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Genetic diversity and population structure analysis of strawberry (*Fragaria* x *ananassa* Duch.) using SSR markers

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Abstract In total, 18 simple sequence repeat (SSR) markers were used to analyze the genetic diversity and population structure of 59 accessions of cultivated strawberry (*Fragaria* x *ananassa* Duch.) from Korea, Germany, United States, United Kingdom, and Japan. In total, 101 alleles were detected with an average of 5.6 per locus and 21 specific alleles were identified. Notably, one genotype (Blonoli from Germany) possessed a maximum of 10 different unique alleles specific to each genotype. The gene diversity varied from 0.027 (EMPaEKO1B) to 0.791 (CFACT110), with an average value of 0.509. PIC values ranged from 0.026 to 0.762 (average 0.454). A model-based structure analysis revealed the presence of two populations. The accessions that were clearly assigned to a single population in which > 70% of their inferred ancestry was derived from one of the model-based populations. However, two accessions (3.4%) in the sample were categorized as having admixed ancestry. Here, we report detailed information on commercially grown strawberry accessions from five different origins using SSR markers. These results couldbe used for broadening the genetic base of commercially grown varieties.

Keywords: genetic diversity, population structure, SSR markers, strawberry

INTRODUCTION

The commercially important strawberries (*Fragaria* x *ananassa* Duch.) belong to the family Rosaceae and the genus *Fragaria*, which comprises 23 species (Rousseau-Gueutin et al*.* 2009). With its high nutritional value, the strawberry is one of the most popular berry fruits in the world. All of these strawberries have seven basic types of chromosomes. However, they exhibit a series of ploidy levels, ranging from diploid species such as *Fragaria vesca* (2n = 2x = 14), to decaploid species, such as some accessions of *Fragaria iturupensis* (2n = 10x = 70). The cultivated strawberry, *F.* x *ananassa,* is an octoploid (2n = 8x = 56) (Nathewet et al. 2010). Because of this, it is difficult to breed it successfully to develop new varieties with differing genetic characteristics. Marker-assisted selection (MAS) will be of benefit with the help of polymorphic markers. However, for MAS, a genome scan of the progeny is

currently not possible because of a lack of evenly distributed markers that are polymorphic in the respective breeding population (Shulaev et al. 2008).

Microsatellites or simple sequence repeats (SSRs) represent a valuable tool for genetic studies (Lewers et al. 2005). Additionally, their transferability among closely related species or genera provides an important tool for molecular ecology and evolutionary biology, such as for addressing the mechanism(s) involved in population divergence and speciation (Barbara et al. 2007) or for studying genome evolution (Rousseau-Gueutin et al. 2008).

Knowledge of the genetic diversity and population structure of germplasm collections is an important foundation for crop improvement. Before performing an association mapping study, it is essential to first define the population structure within the germplasm to avoid spurious associations (Flint-Garcia et al. 2005). Thus, several researchers have developed markers and performed diversity and population structure analyses in different areas, such as Chung and Park (2010) and Cui et al. (2010) in rice, Cho et al. (2010) in Proso millet, Moe et al. (2010) in Cymbidium, and Zhao et al (2010) in garlic. Davis et al. (2006), Shimomura and Hirashima, (2006), Govan et al. (2008) and Gil-Ariza et al. (2009), developed molecular markers and used them to characterize germplasm collections in strawberries. In species with complex genomes, SSRs are the markers of choice in genetic and breeding studies, because of their high polymorphism and reproducibility, multi-allelic nature, and codominant inheritance (Zorrilla-Fontanesi et al. 2011). Several studies have shown a high level of transferability within the *Fragaria* genus (Bassil et al. 2006; Davis et al. 2006; Gil-Ariza et al. 2006; Monfort et al. 2006). Varshney et al. (2005) suggested that genic-SSRs were good candidates for the development of conserved orthologous markers for genetic analysis across related genera. Thus, SSR markers have been increasingly used in molecular and genetic studies in recent years. Here, we evaluated the genetic diversity and population structure of 59 cultivated strawberry accessions collected from five countries, using 18 selected polymorphic SSR markers.

MATERIALS AND METHODS

Plant material and DNA extraction

In total, 59 cultivated strawberry accessions collected from five countries (24 accessions from Japan, 10 from Korea, 10 from USA, 3 from Germany, 1 from UK, and 11 other unpublished accessions) were received from the National Horticultural and Herbal Research Institute, Suwon (Table 1). Genomic DNA samples from all strawberry accessions were extracted from young leaf tissue using the NucleoSpin Plant-II Kit (Macherey-Nagel). The relative purity and concentration of the extracted DNA was estimated with the NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). The final DNA concentration was adjusted to 20 ng/µL for polymerase chain reaction (PCR).

SSR analysis

In total, 18 different polymorphic markers were selected from the manuscript developed by Sargent et al. (2006); Sargent et al. (2007); Sargent et al. (2008) and used for the SSR assays. A three-primer system (Schuelke, 2000) was used that included a universal M13 oligonucleotide (TGTAAAACGACGGCCAGT) labelled with one of the fluorescent dyes 6-FAM, NED, or HEX, which allows PCR products to be triplexed during electrophoresis. A special forward primer, consisting of a concatenation of the M13 oligonucleotide and a specific forward primer, was used with the normal reverse primer for SSR PCR amplification.

PCR amplification was performed in a total volume of 20 µL containing 20 ng genomic DNA, 2 µM of the specific primer, 4 µM M13 universal primer, 6 µM normal reverse primer, 1 x PCR buffer, 0.2 mM dNTP, and 0.5 U *Taq* polymerase. Conditions of the PCR amplification were as follows: 94ºC (3 min), 30 cycles at 94ºC (30 sec), the appropriate annealing temperature (45 sec), and 72ºC (45 sec), followed by 20 cycles at 94ºC (30 sec), 53ºC (45 sec), 72ºC (45 sec), and a final extension at 72ºC for 20 min. The PCR products of three microsatellites were mixed together in a ratio of FAM:HEX:NED = 1:2:2, which was varied depending on the amplification intensity for individual markers as determined using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR products labelled with HEX and NED were added in higher amounts, and those labelled with FAM were added in lower amounts because of the different signal intensities of these fluorescent dyes. The mixed

PCR product (1.5 µL) was combined with 9.2 µL Hi-Di formamide and 0.3 µL of an internal size standard, Genescan-500 ROX. The samples were denatured at 94ºC for 3 min and analyzed using an ABI PRISM 3100 Genetic Analyzer. Molecular weights, in base pairs, of the microsatellite products were estimated with the Genescan software (ver. 3.7; Applied Biosystems) by the local Southern method. The individual fragments were assigned as alleles of the appropriate microsatellite loci with the Genotyper software (ver. 3.7; Applied Biosystems).

Genetic diversity and population structure analysis

Basic statistics were calculated using the genetic analysis package PowerMarker (ver. 3.23; Liu and Muse, 2005) for diversity measurements at each microsatellite locus, including the total number of alleles (N_A), allele frequency, major allele (allele with the highest frequency), accession-specific alleles, gene diversity (GD), and polymorphism information content (PIC). Genetic distances between each pair of accessions were measured by calculating the shared allele frequencies using PowerMarker (ver. 3.23). The same program was used to test the Hardy-Weinberg equilibrium (HWE) and pair-wise linkage disequilibrium (LD). A neighbour-joining (NJ) algorithm was used to construct a phylogram from a distance matrix using the MEGA4 software (Tamura et al. 2007) embedded in PowerMarker. A bootstrap (resampling) test was performed 1,000 times to determine the distances between the accessions using PHYLIP version 3.69 (PHYLogeny Inference Package) programs (Felsenstein, 1989; Felsenstein, 2005).

Population structure and identification of admixed individuals was performed using the model-based software program, STRUCTURE 2.2 (Pritchard et al. 2000). In this model, a number of populations (K) are assumed to be present, each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned to populations (clusters), or jointly to more populations if their genotypes indicate that they are admixed. All loci are assumed to be independent, and each K population is assumed to follow Hardy-Weinberg equilibrium. The posterior probabilities were estimated using the Markov Chain Monte Carlo (MCMC) method. The MCMC chains were run with a 50,000 burn-in period, followed by 50,000 iterations using a model allowing for admixture and correlated allele frequencies. At least five runs of STRUCTURE were performed by setting K from 2 to 10, and an average likelihood value, L (*K*), across all runs was calculated for each K. The model choice criterion to detect the most probable value of *K* was *ΔK*, which is an ad hoc quantity related to the second-order change in the log probability of data with respect to the number of clusters inferred by STRUCTURE (Evanno et al. 2005). An individual having more than 70% of its genome fraction value was assigned to a group.

RESULTS

SSR polymorphism

SSR polymorphisms were measured in terms of the numbers of alleles, gene diversity, and PIC, using PowerMarker software (ver. 3.23; Liu and Muse, 2005). The 18 SSR markers revealed 101 alleles across the 59 accessions, with an average of 5.6 alleles per locus (Table 2). The allele size ranged from 100 (EMFax381869) to 376 (CFACT100) bp. The allelic richness per locus varied widely among the markers, ranging from 2 (CFACT100 and EMPaEKO1B) to 10 (CFACT110) alleles. In total, 21 specific alleles were identified at 18 polymorphic markers. It was noted that at least one rare allele was detected at all loci, except for CFACT100. The maximum specific allele (5) was identified at the locus EMFnCEL2. All rare alleles identified at this locus are genotype-specific alleles. The result showed that rare alleles (frequency < 0.05) comprised 48.5% of all alleles, whereas intermediate (frequency 0.05-0.50) and abundant alleles (frequency > 0.50) comprised 38.6% and 9.9% of all detected alleles, respectively. This result indicated that most alleles concentrated at a low frequency among the accessions studied (Figure 1, Table 2). A maximum of six accession-specific alleles was detected at the loci EMFax381827 (Table 2). Of the 59 genotypes, one (Johong from Korea) possessed three unique alleles and two (Viwa rose from Germany and Wiltguard from USA) possessed two unique alleles for each genotype, while the other four genotypes (Terunoka from Japan, three from Korea; Sakyejul, Seolhong and AC1466) each possessed only one allele. Notably, one genotype (Blonoli from Germany) possessed a maximum of 10 different unique alleles specific to each genotype. A Bonferroni correction for multiple comparisons was applied to the HWE at a significance level of $P < 0.05$, and 15

loci deviated from HWE. Highly significant (P < 0.01) LD values were observed between 14 pairs of loci (Table 2).

The gene diversity and PIC values ranged from 0.027 (EMPaEKO1B) to 0.791 (CFACT110) and from 0.026 (EMPaEKO1B) to 0.762 (CFACT110), with averages of 0.509 and 0.454, respectively. The major alleles frequency per locus varied from 0.302 (CFACT110) to 0.986 (EMPaEKO1B) with the average of 0.586. The highest heterozygosity (1.00) was detected at three loci, EMFax381877, EMFnCEL2, and CFACT084, followed by loci EMFax380097 (0.980), EMFax381869 (0.980), EMFn125 (0.959), CFACT100 (0.927), and EMFxaEKO1Ba (0.906), respectively. Heterozygosity was lowest at locus EMFaxCAD2 (0.018), followed by loci EMPaEKO1B (0.27) and EMFxaPDC2 (0.067; Table 2).

Genetic diversity

A genetic distance-based analysis was performed by calculating the shared allele frequencies among the 59 accessions, and a neighbour-joining phylogram (Figure 2) was computed using PowerMarker (3.23) and the Mega 4 software (Tamura et al. 2007). Colours were used to stain according to the results of a model-based cluster analysis. In the phylogram, all strawberry accessions clustered into two main groups (and GI and GII), except two accessions which are recognized as admixture. Group I consisted of 22 accessions [red colours: 8 unpublished species including 2 from Germany, 2 from USA, 1 each from Germany, Korea, and United Kingdom; green colours: 4 accessions from USA (Prelude, Sweet Charlie, Tamar and Yael), 3 from Japan (Himiko, Akanekko, Sungkang 16), and 1 from Korea (Seolhyang). The other 37 accessions were included in Group II (Figure 2). Group II can be subdivided into four subgroups. GII-1 consisted of 15 accessions (8 from Japan, 5 from Korea, and 2 from USA). Subgroup GII-2 consisted of 15 accessions (10 from Japan, 3 from Korea, and 2 from USA). There are four accessions in subgroup GII-3 (3 from Korea and 1 from Japan). Two accessions, Tochinomine from Japan and Mihong from Korea, belong in subgroup GII-4. One unpublished accession from Germany stands as an outstanding accession; it is distinctly different from all the other accessions examined.

Average diversity statistic values of these five genetic groups (origin-based) showed that three genotypes from Germany were highly diverse, with the highest value in gene diversity (0.59), heterozygosity (0.70), and PIC (0.52). The second highest heterozygosity was found in the group from USA (0.67), followed by the groups from Japan (0.66) and Korea (0.64). The lowest gene diversity (0.46) and PIC (0.39) values were identified in Japan with the largest number of accessions (25) (Table 3).

Population structure analysis

A model-based clustering method was performed for all 59 accessions using 18 SSR markers. However, inference of the exact value of K (gene pool) was not straightforward because the estimated log–likelihood values appeared to be an increasing function of K for all examined values of K. Thus, it may not be possible to determine a true value of K (Figure 3a). Instead, another ad hoc quantity (ΔK) was used (Evanno et al. 2005) to overcome the difficulty in interpreting the real K value. A relatively high value of ΔK for 59 accessions was found for K = 2 (Figure 3b). At K = 2, the highest number of accessions assigned to one specific cluster with a probability higher than 96%. Analysis of these data identified the major substructure groups when the number of clusters was set at 2 with a relatively high value of ΔK and high probability of accessions assigned to one specific cluster.

Of the 59 strawberry accessions, 57 (96.6%) shared > 70% membership with one of two clusters and were classified as members of that cluster, whereas two accessions (3.4%) were categorized as admixture forms with varying levels of membership shared between the two clusters. Cluster 1 consists of 13 accessions (8 unpublished species including 2 from Germany, 2 from USA, 1 each from Germany, Korea, and UK). The remaining 44 accessions were classified as cluster 2 by the STRUCTURE program (Figure 4, Table 1).

DISCUSSION

To assess the genetic diversity and population structure of the cultivated strawberry (*Fragaria* x *ananassa* Duch.), novel SSR markers developed by Sargent et al. (2006); Sargent et al. (2007); Sargent et al. (2008) were selected according to their distribution in map position. Of a total of 101 alleles found in 59 strawberry accessions by 18 selected SSR markers, 21 alleles are accession-specific. One accession, Blonoli from Germany has 10 distinct alleles. The present of much specific allele in this accession might be the cause of identifying it as outstanding in NJ phylogram although it was put in group two in structure analysis. The result showed clear divergence among the 59 accessions. A high value of genetic divergence was found in German accessions though it has least accessions. The present results contrasted with the finding of Cui et al. (2010). They found that the number of alleles detected and gene diversity was strongly correlated with the number of accessions used. However, this result might be influenced by the different accessions used in each study (Zhao et al. 2009).

The model-based structure analysis used here revealed the presence of two populations among the collected genotypes. The grouping patterns of accessions was slightly different when the two results developed by different clustering methods (genetic distance matrix- and model-based membership) were compared (Figure 2, Figure 4). Some accessions from Pop 2, classified by structure analysis, were placed in Pop 1 in the phylogenic tree developed by PowerMarker. This may be because the STRUCTURE software assumed that, within a population, the loci were in Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium; however, in fact, many influences disturb HWE, including non-random mating, mutations, selection, limited population size, random genetic drift, gene flow, and meiotic drive (Hardy, 1908). Indeed, based on our data, 15 loci deviated from HWE.

The distribution of the 59 accessions that shared at least 70% ancestry with one of the two inferred groups is summarized in Table 1. The relatively small value of the alpha parameter ($\alpha = 0.0319$) indicated that most accessions originated from one primary ancestor, with a few admixed individuals (Ostrowski et al. 2006). In addition to the groups indentified by this analysis, 3.4% of accessions showed evidence of mixed population ancestry. The mixture is likely the result of breeding and domestication history, which have had large effects on the diversity structure. The independent population histories of the groups have shaped gene pools (Garris et al. 2005). Only two accessions from Korea (Johong) and Japan (Asuka, unpublished) showed close relationships between the two populations showing derivation from a common ancestor with a 65% inferred value to population one (Pop 1) for Johong and a 56% inferred value to population 2 (Pop 2) for Asuka. This result indicates frequent hybridization and introgressions events within strawberry gene pools. Natural hybridization and introgression occur widely in plants and play important roles in their evolution (Arnold, 1997; Rieseberg and Carney, 1998; Jarvis and Hodgkin, 1999). Introgressive hybridization is of great interest for plant evolutionary studies because it produces considerable numbers of new genotypes, thereby increasing genetic diversity, which may lead to new adaptations (Rieseberg, 1991) and the formation of new ecotypes (Levin et al. 1996; Rieseberg, 1997) or species (Soltis and Soltis, 1999). Although the extent and significance of natural hybridization/introgression is uncertain (Jarvis and Hodgkin, 1998), the new combinations of genes resulting from hybridization and introgression between wild or weedy relatives and their crop cultivars have been important in the evolution of domesticated crop species (Jarvis and Hodgkin, 1999). The presence of unique, accession-specific alleles in many of the strawberry accessions and introgressive hybrid genotypes supports this hypothesis and provides evidence for the evolutionary dynamics of the collected accessions.

Strawberry plants are propagated vegetatively and can be easily misidentified based on phenotype (Bassil et al. 2006). Being a vegetatively propagated species, collected strawberry accessions show low levels of genetic divergence. Although, strawberries have, complicated ploidy levels and different parentages, most of the alleles were shared among the cultivated strawberries. Despite its economic value, the polyploid constitution of the strawberry has been a major barrier to the genetic characterization of the cultivated species and limited information on the genome structure has been published. The diploid *Fragaria vesca*, with a genome size comparable to that of Arabidopsis and sharing a common ancestor with the cultivated strawberry, has become a model system for map development in the genus *Fragaria* (Sargent et al. 2004). Microsatellites, or simple sequence repeats (SSRs) have proved to be locus-specific, codominant, highly reproducible, and usually highly polymorphic molecular markers (Powell et al. 1996). Assessment of genetic diversity is important in germplasm characterization and conservation. A wide genetic diversity is of great importance for the development of improved varieties. The results derived from analyses of genetic diversity could be

used for designing effective breeding programs to broaden the genetic base of commercially grown varieties. Here, we report detailed information on commercially grown strawberry accessions from five different origins using SSR markers. These results will provide necessary information for breeding programs and strawberry improvement practices.

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TABLES

Table 1. List of the 59 strawberry (*Fragaria* x *ananassa* Duch.) accessions and proportion of membership of each pre-defined population in each of the 3 clusters (Cho et al. 2007).

Label	Cultivar	Origin	Parentage	ID/ published year	Reference	P*≥70%	Pop1	Pop2
1	Keumhyang	Korea	Akihime x Tochiotome	Nonsan,	Choi and Latigui, 2008	2	0.066	0.934
2	Nonsan 1	Korea	Tochinomine x Toyonoka	Nonsan,		2	0.016	0.984
3	Terunoka	Japan	Selection of F. x ananassa	PI 616629 616629.	Plant Inventory No. 210	2	0.113	0.887
4	Tochinomine	Japan	(Florida69-266 x Reiko) x Nyoho		Plant Inventory No. 210	2	0.013	0.987
5	Tochiotome	Japan	Kurume 49 x Tochinomine	PI 617008	Plant Inventory No. 210	2	0.112	0.888
6	Dongja 1	Korea	Unpublished			2	0.069	0.931
7	Redpearl	Japan	Aiberry x Toyonoka	PI 618091	Plant Inventory No. 210	2	0.036	0.964
8	Reiyu	Japan	Unpublished			2	0.030	0.970
9	Manhyang	Korea	Nyoho x Akanekko		USDA ARS (Agricultural Research Service)	2	0.020	0.980
10	Maehyang	Korea	Tochinomine x Akihime	Nonsan,	Choi and Latigui, 2008	2	0.044	0.956
11	Myongbo	Japan	Unpublished			2	0.029	0.971
12	Mihong	Korea	Toyonoka x Reiko		USDA ARS (Agricultural Research Service)	2	0.134	0.866
13	Milyang 1	Korea	Unpublished			2	0.080	0.920
14	Bennihoppe	Japan	Akihime x Sachinoka		USDA ARS (Agricultural Research Service)	2	0.055	0.945
15	Hokowase	Japan	Kogyoku(Fairfax salf seedling) x Tahoe	PI 617007	Plant Inventory No. 210	2	0.020	0.980
16	Sakyejul	Korea	Unpublished			1	0.900	0.100
17	Sachinoka	Japan	Toyonoka x Aiberry	1996	http://www.angelfire.com/biz5/0001/variety.html	2	0.017	0.983
18	Seolhyang	Korea	Akihime x Redpearl	Nonsan,	Choi and Latigui, 2008	2	0.029	0.971
19	Seolhong	Korea	Suhong x Toyonoka	Busan	Choi and Latigui, 2008	1	0.809	0.191
20	Sungkang 16	Korea	Unpublished			2	0.048	0.952
21	Sungkang 17	Korea	Unpublished			1	0.705	0.295
22	Sungkang 19	Korea	Unpublished			1	0.865	0.135
23	Suhong	Korea	Hokowase x Harunoka		USDA ARS (Agricultural Research Service)	2	0.016	0.984
24	Sweet charlie	USA	FL 80-456 x Pajaro	1994	http://www.angelfire.com/biz5/0001/variety.html	2	0.264	0.736
25	Shinyurbong	Korea	Unpublished			2	0.072	0.928
26	Summerberry	Japan	Kahou x Reiko	1988	http://www.angelfire.com/biz5/0001/variety.html	2	0.068	0.932

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27	Akanekko	Japan	Aiberry x Hokowase		USDA ARS (Agricultural Research Service)	2	0.049	0.951
28	Amaou	Japan	Fukuoka S6			2	0.087	0.913
29	Asuka	Japan	Unpublished			Admixture	0.645	0.355
30	Eyeberrybusan	Korea	Unpublished			1	0.700	0.300
31	Akasyanomitsuko	Japan	Nyoho x Kunowase		USDA ARS (Agricultural Research Service)	2	0.015	0.985
32	Akihime	Japan	Kunowase x Nyoho		USDA ARS (Agricultural Research Service)	2	0.046	0.954
33	Everberry	Japan	Oishi-Sikinari x Haruyoi		USDA ARS (Agricultural Research Service)	2	0.091	0.909
34	Reiko	Japan	Fukuba x Harunoka		Plant Inventory No. 210	2	0.024	0.976
35	Josaenghongsim	Korea	Unpublished			1	0.927	0.073
36	Johong	Korea	Nyoho x Akihime		USDA ARS (Agricultural Research Service)	Admixture	0.436	0.564
37	Chodong	Korea	Harunoka x Yachio		USDA ARS (Agricultural Research Service)	2	0.018	0.982
38	Haruyoi	Japan	Hokowase x Harunoka	PI 616621	Plant Inventory No. 210	2	0.057	0.943
39	Hongbok	Japan	Unpublished			2	0.022	0.978
40	AC1466	Korea	Unpublished			1	0.830	0.170
41	Donner	USA	CAL 222 x CAL 145.52	1945	ISHS Acta Horticulturae 265	2	0.018	0.982
42	Linn	USA	MDUS 3184 x ORUS 2414		Plant Inventory No. 210	2	0.022	0.978

43	Oso Grande	USA	Parker x (Tioga x Pajaro)		USDA ARS (Agricultural Research Service)	1	0.858	0.142
44	Senga Sengana	Germany	Markee x Sieger		USDA ARS (Agricultural Research Service)	1	0.861	0.139
45	Tamar	USA	Oso Grande x Dorit		USDA ARS (Agricultural Research Service)	2	0.039	0.961
46	Yael	USA	Oso Grande x Dorit		USDA ARS (Agricultural Research Service)	2	0.025	0.975
47	Blonoli	Germany	Unpublished			1	0.995	0.005
48	Viwa rose	Germany	Unpublished			1	0.794	0.206
49	Douglas	USA	(Tioga x Sequoia) x Tufts		USDA ARS (Agricultural Research Service)	2	0.033	0.967
50	Emily	United Kindom	Honeoye x Gea	PI 616854	Plant Inventory No. 210	1	0.710	0.290
51	Harunoka	Japan	Kurume 103 x Donner		USDA ARS (Agricultural Research Service)	2	0.019	0.981
52	Himiko	Japan	Selection of F. x ananassa		USDA ARS (Agricultural Research Service)	2	0.060	0.940
53	Hogyoku	Japan	Open pollinated 'Fukuba'	PI 616622	Plant Inventory No. 210	2	0.086	0.914
54	Pelican	USA	FL 82-1556P x LA 8311		USDA ARS (Agricultural Research Service)	2	0.021	0.979
55	Prelude	USA	Titan x NC 2967		USDA ARS (Agricultural Research Service)	2	0.174	0.826
56	Reikou	Japan	Fukuba x Harunoka	PI 616627	Plant Inventory No. 210	2	0.030	0.970
57	Shizutakara	Japan	(Kurume 103 x Hokowase) x kurume 103	1982	http://www.angelfire.com/biz5/0001/variety.html	2	0.015	0.985
58	Syuukou	Japan	Shizutakara x Haruyoi	PI 616628	Plant Inventory No. 210	2	0.020	0.980
59	Wiltguard	USA	CAL 39.117-4 x CAL 39.96-18		USDA ARS (Agricultural Research Service)	1	0.955	0.045

* = probability value; Pop1, Pop2 = inferred value defined by STRUCTRUE 2.2 software program.

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Table 2. Total number of alleles and the genetic diversity index for 18 simple sequence repeat (SSR) loci in the 59 strawberry (*Fragaria* x *ananassa* Duch.) accessions.

Marker	Reference	Primer sequence		size range	S_A	R_A	H_E	M_{AF}	A_N	GD	PIC	P^1	P^2
		Forward	Reverse										
EMFax380097*	Sargent et al. (2008)	GTTTTGCTTGGAGGTGTAAAGG	GCTGCTGCTCTCTTGTAATGTG	177-200	2	4	0.980	0.450	9	0.733	0.704	0.000	0.000
EMFax381869*	Sargent et al. (2008)	GACTGCATTTTAGGCTGATTGG	ATCCCTCCACGTATCATCACC	100-240	0	1	0.980	0.490	3	0.519	0.403	0.000	0.000
EMFax381827*	Sargent et al. (2008)	AGGTCTATGGTCCTGAAGCAAC	CACTTGCCGCAGAAGAAAAA	158-373	4	6	0.105	0.667	9	0.521	0.490	0.000	0.000
CFACT110*	Sargent et al. (2008)	ATGGAGAAGTTGCGTTTGG	CGTTCCAGACAAAAGAAGCA	168-202	1	5	0.862	0.302	10	0.791	0.762	0.000	0.000
EMFn226*	Sargent et al. (2006)	CGTCAAAGGAACCCTATTTCG	GTGACGGAGGCATCTTGG	232-283	1	4	0.587	0.467	6	0.581	0.492	0.000	0.002
CFACT103	Sargent et al. (2008)	TCTGACTGAAACAACAAACCTG	ATCGCCTCTCATTGCTTCAT	134-160	0	4	0.849	0.557	8	0.639	0.606	0.063	NaN
EMFaxCAD2*	Sargent et al. (2007)	GAATGAATGGGGTTTCTCTACC	GCTCCCTACTTCTGTAACTTCACC	182-212	2	2	0.018	0.982	3	0.035	0.035	0.000	0.992
UFFa02H04*	Sargent et al. (2006)	ATCAGTCATCCTGCTAGGCACT	TACTCTGGAACACGCAAGAGAA	182-227	0	1	0.444	0.567	5	0.616	0.573	0.000	1.000
EMFax381877*	Sargent et al. (2008)	CCACAAATGAGGGAAGATTAGG	CATCTCGAAGTCACTGGTATATGG	188-200	0	1	1.000	0.500	3	0.518	0.402	0.000	0.000
EMFn125*	Sargent et al. (2006)	CCCAACCCTAAACCATACCC	ATGGTTGCCTTTGATTCACG	223-263	1	2	0.959	0.316	7	0.787	0.755	0.000	0.000
EMFnCEL2*	Sargent et al. (2007)	GGTGTTCAGACCCTTGTTGC	ATGCTTACCAGCTTTACCTTGC	232-333	5	5	1.000	0.481	7	0.546	0.442	0.000	0.000
CFACT084	Sargent et al. (2008)	AAAACTAGGCGGTGTTGCAG	GAACAGATCCACCGAGCAGT	121-143	1	3	1.000	0.396	9	0.778	0.755	0.128	0.001
CFACT152*	Sargent et al. (2008)	AACAACAGCTCTCGCATATT	GAACCATCCAGACTATCTCC	115-172	3	5	0.618	0.676	8	0.516	0.491	0.000	0.000
EMFxaPDC2*	Sargent et al. (2007)	AGCCTGTGTATATCAGCATTGG	GCCTCTAGTCCCCACTTATTGC	239-249	0	3	0.067	0.950	4	0.097	0.095	0.000	0.000
EMFxaQR*	Sargent et al. (2007)	CTTCCCTTGGCTATTGAAACTG	CACCCAAGGTTCTCAAGAAATC	107-335	0	1	0.484	0.694	3	0.443	0.369	0.000	0.063
CFACT100*	Sargent et al. (2008)	CTCCCATGATCGACTCTGCT	GTTTCCCACCTCGAGCATT	207-376	0	0	0.927	0.537	2	0.497	0.374	0.000	0.000
EMFxaEKO1Ba*	Sargent et al. (2007)	CACATGAAGACACCCAACTAGG	AAATCTCTCTGGCTTCCATTCC	216-244	0	1	0.906	0.528	3	0.515	0.401	0.000	0.000
EMPaEKO1B	Sargent et al. (2007)	ACAGTCCAGCTCCAATAGTTCC	GCTTTCCCATTGATTCTTGTCC	244-246	1	1	0.027	0.986	2	0.027	0.026	0.934	0.000
Total					21	49	11.813	10.546	101	9.159	8.175		
Mean					1.167	2.722	0.656	0.586	5.611	0.509	0.454		

S_A: specific allele; R_A: Alleles with frequency lower than 5%; H_E: expected heterozygosity; M_{AF}: major allele frequency; A_N: allele number; GD: gene diversity; PIC: polymorphic information content: *Loci deviating from the Hardy-Weinberg equilibrium (HWE); P^1: the probability value (P) for X^2 test of HWE; P^2: the probability value (P) for X^2 test of LD.

Table 3. Gene diversity, heterozygosity and polymorphic information content (PIC) classified according to its origin by 18 simple sequence repeat (SSR).

Origin	Sample Size	Gene Diversity	Heterozygosity	PIC
Germany	3	0.586	0.704	0.519
Japan	25	0.456	0.658	0.394
Korea	20	0.506	0.644	0.450
United Kindom	1			0.375
USA	10	0.471	0.668	0.402

FIGURES

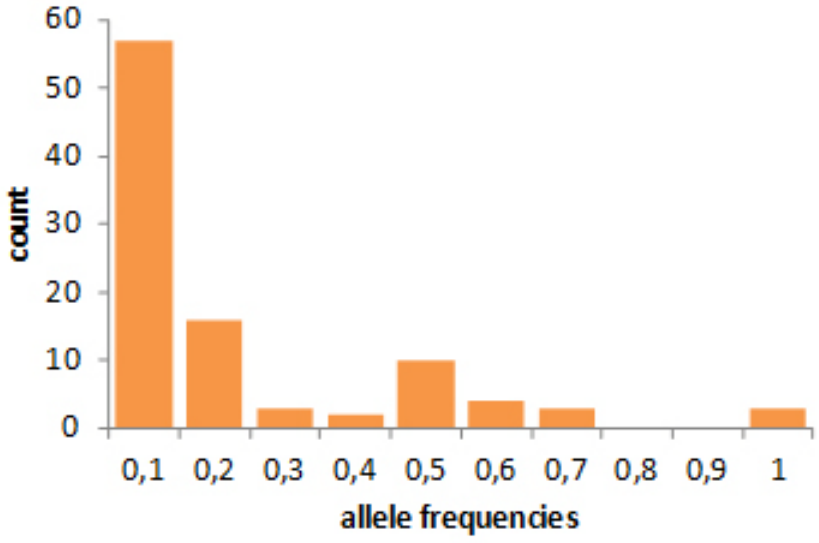

Fig. 1 Histogram of allele frequencies for all 101 alleles in the 59 strawberry accessions.

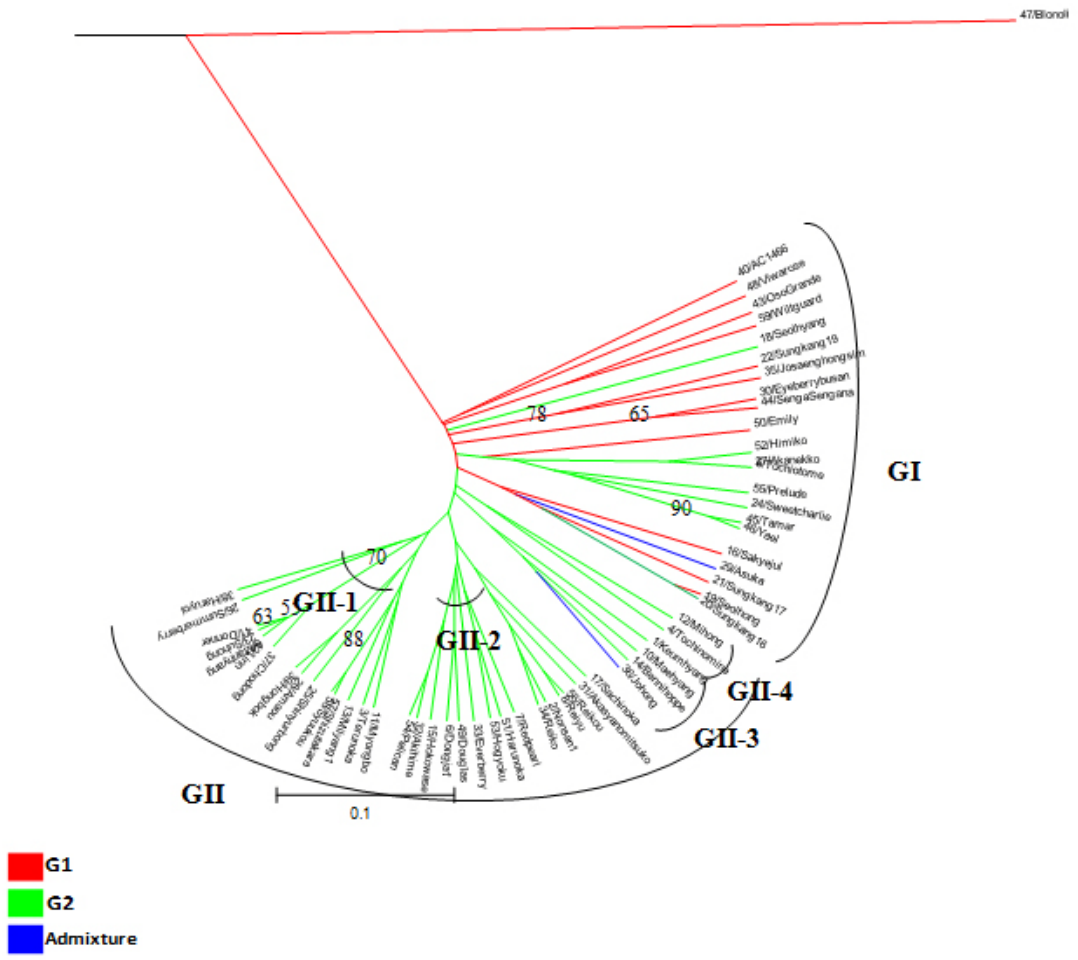

Fig. 2 Unrooted neighbour-joining tree based on a Nei's genetic distance matrix among 59 accessions. The colours correspond to the model-based populations, G1 = group 1 and G2 = group 2. Numbers on branches are bootstrap values obtained for 1000 replicates (only values above 50% are shown).

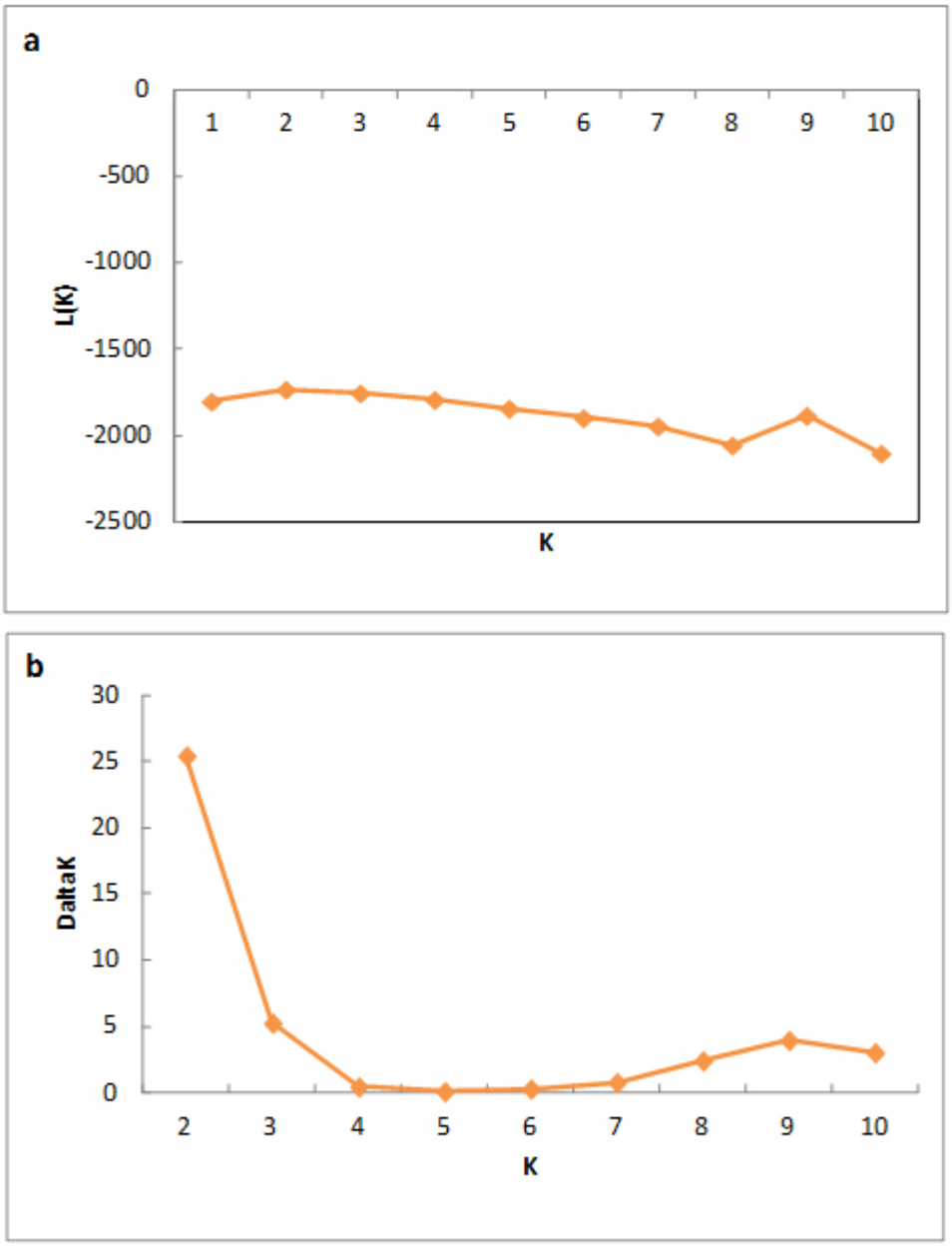

Fig. 3 (a) (Log) Likelihood of the data (*n* = 59), L (K), as a function of *K* (the number of groups used to stratify the sample). For each *K* value, four independent runs (blue diamonds) were considered and data were averaged over the replicates. **(b) Values of *ΔK*, with its modal value detecting a true *K* of the two groups (*K* = 2).**

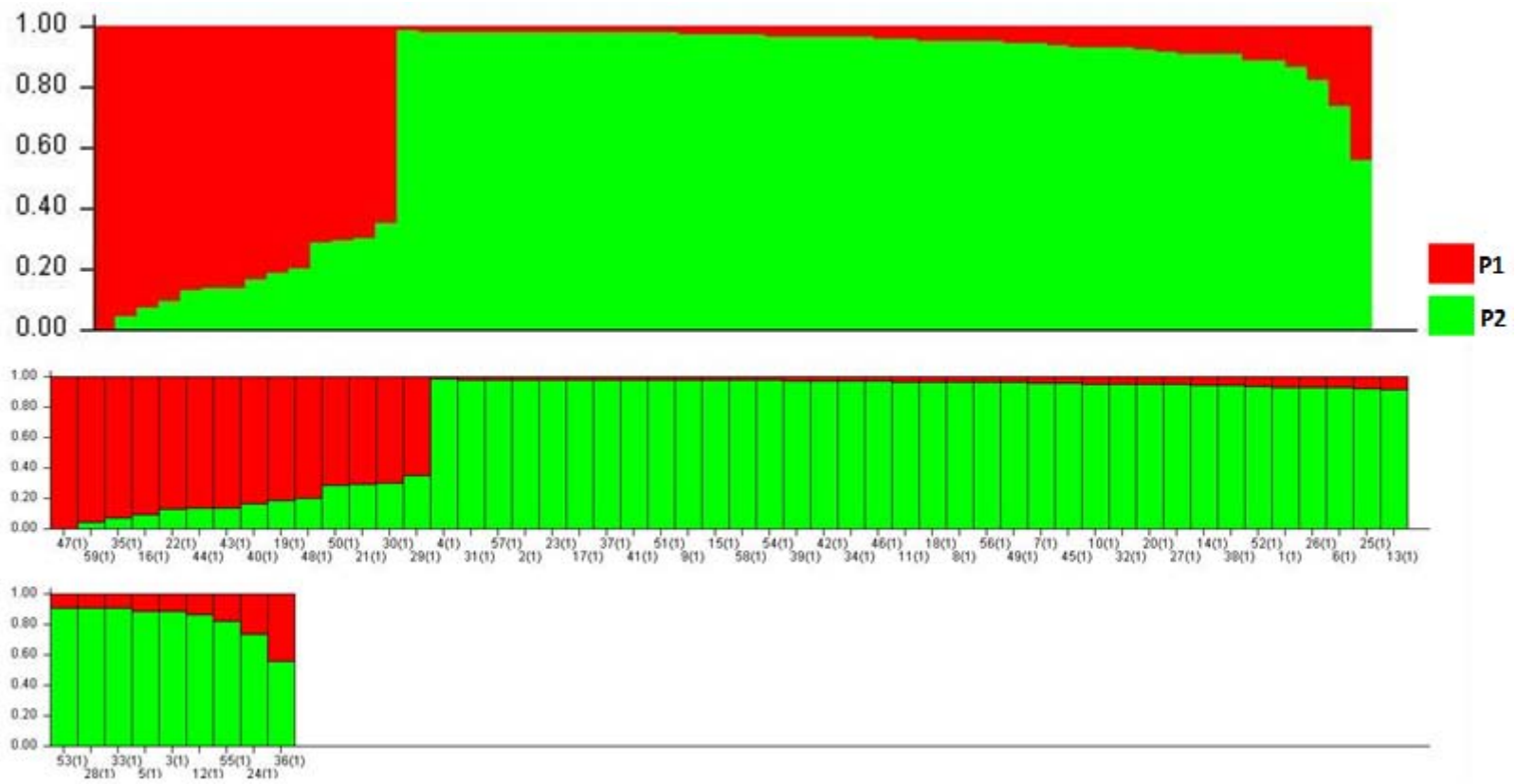

Fig. 4 Model-based ancestry for each of the 59 accessions based on the 20 simple sequence repeat (SSR) markers used to build the Q matrix. The numbers are accession ID numbers, numbers in the bracket are predefined population numbers and P1, P2 are population one and two.

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