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The genetic diversity of strawberry (*Fragaria ananassa* Duch.) hybrids based on ISSR markers

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ABSTRACT. The strawberry is an important agricultural crop in Brazil. However, most of the commercial genotypes currently in cultivation in Brazil were developed in other countries with environmental adaptations often inadequate for the regional conditions. In this work, inter-simple sequence repeat markers were used to determine the genetic variability and the loci segregation profiles of 84 strawberry hybrids obtained from a genetic breeding program at the 'Empresa de Pesquisa Agropecuária de Minas Gerais.' The hybrids were produced from crosses involving the following progenitors: 'Toyonoka' x 'Sweet Charlie', 'Camino Real' x 'Sweet Charlie', 'Oso Grande' x 'Sweet Charlie', 'Oso Grande' x 'Toyonoka', 'Dover' x 'Oso Grande', and 'Camino Real' x 'Toyonoka'. Fourteen genotypes were randomly sampled for each hybrid combination and evaluated. The results showed that the genetic profiles of the hybrids from each test cross were very diverse, most likely due to the high heterozygosity of the genome of each progenitor involved, which might indicate the presence of adequate genetic diversity among the hybrids to allow for the selection of new cultivars with agronomic traits that are more suitable to environmental conditions in Brazil.

Keywords: genetic dissimilarity, pre-breeding, molecular markers.

Diversidade genética de híbridos de morango (*Fragaria ananassa* Duch.) baseado em marcadores ISSR

RESUMO. O morango é uma importante cultura no Brasil. Contudo, a maioria dos genótipos comerciais cultivados foram desenvolvidos em outros países, com pouca adaptação aos ambientes locais. Neste trabalho, o marcador molecular do tipo sequências internas simples repetidas foi utilizado para avaliar a variabilidade genética e a segregação dos perfis genéticos de 84 híbridos de morango obtidos pelo programa de melhoramento desenvolvido pela Empresa de Pesquisa Agropecuária de Minas Gerais. Os híbridos foram obtidos dos cruzamentos entre 'Toyonoka' x 'Sweet Charlie', 'Camino Real' x 'Sweet Charlie', 'Oso Grande' x 'Sweet Charlie', 'Oso Grande' x 'Toyonoka', 'Dover' x 'Oso Grande' e 'Camino Real' x 'Toyonoka', sendo avaliados 14 genótipos escolhidos aleatoriamente para cada combinação híbrida. Os resultados obtidos indicam que os perfis genéticos entre os genótipos híbridos dentro de cada cruzamento são bem diversos, provavelmente devido a elevada heterozigosidade da constituição genômica de cada parental envolvido. Isso indica a existência de suficiente diversidade genética entre os híbridos que pode facilitar a seleção de novos cultivares com características agronômicas adequadas.

Palavras-chave: dissimilaridade genética, pré-melhoramento, marcadores moleculares.

Introduction

The cultivated strawberry (*Fragaria ananassa* Duch.) is an octaploid species of the family Rosaceae that has economic importance related to fruit yield. The cultivated species is a hybrid between (*F. virginiana* Duch.) and (*F. chiloensis* (L.) Duch) (HANCOCK et al., 1991), and it has been produced in various regions and climate types, including temperate, Mediterranean, and subtropical zones and taiga areas (HANCOCK; LUBY, 1993). Worldwide production of the strawberry recently reached four million tons (ZORRILLA-FONTANESI et al., 2011), and the state of Minas Gerais is the biggest strawberry producer in Brazil.

Strawberries are vegetatively propagated, with commercial plantations preferably using plants from meristem culture, and these methods aim to maintain the genetic constitution and desirable

characteristics of strawberries and to select for healthy seedlings. The strawberry has a moderate level of genetic variability, which can be exploited by breeding, which has allowed for the selection of promising genotypes adapted to several Brazilian conditions. These hybrids are propagated through the culture of meristems to be used in commercial plantations.

The evaluation of cultivars through genetic traits, such as studies on the characterization, identification, diversity and selection of markers linked with high performance traits (superior qualitative traits), can directly influence the quality and productivity of strawberry plants in the field. Furthermore, the use of molecular markers, which are highly effective and sensitive tools for performing such studies, is important to ensure the quality of materials that are introduced every day into productive fields. Moreover, in contrast to morph-agronomic traits, studies based on molecular genetics are not influenced by environmental effects.

DNA marker-based analyses can evaluate the genetic diversity among cultivars regardless of the physiological stage or organs being analyzed. This type of analysis has additional advantages, such as a short turnaround time for results and being highly informative (ARNAU et al., 2002). For example, ISSR (inter-simple sequence repeat) markers are routinely applied to the genetic characterization of a large number of biological samples. Such markers have been widely used with this approach because they are efficient, reproducible, and fast and generate high numbers of polymorphisms (KURAS et al., 2004; REDDY et al., 2002). In addition, this technique has a low associated cost.

The molecular marker techniques, including ISSR markers, are useful in the varietal identification of strawberry cultivars, can be used at any time of the breeding process and can be used to check the genetic fidelity of the steps during the clonal multiplication of genotypes. Debnath (2009) has used ISSR markers to track the clonal fidelity of micropropagated strawberry plants.

The use of ISSR markers for the evaluation of strawberry genotypes contributes a great deal of data on genetic polymorphisms. Arnau et al. (2002) verified that the ISSR technique requires only a few markers to achieve the correct fingerprinting pattern of strawberry cultivars, demonstrating the potential for this technique for genetic studies in strawberries. The studies performed by Kuras et al. (2004) and Debnath et al. (2008), with the goals of evaluating the genetic similarity and genetic diversity, respectively, of strawberry cultivars, also confirmed the technical feasibility of ISSR markers. Furthermore, the study performed by Hussein et al. (2008) developed an efficient fingerprinting pattern in six strawberry cultivars monitored with nine ISSR markers.

In the present study, the ISSR markers were used to assess the genetic variability of 84 strawberry hybrids obtained from the crosses of 5 commercial strawberry cultivars generated in a breeding program carried out by the Agricultural Research Agency of Minas Gerais (Epamig). This study used the Upgma dendrogram, principal coordinate analysis method and Bayesian genetic structure to analyze the data. The results presented here demonstrate that ISSR markers are a suitable tool for evaluating genetic polymorphisms and structure and can assist in the selection of new strawberry cultivars.

Material and methods

Strawberry genitors and hybrids:

The strawberry (*Fragaria ananassa* L.) plants were cultivated on the experimental farm of Epamig located in Nova Porteirinha, north of Minas Gerais State. Five strawberry genitors were used ('Toyonoka,' 'Sweet Charlie,' 'Camino Real,' 'Oso Grande,' and 'Dover'), and 84 strawberry hybrids were obtained from the crosses involving 'Toyonoka' x 'Sweet Charlie' (population1-pop1), 'Camino Real' x 'Sweet Charlie' (pop2), 'Oso Grande' x 'Sweet Charlie' (pop3), 'Oso Grande' x 'Toyonoka' (pop4), 'Dover' x 'Oso Grande' (pop5), and 'Camino Real' x 'Toyonoka' (pop6). Fourteen individuals were randomly sampled for each hybrid combination (Table 1).

DNA isolation:

Five young, completely expanded leaves were collected from each strawberry hybrid and each strawberry progenitor from plants cultivated in the field. The leaves were labeled, frozen in liquid nitrogen and stored at -80°C. The genomic DNA was extracted using the method described in Nunes et al. (2011).

Primers and PCR amplification:

The experiment was based on 5 ISSR markers (Table 2) that were previously selected by Arnau et al. (2002). These markers were anchored in the 5' end by three partially degenerated selective nucleotides.

Table 1. The strawberry progenitors and the 84 strawberry hybrids (14 plants for each hybrid combination). The hybrids are described by the following abbreviations and the individual hybrid number: TO, 'Toyonoka'; SC, 'Sweet Charlie'; CR, 'Camino Real'; OG, 'Oso Grande'; and DO, 'Dover'. *Source and characteristics of the fruit. Dover: United States of America (USA), firm fruit, acid, red color and good post-harvest storage; Oso Grande: USA, large fruit, firm and sweet; Camino Real: USA, large fruit, firm, dark-red coloration; Sweet Charlie: USA, early fruit, firm and sweet; Toyonoka: Japan, fruit with excellent aroma, flavor and average production (DUARTE-FILHO et al., 2007).

Progenitors			
ID	Name	ID	Name
85	Camino Real	88	Sweet Charlie
86	Dover	89	Toyonoka
87	Oso Grande		

Progeny							
ID	Name	ID	Name	ID	Name	ID	Name
H1	TO X SC 103	H22	CR X SC 16	H43	OG X TO 30	H64	DO X OG 8
H2	TO X SC 45	H23	CR X SC 19	H44	OG X TO 44	H65	DO X OG 7
H3	TO X SC 154	H24	CR X SC 123	H45	OG X TO 3	H66	DO X OG 28
H4	TO X SC 61	H25	CR X SC 56	H46	OG X TO 2	H67	DO X OG 80
H5	TO X SC 88	H26	CR X SC 50	H47	OG X TO 9	H68	DO X OG 29
H6	TO X SC 171	H27	CR X SC 21	H48	OG X TO 95	H69	DO X OG 30
H7	TO X SC 3	H28	CR X SC 29	H49	OG X TO 32	H70	DO X OG 71
H8	TO X SC 52	H29	OG X SC 146	H50	OG X TO 13	H71	CR X TO 105
H9	TO X SC 57	H30	OG X SC 169	H51	OG X TO 16	H72	CR X TO 115
H10	TO X SC 63	H31	OG X SC 164	H52	OG X TO 33	H73	CR X TO 134
H11	TO X SC 69	H32	OG X SC 9	H53	OG X TO 38	H74	CR X TO 6
H12	TO X SC 77	H33	OG X SC 25	H54	OG X TO 51	H75	CR X TO 13
H13	TO X SC 82	H34	OG X SC 172	H55	OG X TO 42	H76	CR X TO 91
H14	TO X SC 89	H35	OG X SC 35	H56	OG X TO 55	H77	CR X TO 142
H15	CR X SC 94	H36	OG X SC 179	H57	DO X OG 68	H78	CR X TO 8
H16	CR X SC 83	H37	OG X SC 170	H58	DO X OG 55	H79	CR X TO 56
H17	CR X SC 135	H38	OG X SC 162	H59	DO X OG 62	H80	CR X TO 43
H18	CR X SC 101	H39	OG X SC 38	H60	DO X OG 3	H81	CR X TO 23
H19	CR X SC 1	H40	OG X SC 30	H61	DO X OG 56	H82	CR X TO 47
H20	CR X SC 24	H41	OG X SC 20	H62	DO X OG 188	H83	CR X TO 52
H21	CR X SC 37	H42	OG X SC 6	H63	DO X OG 59	H84	CR X TO 2

Table 2. List of the ISSR markers used in this study.

Primer sequence	Annealing temperature (°C)
5'VBVACACACACACACAC3'	51
5'BDBCACACACACACACA3'	51
5'HBHCTCTCTCTCTCTCT3'	47
5'GCVTCTCTCTCTCTCTC3'	49
5'BDVAGAGAGAGAGAGAG3'	47

V is non T, B is non A, H is non G, and D is non C.

The DNA amplifications were performed in a gradient thermocycler (MultiGene, USA) at a final volume of 25 μL for each reaction, which contained 20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 100 mM KCl, 0.10% Triton X-100, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 0.8 μM primer, 2.4 units of GoTaq Flexi DNA polymerase (Prodimol, USA) and 50 ng of DNA. In the PCR reactions, the samples were initially subjected to 95°C for 2 min. and then 40 cycles of amplification. Each cycle involved the following steps: 95°C for 45 s, annealing at 47-51°C (depending on the primer used) for 1 min and extension at 72°C for 2 min. After the 40 cycles, the samples were kept at 72°C for 5 min., for a final extension step. The products obtained from the PCR were separated by electrophoresis in a 1.5% agarose gel with 0.2 μg mL^{-1} EtBr under a 1X TBE buffer that was run at 100 mV for at least 3h. All gels were visualized by and recorded with a UV image digitalizer (Uvitec, USA).

Data analysis:

The ISSR-amplified fragments behave as dominant markers and were tabulated as binary haploid data, scored as present (1) or absent (0) across all genotypes for each locus. The molecular weight of each fragment was estimated based on the DNA marker (50 bp DNA Ladder, 50 μg [1.0 μg μL^{-1}], Invitrogen, USA). The markers were analyzed using the program "Genealex 6" (PEAKALL; SMOUSE, 2006), which performed the principal coordinate analysis (PCoA). Additionally, we used the software "Powermarker" (LIU; MUSE, 2005) to examine the parameter of average expected heterozygosity or gene diversity in each progeny for all five ISSR primers. Dendrograms were constructed using the Upgma clustering method based on the dissimilarity matrix generated by simple matching coefficients for all individual progeny and their five parents; this analysis was performed with the software "GENES" (CRUZ, 2006). The bootstrap values were obtained with the software "dBood" (COELHO, 2001), and the analysis was performed with 10,000 bootstraps.

The application "Structure 2.3.3" (PRITCHARD et al., 2000) was used to determine the genetic structure of the 89 genotypes analyzed in this study; the Bayesian method was applied, and the number of groups (k) was set as the most liable adjustable parameter. The admixture model with independent allele frequencies was applied using the Markov Chain Monte Carlo algorithm, and it was run at a burn-in period of 10,000 steps and a chain length of 100,000 replicates during the analysis. Twenty simulations were performed for each value of k, with the k values ranging from one to twelve.

The Δk statistical test was performed using the "Structure Harvester" program (EARL; VONHOLDT, 2012) based on the criterion suggested by Evanno et al. (2005). This criterion is based on the mean and standard deviation of the log probability of the data [lnP(D)] obtained for each value of k during the 20 simulations. The Δk value was estimated for each k to obtain the greatest value. After the optimum Δk value was selected, the lower lnP(D) value was selected from among the 20 simulations for each value of k. A graph for each replicate run was generated, and each color generated represented a group of structured individuals.

Results and discussion

The results of the 5 ISSR markers optimized for strawberry identification showed that they could efficiently individualize all genitors and hybrids, generating unique profiles of all the sibling progenies except H39 $H40^{-1}$ and H50 $H55^{-1}$. To ensure the reproducibility of the ISSR band patterns, two independent amplifications were performed for each ISSR marker (Figure 1). The results observed in this study agree with those of Arnau et al. (2002), who showed a high reproducibility of their ISSR markers even though they used a different DNA extraction method to perform the amplification. The 5 ISSRs were capable of screening 41 loci, generating 100% polymorphic bands considering all individuals assessed. Dhanorkar et al. (2005), working with grape varieties, obtained similar results using ISSR markers. These researchers observed an average of 10.69 bands per ISSR primer in the agarose gels. The molecular profile generated by each ISSR primer used is presented in Figure 2. Considering all the data, three and four specific absence of bands, respectively to 'Camino Real' and 'Dover', gives a clue of their differences in relation to the other parental individuals (data not shown). The next analyzes in this article will show the agreement with this observation.

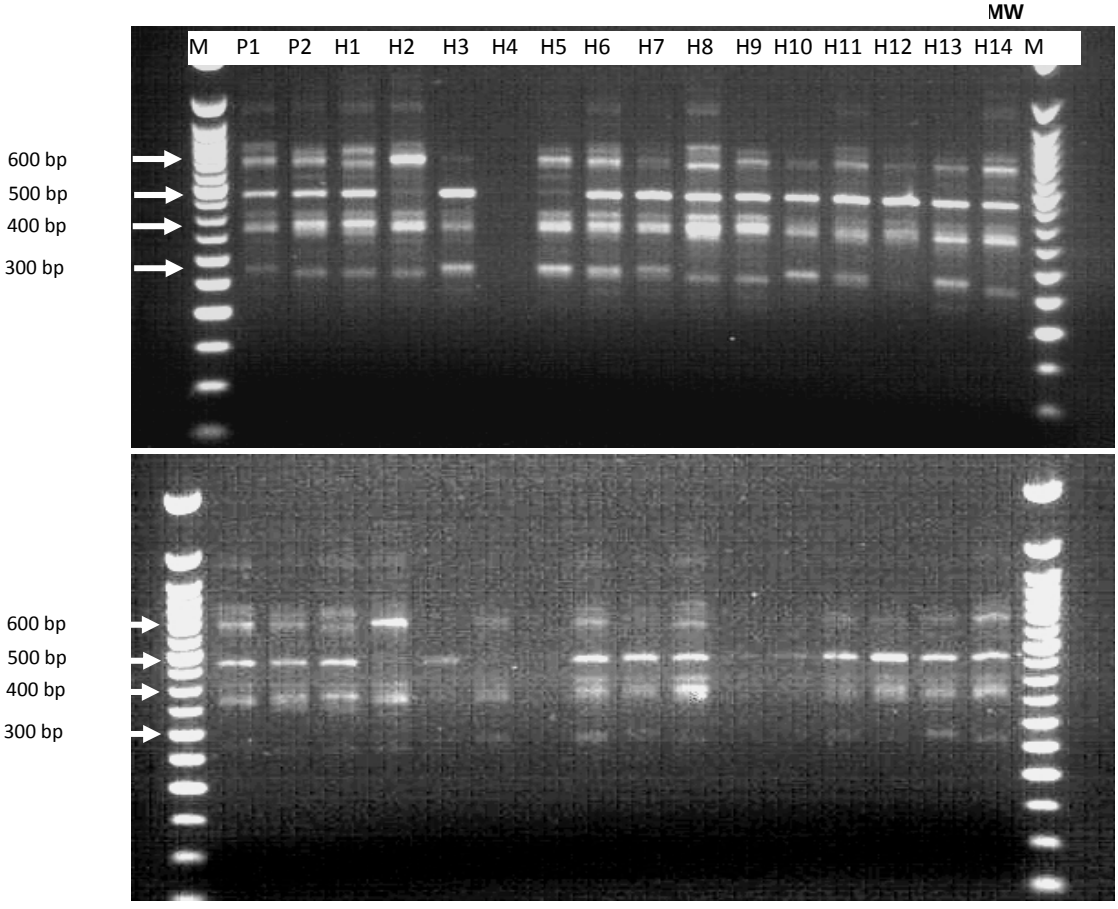

Figure 1. The ISSR reproducibility of PCRs with the primer BDV $(AG)_7$ on gels A and B. The arrows indicate scorable DNA bands, and the samples were identified according to the description in Table 1. M represents molecular weight (50 bp DNA ladder).

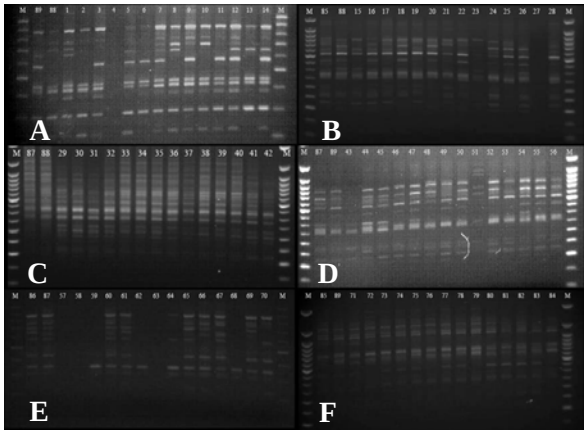

Figure 2. The molecular profiles of the ISSR markers in strawberry samples in agarose gels (1.5%) stained with EtBr (0.2 μg mL^{-1}). A) Primer VBV-$(AC)_7$; B) primer HBH-$(CT)_7$; C) primer BDV-$(AG)_7$; D) primer HBH-$(CT)_7$; E) primer BDB-$(CA)_7$ and F) primer GCV-$(TC)_7$. M represents molecular weight (50 bp DNA ladder).

A UPGMA dendrogram was generated based on a simple matching dissimilarity coefficient for all full-sibling progenies, including the parental individuals (Figure 3). Importantly, each individual had a clearly distinct location on almost all accessions evaluated, except two profiles, indicating the efficiency of these markers in fingerprinting the strawberry samples. These results are congruent with those of Arnau et al. (2002), who showed the highly discriminatory nature of their ISSR markers. The data presented by Hussein et al. (2008) are also congruent, showing the efficiency of a few ISSR markers in correctly identifying and discriminating among strawberry genotypes. The dendrogram divided our panel into two basic groups. The small group included the progeny from the crosses 'Toyonoka' x 'Sweet Charlie' and 'Oso Grande' x 'Sweet Charlie' and the parents 'Oso Grande,' 'Sweet Charlie,' and 'Toyonoka.' The large group included the progeny from the remaining crosses and the parents 'Camino Real' and 'Sweet Charlie.'

Zorrilla-Fontanesi et al. (2011), studying QTLs for agronomic traits and fruit quality, observed a highly transgressive pattern for most of the evaluated traits over three years in the strawberry. Strawberry cultivars preserve a high level of heterozygosity due to their vegetative propagation, and outcrossing between distinct cultivars is expected to have a high potential to create progeny with new allele combinations, generating genotype diversity and, consequently, new phenotypes that might show better performance for ambient adaptation, including environments where plants may be exposed to biotic and abiotic stresses.

However, the selection of superior individuals based on their agronomic and morphologic behaviors, and thus of the most promising progenitors, is a crucial step in any breeding program. Approaches that allow the previous knowledge of genetic potential to generate variability can optimize breeding programs by selecting the best progenitors to use for the hybrid crosses.

Therefore, the bi-dimensional dispersion PCoA was applied to all strawberry genotypes (including the progeny and the 5 progenitors used in this study) to visualize the genetic distance of each individual (Figure 4). Considering all 89 individuals, the first two axes together explained 46.85% of the variance. Again, there is high divergence among the full-sibling individuals from their respective parents. The level of explanation derived from the first two axes of the PCoA support an unambiguous high genetic divergence of the progenies. In addition, the explanation of less than 60% suggested the need to add more loci to more fully scatter the individuals in the dispersion using molecular data (ANDERSON; WILLIS, 2003). Debnath et al. (2008) also used this approach to understand and establish the genetic relationships among strawberry cultivars in Canada. These authors obtained 8.0, 6.7, and 6.5% explanation from the first, second and third axes, respectively. In our results, the first coordinate was able to scatter the individuals originating from the crosses 'Toyonoka' x 'Sweet Charlie' (Pop1) and 'Oso Grande' x 'Sweet Charlie' (Pop3), the five parents, and some individuals from the cross 'Dover and Oso Grande' (Pop5) on the right side. This allocation agrees partially with the dendrogram grouping. The left side also agrees with the large group in the dendrogram, with some exceptions. The PCoA approach provides the relative location of the individuals according to their dissimilarities and serves as a useful complementary analysis to understand and visualize the genetic diversity of the analyzed individuals. Kuras and Korbin (2010) also applied the PCoA method to data from RAPD, ISSR, SSR, and AFLP markers to determine the extent of the genetic relationships among 96 strawberry cultivars across several countries in the world, and they successfully detected nine clusters, with the clusters formed mainly between cultivars originating from the same breeding center.

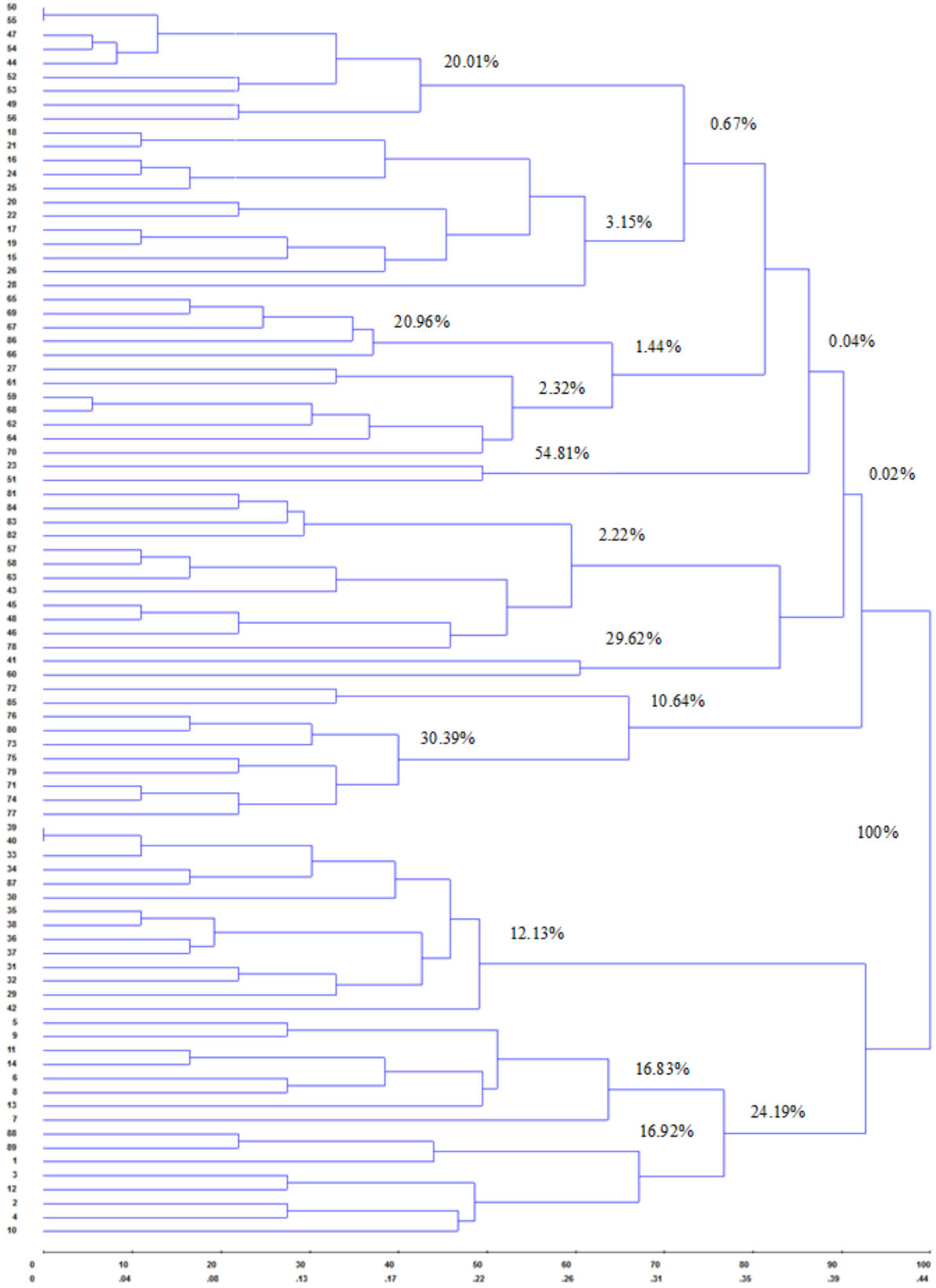

Figure 3. The dendrogram obtained by the simple matching dissimilarity coefficient showing the genetic relationships among the 5 parents and 14 individuals per hybrid combination. All individuals are identified according to the description given in Table 1.

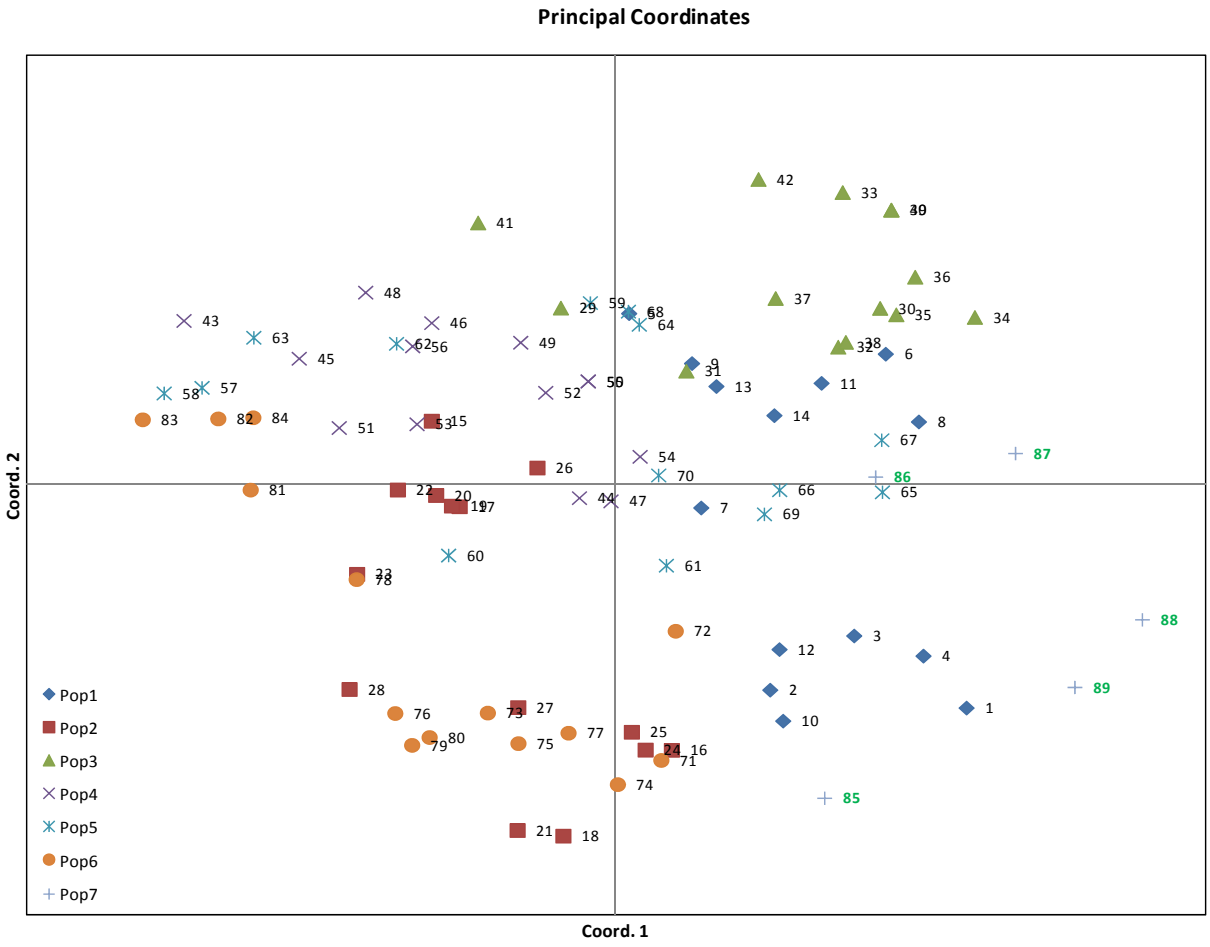

Figure 4. The bi-dimensional dispersion according to the principal coordinates analysis obtained using the GenAlex software. The progeny are represented by population 1 'Toyonoka' x 'Sweet Charlie', population 2 'Camino Real' x 'Sweet Charlie', population 3 'Oso Grande' x 'Sweet Charlie', population 4 'Oso Grande' x 'Toyonoka', population 5 'Dover' x 'Oso Grande', and population 6 'Camino Real' x 'Toyonoka', and the 5 progenitors are represented by blue crosses (identified as Pop 7). All individuals are identified according to the description in Table 1.

A comparison of the data of the expected heterozygosities for all progenies revealed that the range varied from 18.44% ('Oso Grande' x 'Sweet Charlie') to 26.38% ('Dover' x 'Oso Grande') (Table 3), showing that crosses between some parents were more effective in generating genetic variability than others. The expected heterozygosity, also known as gene diversity, may indicate their potential use for hybrid vigor. Therefore, we might conclude that these two cultivars are the best choices for generating better progeny.

However, comparing only the five genitor genotypes, Table 4 shows that the genetic distance between 'Dover' and 'Oso Grande' (0.1129) is not the greatest. Using ISSR markers, Graham et al. (1996) reported low levels of genetic diversity found in cultivars developed independently in several breeding programs across the world. Debnath et al. (2008) also used ISSR markers to monitor both the level of genetic diversity between cultivars and the low selection advances in the Canadian breeding programs. In addition to the general low level of dissimilarity (0.1435) between the cultivars used as parents in our breeding program, the overall analysis, including all 84 hybrids originating from these five parents, revealed that the mean level of dissimilarity increased to 0.3937.

Table 3. The genetic parameters used in the identification and characterization of the progeny of crosses between 'Toyonoka' x 'Sweet Charlie', 'Camino Real' x 'Sweet Charlie', 'Oso Grande' x 'Sweet Charlie', 'Oso Grande' x 'Toyonoka', 'Dover' x 'Oso Grande', and 'Camino Real' x 'Toyonoka'. Each cross is represented by 14 individuals.

Individuals	Band presence	Expected Heterozygosity
H1 to H14	0.7909	0.2563
H15 to H28	0.8275	0.2227
H29 to H42	0.8659	0.1844
H43 to H56	0.8554	0.1949
H57 to H70	0.7840	0.2638
H71 to H84	0.8171	0.2427
Parents	0.8439	0.2029

Table 4. The genetic divergence between the 5 strawberry parents by a simple matching dissimilarity coefficient.

Crosses	Dissimilarity
'Camino Real' x 'Dover'	0.2419
'Camino Real' x 'Oso Grande'	0.1935
'Camino Real' x 'Sweet Charlie'	0.1612
'Camino Real' x 'Toyonoka'	0.1290
'Dover' x 'Oso Grande'	0.1129
'Dover' and 'Sweet Charlie'	0.2419
'Dover' x 'Toyonoka'	0.2419
'Oso Grande' x 'Sweet Charlie'	0.1290
'Oso Grande' x 'Toyonoka'	0.1612
'Sweet Charlie' x 'Toyonoka'	0.0645

The highest dissimilarity was 0.8293, between hybrid #43 and the accession 'Sweet Charlie' (ID 88) (data not shown). This survey achieved our goal, which was the liberation of the genetic variability via these artificial crosses for further selection.

Although two genotypes may share many bands, the presence of a band in both genotypes does not necessarily indicate that they have the same genetic constitution. Indeed, because the ISSR is a dominant marker, the presence of a band indicates that a specific locus is either homozygous or heterozygous. Thus, the dominant nature of the marker generally reduces the informativeness of that marker by up to one-tenth compared with a co-dominant marker (EVANNO et al., 2005).

Another aspect of this approach that should be emphasized was the sample size. Most likely, 14 individuals per outcrossing do not represent all combinations possible in the offspring, but they were representative enough to indicate the potential of the crosses between two hybrid parental genotypes, as indicated by the results presented here.

Another interesting approach used was the Bayesian statistics performed with the Structure 2.3.3 software. The use of this extraordinarily robust technique is becoming very common in plant genetic diversity studies, such as in the common bean (BURLE et al., 2010) and coffee (SETOTAW et al., 2010). The results of this analysis were assessed by the Delta K statistical test, implemented in Structure harvester. The highest Delta K value (298.97) was generated by K = 2, indicating that the genetic structure produced by dividing all genotypes into two structured groups is the most likely. Among the twenty independent runs with K = 2, run #29 had the lowest module value of LnP(D) (|-1886.1|). Thus, this run was selected to observe the genetic structure of all the genotypes presented in Figure 5.

The results clearly demonstrate the genetic structuration of the progeny (each progeny was labeled according to the material and methods used, with the corresponding number inside the brackets). The authentication of the crosses is also potentially confirmed by the additional support of the Bayesian statistic, i.e., the progeny individuals are well structured. Importantly, the progeny from the cross 'Dover' and 'Oso Grande' are not well structured, presenting two types of genetic structure and an intermediate structure (membership coefficient lower than 0.8) in some genotypes. Therefore, this progeny has the most diverse genotype according to the Bayesian structure; furthermore, these results indicate that the cross between 'Dover' and 'Oso Grande' is the most promising for the generation of new hybrid combinations and heterotic groups. In addition, the first two groups formed by the dendrogram are almost congruent with the Bayesian approach, with only a few (9 out 89) differences inherent to each analysis.

Adequate correlation between the molecular data and the morphological and agronomic traits was lacking because these hybrids were still under field evaluation. However, the ISSR results generated for these outcrosses were valuable because they provided effective knowledge of the genetic constitution of each hybrid compared with their progenitors and siblings. Therefore, these results open opportunities to conduct field trials in a more directed way, focusing mainly on the most divergent genotypes.

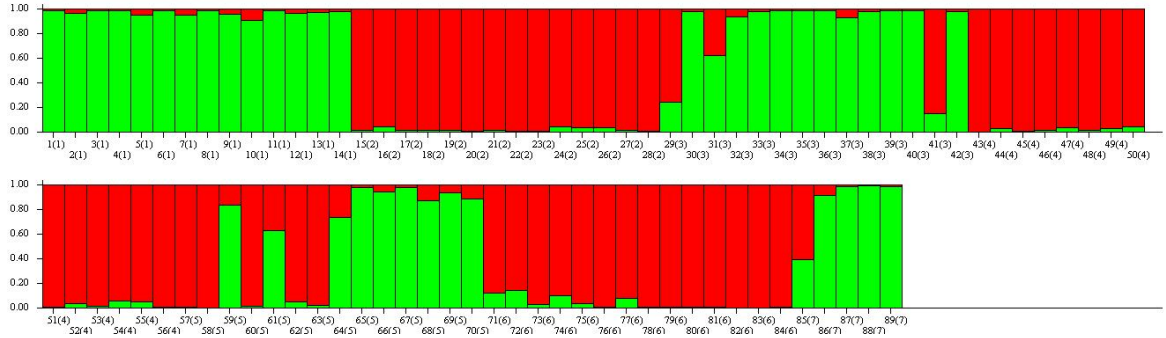

Figure 5. The Bayesian genetic structure of strawberry individuals assessed by ISSR markers. The number outside the brackets represents the individual ID, and the number inside the brackets represents the population (cross – according to Table 1), except for population 7, which was represented by the 5 parents. The vertical bars indicate the relative amount of membership coefficient of each individual in that structured group.

Therefore, the chance of selecting phenotypes with better agronomic performance increases, based not only on their environmental responses but also their genetic constitution.

Conclusion

The ISSR markers used showed suitability and efficiency in discriminating among 82 of the 84 hybrids and all progenitors, revealing the genetic variability of each strawberry genotype. This genetic diversity among strawberry genotypes may be useful for the selection of new varieties with improved performance in the field.

Additionally, the results showed that the hybrids from the cross between 'Dover' and 'Oso Grande' were the most genetically divergent.

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