypes were named for their status as accessions from the Biology Department of Universidad Nacional (cod: BUN). In addition, 14 accessions from the national germplasm bank of AGROSAVIA, La Selva Experiment Station in Rionegro, Antioquia, were used in the study and denominated “genebank” accessions.
2.2. DNA Extraction and Quality Check

In this study, each accession was represented by an individual seedling. To obtain the seedlings, seeds of the P. edulis accessions were treated with 20% \( \text{H}_2\text{SO}_4 \) for 15 min, then washed twice with water, and placed to germinate in sterile soil under greenhouse conditions (18 °C, RH 70%, photoperiod 12 h/12 h) and with optimum conditions of fertilization and humidity. Subsequently, DNA extraction was performed for each plant according to the protocol of [18]. Briefly, 100 mg of fresh leaf tissue was ground in liquid nitrogen with a hand mortar and pestle and then extracted with a modified Dellaporta technique using CTAB (Cetyl Trimethyl Ammonium Bromide) as an extraction buffer. The DNA quality was evaluated in a 1% agarose gel by comparing plant DNA bands to a lambda (\( \lambda \)) phage gradient at three different total concentrations of 25, 50, and 100 ng and confirmed by a NanoDrop®ND-1000 (Thermo-Fisher, Waltham, MA, USA) using fluorescence emission. Digestibility of DNA was tested by restriction with the enzyme \( \text{HindIII} \) (Promega, Madison, WI, USA) and visualization on a 1% agarose gel with ethidium bromide (Sigma Aldrich, St. Louis, MO, USA). Poor-quality DNA from four genotypes (BUN25, 43, 61, and 76) were not used.

2.3. Genotyping by Sequencing

After DNA evaluation, DNA extractions with the best quality values based on absorbance (A) readings at 260/280 nm wavelengths ratios of >1.8 were adjusted to a genomic DNA concentration of 100 ng/\( \text{uL} \) for each genotype, and 20 \( \mu \text{L} \) was aliquoted for the GBS experiment. Subsequently, the DNAs of the genotypes were sent lyophilized to the Elshire Group Limited in New Zealand. GBS library preparation, DNA fragment selection, and sequencing were according to the method proposed by [13] using the restriction enzyme \( \text{PstI} \) (Promega) for digestion of DNAs and 1.44 ng of adapters per reaction. An Illumina 1.9 sequencing system (Illumina Inc., San Diego, CA, USA) was used to sequence fragments at both ends of each molecule (double reads).

2.4. Demultiplexing and SNP Calling

Because purple passion fruits do not yet have a reference genome, the alignment of sequences was made de novo. For this, DNA sequences of each genotype were evaluated looking for sites that were polymorphic and informative using the STACKS program (http://creskolab.uoregon.edu/stacks/ (accessed on 30 March 2020)). The reads were demultiplexed and cleaned by means of the software subprogram tool “process_radtags”. Read quality was checked with FastQC from the Illumina bioinformatics website (https://www.illumina.com (accessed on 30 March 2020)). After this, data of all genotypes were grouped, and polymorphic SNP sites were identified using tools “ustacks” and “pstacks” for alignment and misalignment, respectively. The loci that were of high quality whose sequences had no discrepancies were grouped, and the catalogs were constructed using the final tool called “cstacks”. All these are part of the denovo.pl package.

The polymorphic loci within these first quality sequences for each accession were linked between the catalogs to determine the allelic status of each locus per accession with “sstacks” according to [19]. The cataloged sequences were cut to a length of 80 bp using the t parameter in “process_radtags” as recommended by [20]. The number of GC cut sites was estimated, and the data was visualized crossing the sample populations using the “stacks populations” module. Subsequently, the following parameters were used to determine the total number of SNPs: \( m = 3; \ m = 2; \ n = 1; \ -p = 10; \ -r = 0.8; \ -\text{min} \_\text{maf} = 0.05 \) as recommended by [19]. The number of transversions and transitions was determined, and the SNPs were then arranged in a .vcf file for further analysis.

2.5. Data Analysis

After sequencing and SNP calls, observed heterozygosity, expected heterozygosity, and polymorphism information content (PIC) were evaluated for each marker discovered in the GBS experiment according to [21] using PowerMarker software [22]. The Dice coeffi-
cient of similarity for each pair of genotypes and the overall genetic diversity according to Weir (1996) were determined with the same program. The genetic structure of the purple passion fruit accessions was established through the program STRUCTURE Version 2.3.4 [23]. In this software, we determined the optimal subpopulation number and grouped each genotype without a priori group assignment into the most appropriate subpopulation using an admixture model assuming correlated allele frequencies and using 20,000 iterations for burn-in and 20,000 Markov chain Monte Carlo (MCMC) repetitions. We performed structural analysis twice, once for the entire set of purple and yellow passion fruits and one with only purple passion fruits. The best K values were determined separately for the two sets using the ΔK method, and the results of the Evanno test were graphed on XY plots. Membership coefficients for individuals were calculated and visualized using DISTRUCT [24] for K = 2 for the full set of genotypes, for K = 3 for just the purple passion fruits, and for K = 4 for the combination of the two forms. Genotype relationships were then graphed using a neighbor-joining (NJ) tree, generated with DarWin software [25]. Finally, a multidimensional scaling analysis (MDS) was performed with RWizard [26] to see the distribution of the accessions in a two-dimensional plot.

3. Results

3.1. Success of the GBS Technique in Purple Passion Fruit

The quality of the DNA was sufficient for the GBS technique to succeed for the purple passion fruit genotypes, and a total of 456,505,522 genome-based amplicons were sequenced in both the forward and reverse directions. Sequence length in base pairs (bp) ranged between 129 and 150 bp for forward sequences and between 35 and 150 bp for reverse sequences (Figure S1). The forward sequences presented more high-quality bases (31 to 40%) than the reverse sequences (12 to 40%). No significant variations were found for GC content between forward (47%) and reverse (46%) sequencing. For the full set of passion fruit (P. edulis) entries, 29,758 SNP loci were identified, and the sequences were uploaded to the NCBI database under SRA entry PRJNA699284.

When stacking was performed across aligned sequences, a total of 3820 verified SNP loci were found. These SNPs were 67% transitions and 33% transversions. C/T transitions were slightly more frequent than A/G transitions, while the four types of transversions were very equal in their frequency (Table 1). Because paired end reads were used for GBS sequencing, the informative SNPs were further reduced on a per fragment basis to a total of 1706 loci, each one on a separate sequence contig.

Table 1. Number of single-nucleotide polymorphism (SNPs) and frequency of transitions and transversions present in the passion fruit (P. edulis f. edulis and f. flavicarpa) in this study.

<table>
<thead>
<tr>
<th>SNP Type</th>
<th>Number of SNPs</th>
<th>Frequency (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transition (subtotal)</td>
<td>2568</td>
<td>67.23</td>
</tr>
<tr>
<td>A/G</td>
<td>1210</td>
<td>31.68</td>
</tr>
<tr>
<td>C/T</td>
<td>1358</td>
<td>35.55</td>
</tr>
<tr>
<td>Transversions (subtotal)</td>
<td>1252</td>
<td>32.77</td>
</tr>
<tr>
<td>A/C</td>
<td>294</td>
<td>7.70</td>
</tr>
<tr>
<td>A/T</td>
<td>368</td>
<td>9.63</td>
</tr>
<tr>
<td>C/G</td>
<td>278</td>
<td>7.28</td>
</tr>
<tr>
<td>T/G</td>
<td>312</td>
<td>8.17</td>
</tr>
<tr>
<td>Grand total SNP verified</td>
<td>3820</td>
<td>100.00</td>
</tr>
<tr>
<td>Number of independent loci</td>
<td>1706</td>
<td>44.65</td>
</tr>
<tr>
<td>Number of polymorphic loci in P. edulis</td>
<td>966</td>
<td>25.28</td>
</tr>
</tbody>
</table>

* As a percentage of the total SNPs identified.

Out of the total number of SNPs found for the purple and yellow passion fruit comparisons, a subtotal of 966 were identified as informative and polymorphic for the P. edulis f. edulis accessions evaluated. For these SNPs, the average expected heterozygosity across the purple passion fruits was 0.47, and the average polymorphism information
content (PIC) was 0.42. In terms of SNP numbers, 437 loci had genetic diversity ≥0.5 and 347 had PIC ≥0.5. Average observed heterozygosity was 0.26. Only 140 SNPs had an observed heterozygosity greater than 0.5. The expected and observed heterozygosity as well as PIC value were significantly higher in semiwild landrace genotypes than in commercial cultivars or genebank accessions (Table 2).

Table 2. Average expected heterozygosity and polymorphism information content (PIC) values as well as average observed heterozygosity for the accessions of purple passion fruit \((P. edulis\ f. edulis)\), grouped according to their origin.

<table>
<thead>
<tr>
<th>Type of Accession</th>
<th>Exp. Het.</th>
<th>Obs. Het.</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landrace</td>
<td>0.78 ± 0.54 a</td>
<td>0.45 ± 0.53 a</td>
<td>0.75 ± 0.12 a</td>
</tr>
<tr>
<td>Commercial cultivar</td>
<td>0.41 ± 0.56 b</td>
<td>0.18 ± 0.48 b</td>
<td>0.38 ± 0.95 b</td>
</tr>
<tr>
<td>Genebank entry</td>
<td>0.43 ± 0.47 b</td>
<td>0.22 ± 0.87 b</td>
<td>0.41 ± 0.87 b</td>
</tr>
</tbody>
</table>

Superscripts indicate \(p < 0.05\) significant differences between groups based on Tukey’s test. Different letter subscripts represent results of Tukey’s range test grouping.

3.2. Population Structure of Purple and Yellow Passion Fruit

The population structure of the purple passion fruit accessions was established with the selected sets of SNPs for the entire set of genotypes, including the yellow passion fruit outgroup (Figure 1a), and for the purple passion fruit genotypes alone (Figure 1b). The most likely number of subpopulations was found to be \(K = 2\) for the full set of \(P. edulis\) and \(K = 3\) for the subset of only purple passion fruit. In the full analysis, all \(f. flavicarpa\) were separated from all \(f. edulis\). In the purple passion fruit analysis, admixture was highly evident among all genotypes.

![Figure 1](image_url)

Figure 1. Analysis of (a) population structure for all passion fruit \((P. edulis)\) accessions, including purple \((f. edulis)\) and yellow \((f. flavicarpa)\) genotypes, compared to (b) population structure for only purple passion fruits \((P. edulis\ f. edulis)\). In each vertical panel, the Evanno test for most likely number of subpopulations or Delta K value is shown above, and the structure analyses at various K values are shown below. Each color within the subpopulations represents Q value genomic admixture.
Meanwhile, no admixture was observable between the two forms of passion fruits: purple (f. edulis) and yellow (f. flavicarpa). At K = 4, there was possible evidence of introgression between the two types of P. edulis, but this was only evident with the multiple SNPs per fragment discarded. It was notable that landraces were mixed with genebank accessions and commercial cultivars in the structure analysis, indicating the reduced diversity for purple passion fruits. Even landraces from the mid-elevation sites, such as in Quindío, were genetically similar to those from highland sites in Boyacá, Cundinamarca, and Nariño.

Further analysis was done with Darwin’s NJ analysis (Figure 2), which showed the formation of highly separated groups in the comparison of the two passion fruit types: purple (P. edulis f. edulis) and yellow (P. edulis f. flavicarpa). This can be clearly seen in the left-hand panel of the figure (Figure 2a), where the f. flavicarpa genotypes are highly distributed compared to the f. edulis genotypes. The diversity was much higher in the yellow passion fruits than in the purple passion fruits. We concentrated on purple passion fruit with a second NJ analysis using only f. edulis genotypes, as shown in the right-hand panel of the figure (Figure 2b).

Figure 2. Dendogram of relationships in a neighbor-joining analysis between accessions of (a) passion fruit (P. edulis) showing division between purple passion fruit (f. edulis) and yellow passion fruit (f. flavicarpa) types and (b) only purple passion fruits (P. edulis f. edulis). The latter genotypes are color coded to represent landraces in blue circles, commercial cultivars in red squares, and genebank accessions in green triangles.

Within the purple passion fruit, we saw multiple but less well-defined groups with just the genotypes of P. edulis f. edulis. Four diffuse groups could be observed and were distinguished by the types of genotypes contained within them. One of the purple passion fruit groups was in the upper part consisting mainly of landraces (86%) and a few
cultivars (14%). A slightly differentiated group in the lower region comprised genebank and cultivated accessions and only one landrace. Two intermediate groups had mixtures of landraces (BUN40, BUN46, BUN47, BUN48, and BUN59) and commercial cultivars (BUN63 and BUN64). Of these, BUN48 and BUN59 landraces shared the same node and coincided with the same geographical origin in the mid-elevation site mentioned above in the department of Quindío.

3.3. Multidimensional Scaling Analysis

To confirm the structure of only the purple passion fruit genotypes, a MDS analysis was performed (Figure 3) using the Kruskal stress criterion as an adjustment to find the average deviation between distances [27]. In this case, the greater the stress, the lower the adjustment [28]. For all *P. edulis* f. *edulis* and the 966 SNPs selected as most informative, a mean adjustment stress value of 0.16 was obtained. Therefore, the MDS explained 16% of the population structure for purple passion fruits. Four groups were found and are shown by large concentric containing separate symbols for each group.

![Multidimensional scaling of four groups proposed by population structure analysis for genotypes of purple passion fruit](image)

**Figure 3.** Multidimensional scaling of four groups proposed by population structure analysis for genotypes of purple passion fruit (*P. edulis* f. *edulis*) based on 966 SNP markers generated through genotyping by sequencing (GBS). Group 1 in red, 2 in light green, 3 in dark green, and 4 in purple. The central trend is represented by a blue line. The major SNPs are highlighted with a lowercase “s” followed by a number. The central tendency is represented by a dark blue line and distance from the centroid spot on the line.

Several observations from the MDS analysis provided new information. Group 1 was mostly made up of landraces and some cultivars and was the most different from others, which meant that within the accessions of this group, there were a certain number of characteristics distinguishing SNPs. Groups 2 and 3 had the lowest number of accessions and were mainly represented by landraces. Group 4 was mainly made up of commercial cultivars and genebank accessions but was not spatially differentiated in a significant way from Groups 1, 2, and 3. For purple passion fruits, this provided evidence of more diversity in the in situ collected landraces than in commercial cultivars and the small number of genebank accessions found in ex situ collection available to date. Notably, some SNPs were more informative for the MDS analysis.

4. Discussion

The lack of genomic resources, such as SNPs, for the species *P. edulis* or its Passifloraceae relatives is a serious limitation to marker studies in these crops. We found the
GBS technique to be useful for the study of purple and yellow passion fruit genetic diversity, as has been found recently for some fruit species [29] and previously for many row crops [30]. Comparing *P. edulis* f. *edulis* and f. *flavicarpa*, we identified 3820 high-quality SNPs. However, only one third of these SNPs were highly informative in the evaluation of the genetic diversity of purple passion fruits (*f. edulis*). These were valuable as they were on single contigs and amenable to SNP assay design. The relatively low heterozygosity found for our markers in purple passion fruits agrees with studies that have used single-copy SSR markers [11,31,32] as well as multicopy markers. Low diversity estimates have been reported for RAPD [33] and AFLP [34,35] markers or internal transcribed spacer region sequencing [36]. Meanwhile, ISSR markers have been more polymorphic for purple passion fruits, indicating that the genome probably has many inverted repeats [37]. Organellar DNA fingerprinting also shows low diversity [38]. Overall, diversity estimates for *P. edulis* f. *edulis* were lower than for yellow passion fruits or other *Passiflora* species [5,39]. To date, no reference genome is available within the family or for *P. edulis* itself, perhaps because of its large 3 Gb plus genome [40,41], making our study very relevant. Low-pass sequencing of passion fruit accessions has been conducted for microsatellite discovery [11] but not SNPs like in this study.

Our results with SNP markers derived from GBS analysis have some implication on the mating system and the resulting breeding of purple passion fruits. The low observed heterozygosity we found with GBS in purple passion fruits may indicate high levels of self-pollination. The species *P. edulis* as a whole is cataloged as being an outcrossing species [9]; however, more studies are needed in pollination behavior in purple passion fruits. High level of cross-pollination and low tolerance for autogamy is more typical of yellow passion fruits (*P. edulis* f. *flavicarpa*) than of purple passion fruits (*P. edulis* f. *edulis*), which tolerates a much higher level of inbreeding [14]. This suggests that while yellow passion fruits are mainly allogamous and have higher heterozygosity, it is likely that self-fertilization and autogamy has been favored in purple passion fruits, resulting in lower heterozygosity. The result of self-pollination might be a narrowing of genetic diversity in purple passion fruits, especially those that are selected for cultivation, as we observed for both expected and observed heterozygosity of cultivars compared to landraces. Purple passion fruits in the mid-altitude valleys of northern South America may have also suffered from isolation, with distance spurring on the selection for self-compatibility and self-pollination. In this regard, purple passion fruits may show evidence of a genetic bottleneck during domestication or adaptive pressure for highland production in the Andes Mountains compared to the Amazon and surrounding foothills and lowlands where the distinct yellow passion fruit is grown [8]. A distinct difference might have been the natural pollinators available to purple passion fruits growing in higher altitudes compared to yellow passion fruits growing in lower elevations. Pollinators would be less abundant in cooler climes further up the mountains, making self-pollination more likely [39]. The reduced need for large populations of pollinating species was proposed by [40] as a distinguishing feature of highland purple passion fruits compared to lowland yellow passion fruits.

For pollination, various authors [41–43] have determined that large bees of the genus *Epicharis* sp. have greater efficiency in fertilizing purple passion fruits than the second most common insect pollinators *Xylocopa* sp. and *Apis mellifera*. However, the pollinator populations change regionally and can vary from one area to another [44]. Overall, pollinator populations have been on the decline due to natural processes of inter and intraspecific competition as well as anthropogenic phenomena, such as the use of agrochemicals leading to pesticide residues, as well as forest loss, reduction in perennial cropping, habitat destruction, and climate change [14]. Indeed, given a reduction in the population density of pollinating insects in some crops, artificial pollination has been utilized to increase yield [45]. Correspondingly, a decrease in productivity for bee-dependent crops, such as sunflowers, has led plant breeders to select for genotypes with higher rates of self-fertilization [46], allowing the creation of inbreeding crops with implications for exploiting hybrid vigor. Passion fruit seed supply in most tropical countries and even in the US state...
of Florida have been generated from a few nurseries, which narrows the genetic base of the cultivars in use.

Although only a hypothesis, the possibility of increasing outcrossing rates in purple passion fruits would be interesting for creating hybrids in the crop. Positioning of accessions into separate genetic groups might allow for the exploitation of heterozygosity. With an open flower structure, the position of the style above the stamens, and an abundance of anthers and resulting pollen, passion fruits could be easy to manually cross. Using controlled crosses or self-incompatibility if found, the resulting seed could be sold as hybrids for the establishment of fruit orchards. Selected phenotypic and genetic markers from this study or others could also be used to ensure that seeds do indeed result from the intended cross-hybridization and not from self-fertilization. Many descriptor traits have been evaluated in purple passion fruits that could serve as these stable markers [7,17]. SSR and SNP markers are codominant for F1 detection [11].

The high-value nature of this fruit crop, their high biomass and fruit yield per plant, and the semiperennial nature of passion fruit vines [6] would make them good candidates to turn into hybrid crop plants if the tendency to self-pollinate can be overcome. Seeds could be specially produced to retain valuable traits from each parent, most of which are currently highly homozygous, thus exploiting the specific and general combining ability of crosses and heterosis if observed. However, further studies are needed for determining heterotic groups and the best combinations for higher fruit production and stress tolerance. For breeding, our diversity evaluation showed the number of alleles was higher in landraces than among cultivars. Further genomic studies are also needed.

The high similarity between some cultivars and landraces shows that commercial varieties have been derived from in situ variability but do not represent all the diversity within the landraces. In turn, similarity between some cultivars and some landraces may show that some of these correspond to cultivar escapes. Genebank accessions represented the least diversity among the three groups, which corroborates the homogeneity in these genotypes. Therefore, the greatest genetic gain would be from further exploitation of in situ diversity rather than relying on current commercial types or ex situ collections for crosses. This conclusion highlights the need for further collection and preservation of germplasm beyond the purple passion fruit genotypes we describe here.

Use of landraces in breeding would allow for increased diversity, heterozygosity, and potentially heterosis for development of purple passion fruits as a higher yielding crop as it implies an increase in variability. Purple passion fruits must rely on their own genetic base as they are very distinct from yellow passion fruits. Finally, few purple passion fruit breeding programs exist and are limited by a lack of diversity; therefore, more germplasm collection should be undertaken. Some work has been carried out successfully in yellow passion fruits for improved response to pests and diseases [33], so similar work in purple passion fruits would be desirable.

Therefore, this molecular study is very important for the improvement of this orphan fruit crop species that is of great importance in international and local juice markets. A final observation from our study was the utility of low-coverage sequencing in the GBS experiments for the discovery of SNP markers, which could be useful in the breeding of purple passion fruits. Among the many SNP loci we found, many could be converted into single-copy markers, such as single-base extension (e.g., Kompetitive Allele Specific PCR (KASP) and Taqman) assays or elements of bead arrays (e.g., Illumina). A recent study of expressed sequence tags (ESTs) has provided a transcriptome [15] that would be useful for gene annotation of the GBS results we obtained.

Despite these efforts, there are still very few single-copy markers for the purple passion fruit or its more important cousin, the yellow passion fruit. SSR containing loci were shown by [11,33,34] to be useful in distinguishing between various Passiflora species as should our SNP markers. In our laboratory, we are conducting preliminary analysis of marker x trait associations to identify the SNPs that would be most important for tagging agronomic, eco-physiological, or morphological traits from analyses of a set of genotypes.
under multienvironment tests [6,7]. In conclusion, a new era of genomic breeding using SNP markers for cultivar development in purple passion fruits should be possible because of our current study.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13040144/s1, Figure S1: Raw data of paired-end (PE) sequence lengths (Forward and Reverse) found in Genotyping by Sequencing (GBS) of passion fruit (P. edulis) samples used in this study. Table S1: Accessions of Passiflora edulis forms “edulis” and “flavicarpa” collected in different departments of the Colombian Andean region, geographical characteristics of collection, origin and the group to which they were assigned according to the present study.

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