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Characterization of Genetic Diversity in Accessions of *Prunus salicina* Lindl: Keeping Fruit Flesh Color Ideotype While Adapting to Water Stressed Environments

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Abstract: The genetic diversity of 14 Japanese plum (*Prunus salicina* Lindl) landraces adapted to an ecosystem of alternating flooding and dry conditions was characterized using neutral simple sequence repeat (SSR) markers. Twelve SSRs located in six chromosomes of the *Prunus persica* reference genome resulted to be polymorphic, thus allowing identification of all the evaluated landraces. Differentiation between individuals was moderate to high (average shared allele distance (DAS) = 0.64), whereas the genetic diversity was high (average indices polymorphism information content (PIC) = 0.62, observed heterozygosity (Ho) = 0.51, unbiased expected heterozygosity (uHe) = 0.70). Clustering and genetic structure approaches grouped all individuals into two major groups that correlated with flesh color. This finding suggests that the intuitive breeding practices of growers tended to select plum trees according to specific phenotypic traits. These neutral markers were adequate for population genetic studies and cultivar identification. Furthermore, we assessed the SSR flanking genome regions (25 kb) in silico to search for candidate genes related to stress resistance or associated with other agronomic traits of interest. Interestingly, at least 26 of the 118 detected genes seem to be related to fruit quality, plant development, and stress resistance. This study suggests that the molecular characterization of specific landraces of Japanese plum that have been adapted to extreme agroecosystems is a useful approach to localize candidate genes which are potentially interesting for breeding.

Keywords: Japanese plum; SSR; diversity; genetic structure; candidate genes

1. Introduction

The genus *Prunus* consists of more than 400 species, of which plum, peach, almond, and cherry trees stand out for their commercial importance worldwide. The Japanese plum (*Prunus salicina* Lindl) is one of the most commercially important plum species. This diploid ($2x = 2n = 16$) species has been

cultivated in different environments and was introduced to North America from China in the 19th century [1]. Today, most Japanese plum cultivars grown worldwide come from the early selections originated in California at the end of the 19th century [2]. Besides, most planted plum orchards in other extensive production regions of Argentina derive from a few introduced cultivars of global distribution.

By contrast, European immigrant growers early in the last century who settled in the Paraná River Delta (PRD) introduced old traditional cultivars from their European countries. They selected wild plants emerged by the spontaneous sowing of seeds of the introduced varieties that they cultivated based on good performance and high-quality traits. Growers from the PRD had to face a particularly harsh environmental ecosystem.

The PRD is a unique ecosystem dominated by floods because of water discharges mainly from the Paraná River followed by discharges from the Uruguay and Gualeguay rivers. Tidal and storm surges from the Río de la Plata estuary as well as local rainfalls also contribute to generate these wetlands [3]. Precipitations in this region are influenced by the El Niño Southern Oscillation (ENSO) [4]. Indeed, three important floods associated with El Niño took place in PRD in 1982, 1998, and 2007 [3,5]. Furthermore, the alternation of wet and dry periods in the PRD influences the variability of the ecosystem. All these features give place to at least 15 landscape units [3] with a high ecological diversity distinctly adapted to annual hydrological cycles [6].

In this climate context, growers from the PRD generated local plum landraces that were cultivated after multiplication by grafting or from seeds, and therefore obtained the best wetland-adapted plants. In this way, they intuitively created a specific fruit tree germplasm for fruit production in an ecosystem with harsh environmental conditions that alternates river flooding periods with extreme dry season. The generated germplasm, which presents different harvest times (November to February, Table 1), is highly variable regarding pulp and skin coloration, as well as in its organoleptic characteristics.

Table 1. Characteristics of the landraces collected from the Paraná River Delta (PRD).

Landrace	Origin	Flesh Color	Harvest
Ratto	Seedling Delta	red	Very late season—End of January
Severiana	Seedling Delta	red	Very late season—End of January to mid-February
Remolacha de Berisso	Seedling Delta	red	Very late season—End of January to early February
Fragata	Seedling Delta	red	Very late season—End of January
Remolacha de Leber	Seedling Delta	red	Middle season—Early/middle December
Gigaglia	Seedling Delta	red	Middle season—Mid-November to early December
Capri	Seedling Delta	red	Late season—Late December
Giordano	Seedling Delta	red	Middle season—Late November to early December
Ciervita	Seedling Delta	pink	Early season—Mid-to late November
Reina de oro	Seedling Delta	yellow	Late season—Early January
Juanita	Seedling Delta	yellow	Early season—Mid-November to early December
Gascón	Seedling Delta	yellow	Middle season—Late November to early December
Tricerri	Seedling Delta	yellow	Mid-November
“X”	Seedling Delta	yellow	Mid-November

Thus, Japanese plum landraces selected in the PRD are a unique genetic resource in the world, and one of the few adapted to delta edaphic conditions and to humid temperate climate [7]. These landraces could constitute the closest reservoirs of “useful” alleles for future genetic improvement in the context of climate change. They contain the genetic variants naturally or artificially selected by growers because of their adaptation, productivity, or resistance to different stresses in the territory [4,5]. The knowledge of this interesting germplasm collection’s diversity is essential for its characterization, conservation, and maintenance, as proposed for sweet cherry [8].

Genomics has triggered a revolution in the study of diversity [9,10] and conservation [11] by providing methods for the genetic characterization of individuals, populations, and species. The availability of *Prunus* genomic resources such as peach whole-genome sequences [12] allows the search for sequences of interest for breeding purposes (see for review [13]).

Mnejja et al. [2] isolated 27 microsatellites or simple sequence repeats (SSRs) in *P. salicina*, whereas Carrasco et al. [14] analyzed genetic diversity and correlation among 29 Japanese plum cultivars using a combination of inter simple sequence repeat (ISSR) and SSR markers. On the other hand, Klabunde et al. [15] genotyped 47 cultivars with eight microsatellite markers. A unique genetic map based on Restriction Fragment Length Polymorphism (AFLP) has been generated for this species [16]. Recently, González et al. [17] reported a set of EST-SSR markers for *P. salicina* developed from specific genes, which determine different organoleptic properties of the fruit, and analyzed 29 cultivars with these markers.

Microsatellite markers generate genotype profiles that can allow the identification and traceability of different cultivars in the diverse stages of their breeding. Therefore, the use of microsatellite markers could be a valuable tool to describe and protect Japanese plum germplasm. In addition, the genome position of SSR on a reference genome, together with potential candidate genes for fruit properties and self-incompatibility systems, could also be a useful tool for breeding purposes [13].

This unique plum germplasm was studied through the use of SSR markers to assess the diversity, genetic structure, and relationships between 14 landraces of Japanese plums from the PRD. Therefore, we also analyzed the flanking regions of the polymorphic SSR with bioinformatics tools to search for candidate genes. These candidate genes were in linkage disequilibrium with polymorphic SSR markers in an adaptive evolution to water stress conditions and fruit quality.

2. Materials and Methods

2.1. Plant Material

Fourteen landraces of *P. salicina* and two introduced commercial cultivars were assessed to evaluate amplification and polymorphic status of the 12 selected pairs of primers from Mnejja et al. [2]. The analyzed landraces were “Ratto”, “Severiana”, “Remolacha de Berisso”, “Fragata”, “Remolacha de Leber”, “Gigaglia”, “Capri”, “Ciervita”, “Giordano”, “Reina de oro”, “Juanita”, “Tricerri”, “X” (unknown origin), “Gascón”. The introduced cultivars consisted of one Japanese plum of American origin (“Santa Rosa”) and one European plum cultivar (*P. domestica*; “Reina Claudia”). Two peach cultivars (*P. persica*) “Sol de Mayo” and “Zelanda” were included as outgroups (Table 1). All samples were collected from one representative tree of each clonally reproduced genotype located in the Pacífico River, San Fernando Island of the PRD, Province of Buenos Aires, Argentina (34°12′01.62″ S, 58°40′07.66″ W).

2.2. DNA Extraction, PCR, and Gel Electrophoresis Conditions

Total genomic DNA from 20 mg of leaf samples was extracted with a Nucleo SpinR Plant II kit (Macherey-Nagel, Düren, Germany).

The samples were screened for polymorphisms with 12 SSR markers [2]. PCR was performed according to Mnejja et al. [2]. The amplification products were denatured for 5 min in denaturing loading buffer at 95 °C and separated by a 6% polyacrylamide gel electrophoresis (6% acrylamide/bisacrylamide 20:1, 7.5 M urea, 0.5 × TBE) along with a 25 bp DNA ladder standard (Invitrogen, Waltham, USA). The DNA silver-staining procedure of Promega (Madison, WI, USA) was used for visualization.

2.3. Genetic Analyses

The number (N_a), effective number (N_e) and frequency of alleles, as well as the observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e), and private alleles by population

were determined for the 14 local Japanese plums samples using the GenAlEx 6.5 program [18]. The polymorphism information content (PIC) of each marker was calculated according to Botstein et al. [19].

Genetic diversity analysis was performed on plum and peach genotypes. Shared allele distance (DAS) [20] was implemented between individuals using POPULATIONS 1.2.28 [21]. The unweighted pair group method using arithmetic averages (UPGMA) algorithm was used for cluster analysis and the development of the corresponding dendrogram.

2.4. Population Structure. The population structure pattern was assessed by performing a Bayesian analysis with the software STRUCTURE v.2.3.3 [22]. Assignment of individuals to a group was evaluated according to the membership coefficient Q criteria ($Q \geq 0.8$). The model used was an admixture model with ten replicates for each number of genetic groups ($K = 1-10$) and 100,000 iterations of burn-in followed by 250,000 Markov chain Monte Carlo (MCMC) iterations. The outputs of the genetic group analysis were extracted in Structure Harvester [23]. The optimal K -value was determined using the delta K method, as described by Evanno et al. [24].

Because the assumptions underlying the population genetic model in STRUCTURE (e.g., Hardy–Weinberg or linkage equilibrium (LD)) may limit its use, this analysis was complemented with a discriminant analysis of principal components (DAPC) [25]. The value of K was determined using the Bayesian Information criterion (BIC) value given by the software [26]. The critical membership value was set at 0.8. The DAPC was implemented in the R package adegenet 2.0 [27,28].

2.4. Mapping of SSR Markers on the *Prunus persica* Genome and Identification of Their Flanking Genes

The obtained amplification sequences for the 12 SSR were mapped to the *P. persica* genome [29] (Phytozome–*Prunus persica* v2.1 (phytozome.jgi.doe.gov)). Mapping was performed using the Bowtie2 Alignment tool with default settings [30]. A custom Perl script (Higgins J., personal communication) was used to determine the annotated genes of the *P. persica* genome within a flanking region of 50 kb (± 25 kb adjacent to each SSR locus). This window size was selected based on the high macrosynteny found between *Prunus* species [31] and studies in sweet cherry (*P. avium* L), which presents the lowest LD in *Prunus* genus. Indeed, in sweet cherry, the intra-chromosomal LD is lower than peach, therefore declining among 0.05 and 0.1 Mb [8].

3. Results

3.1. Diversity Study

All SSRs generated polymorphic bands in the 14 *P. salicina* landraces (Table 2). The alleles were clearly differentiated, with no discrepancies in the banding pattern.

The 12 polymorphic SSRs in the 14 evaluated local landraces of Japanese plums generated 66 different alleles (Electronic Supplementary Material, Table S1). The N_a per locus ranged from four to seven, with a mean of 5.5 (SD 1.0), and the N_e ranged from 2.2 to 4.2, with a mean of 3.1 (SD 0.6). Additionally, PIC, H_o , and uHe ranged from 0.51 to 0.73, 0.15 to 0.86, and 0.56 to 0.79, with global means of 0.62 (SD 0.06), 0.51 (SD 0.23), and 0.70 (SD 0.06), respectively (Table 2). Thus, the genetic diversity levels were moderate to high, and kept 37 private alleles in 12 SSRs (20 with a frequency below 0.08) with respect to 4 private alleles in 4 SSRs (all of them with a frequency of 0.25) found in the analyzed outgroups.

3.2. Cluster and Genetic Structure Analyses

These 12 SSRs allowed the unambiguous genotype differentiation of the 14 landraces studied. DAS values between plum individuals fluctuated from 0.14 (between “Ratto” and “Severiana”, which shared 19 alleles out of 24) to 1 (between “Reina de oro” and “Remolacha de Berisso”; with no shared alleles), with an average DAS of 0.64 among all the landraces (Electronic Supplementary Material, Table S2).

In order to easily represent the genetic relationship among all samples, a DAS-based dendrogram was developed. Therefore, the genotypes were grouped in three main groups (Figure 1). One group contained nine accessions with red flesh fruits (Group 1: “Ratto”, “Severiana”, “Remolacha de Berisso”, “Fragata”, “Remolacha de Leber”, “Gigaglia”, “Capri”, “Ciervita”, and “Giordano”) and within this group “Ratto” and “Severiana” were the closest genotypes (DAS = 0.14). A second group consisted of seven accessions with yellow flesh fruits (Group 2: “Reina de oro”, “Juanita”, “Tricerri”, “X”, “Gascón”, and the two introduced commercial genotypes “Santa Rosa” and “Reina Claudia”). The sample from the “X” landrace showed higher genetic similarity with “Tricerri” (DAS = 0.31) but differed from all the other samples. Finally, the third group contained the outgroup varieties, that is, the two peach samples (Group 3: “Sol de Mayo” and “Zelanda”).

Table 2. Information of the 12 analyzed polymorphic simple sequence repeat (SSR) markers in 14 local landraces of Japanese plums.

SSR Marker	Location	AR (pb)	N	Na	Ne	Ho	uHe	PIC
CPST011 (AY426199.1)	C 5 (4163117–4164052)	171–189	13	6	3.04	0.38	0.70	0.63
CPST018 (AY426204.1)	C 8 (123199–124139) 3e-	151–172	13	6	2.18	0.23	0.56	0.51
CPST021 (AY426206.1)	C 2 (27308766–27309699)	132–157	11	6	3.66	0.36	0.76	0.69
CPST022 (AY426207.1)	C 5 (16620819–16621314)	159–179	13	7	2.91	0.77	0.68	0.62
CPST024 (AY426209.1)	C 1 (28058204–28058903)	157–179	13	4	2.89	0.61	0.68	0.60
CPST025 (AY426210.1)	C 3 (6709070–6709740)	181–223	12	5	2.51	0.66	0.64	0.53
CPST026 (AY426211.1)	C 7 (11365276–11365753)	176–195	14	4	2.78	0.43	0.66	0.60
CPST027 (AY426212.1)	C 1 (23010058–23010528)	137–156	13	5	2.66	0.31	0.65	0.57
CPST030 (AY426215.1)	C 5 (15121388–15122031)	179–200	14	7	4.21	0.86	0.79	0.73
CPST034 (AY426219.1)	C 2 (29946149–29946699)	177–224	14	6	3.70	0.64	0.76	0.68
CPST042 (AY426226.1)	C 7 (16682143–16682742)	167–185	14	5	3.53	0.71	0.74	0.67
CPST044 (AY426228.1)	C 2 (20793617–20794126)	218–241	13	5	3.35	0.15	0.73	0.65
Mean				5.50	3.12	0.51	0.70	0.62
SD				1.00	0.58	0.23	0.06	0.06

Location on the reference genome of *P. persica*, C: chromosome, AR: allele size ranges (pb), N: sample size, Na: number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, PIC: polymorphism information content, SD: standard deviation.

Subsequently, the Bayesian analysis [22] supported the existence of a genetic structure among the studied genotypes, with a most probable value of $K = 3$ subpopulations. All the accessions except for “Ciervita” ($Q = 0.77$) were assigned to a group with Q values above 0.85. The low membership value (Figure 2a) for “Ciervita” suggests a possible genetic mixed origin of this variety.

Similarly, the DAPC analysis also retrieved $K = 3$ genetic groups according to cultivar flesh color (Figure 2b). This analysis supported the results obtained by the Bayesian model and the genetic distance results.

These three main groups had several private alleles, and half of these alleles were at a very low frequency in plums. For instance, the red pulp group (9 genotypes) presented 13 private alleles (9 SSRs) and 5 alleles with a frequency below 0.06. The yellow pulp group (7 genotypes) contained 26 private alleles (11 SSRs), of which 10 had a frequency below 0.08. Both peaches had 10 private alleles (8 SSRs), and all these alleles showed a frequency above 0.25.

Comparison of the genotypic and phenotypic data enabled the identification of a marker/allele related to flesh color phenotype. Marker CPST25 revealed an allele of 193 bp that was exclusively present in all the red flesh plums. Allele “193-bp” was present in a heterozygous or homozygous

state (Electronic Supplementary Material, Table S1). BLASTx search using the sequences harboring CPST25 against the GenBank non-redundant protein database revealed significant matches with *P. persica* protein FAR1-Related sequence 6.

Another SSR marker, CPST11, presented a private allele, allele “171-bp”. This allele was present in all the yellow flesh plums, except for the “Reina de Oro” cultivar, but absent from the red flesh plums (Electronic Supplementary Material, Table S1). BLASTx search using the sequences harboring CPST11 revealed significant matches with *P. mume* abscisic acid receptor PYR1 isoform X1. Both FAR1 and PYR1 are related to the abscisic-acid-mediated signaling pathway and to seed germination regulation [32].

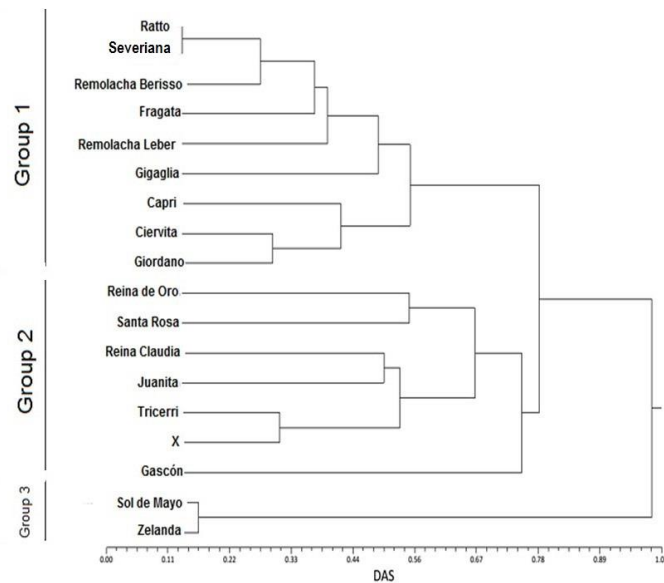


Figure 1. Cluster analysis. Dendrogram obtained by unweighted pair group method using arithmetic averages (UPGMA) calculated by shared allele distance (DAS) based on 12 SSRs. Group 1: red flesh plums, Group 2: yellow flesh plums, Group 3: outgroups (peach samples).

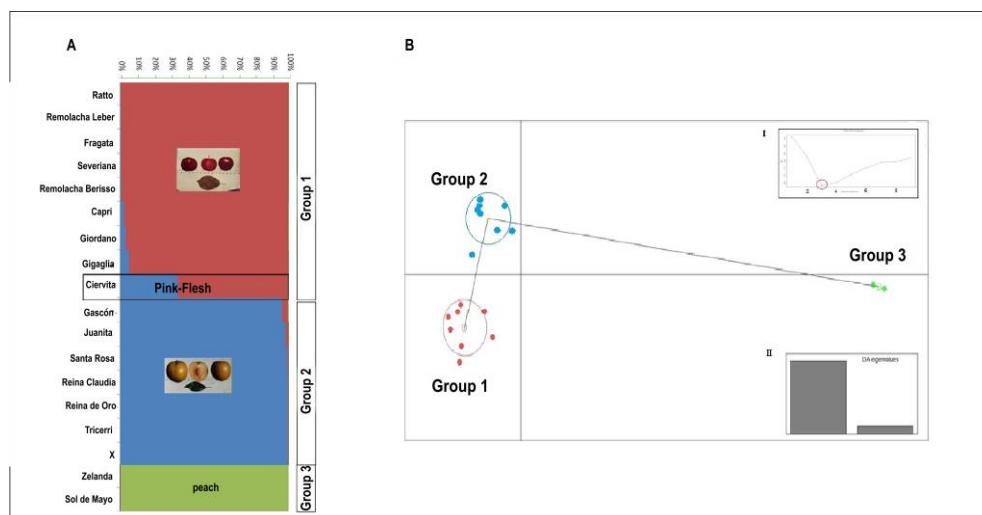


Figure 2. Estimated population structure. Genetic structure of 16 plum genotypes (14 landraces and two introduced cultivars) analyzed as estimated by (A) model-based Bayesian cluster analysis implemented in the program Structure; and (B) discriminant analysis of principal components (DAPC) implemented in the adegenet package of R software. Inset I: Inference of the number of clusters (Clusters vs. Bayesian information criterion (BIC)). Inset II: Relative importance of the two most important axes.

3.3. Mapping of SSR Markers on the *Prunus persica* Genome and Identification of Their Flanking Genes

The sequences containing polymorphic SSR were mapped and annotated on the reference genome of *P. persica*. The loci were physically mapped to a unique position on six out of eight chromosomes (1, 2, 3, 5, 7, and 8) (Table 2) with sequence similarities above 85%.

By an in-silico analysis, we identified 118 genes located within the 50 kb windows flanking the SSRs.

Interestingly, within these genes, some candidates were related to stress resistance (NAC transcription factor and glutathione S-transferases (GSTs)), fruit quality (Squamosa, AGAMOUS-like MADS box, Transducin/WD40 repeat-like superfamily protein and possibly NAC), plant growth and development (VQ motif-containing proteins and microspore-specific promoter proteins) (Electronic Supplementary Material, Table S3).

4. Discussion

In this study, we detected high levels of diversity within 14 different plum landraces from the PRD region that have been selected through the years by local growers. The diversity indexes were lower than those described by Carrasco et al. [14] ($He = 0.80$ and $Ho = 0.90$) and Ferrero Klabunde et al. [15] ($PIC = 0.80$ and $Ho = 0.77$) with eight SSR markers. However, this was expected due to a higher number of *P. salicina* cultivars analyzed (29 and 47, respectively). On the other hand, the PIC value detected in the present study was closer to the value obtained in a study of 24 cultivars from different areas in China evaluated with 16 SSR markers ($PIC = 0.7$) [33]. Likewise, the levels of diversity in our study were similar to or higher than those of other *Prunus* species, such as peach ($Ho = 0.35$; $He = 0.55$ [34]), apricot ($Na = 3.5$; $Ho = 0.58$ [35]), and cherry ($He = 0.66$; [36]).

Although the number of genotypes analyzed in this study was small, the degree of diversity was high. This could be due to the mating system of *P. salicina*. Indeed, *P. salicina* requires cross-pollination because of its strong gametophytic self-incompatibility system, and this characteristic makes it more diverse [37] and heterozygous. Cross-pollination may have played an important role in the evolution of landraces in the PRD region because early 20th century producers very likely multiplied their trees by seed planting instead of vegetative cloning.

The 12 SSRs allowed the unambiguous identification of the genotypes studied (DAS values between plum individuals ≥ 0.14). Thus, the 14 evaluated plum landraces were genetically unique (i.e., with SSR genotypes different from the rest). Cases of synonyms among samples with different names but identical SSR profiles at the 12 analyzed loci were discarded.

The cluster analysis (UPGMA) grouped the plum genotypes into two groups: plums with red and yellow flesh, respectively. Half of the private alleles of both groups occurred in a low frequency. Furthermore, the genetic relationship analysis supported the existence of genetic structure within the studied landraces. The two different methods of accessing genetic structure (Bayesian and DAPC analyses) coincided in differentiating the landraces based on the color of fruit flesh. The high Q values in the Bayesian study could reflect the selection history in response to flesh color.

Particularly, the membership of “Ciervita” to a group varied depending on the software applied. This characteristic suggests a genetic mixed origin in this landrace. Interestingly, “Ciervita” has fruits with a pink flesh phenotype.

Another interesting result is that “Santa Rosa” and “Reina Claudia” were grouped within Group 2, that is, based on a phenotypic characteristic of fruits (yellow flesh fruits). Because both are introduced commercial cultivars and have some private alleles, we would have expected that these cultivars were within another group, or at least that they would differ more from the rest of the genotypes within this group. With the use of more (or different) SSRs, we may obtain different results, and these independent cultivars would be separated from these groups.

By contrast, González et al. [17] found that the cultivars they evaluated in their study were grouped based on other characteristics. They used SNPs and EST-SSR markers developed from the putative flavonoid pathway transcription factors to study the genetic structure. Only when using

the specific markers EST-SSR (PsMYB10, PsMYB1, and PsbHLH35) did they obtain three clusters of cultivars related to the skin color: two cultivars with red skin fruits and a third cluster that grouped all the cultivars with yellow skin fruits.

Although nutritional traits like the anthocyanin content of plum fruit flesh are interesting, the reason for characterizing PRD landraces was because of their unique adaptation to a peculiar water-stressed ecosystem. Many genes may contribute to this adaptation, and for this reason the first goal was to represent-as much as possible-a plum genome with this rather limited number of molecular markers. The genetic mapping of SSR loci to the *P. persica* v2.1 genome enabled us to establish the position of the 12 polymorphic SSR markers used. The next step was to evaluate if these polymorphic markers were flanked, probably linked, to genes that could be associated to water stress conditions. The information of annotated genes nearby polymorphic SSRs (< 25 kb) allowed us to describe the context of the markers and provided a first step to future association studies by identifying candidate genes for the expression of important agronomical characteristics.

We identified 118 genes located within the 50-kb windows. Of these genes, 106 corresponded to homologous genes described in *Arabidopsis*. Anatomical and physiological comparisons between *Arabidopsis* and *Prunus* species indicated marked similarities between them [35]. Cautiously taking into account the phylogenetic distance between *Prunus* and *Arabidopsis*, as well as their enormous biologic differences, we found some similarities in predicted gene functions that were also found in various plant taxonomic groups. Therefore, we detected candidate genes related to stress resistance, fruit quality, and other interesting candidate genes (Electronic Supplementary Material, Table S3).

For instance, genes encoding Acyl-CoA N-acyltransferases (NAT) superfamily proteins found close to marker CPSCT021 are associated with flavor generation during ripening in some fruit species, such as apricot [38] and peach [39]. Marker CPSCT022 was also close to other genes associated with fruit ripening and quality: SQUAMOSA MADS Box [40] and AGAMOUS-like MADS box genes [41,42]. Interestingly, these two genes have been also associated with the metabolic pathway of anthocyanins responsible for fruit color. Indeed, they were associated with the regulation of anthocyanin accumulation or synthesis in pear and *Arabidopsis* [43,44], as well as in bilberry [45]. Anthocyanin biosynthesis is cooperatively regulated by transcriptional regulators, including WD40 proteins (adjacent to CPSCT042). These regulators form a complex that binds to promoters and activates the transcription of structural genes of the anthocyanin biosynthetic pathway [46]. In addition, an alpha/beta-hydrolases superfamily protein involved in delaying fruit senescence under low temperature in strawberry fruit [47] was close to marker CPSCT025 (Electronic Supplementary Material, Table S3).

Stress-related genes corresponding to the NAC (No Apical Meristem) domain transcriptional regulator superfamily protein and tau-type glutathione S-transferases (GSTs) [48,49] were located close to CPSCT024. Additionally, stress-related genes encoding reversibly glycosylated polypeptide (RGP family) [50] and the bZIP transcriptional regulator gene [51] were adjacent to CPSCT022 and CPSCT042, respectively.

The NAC protein consists of a large and complex family of transcription factors that are involved in multiple biological processes in plants, including perception of biotic/abiotic stress, signal transduction, transcription control, and gene activation. The NAC transcription factors regulate the differentiation of cells specialized for water conduction in vascular plants, and their conserved genetic basis suggests roles for NAC proteins in the adaptation of plants to land [52].

Interestingly, NAC (close to CPSCT024) was also related to cold-stress responses in *P. mume* [53], and to the metabolic pathway of anthocyanins responsible for fruit color in different plants such as peach, plum, and *Arabidopsis* [54–56].

On the other hand, the bZIP families described by Janiak et al. [51] have a role in gene expression regulation in roots and may have an impact on root development under drought stress conditions.

Furthermore, genes involved in plant growth and development encoding VQ motif-containing proteins and microspore-specific promoter proteins [57,58] were close to CPSCT011.

Although the evaluated sample size was too small to define associated markers by association mapping analysis through haploblocking definition (genetic blocks where alleles are grouped in linkage disequilibrium without recombination), pedigree history could also explain this result [13].

Further analyses are needed to assess the potential contribution of these genes to the specific adaptation of the local landraces to water-stressed environments. Some, or even all, of the allele associations described above, could still be attributed to demographic or management reasons that have nothing to do with adaptation to the environment.

In peach, an analysis of 53 SSRs distributed through the genome was carried out in 104 landraces from six Chinese geographical regions, determining an LD decay across all populations of 2500 kb [59]. However, lower LD decay was found in grape (~1300 kb) [60] and in sweet cherry (~100 kb) [8], independently of the number and type of molecular markers used. In our work, the evaluated sequences for searching interesting candidate genes were up to 25 kb distance from the SSR markers, so we expect that linkage between them is high enough to be considered in future breeding studies with association markers.

The findings described here could provide an interesting working hypothesis for future research in plum molecular breeding. The incorporation of the markers linked to the annotated genes in this study could be of high value in marker-assisted selection breeding programs and in future genome-wide association studies (GWAS).

5. Conclusions

This is the first study characterizing a representative sample of different plum landraces adapted to water-stressed environments, particularly in the PRD region of Argentina.

Twelve SSRs allowed us to estimate the genetic diversity and structure of PRD plums. These landraces showed a high degree of diversity and a differentiation between accessions with red and yellow flesh.

The genetic fingerprinting profiles could allow the identification and traceability of different genotypes in the various stages of breeding. Furthermore, these profiles could contribute to registering the assessed genotypes in the National Register of Cultivars. The results highlight the utility of bioinformatics to identify genes involved in complex characters to contribute to the understanding of the genetics behind the phenotypic variation. Potentially, the markers close to candidate genes are suitable for comparative QTL mapping, molecular-marker-assisted breeding, and for population genetic studies across different species within the genus *Prunus*.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/9/9/487/s1>. Table S1 SSR Genotyping Matrix. Table S2 Shared allele distance (DAS) Matrix. Table S3 Mapping and annotation of genes nearby (< 25 kb window) to polymorphic SSR on the *Prunus persica* genome.

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