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Recurrent genome recovery in backcross breeding programs of *Passiflora edulis* Sims based on SNP DArTseq sequencing

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ABSTRACT

The backcross method in *Passiflora edulis* Sims is used for genetic improvement with the aim of transferring disease-resistance from wild to commercial species. Recurrent genome recovery and the characterization of hybrids and potential parents with desirable traits are essential for the design of intra and interspecific crosses. In the present study, recurrent genome recovery and genetic variability of three genealogies comprising hybrids and wild species of *Passiflora* were evaluated. Eighty-four genotypes were evaluated using 3.717 biallelic codominant SNP markers generated through DArTseq (NGS) sequencing. To characterize the structure of the genotype panel, the three genealogies were independently analyzed by calculating the genetic distance and by hierarchical clustering analysis (UPGMA) principal coordinate analysis (PCoA), and intersection analysis (UpSetR). The genetic availability of SNPs was evaluated using Polymorphism Information Content (PIC), one ratio proportion, Minor Allele Frequency (MAF), and reproducibility with the adegenet package (R software). The results identified well-defined similarity groups between hybrids and parents, with a clear trend of clustering of accessions of the same species and of backcrossed genotypes closer to the recurrent parent. The present study emphasizes the efficiency of backcrossing to recover recurrent genome and the preservation of genetic variability to improve the sour passion fruit.

Keywords: diversity matrix technology, molecular markers, next-generation sequencing, passion fruit, plant breeding.

INTRODUCTION

The sour passion fruit (*Passiflora edulis* Sims) holds significant economic and social importance in Brazil, accounting for more than 90% of the country's passion fruit orchards due to its high productivity, pulp yield, and nutritional benefits.[1-5] Brazil is the world's leading producer and consumer[3] with a national output of 736.585 tons in 2024.[6] Despite establishing a strong production chain since the 1970s, most of the output is destined for the domestic market, meaning that export markets have remained incipient over the decades.[3,7,8] The development of new adaptable cultivars has been a priority in passion fruit breeding research.[1,2,9,10] Embrapa and its partners have already released hybrids, such as BRS Gigante Amarelo and BRS Sol do Cerrado,[11] which can be used as sources of genetic variability for other breeding programs.[12]

Commercial breeding programs mostly focus on *P. edulis*. However, wild *Passiflora* relatives are an essential source of genetic diversity for crop improvement. To use this diversity and create fertile hybrids,[13] breeders employ recurrent selection. This approach increases favorable genes and boosts hybrid vigor[7,14] through continuous cycles of evaluation, selection, and recombination.[12,15,16] Backcrossing is also used as a targeted method to transfer specific genes from wild species into elite *P. edulis* lines. It focuses on key traits like disease resistance, self-compatibility, orange pulp, and photoperiod insensitivity.[17] For example, the North American *P. incarnata* L. provides tolerance to environmental stress and broad disease resistance. Similarly, wild relatives like *P. caerulea* L. and *P. amethystina* J.C. Mikan offer self-compatibility and resistance against bacteriosis and anthracnose.[18] In addition, Brazilian native species like *P. quadrifaria* Vanderpl. and *P. hatschbachii* Cervi act as donors of other valuable agronomic traits. By incorporating these wild genomes, breeders can expand the genetic base of cultivated passion fruit and develop superior, resilient cultivars.

The analysis of recurrent genome recovery and the characterization of hybrids and parents are essential for the planning of breeding programs. Molecular markers confirm hybridization and quantify recurrent genome recovery in backcrosses. Selecting individuals genetically closer to the parent accelerates genomic recovery and development of new cultivars. DNA analysis is a fast and reliable technology that allows the early confirmation of hybridization.[19] The goal in this present study was to analyze the recurrent genome recovery and genetic variability of *P. edulis* multispecific hybrids and their receptive parents used in the sour passion fruit breeding program performed by Embrapa and its partners based on SNP-DArT.

MATERIAL AND METHODS

Selection of accessions

Eighty-four accessions of species and inter and intra-specific crosses of the genus *Passiflora* spp kept at the Active Germplasm Bank of Passionfruit (Banco Ativo de Germoplasma de *Passifloras*) at Embrapa Cerrados were selected for SNP DArTseq sequencing (Table 1).

The number of accessions varied across the evaluated groups. However, this variation reflects the real composition and selection stages of the Embrapa breeding program. Unequal group sizes are naturally expected because parental lines, commercial cultivars, and wild donors play different roles. To prevent bias from this unbalanced sampling, we analyzed each genealogy independently using complementary methods (UPGMA, PCoA, and UpSetR). These approaches focus on genetic relationships and population structure rather than group size. As a result, they provide a reliable assessment of both recurrent genome recovery and genetic diversity.

Genetic Material

In order to obtain genomic DNA, leaf samples from *Passiflora* were collected at Unidade de Apoio à Fruticultura da Embrapa Cerrados, in Planaltina, Federal District, Brazil. A total of 84 accessions was selected for genotyping and delivered to DArT Pty® in Yarralumla, Australia (diversityarrays.com). Three genealogies were analyzed: genealogy A - {[BRS Minimaracujá Roxo (roxo típica) × BRS Sol do Cerrado (*P. edulis* flavicarpa: commercial sour cultivated today) × BRS Sol do Cerrado] × VAO RC4 (*P. caerulea* x *P. edulis* "flavicarpa")}; genealogy B - : RJB [BRS mini-roxo (*Passiflora edulis* Sims "roxo típica")], GA (Matriz da BRS Gigante Amarelo) and LD4 (Matriz da cultivar BRS Sol do Cerrado); genealogy C - ML1 (*P. edulis* Sims "flavicarpa"x *P. amethystina* "macrocarpa") and PL5 {[(*P. hatschbachii* x *P. quadrifaria*) x *P. incarnata*] x *P. edulis*, with their respective crossings and probable parents (Table 1).

Genotyping by sequencing DArTSeq platform

Leaf samples from 84 *Passiflora* accessions were collected from the Active Germplasm Bank at Embrapa Cerrados, in Planaltina, Federal District, Brazil, for

Table 1. Accessions of species and inter and intra-specific crosses of the genus *Passiflora* spp kept at the Active Germplasm Bank of Passionfruit (Banco Ativo de Germoplasma de *Passifloras*) at Embrapa Cerrados selected for SNP DArTseq sequencing for clustering analysis. Embrapa Cerrados/ UnB, Brasília, Federal District, 2025

Group	Pedigree / Description	Role in the breeding program	No. of accessions
RV0	{[(PL5 (*Passiflora incarnata* L.) × VAO)] × VAO}	Advanced backcross line developed to recover the recurrent *P. edulis* genome while introgressing favorable alleles from *P. incarnata*.	1
BRCE	Rubi do Cerrado (*P. edulis* × *P. alata*) × GA matrix	Commercial breeding line combining elite fruit quality and agronomic performance.	1
RLC	[RC1 AC1 (RJB × *P. edulis*) × LD4 × RC1 AC]	Advanced backcross population selected for recurrent genome recovery and self-compatibility.	2
RL	[RC1 AC1 (RJB × *P. edulis*) × LD4 × RC1 AIC]	Advanced backcross population for recurrent genome recovery.	44
RM	{[(BRS Mini Roxo × BRS Sol do Cerrado) × BRS Sol do Cerrado] × VAO} AC	Advanced backcross population selected for self-compatibility and recurrent genome recovery.	3
RR0	{[(BRS Mini Roxo × BRS Sol do Cerrado) × BRS Sol do Cerrado] × VAO} AIC	Advanced backcross population selected for self-incompatibility and recurrent genome recovery.	6
RI	{ML-1 (*P. edulis* × *P. amethystina*) × PL-5 [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis*}	Interspecific breeding population developed for introgression of favorable alleles from multiple wild species.	15
RJB	BRS Mini Roxo (*Passiflora edulis* "roxo típica")	Elite recurrent parental cultivar used as a breeding reference and donor of agronomic traits.	1
HVAP	BRS Polpa Forte VAO [(RC5 (*P. caerulea* × *P. edulis*)) × 325 (RC4 (*P. caerulea* × *P. edulis*))]	Advanced pre-release breeding line combining recurrent genome recovery, self-compatibility and disease resistance.	1
BSCE	BRS Sol do Cerrado (*Passiflora edulis*)	Commercial recurrent parent used as a breeding reference.	1
BGA	BRS Gigante Amarelo (*Passiflora edulis*)	Commercial recurrent parent used as a breeding reference.	2
BFOR	BRS Polpa Forte matrix (*P. caerulea* × *P. edulis*) RC4	Interspecific donor parent used as a source of self-compatibility and disease resistance.	1
PAME	*Passiflora amethystina*	Wild donor species providing genetic variability and favorable alleles for breeding.	1
PEDM	*Passiflora edulis* ML1	Recurrent parental genotype used as a reference for genome recovery analyses.	1
PHAT	*Passiflora hatschbachii* Cervi	Brazilian wild donor species used for introgression of favorable agronomic traits and disease resistance.	1
PINC	*Passiflora incarnata* L.	North American wild donor species used as a source of disease resistance and abiotic stress tolerance.	1
PQDR	*Passiflora quadrifaria* R. Vanderpl.	Brazilian wild donor species contributing genetic diversity and favorable agronomic alleles.	1
RVI	PL-5 [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*]	Wild interspecific hybrid serving as a donor of favorable alleles from multiple wild species.	1
HRJB	RJB (*P. edulis* "roxo-típica") × "flavicarpa"	Elite intraspecific hybrid used as a breeding reference for comparison with backcross populations.	1

genomic DNA extraction. DNA concentration and purity were analyzed using a sorbitol-CTAB–based protocol[19] and a Nanodrop 2000 spectrophotometer (Thermo Scientific), respectively. The samples were then sequenced through GBS using the DArTSeq platform (diversityarrays.com)[20] according to the Sansaloni *et al.* methodology (2010).[21] The combination of PstI/TaqI enzymes was used for the complexity reduction. Site-specific adapters for *PstI* were marked with 96 distinct barcodes for sample identification, allowing pooled sequencing on an Illumina HiSeq 2500 system. The generated sequences (75 bp) were filtered by quality (95% confidence for ≥ 50% of bases) and with barcodes. The filtered data were aligned against the reference genome of *Passiflora edulis*[22] and a DArT analytical pipeline resulted in two types of markers: single nucleotide polymorphisms (SNP) and presence/absence of variations (PAV).[23]

Mapping SNPs against the *P. edulis* reference genome presents an inherent limitation when analyzing complex hybrids. Because wild species like *P. amethystina*, *P. hatschbachii*, and *P. quadrifaria* are highly divergent, their genomic regions align less efficiently to the reference. Consequently, the overall contribution of these wild species may be slightly underestimated. Nevertheless, since our

main objective was to evaluate the recurrent genome recovery toward the *P. edulis* background, this reference-based approach is appropriate. It ensures robust and reliable estimates of both the recurrent genome recovery and the genetic relationships among the evaluated accessions.

Filtering of Genotypical Data

The SNPs were subjected to strict filters: Call Rate (≥ 0.80), Minor Allele Frequency (MAF > 0.01) and Q-value (> 2). Accessions with more than 40% missing SNP data were not included. The selected markers were aligned to the *P. edulis* reference genome using BLASTN (default parameters),[22] only accepting sequences located in single genomic regions and allowing a maximum of two gaps per sequence.

Genetic Variability Analysis for SNPs

The averages of Polymorphism Information Content (PIC), one ratio proportion, Minor Allele Frequency (MAF) and reproducibility were calculated for all 84 accessions. Mean allele frequencies were estimated considering all accessions and genealogies A, B, and C. Allele frequencies were calculated using genotype frequencies, including homozygosity for the reference allele (*P. edulis*), homozygosity for the alternative allele (SNP), heterozygosity and null genotypes. All estimates were obtained using the adegenet package, with the functions genind and genpop, in R software (version 4.3.1).[24]

Clustering and Genetic Similarity Analysis

The genetic distances among accessions were calculated based on the allele coincidence index (SNPs) using the following formula: DGij = 1 – (NLC/NTL), where *DGij* is the genetic distance between accessions *i* and *j*, *NLC* corresponds to the number of coincident loci (1, 0,5 or 0) and *NTL* being the total number of loci. Genetic distance matrices were used to perform hierarchical clustering with the UPGMA method (Unweighted Pair Group Method Using Arithmetic Averages[25] and Principal Coordinates Analysis (PCoA)[26] to visualize the difference among accessions.[26]

The packages ggplot2 and adegenet, respectively (software R v 4.3.1) were used for these analyses.[24] The accessions genetic similarity was investigated using the ggplot2 package through the UpSetR function (software R v. 4.3.1),[24] based on the UpSet technique.[27] This function provides plots that calculates the SNP frequency showing the higher intersection among the accessions, which purpose is to identify the genetic similarity.[28] Three separate analyses corresponding to genealogies A, B and C, including their respective parents were performed.

RESULTS AND DISCUSSION

Quality of SNP Markers and Genetic Variability Analysis

A total of 109.404 SNPs were identified across the 84 passion fruits (*Passiflora* spp.) accessions. After filtering and alignment 3.717 SNPs were selected to perform the statistical analysis. The PIC average was 0.381, suggesting that the markers used are informative, being higher than the values previously reported by Reis et al.[29] for two populations of *P. edulis* (0.16 and 0.18). The average *one ratio* was 0.294, showing high genetic variability. The average values for *call rate* and MAF were, respectively, 0.841 and 0.382, demonstrating the reliability of data and a good allele frequency. The reproducibility for SNP marker was 99.6%, indicating the reliability of the generated data and suggesting that the results might be replicated across different analyses and conditions.

In the past, passion fruit breeding relied on traditional molecular markers like SSRs and RAPDs.[30-36] These markers covered little of the genome and had low density. They helped a lot in characterizing germplasm. But panels usually had fewer than 10 to 20 loci per assay. This limited the ability to tell closely related genotypes apart, estimate genetic parameters accurately, and track introgressed chromosome segments.[37-39]

This problem is not unique to passion fruit. Studies comparing methods in other crops found the same pattern: SNP panels outperform SSRs in resolving population structure and estimating genetic diversity. In rice, for example, SNP markers explained 45.2% of the variance in principal coordinates analysis, compared to only 13.3% for SSRs. SNPs also detected population structure at a finer scale (K = 15 versus K = 5).[40] A PRISMA-compliant meta-analysis across several plant species confirmed that SNPs have greater discriminatory power than SSR, AFLP, and RAPD.[41]

Next-generation sequencing (NGS) has changed this picture. It is now possible to genotype thousands of SNPs across the whole genome at once.[42-44] An important step

in this direction was the use of genotyping-by-sequencing (GBS) in *Passiflora edulis* f. *edulis*. This study showed that high-density SNP panels reveal patterns of population structure and genetic diversity that low-density traditional markers cannot detect.[45]

In the present study, the DArTseq platform was used, an upgrade over older methods. This technology has already proven its ability to generate thousands of high-quality SNPs across the genome in tropical fruit species.[46]

With a dataset of more than 3.000 high-quality SNPs, unprecedented resolution was achieved for mapping genetic diversity and introgression. This high-throughput approach allowed recurrent genome recovery to be characterized with a level of precision that traditional SSR markers cannot match.[47-48]

When considering genealogies A, B and C, the mean genotypic frequencies of homozygosity for the reference allele (*P. edulis*) and the alternative allele (SNP) showed little variation among genealogies: Genealogy A, 72.7% and 24.4%, respectively; Genealogy B, 68.6% and 22.8%; and Genealogy C, 73.3% and 26.7%. These small differences may have implications for the functional diversity of the genealogies, particularly when associated with specific agronomic traits. The mean genotypic frequency of null alleles (absence of amplification) was higher in Genealogy B (7.2%) and lower in Genealogies A and C, at 1% and 0.8%, respectively. The higher number of null alleles in Genealogy B may be related to structural mutations, such as the self-compatibility trait observed in the accessions of this genealogy.

The analysis of allele frequencies across the genealogies showed a predominance of *P. edulis* reference alleles, with an average recurrent genome recovery of 74.4% compared to 25.6% for the alternative alleles (SNPs). This result closely matches the 75% theoretical expectation for a first backcross generation, confirming that the breeding strategy restored the recurrent genetic background while securing essential segments from the wild donors. The slight deviation from the strict 75% expectation is not a shortcoming; rather, it highlights the selection pressure deliberately applied to retain favorable introgressed alleles for disease resistance and key agronomic traits. The Minor Allele Frequency (MAF = 0.382), demonstrating that the program preserved substantial genetic variability despite repeated backcrossing. Ultimately, this variability safeguards against excessive genetic uniformity and provides a foundation for future selection cycles.

Cluster and Genetic Similarity Analysis

The cluster analysis of 14 accessions from Genealogy A – {[(BRS Minimaracujá Roxo × BRS Sol do Cerrado) × BRS Sol do Cerrado] × VAO} (Figure 1A) demonstrated that the hybrid RJB (*P. edulis* Sims "mini-roxo") × *P. edulis* Sims "flavicarpa", which is self-compatible, stood out for being isolated from the other accessions. In contrast, the accession (BSCE) LD4 (BRS Sol do Cerrado, *P. edulis*) showed a lower distance compared to the other genotypes, as it essentially contains the genome of *P. edulis* (the recurrent parent species).

The accessions resulted from the RR (self-incompatible) and RM (self-compatible), [(BRS Mini Roxo x Matriz BRS Sol do Cerrado) x Matriz BRS Sol do Cerrado] x VAO backcrossing, formed a relatively cohesive clade with short ramifications indicating that they are genetically similar due to the recurrent genome recovery. The clustering of these self-compatible accessions reinforces this observation; however, one backcross accession (RM01) stood out for being more isolated from the rest of the backcross clade.

The HVAP accession originated from BRS Polpa Forte, a cultivar still to be released, obtained by the backcross between CPAC-VAO [(RC5) (*P. caerulea* L. x *P. edulis* Sims)] x 325 [(RC4) (*P. caerulea* L. x *P. edulis* Sims)] matrix, was very close to the backcrossing, as it essentially carries the genome of the recurrent species. By contrast, BGA (*P. edulis*) and BFOR accessions, the female parent of BRS Polpa Forte [(*P. caerulea* L. × *P. edulis* Sims) RC4], appeared slightly more distant from the others because it still retains part of the resistant parent genome. This result was expected, since *P. caerulea*, even when diluted in the backcrosses with the recurrent *P. edulis*, still maintains specific genetic traits, such as resistance to bacteriosis and tolerance to viruses.

This pattern reflects a mixture of materials: BGA (*P. edulis*), BFOR (interspecific hybrid, RC4), HVAP (backcross of interspecific hybrid) and RM01 (based on self-compatible *P. edulis*). The presence of BFOR and HVAP indicates that this cluster contains materials with some proportion of *P. caerulea*, although this contribution is probably very diluted due to the backcross with *P. edulis*.

The PCoA analysis (Figure 1B) demonstrated a clear separation along Axis 1 to the left, with most accession clusters showing strong uniformity and with a highlight to (HRJB) RJB [*P. edulis* Sims "mini-roxo" × *P. edulis* Sims "flavicarpa"] accession. Along Axis 2, a compact central

cluster was observed except for RM01 backcross {[(BRS Mini Roxo × BRS Sol do Cerrado parent) × BRS Sol do Cerrado parent] × VAO}, which is self-compatible, and (BSCE) LD4 – BRS Sol do Cerrado (*P. edulis*), which is self-incompatible. RM01 accession remained more isolated corroborating the information obtained through the dendrogram clustering, possibly due to the selection process for resistance within the backcross population. This represents an important source of genetic variability for the group's genetic base and holds potential for the breeding program.

According to the UpSet analysis (Figure 2), all accessions share a high number of SNPs (708), indicating that despite phenotypic differences, they exhibit genetic similarities due to the predominance of the recurrent genome. The backcross accessions that are self-compatible [RM1 (928), RM2 (913) and RM3 (917)] and those that are self-incompatible [RR03 (919) and RR05 (912)] show a higher number of shared SNPs, suggesting strong genetic similarity. The RM group accessions (self-compatible) should be highlighted suggesting that repeated self-fertilization or crosses within the RM group may have accelerated allele fixation, explaining the strong overlap observed. The accession with the lowest number of SNP intersections (829) was HRJB – RJB (*P. edulis* Sims. "mini-roxo" × *P. edulis* Sims. "flavicarpa"), although it is not completely isolated. This accession showed the highest genetic divergence compared to the others, while still maintaining a single subset that could be useful for increasing diversity in future crosses.

Although the three analyses evaluate different aspects, they present some similarities concerning the results. All of them revealed genetically similar clusters, such as the one found on RM and RR backcrosses, that tend to cluster and share intersections like the SNPs. The HRJB accession also consistently stood out as the most distinct or divergent. Thus, the three analyses provide a complementary view of the accessions genetic structure supporting the conclusions regarding diversity and genetic parenthood in the materials studied.

The dendrogram analysis of 50 accessions using genealogy B [RC1 AC1 (RJB × *P. edulis* Sims.) × LD4 × RC] (Figure 3A), demonstrated that the self-compatible (RLC) and self-incompatible (RL) backcross accessions showed no differences. They were genetically similar and shared higher similarity with the accessions HRJB [RJB (*P. edulis* "mini-roxo") × GA (Gigante Amarelo – *P. edulis*)], BGA (Gigante Amarelo – *P. edulis*), and BSCE – BRS Sol do Cerrado (*P. edulis*), which are more distant. On the other hand, RL04, RL31 and RL20 backcrosses are more closely

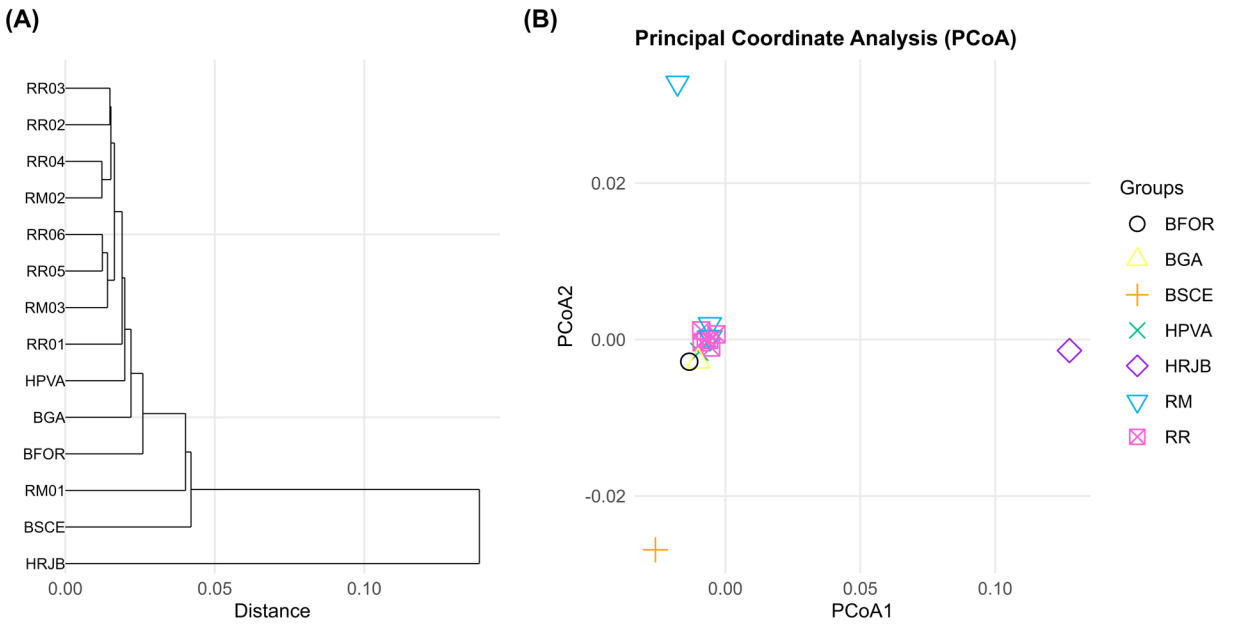

Figure 1. Genetic structure of 14 accessions from Genealogy A assessed via DArTseq SNPs. (A) UPGMA dendrogram based on the allele coincidence index. (B) Principal Coordinate Analysis (PCoA). Abbreviations: RM/RR: self-compatible and self-incompatible advanced backcrosses; BSCE: BRS Sol do Cerrado; BGA: BRS Gigante Amarelo; HVAP: advanced line BRS Polpa Forte; BFOR: interspecific parent (*P. caerulea* × *P. edulis*); HRJB: elite hybrid.

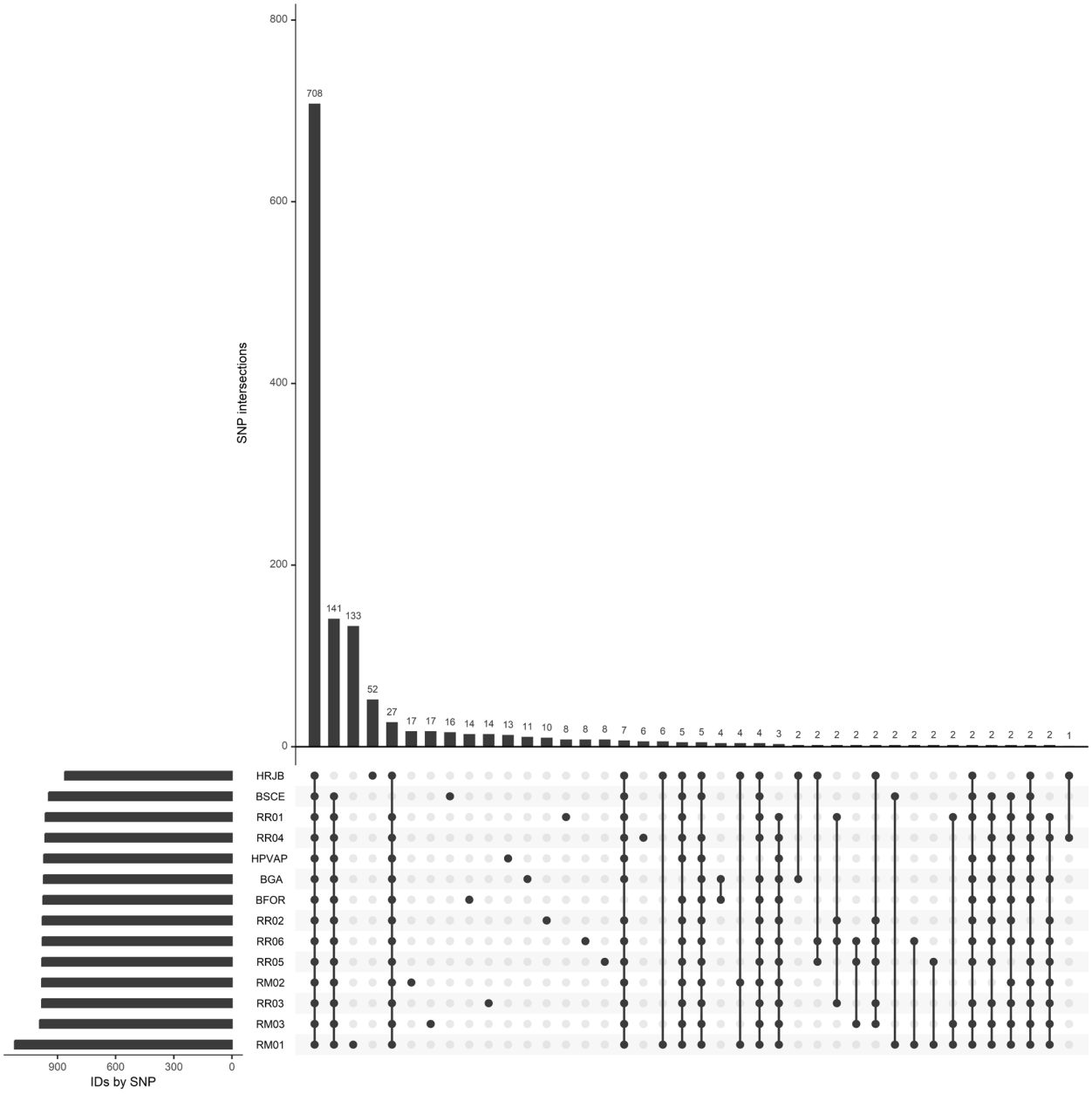

Figure 2. UpSet set present the intersections of SNP sets among the accessions of Genealogy A. The analysis highlights the extent of SNP sharing among advanced backcross populations self-compatible (RM) and self-incompatible (RR) and their recurrent parental lines.

clustered when compared to the RJB parent [BRS mini-roxo (*P. edulis*)]. RL05, RL15, RL28 and RL29 accessions are in another cluster, which is distant from the others.

The PCoA analysis (Figure 3B) along Axes 1 and 2 demonstrated that the accessions were clustered. However, a dispersion was observed in the RL backcrosses, which may suggest a variation in the proportion of the recurrent genome recovered. This is a process that can be observed in successive backcrosses with genetic recombination. Many accessions are more closely clustered to HRJB and BGA indicating significant recovery toward BGA (*P. edulis*).

The UpSet analysis (Figure 4) showed that all accessions share SNPs but in small amount - only 14 SNPs. The intersection with the highest number of SNPs was 53, suggesting that despite the similarities, the genetic divergences remain due to the involvement of interspecific crosses, since few SNPs are shared by all accessions. RJB (411) showed more SNPs and RL05 (50) presented fewer SNPs in the intersections. The RL and RLC accessions displayed little variation in the number of intersecting SNPs, indicating that these SNPs are largely conserved or fixed within the backcross population. The variability

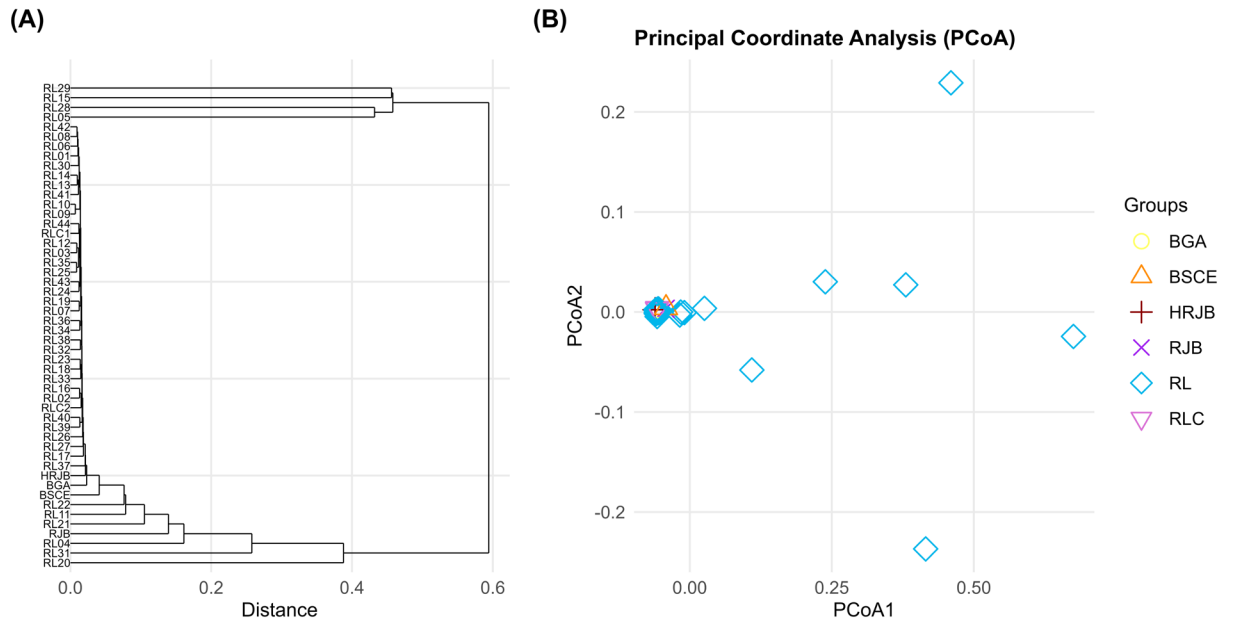

Figure 3. Genetic structure of 50 accessions from Genealogy B assessed via DArTseq SNPs. (A) UPGMA dendrogram based on the allele coincidence index. (B) Principal Coordinate Analysis (PCoA). Abbreviations: RL/RLC: advanced backcross populations for recurrent genome recovery; BSCE: BRS Sol do Cerrado; BGA: BRS Gigante Amarelo; HRJB: elite hybrid. The spatial separation reflects the genetic relationships and recurrent genome recovery toward the elite parental lines.

observed in the intersections highlights the dynamics of genetic introgression and the importance of a detailed analysis for genetic improvement.

The Principal Coordinate Analysis (PCoA) and the UPGMA dendrogram provide a clearer visualization of the genetic dynamics. In Genealogy B, for instance, most backcrossed accessions (RL) clustered closely with the recurrent parent: Gigante Amarelo (BGA), visually confirming the efficiency of the backcrossing method in recovering the *P. edulis* genome. However, specific accessions - such as RL05, RL15, RL28, and RL29 - remained genetically distant in the dendrogram, indicating that they retained greater genetic divergence from the recurrent parent, which likely reflects the persistence of genomic regions inherited from the wild donor species. Because they represent important reservoirs of introgression, these distinct accessions deserve particular attention in future breeding cycles, as they may serve as valuable sources of favorable alleles for disease resistance and other agronomic traits.

The dendrogram for genealogy C - {ML-1 (*P. edulis* Sims "flavicarpa" × *P. aff. amethystina* "macrocarpa") × PL-5 [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis*} (Figure 5A) including 23 accessions, showed that the *P. incarnata* (PINC) accession was in a branch isolated from the others. *P. amethystina* (PAME), *P. quadrifaria* (PQDR) and *P. hatschbachii* (PHAT) were more distant and in separate branches indicating a higher genetic distance from the backcrosses (RI). The backcrosses, with few variations, demonstrated to be clustered together close to Gigante Amarelo - *P. edulis* (BGA2) and *P. edulis* ML1 (PEDM), confirming that the backcrossing process led to genetic convergence.

As observed in the dendrogram, in the PCoA (Figure 5B), PINC, PAME, PQDR and PHAT accessions were on Axes 1 and 2, positioned farther from the backcrosses, that are genetically closer to the parent (PEDM) and the recurrent parent (BGA2). It was observed that in the backcrosses (RI) there was an increase in the proportion of the recurrent parent genome while still preserving specific segments of interest from BGA2.

The intersection analysis (Figure 6) showed that 296 SNPs were shared among all accessions indicating significant genomic conservation. Few SNPs presented exclusive intersections with minimal counts around 5 SNPs, suggesting low singularity per individual accession. The backcrosses (RI), PEDM and the BGA2 genotype (*P. edulis*) showed the same number of SNPs shared among them (821). On the other hand, the accession with the lowest number of shared SNPs was PINC (*P. incarnata*) (548).

The dendrogram analysis, PCoA and SNPs intersections also demonstrated the genetic structure of RI backcrosses

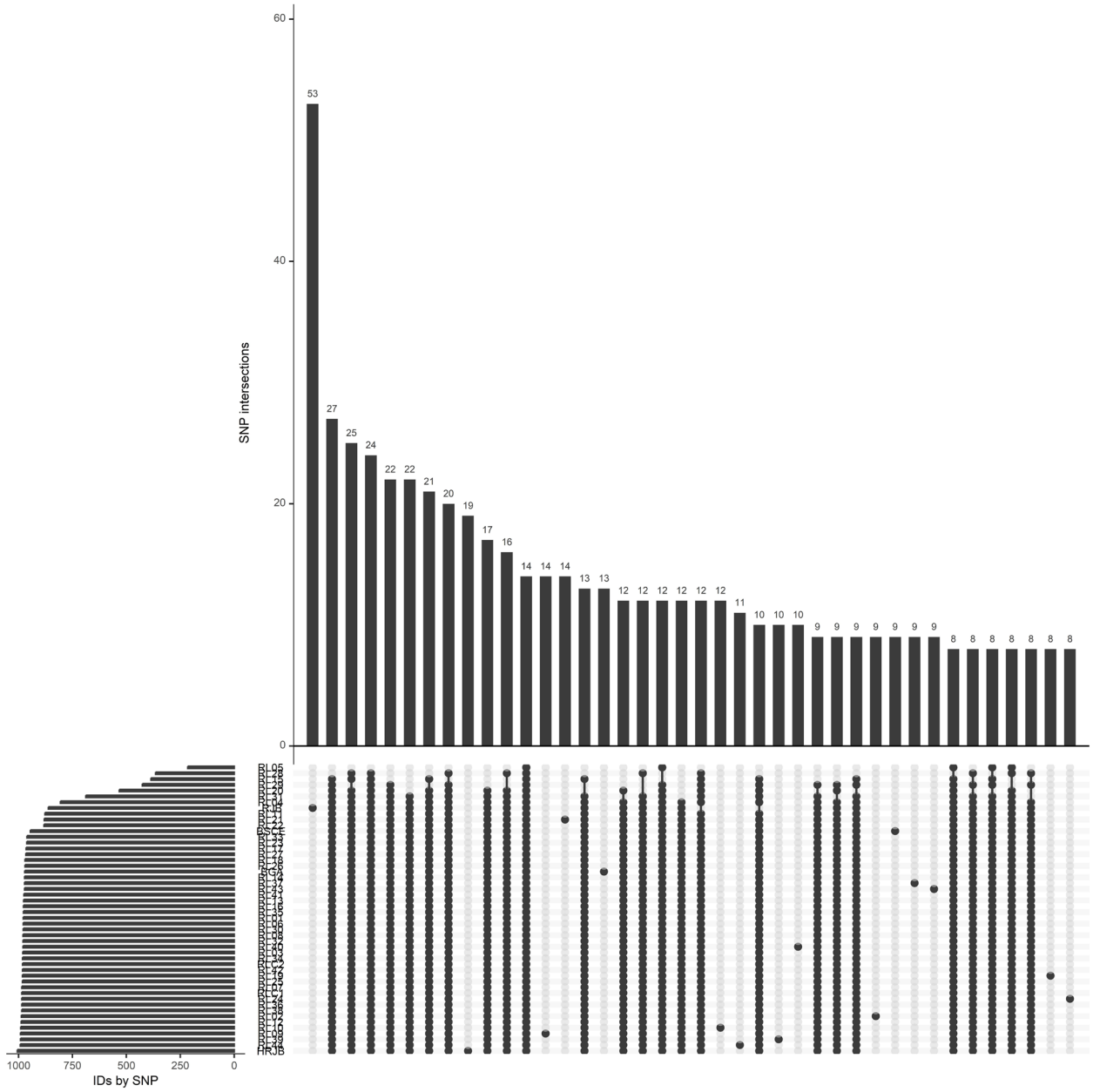

Figure 4. UpSet set present the intersections of SNP sets among the accessions of Genealogy B. The analysis highlights the extent of SNP sharing among advanced backcross populations (RL and RLC) and their recurrent parental lines.

and their respective recurrent genomes. The results indicated that the backcross used in this present study was effective in recovering the recurrent genome due to the high backcross genetic proximity with the parental base (BGA e Sol do Cerrado) and the high number of shared SNPs.

The analyses also showed that backcrosses involving fewer wild species, with the purpose of fixing agronomic traits, led to genetic clustering with a more uniform genetic composition. Recurrent genome recovery is important for fixing traits related to fruit yield and quality. However, it can result in the loss of resistance genes from wild species during successive backcrosses. This loss of resistance genes can be lowered by selecting resistant individuals for new backcrossing cycles.[49] For instance, the analysis of genealogy A revealed that RM01 accession ([(BRS Mini Roxo x Matriz BRS Sol do Cerrado) x Matriz BRS Sol do Cerrado] x VAO, self-compatible), resulted in an isolation compared to the others suggesting that the self-compatibility selection may have preserved the wild species genomes. In many backcrosses that clustered together traces of *P. caerulea* wild species genome were found.

The *UpSet* analyses (Figures 2, 4 and 6) showed that the SNPs accessions were shared, which might reflect introgression events. Shared SNP intersections allow

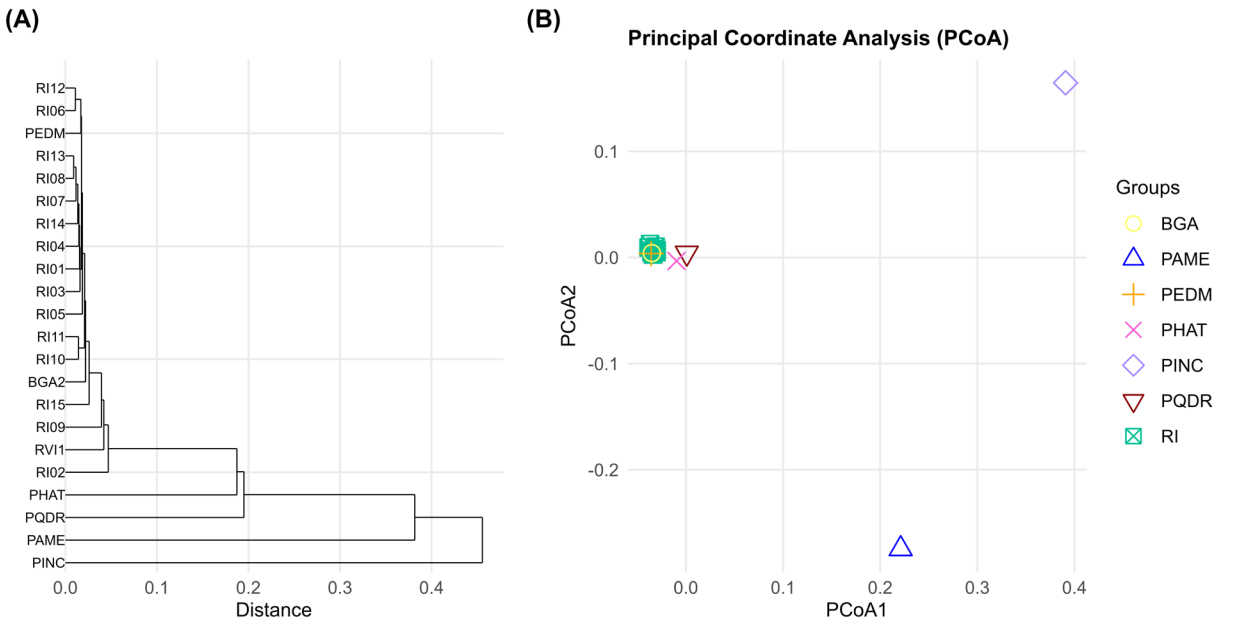

Figure 5. Genetic structure of 23 accessions from Genealogy C assessed via DArTseq SNPs. (A) UPGMA dendrogram based on the allele coincidence index. (B) Principal Coordinate Analysis (PCoA). Abbreviations: PEDM: recurrent parent (*P. edulis*); RI: interspecific backcrosses; PAME: wild donor (*P. amethystina*); PHAT: wild donor (*P. hatschbachii*); PQDR: wild donor (*P. quadrifaria*); PINC: wild donor (*P. incarnata*). The spatial separation reflects the recurrent genome recovery and the integration of these wild species as sources of disease resistance and favorable agronomic traits.

the inference of genomic regions potentially affected by introgression, especially when mapped to specific positions in the reference genome.[50] In genealogy B, only 14 SNPs were shared in all accessions suggesting that genetic recombination during successive backcrosses resulted in a high variation in the proportion of the recovered genome. According to Enggarini *et al.* (2012),[51] in rice backcrossed populations, the length and size of the donor genome segment significantly decreased during the successive generations demonstrating the role recombination in lowering the presence of the donor genome. In the PCoA plot (Figure 3B), this variation in dispersion was observed among the backcrosses (RL) indicating possible differential gene flow among the accessions.

As expected, *P. incarnata*, *P. loefgrenii* and *P. amethystina* appeared isolated in the analyses (Figures 5A and B) suggesting a higher genetic distance when compared to *P. edulis* cultivars and the backcrosses. The simple hybrids were in intermediate positions in relation to the backcrosses, which were closer to the recurrent parent. Li *et al.*[52] also observed this fact when studying two invasive species: *Cakile edentula* (self-compatible) and *Cakile maritima* (self-incompatible).

The recurrent genome recovery in backcrosses was evidenced by the cluster analyses (dendrogram and PCoA). In all genealogies A, B and C, the backcrosses (RI, RL, RR) were located close to their respective recurrent parents (HVAP, RJB, BGA2), indicating a significant genomic recovery. However, the recurrent genome recovery was different according to the accessions, as demonstrated by the varied dispersion of the backcrossed individuals in the PCoA plots and by the differences in SNPs sharing identified in the *UpSet* analysis.

Studies using DArT-seq sequencing have demonstrated that these markers facilitate the reliable identification of hybrids, as in the study performed by Vašut *et al.*,[53] that identified *Salix* (willow) hybrids. Similarly, Bocianowski *et al.*[54] carried out a study that highlights the versatility of DArT-seq markers as tools for diagnosing heterosis in maize by analyzing traits, such as ear length and yield. In the present study, the complex genetic structure of *Passiflora* hybrids was also efficiently observed, validating the effectiveness of backcrossing for recurrent genome recovery. It also was effective in identifying the genetic variability of accessions. This variability enables the selection of accessions that combine the productivity of *P. edulis* with the desirable genes of donor species.

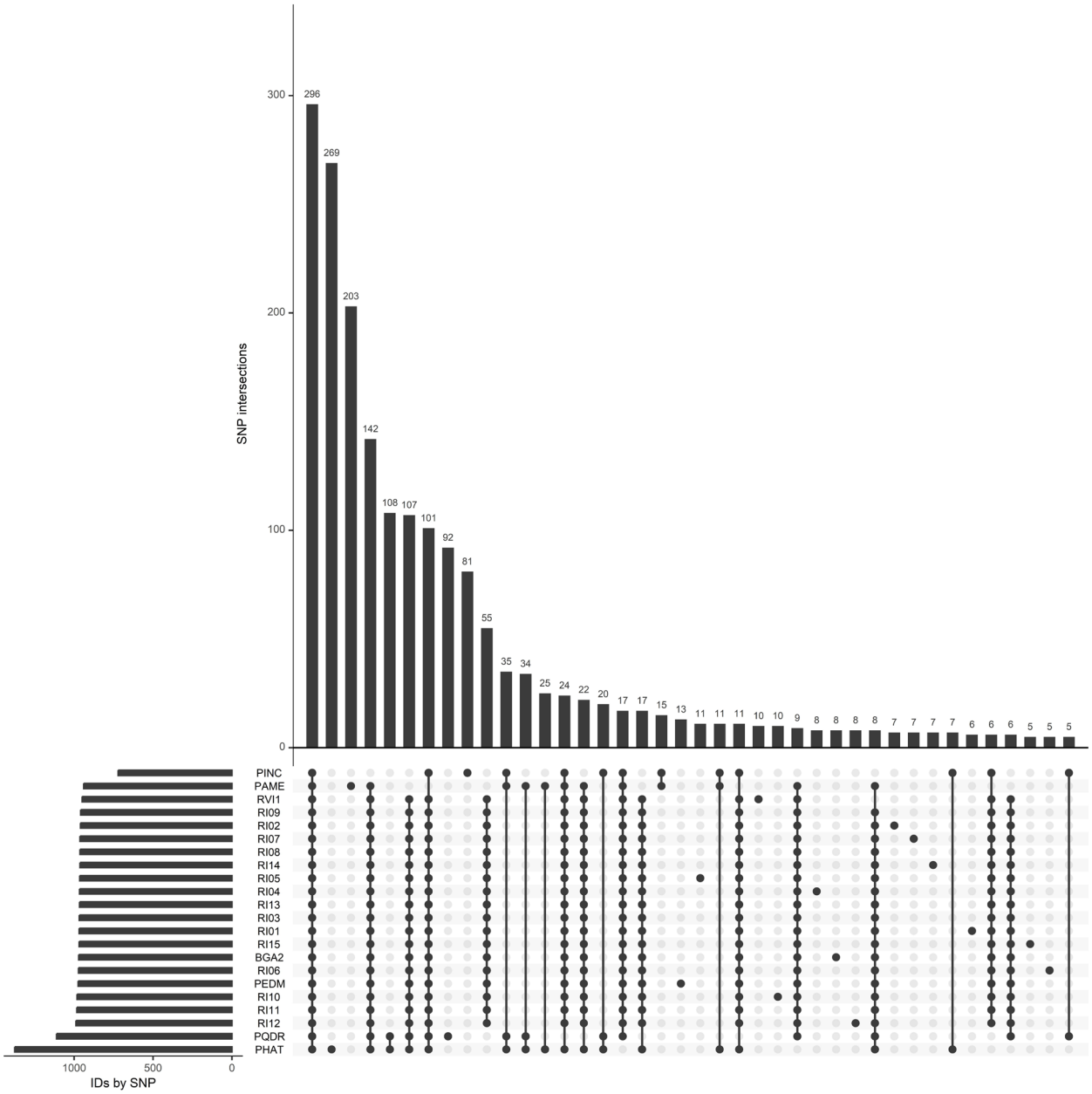

Figure 6. UpSet plot set present the intersections of SNP sets among the accessions of Genealogy C, including recurrent parents, interspecific hybrids, and wild donor species used in the breeding program.

CONCLUSION

The analyses of A, B and C demonstrate that the backcross-based breeding programs in passion fruits performed at Embrapa Cerrados have effectively recovered the recurrent genome, preserving both allelic diversity wealth of important commercial *P. edulis* cultivars and genes of interest of wild species.

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DATA AVAILABILITY STATEMENT

All datasets supporting the findings of this study are available upon request from the corresponding author, Tais Barbosa. The datasets are not publicly available because they are part of an ongoing breeding program and institutional research activities.

AUTHOR CONTRIBUTIONS

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