Population Structure and Diversity in Valencia Peanut Germplasm Collection

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ABSTRACT

Valencia peanuts [*Arachis hypogaea* L. subsp. *fastigiata* Waldron var. *fastigiata* (Waldron) Krapov. & W. C. Greg.] are well known for their in-shell market value. Assessment of genetic diversity is key to the success of developing improved cultivars with desirable agronomic and quality traits. Seventy-eight U.S. Valencia core collection accessions together with 36 Valencia accessions representing the global peanut mini-core collection were used to study population structure and diversity and to identify genetically diverse Valencia germplasm for use in peanut breeding. Fifty-two simple sequence repeats loci amplified 683 alleles, with an average of 13 alleles per locus. The mean polymorphism information content and gene diversity, respectively, were 0.270 and 0.335. The pairwise genetic distance ranged from 0.143 to 0.474, with an average of 0.631. Neighbor-joining clustering, principal coordinate analysis, and STRUCTURE analysis consistently separated the Valencia germplasm into five clusters with two distinct major groups. The first major group consisted of genotypes from South America (64%) with few accessions from Africa, North America, Caribbean, and European regions. The second group consisted of accessions mostly from diverse regions of Africa, North and South America, Asia, and the Caribbean. However, the structuring was not related to the geographic origin and several admixtures were observed. The information generated in this study and phenotyping of this material for biotic and abiotic stress responses and yield-quality traits will facilitate selection of trait-specific, genetically diverse parents for developing Valencia peanut cultivars with a broad genetic base.

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Abbreviations: NJ, neighbor joining; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; PIC, polymorphism information content; SSR, simple sequence repeats.

Cultivated peanut (*Arachis hypogaea* L.), also known as groundnut, is an important food and oil-producing legume, grown annually on 23.51 million ha with a production of 35.5 Mt (FAOSTAT, 2008) in tropical and subtropical regions of the world (mainly in the Americas, Asia, and Africa). China, India, Nigeria, and the United States are the major peanut producers, accounting for 68.7% of global production (FAOSTAT, 2008). Peanut ranks sixth among the major oil-producing crops after soybean [*Glycine max* (L.) Merr.], oil palm (*Elaeis guineensis* Jacq.), cotton (*Gossypium hirsutum* L.), coconut (*Cocos nucifera* L.), and rapeseed (*Brassica napus* L.), and has the potential to be used as a biodiesel crop. Peanut is rich in oil, protein, minerals, and vitamins, making it a valued crop for direct human consumption, while its fodder is highly nutritious and palatable animal feed. Peanut production in the United States is primarily targeted to the confectionary industry and for production

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of peanut butter. The four market types (i.e., Virginia, Runner, Spanish, and Valencia) grown in the United States are characterized by distinct growth habit, pod/seed size, and flavor attributes (Isleib et al., 2001). Valencia [*Arachis hypogaea* L. subsp. *fastigiata* Waldron var. *fastigiata* (Waldron) Krapov. & W. C. Greg.] peanuts, which constitute only 1–2% of the total U.S. market and are almost exclusively cultivated in New Mexico and Texas, have high in-shell market value, possess sweet flavor as desired by the confectionary industry, and remain a viable niche crop for production in these regions.

Cultivated peanut ($2n = 4x = 40$) is an allotetraploid (AABB), native to South America, whose origin is attributed to a single hybridization event involving diploid wild species *Arachis duranensis* Krapov. & W. C. Greg. (AA-genome) and *A. ipaensis* Krapov. & W. C. Greg. (BB-genome) followed by a spontaneous chromosome duplication (Kochert et al., 1996; Hopkins et al., 1999; Fávero et al., 2006; Seijo et al., 2007). Cultivated peanut has six botanical varieties, each differentiated by the presence or absence of flowers on the main axis and unique pod and seed characteristics (Gregory et al., 1980; Krapovickas and Gregory, 1994). Although cultivated peanut has considerable variability in morphological traits, several studies reported a low level of molecular diversity (Baichi-Hall et al., 1991; Lacks and Stalker, 1993; Lanham et al., 1994; Halward et al., 1991, 1992; Kochert et al., 1991; Paik-Ro et al., 1992; Gimenes et al., 2002; Herselman, 2003; Milla-Lewis et al., 2010a, 2010b). Microsatellites or simple sequence repeats (SSRs), the tandem repeats of short (2–6 bp) DNA sequences distributed abundantly throughout eukaryotic genomes, are often highly polymorphic, multi-allelic, and amenable to high-throughput genotyping, making them the markers of choice in diversity assessment, construction of linkage maps, and for assessing marker-trait association (Litt and Lutty 1989). At present, >1000 SSR markers are available in peanut (Hopkins et al., 1999; He et al., 2003; Ferguson et al., 2004b; Moretzsohn et al., 2005; Palmieri et al., 2005; Gimenes et al., 2007; Proite et al., 2007; Cuc et al., 2008), some of which have been used to assess genetic diversity in peanut germplasm, including wild species (Hopkins et al., 1999; Moretzsohn et al., 2004; Krishna et al., 2004; Freitas et al., 2007; Tang et al., 2007; Naito et al., 2008; Barkley et al., 2007; Jiang et al., 2007; Mace et al., 2006, 2007; Kottapalli et al., 2007) and construction of genetic maps for the diploid AA- and BB-genomes as well as the AABB-genome (Moretzsohn et al., 2005, 2009; Varshney et al., 2008; Hong et al., 2008; Khedikar et al., 2010), thereby providing tremendous opportunity to identify allelic variation associated with beneficial traits and conduct marker-assisted genetic enhancement in peanut. However, the success of any crop improvement program depends on the researcher's ability to detect and exploit the genetic diversity locked in germplasm, including wild relatives that are conserved in various gene banks worldwide. Core collections have been suggested as a gateway to enhance the use of germplasm in crop improvement programs (Frankel, 1984; Brown, 1989). In peanut, both core and mini-core collections have been developed (Holbrook et al., 1993; Upadhyaya et al., 2002, 2003; Holbrook and Dong, 2005; Dwivedi et al., 2008). These subsets have been used to identify new sources of genetic variation for traits of interest (Upadhyaya et al., 2009; Kottapalli et al., 2009) and to assess levels of molecular diversity (Kottapalli et al., 2007).

The objectives of the present investigation were to characterize population structure and diversity in a set of Valencia peanut core collection accessions using SSR markers to identify genetically diverse accessions to broaden the genetic base of Valencia peanut germplasm/cultivars.

MATERIALS AND METHODS

Plant Materials

A total of 114 Valencia peanut accessions, including 78 Valencia core collection accessions (Dwivedi et al., 2008), 35 Valencia accessions from global mini-core collection (Upadhyaya et al., 2002), and a control cultivar Gangapuri (Ramamurthy, 1974), constituted the experimental material (Table 1). The majority of accessions (68.4%) were from South America, followed by Africa (12.2%), North America (6.1%), and Asia (6.1%). Other regions were represented by 1 to 2%.

Isolation of Genomic DNA and SSR Genotyping

Total genomic DNA was isolated from leaves of 14-d-old seedlings using acetyl trimethyl ammonium bromide–based high-throughput protocol (Mace et al., 2003), with the modification of using a 48-well Retch (Newtown, PA) Mixer Mill MM300 grinder, in which tissue was ground three times at 25 Hz for 1 min. DNA quantification was done with a ND-1000 Spectrophotometer (Nano Drop Technology, Wilmington, DE) and the samples were diluted to a concentration of 10 ng μL^{-1} for the SSR genotyping. A modified M13-tagged forward, concatenated with a 20-mer M13 oligo (GAC GTT GTA AAA CGA CGGCC) to the 5′ end of each forward primer and normal reverse primer were used for each marker. To facilitate detection, a 20-mer M13 oligo labeled with one of four fluorescent dyes, 6FAM, VIC, NED, or PET, was added to the polymerase chain reaction (PCR) mix to label SSR products of each marker. Initially 100 SSR primers were optimized with four genotypes (PI 493631, PI 565461, PI 576604, and ICG 2738) to identify 52 polymorphic SSR primers (Table 2) that were used for molecular profiling of 114 Valencia accessions. A reference for the PCR conditions used for primer sets obtained from previous studies (Hopkins et al., 1999; Ferguson et al., 2004b; He et al., 2003, 2005; Moretzsohn et al., 2004, 2005) is briefly summarized. Template DNA was amplified using a PTC-225 (MJ Research, Waltham, MA) Peltier thermal cycler with the following conditions: 94°C initial denaturation for 15 min (1 cycle); followed by 10 cycles of denaturation at 94°C for 45 s, annealing 65 to 55°C touchdown temperature with decrease of 1°C per cycle and

Table 1. Information of 114 Valencia peanut accessions used for the assessment of genetic diversity.

Serial no.	Code†	Country of origin	Geographic region	Biological status	NJ‡	PCoA§	STRUCTURE¶
1	PI 259601	Australia	Australia	Landrace	I	B	1/4
2	PI 493536	Brazil	South America	Advanced cultivar	I	A	1/4
3	PI 259580	Jamaica	Caribbean	Landrace	I	A	1/3
4	PI 493501	Brazil	South America	Advanced cultivar	I	A	1/3/4/5
5	PI 497447	Bolivia	South America	Landrace	I	A	1/3
6	PI 602494	Argentina	South America	Landrace	I	A	1/2
7	PI 493630	Paraguay	South America	Advanced cultivar	I	A	1/2/3/4/5
8	PI 493518	Brazil	South America	Advanced cultivar	I	B	1/3/4/5
9	PI 576604	Bolivia	South America	Unknown	I	B	1/2/4/5
10	PI 493446	Paraguay	South America	Advanced cultivar	I	A	1/3/4/2
11	PI 365564	Bolivia	South America	Landrace	I	A	1/2
12	PI 406718	Costa Rica	Central America	Landrace	I	A	1/2/3
13	PI 429430	Zimbabwe	Africa	Landrace	I	A	1/2/3
14	PI 475913	Bolivia	South America	Landrace	I	A	1/2
15	PI 476078	Brazil	South America	Landrace	I	A	1/2
16	PI 429427	Zimbabwe	Africa	Landrace	I	A	1/2/3/4/5
17	PI 493344	Paraguay	South America	Advanced cultivar	III	B	1/2/4/5
18	PI 493688	Paraguay	South America	Advanced cultivar	I	A	3/4/5
19	PI 476089	Brazil	South America	Landrace	III	B	1/2/3/4
20	PI 493339	Bolivia	South America	Advanced cultivar	I	A	3/4/5
21	PI 493458	Paraguay	South America	Advanced cultivar	II	E	1/2/3/4
22	PI 493507	Brazil	South America	Advanced cultivar	I	A	4/5
23	PI 476079	Brazil	South America	Landrace	I	A	1/2/3
24	PI 476074	Brazil	South America	Landrace	I	A	1/2/3
25	PI 493562	Brazil	South America	Advanced cultivar	I	A	2/3
26	PI 497459	Bolivia	South America	Landrace	III	B	2/3/4
27	PI 493405	Paraguay	South America	Advanced cultivar	II	E	1/2/3/4/5
28	PI 493461	Brazil	South America	Advanced cultivar	I	A	3/4
29	PI 493865	Paraguay	South America	Advanced cultivar	II	A	1/2/3/4
30	PI 493415	Paraguay	South America	Advanced cultivar	I	A	2/3/4
31	PI 493470	Brazil	South America	Advanced cultivar	I	A	1/2/3
32	PI 315612	Africa	Africa	Advanced cultivar	II	A	1/2/4
33	PI 493325	Paraguay	South America	Advanced cultivar	II	B	2/4/5
34	PI 493340	Bolivia	South America	Advanced cultivar	I	A	2/3/4
35	PI 493624	Paraguay	South America	Advanced cultivar	I	A	1/2/3/4
36	PI 493810	Brazil	South America	Advanced cultivar	I	A	1/2/3
37	PI 501985	Peru	South America	Landrace	II	A	1/2/3
38	PI 338337	Venezuela	South America	Advanced cultivar	III	B	2/3/4/5
39	Breedingline	USA	North America	New breeding line	I	A	3/4/5
40	PI 493382	Paraguay	South America	Advanced cultivar	I	A	2/3/5
41	PI 493360	Paraguay	South America	Advanced cultivar	I	A	1/2/3
42	PI 493523	Brazil	South America	Advanced cultivar	I	A	2/4
43	PI 475925	Bolivia	South America	Landrace	III	B	1/2/3/4/5
44	PI 494019	Brazil	South America	Advanced cultivar	II	A	2/3/4
45	PI 493565	Brazil	South America	Advanced cultivar	II	A	2/3/4
46	PI 493584	Brazil	South America	Advanced cultivar	I	A	2/3
47	PI 536300	Uruguay	South America	Landrace	III	A	1/2
48	PI 493660	Paraguay	South America	Advanced cultivar	I	A	2/3/4/5
49	PI 536307	Uruguay	South America	Landrace	III	B	2/3
50	PI 493373	Paraguay	South America	Advanced cultivar	I	A	2/3/4/5
51	PI 497642	Ecuador	South America	Landrace	I	A	1/2/3/4
52	PI 493612	Bolivia	South America	Advanced cultivar	I	A	1/2/3/5
53	PI 493484	Brazil	South America	Advanced cultivar	I	A	1/2/3/4
54	PI 493816	Paraguay	South America	Advanced cultivar	I	A	1/2
55	PI 493566	Brazil	South America	Advanced cultivar	I	A	2/3/4

(cont'd)

Table 1. Continued.

Serial no.	Code†	Country of origin	Geographic region	Biological status	NJ‡	PCoA§	STRUCTURE¶
56	PI 493451	Paraguay	South America	Advanced cultivar	I	A	1/2/3
57	PI 501269	Peru	South America	Landrace	I	A	1/2/3
58	PI 536121	Brazil	South America	Breeding material	I	A	3/4/5
59	PI 493514	Brazil	South America	Advanced cultivar	I	A	2/3/4/5
60	PI 468208	Bolivia	South America	Advanced cultivar	I	A	1/2
61	PI 493666	Paraguay	South America	Advanced cultivar	I	A	1/2/3/4
62	PI 493381	Paraguay	South America	Unknown	I	A	1/2/3
63	PI 502023	Peru	South America	Unknown	I	A	1/2/3
64	PI 475921	Bolivia	South America	Landrace	I	A	1/2/3
65	PI 501293	Peru	South America	Breeding material	I	A	1/2/3/5
66	PI 306361	Israel	Asia	Advanced cultivar	I	A	2/3
67	PI 493629	Paraguay	South America	Advanced cultivar	I	A	1/2
68	PI 493442	Paraguay	South America	Advanced cultivar	I	A	2/3/4
69	PI 407451	Ecuador	South America	Unknown	I	A	1/2/3
70	PI 390432	Ecuador	South America	Landrace	I	A	1/2/3
71	PI 409037	Zimbabwe	Africa	Landrace	I	A	1/2/3/4
72	Grif13802	Ecuador	South America	Unknown	III	B	2/3/4
73	PI 599612	Bolivia	South America	Advanced cultivar	I	B	3/4/5
74	PI 508278	USA	North America	Breeding material	I	A	2/3/4/5
75	PI 493521	Brazil	South America	Advanced cultivar	I	B	2/3/4
76	PI 314980	Russia	Europe	Landrace	I	B	2/3/4
77	PI 468225	Bolivia	South America	Landrace	–	A	2/3/4
78	PI 493631	Paraguay	South America	Advanced cultivar	I	B	1/2/3/4
79	ICG 5609	Sri Lanka	Asia	Landrace	–	E	3/4/5
80	ICG 8517	Bolivia	South America	Landrace	II	B	3/4
81	ICG 15042	UK	Europe	Other	IV	A	3/4/5
82	ICG 6646	UK	Europe	Landrace	IV	B	1/2/3/4/5
83	ICG 5475	Kenya	Africa	Other	–	E	1/3/4/5
84	ICG 7181	India	Asia	Landrace	II	E	3/4
85	ICG 6022	Sudan	Africa	Advanced cultivar	II	B	3/4
86	ICG 10092	Zimbabwe	Africa	Landrace	–	B	1/2/3/4/5
87	ICG 6888	Brazil	South America	Breeding material	IV	B	3/4/5
88	ICG 8106	Peru	South America	Landrace	IV	B	3/4/5
89	ICG 332	Brazil	South America	Landrace	IV	B	1/2/3/4/5
90	ICG 13856	Uruguay	South America	Landrace	IV	B	2/3/4/5
91	ICG 397	USA	North America	Breeding material	IV	B	2/4/5
92	ICG 9315	USA	North America	Breeding material	IV	B	1/2/3/4/5
93	ICG 3681	USA	North America	Other	IV	B	3/2/5
94	ICG 14127	Zaire	Africa	Other	IV	B	1/2/4/5
95	ICG 14630	Brazil	South America	Landrace	IV	B	1/2/4/5
96	ICG 10566	Congo	Africa	Other	IV	B	3/4/5
97	ICG 115	India	Asia	Landrace	IV	C	1/4/5
98	ICG 13858	Uruguay	South America	Landrace	V	C	3/4/5
99	ICG 10890	Peru	South America	Landrace	V	C	1/3/4/5
100	ICG 11144	Argentina	South America	Breeding material	V	C	3/4/5
101	ICG 1399	Malawi	Africa	Breeding material	V	C	3/5
102	ICG 15309	Brazil	South America	Landrace	V	C	2/3/5
103	ICG 4670	Sudan	Africa	Other	V	C	2/3/4/5
104	ICG 5221	Argentina	South America	Landrace	V	C	3/4/5
105	ICG 1274	India	Asia	Other	V	D	3/4/5
106	ICG 3673	Korea	Asia	Landrace	V	D	3/4/5
107	ICG 14106	Zaire	Africa	Other	V	D	3/4/5
108	ICG 1142	Benin	Africa	Breeding material	V	C	3/4/5
109	ICG 10554	Argentina	South America	Breeding material	V	C	1/3/4/5
110	ICG 10474	Cuba	Caribbean	Advanced cultivar	V	–	2/3/5

(cont'd)

Table 1. Continued.

Serial no.	Code†	Country of origin	Geographic region	Biological status	NJ‡	PCoA§	STRUCTURE¶
111	ICG 6201	Cuba	Caribbean	Landrace	V	D	3/4/5
112	ICG 297	USA	North America	Advanced cultivar	V	B	3/4/5
113	ICG 14710	Cameroon	Africa	Landrace	III	B	3/4/5
114	ICG 2738	India	Asia	Advanced cultivar	II	A	1/3/4/5

† Plant Introduction (PI) accession code refers to U.S. Valencia Mini-core collection; ICG refers to groundnut from ICRISAT gene bank.

‡ Classification by neighbor-joining (NJ) tree using Nei's coefficient based on 52 simple sequence repeat (SSR) markers.

§ Classification based on principal coordinate analysis (PCoA) based on 52 SSR markers.

¶ Classification based on STRUCTURE analysis based on 52 SSR markers.

extension at 72°C for 30 s, followed by 35 cycles of 94°C for 45 s, 55°C for 60 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR amplifications were achieved in a volume of 5 μL, containing 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.25 units Hotstart *Taq* DNA polymerase (Qiagen Inc., Valencia, CA), 5 pmol of each M13-tagged forward and normal reverse primer, 0.02 pmol of fluorescently labeled 20-mer M13 primer (labeled with either 6FAM, VIC, NED, or PET), and 10 ng of template genomic DNA. The PCR-amplified products (1 μL each) were multiplexed into a mixture of Hi-Di formamide (10 μL) containing an internal standard GeneScan-500 LIZ (Applied Biosystems, Foster City, CA), denatured at 94°C for 5 min, and separated by capillary electrophoresis on an ABI Prism 3130 DNA Analyzer (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Fragments were called using Gene Mapper V4.0 software (Applied Biosystems Inc., Foster City, CA).

Data Analysis

The amplification products were scored as present (1) or absent (0) for each of the accessions to compute the binary data matrix that was analyzed using NTSYS-PC version 2.2 (Numerical Taxonomy and System) (Rohlf, 2000). A genetic distance matrix was developed and subsequently used to construct dendrograms based on the neighbor-joining (NJ) clustering method and visualized using the tree program of the NTSYS-PC version 2.2. To measure the goodness of fit for cluster analysis, a cophenetic correlation value between the original similarity matrix and the cophenetic matrix was compared using the MXCOMP procedure of NTSYS-PC version 2.2. The significance of correlation between the matrices was tested using the normalized Mantel *Z*-statistics (Mantel, 1967). The binary data were also subjected to principal coordinate analysis (PCoA) to partition the variance using NTSYS-PC version 2.2 and the first three components were plotted into MOD three-dimensional scatter plots.

Power Marker V 3.25 (Liu and Muse, 2005) was used to detect polymorphic information content (PIC) and gene diversity. The PIC measures allelic diversity at a locus (Botstein et al., 1980), while gene diversity is defined as the probability that two randomly chosen alleles from the population are different (Nei, 1973). A distance matrix was generated based on the number of shared alleles between different countries and unrooted NJ tree was constructed. Pairwise comparisons of genetic distances among the accessions were calculated using Nei's formula (Nei, 1972).

The model-based program, STRUCTURE version 2.3.1 (http://pritch.bsd.uchicago.edu/software/structure_v.2.3.1.html [verified 15 Feb. 2011]) was used to determine *K*, the number of structured groups (Pritchard et al., 2000; Falush et al., 2003, 2007). The ancestry model with admixture was chosen and using the correlated allele frequency option, multiple runs of STRUCTURE were performed by setting *K* from one to 10 groups. The length of burn-in was set at 10,000 followed by 100,000 iterations, and each run was replicated 10 times. Runs with the highest *Ln* P(D) probability were considered for each *K* and graphical outputs were visualized to determine the most appropriate number of clusters.

RESULTS

Molecular Marker Diversity

The 52 SSRs detected a total of 683 alleles, with an average of 13 alleles per primer pair (Table 2). The number of alleles ranged from two (TC4H2) to 28 (PM3), with large number of SSRs detecting 11 to 15 alleles (Fig. 1). Amplicon sizes ranged from 100 (TC11E4) to 499 bp (Ah193). The PIC ranged from 0.129 (gi716) to 0.361 (pPGPseq4D4) with a mean of 0.270, which revealed low to moderate levels of polymorphism. Gene diversity ranged from 0.1427 to 0.4743 with a mean of 0.335, indicating a moderate level of diversity in the Valencia peanut germplasm. Based on estimates of gene diversity, the most informative markers were pPGPseqD4, pPGPseq2A6, pPGPseq7G2, TC9H8, and PM238, while the least informative were gi716 and pPGSseq17E3.

Geographical Diversity

The 114 accessions included in this study originated from 27 countries belonging to different geographic regions of the world (Table 1). To understand the geographic diversity and underlying genetic diversity of the Valencia genotypes, the binary data from SSR markers were used to generate a distance matrix based on number of shared alleles between different countries and an unrooted NJ tree was constructed. The clustering based on shared SSR alleles between countries could be useful in understanding pattern of dispersal of the Valencia germplasm across the world from its source of origin in South America. The 27 countries were grouped into five clusters (Fig. 2). Cluster 1 from Malawi and Benin shared common alleles with Korea and Cuba. Kenya, Sri Lanka, and Venezuela shared common alleles with the Congo in Cluster 2. Accessions from Costa Rica, Jamaica, and Australia shared common alleles with countries from Africa, Russia, and Israel in Cluster 3. The accessions from Cameroon separated

Table 2. List of simple sequence repeat primers, number of alleles, size range, and summary of genetic diversity analysis components analyzed.

Marker	No. of alleles	Size range	Availability†	Gene diversity	PIC‡
		bp			
Ah41	12	277–329	1.0000	0.4221	0.3295
Ah193	22	292–499	0.9825	0.4130	0.3231
Ah229	7	121–238	0.9912	0.3715	0.2966
Ah4_20	16	220–227	0.9912	0.3297	0.2711
Ah4_26	13	170–296	0.9474	0.3928	0.3141
gi716	11	145–347	0.9912	0.1427	0.1298
PM3	28	187–255	0.9825	0.3740	0.2989
PM32	14	106–224	1.0000	0.3805	0.3048
PM35	27	121–182	1.0000	0.3671	0.2955
PM36	19	117–228	1.0000	0.3342	0.2725
PM45	14	103–163	0.8772	0.3773	0.3012
PM65	23	214–265	0.9825	0.4332	0.3363
PM137	11	148–223	0.9298	0.3356	0.2660
PM183	16	124–174	0.9912	0.3652	0.2888
PM188	12	105–140	0.9912	0.3366	0.2753
PM204	19	211–251	0.9912	0.4160	0.3246
PM238	22	162–221	0.9825	0.4504	0.3473
PM375	14	117–178	0.9912	0.2785	0.2322
pPGPseq1B9	15	278–320	1.0000	0.3685	0.2928
pPGPseq2A5	11	103–180	1.0000	0.2024	0.1807
pPGPseq2A6	14	200–288	1.0000	0.4662	0.3557
pPGPseq3C2	19	297–340	0.9649	0.4333	0.3351
pPGPseq3E10	7	119–235	0.9912	0.3146	0.2540
pPGPseq4D4	12	200–253	0.9905	0.4743	0.3616
pPGPseq4H11	14	232–296	1.0000	0.2041	0.1828
pPGPseq5D5	6	270–301	0.9912	0.3502	0.2831
pPGPseq6B8	8	185–264	0.9912	0.4150	0.3270
pPGPseq7G2	9	233–267	0.9561	0.4638	0.3556
pPGPseq7H6	18	146–317	1.0000	0.4096	0.3220
pPGPseq8E12	8	199–221	0.9035	0.4308	0.3346
pPGSseq9F1	11	243–284	0.9825	0.3357	0.2747
pPGSseq13E11	8	145–338	0.9912	0.1836	0.1629
pPGSseq16G8	17	211–477	0.8509	0.3317	0.2727
pPGSseq17E3	5	194–217	1.0000	0.1546	0.1409
pPGSseq18C5	7	138–239	0.8509	0.2246	0.1910
pPGSseq19A5	12	138–281	0.9825	0.1953	0.1681
pPGSseq19D6	5	306–365	0.8509	0.2017	0.1661
pPGSseq19G7	19	129–198	0.9825	0.3204	0.2621
TC1E6	16	141–173	1.0000	0.3723	0.2966
TC3G1	13	184–279	0.9825	0.3440	0.2732
TC4D9	16	128–257	0.9825	0.3538	0.2843
TC4F12	8	235–256	1.0000	0.2868	0.2452
TC4G10	10	141–158	1.0000	0.2968	0.2484
TC4H2	2	122–138	1.0000	0.1668	0.1480
TC6E1	15	126–189	0.9825	0.2031	0.1789
TC6H3	12	121–253	0.9825	0.2801	0.2343
TC7E4	18	110–175	0.9825	0.2893	0.2406
TC9F10	7	264–298	0.9825	0.3513	0.2788
TC9H8	12	234–282	1.0000	0.4417	0.3380
TC11A2	10	155–295	1.0000	0.3526	0.2876
TC11A4	12	186–217	0.7807	0.3598	0.2920
TC11E4	7	100–116	1.0000	0.3264	0.2696
Mean	13.1346		0.9727	0.3351	0.2701
Total	683				

† Percentage available data.

‡ PIC, polymorphism information content.

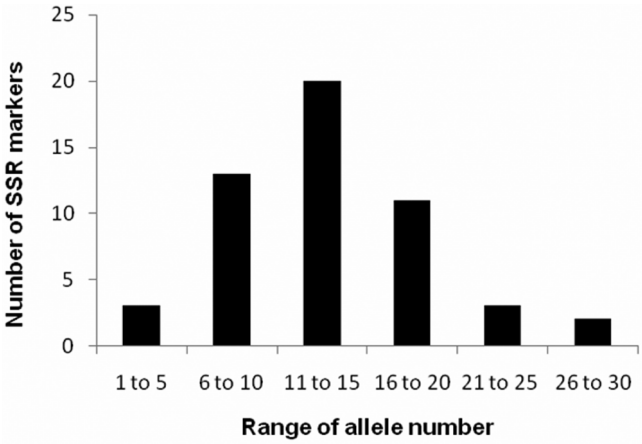

Figure 1. Distribution of allele numbers of 52 polymorphic simple sequence repeat (SSR) loci in 114 Valencia peanut genotypes representing global Valencia peanut germplasm.

as an individual group in Cluster 4. Accessions from Brazil, Paraguay, Bolivia, Ecuador, Peru, Argentina, and Uruguay grouped in Cluster 5 and shared common alleles with those accessions from the United States, United Kingdom, Zaire, Sudan, Zimbabwe, and India.

Neighbor-joining Clustering

Six hundred eighty-three alleles from 52 SSR loci were used to estimate pairwise genetic distances among the accessions based on Nei's coefficient. A high correlation (r = 0.88) between the cophenetic matrix and the original matrix reveals a good fit of the genotype clustering. The average genetic distance was 0.631, representing high variability among the Valencia peanut accessions. A dendrogram constructed using NJ method based on Nei's genetic distance grouped 114 accessions into five distinct clusters, with two major groups (Fig. 3 and Table 1). Group A was the largest cluster, with 72 accessions originating mostly from South America, with few from Africa, North and Central America, Asia, and Caribbean regions. Further, two clusters (I and II) in this group separated at a genetic distance of 0.91. Group B consisted of 38 accessions from diverse regions (Africa, North and South America, Asia, Europe, and the Caribbean), separated into three clusters (III, IV, and V). Furthermore, four accessions, ICG 5609, ICG 5475, PI 468225, and ICG 10092, were not the part of either of the two major groups.

Principal Coordinate Analysis

The molecular genetic diversity of the SSR loci was also subjected to PCoA to validate the clustering pattern. The first three components were plotted into MOD three-dimensional scatterplots (Fig. 4). Principal Component (PC) 1 and PC 2 accounted for 54.4 and 5.3% of the total variation, respectively. The first three components together explained 62.4% of the total variation. The accessions were clustered into five distinct groups, A, B, C, D, and E (Table

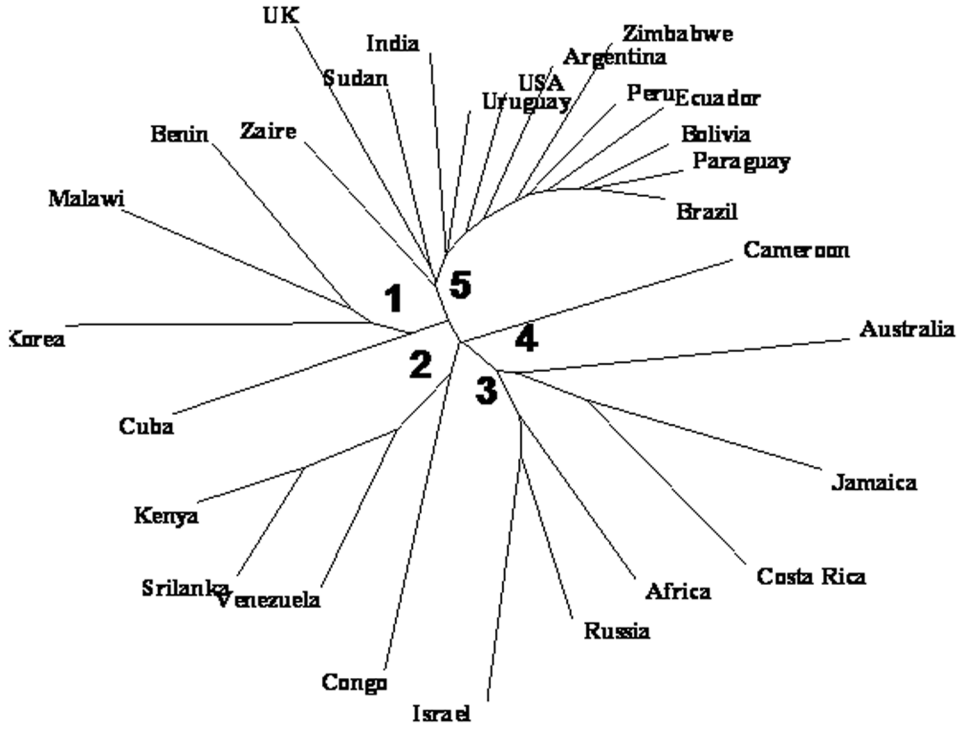

Figure 2. Unrooted neighbor-joining tree constructed based on number of shared alleles among different source countries.

1 and Fig. 4), except for ICG 10474, an advanced cultivar from Cuba, which was placed far apart from the rest of the accessions. Groups A and B consisted of 63 and 31 accessions, respectively. Group A consisted of 20 accessions from Brazil and 16 accessions from Paraguay, mostly advanced cultivars. It also had representation from Bolivia (nine accessions), Peru (four accessions), Ecuador (two accessions), Zimbabwe (three landraces), United States (two accessions), one cultivar each from India, Israel, and in Africa, and one each from the United Kingdom, Uruguay, Costa Rica, and Argentina. Cluster B consisted of 19 accessions from South America, (six from Brazil, five from Bolivia, three from Paraguay, two from Uruguay, and a single accession each from Ecuador, Peru, and Venezuela), four accessions from the United States, five accessions from Africa, one each from Cameroon, Congo, Sudan, Zaire, and Zimbabwe, and single accessions from Australia, United Kingdom, and Russia. Group C consisted of 10 accessions, six from South America and one each from India, Benin, Malawi, and Sudan. Four accessions from India, Korea, Zaire, and Cuba formed the minor Group D. Two cultivars from Paraguay showed close affinity toward accessions from Sri Lanka, Kenya, and India, and grouped together in Cluster E.

STRUCTURE Analysis

STRUCTURE 2.3.1 was used to find appropriate numbers of clusters with no prior information about the geographical origin of the genotypes. Population STRUCTURE analysis revealed that LnP (D) estimates increased from $K = 1$ to $K = 5$ and then began to plateau, suggesting the genetic diversity should be structured into five subpopulations. The overall proportion of membership in each of the five clusters has 15.2% (1—red), 30.7% (2—green), 13.3% (3—blue), 25.2% (4—yellow), and 15.6% (5—pink) (Fig. 5 and Table 1). Admixtures were observed among all the clusters, with eight accessions (PI 493630, PI 429427, PI 493405, PI 475925, ICG 6646, ICG 10092, ICG 332, and ICG 9315) forming admixtures in all five clusters (Table 1). STRUCTURE analysis also identified the two major clusters, obtained by NJ clustering (Fig. 3 and Fig. 5). The five *K*s structuration also corresponded to the genetic clusters revealed by the PCoA plots (Fig. 4). However, the structuring was not related to the geographic origin of the populations (Table 1).

DISCUSSION

Cultivated peanut has a narrow genetic base (Knauft and Gorbet, 1989). In the United States, the few Valencia peanut cultivars that are commercially grown were primarily based on selections from plant introductions (Isleib et al., 2001). Identification of genetically diverse germplasm with beneficial traits and their use in peanut breeding is the key to broaden the genetic base of peanut cultivars. The researchers at New Mexico State University are engaged in a systematic evaluation of Valencia germplasm to identify genetically diverse germplasm with beneficial traits. To this end, a core collection of 78 Valencia accessions (Dwivedi et al., 2008) and 36 diverse Valencia germplasm accessions from global mini-core collection (Upadhyaya et al., 2002) were assessed for genetic diversity in this study. Characterization of genetic

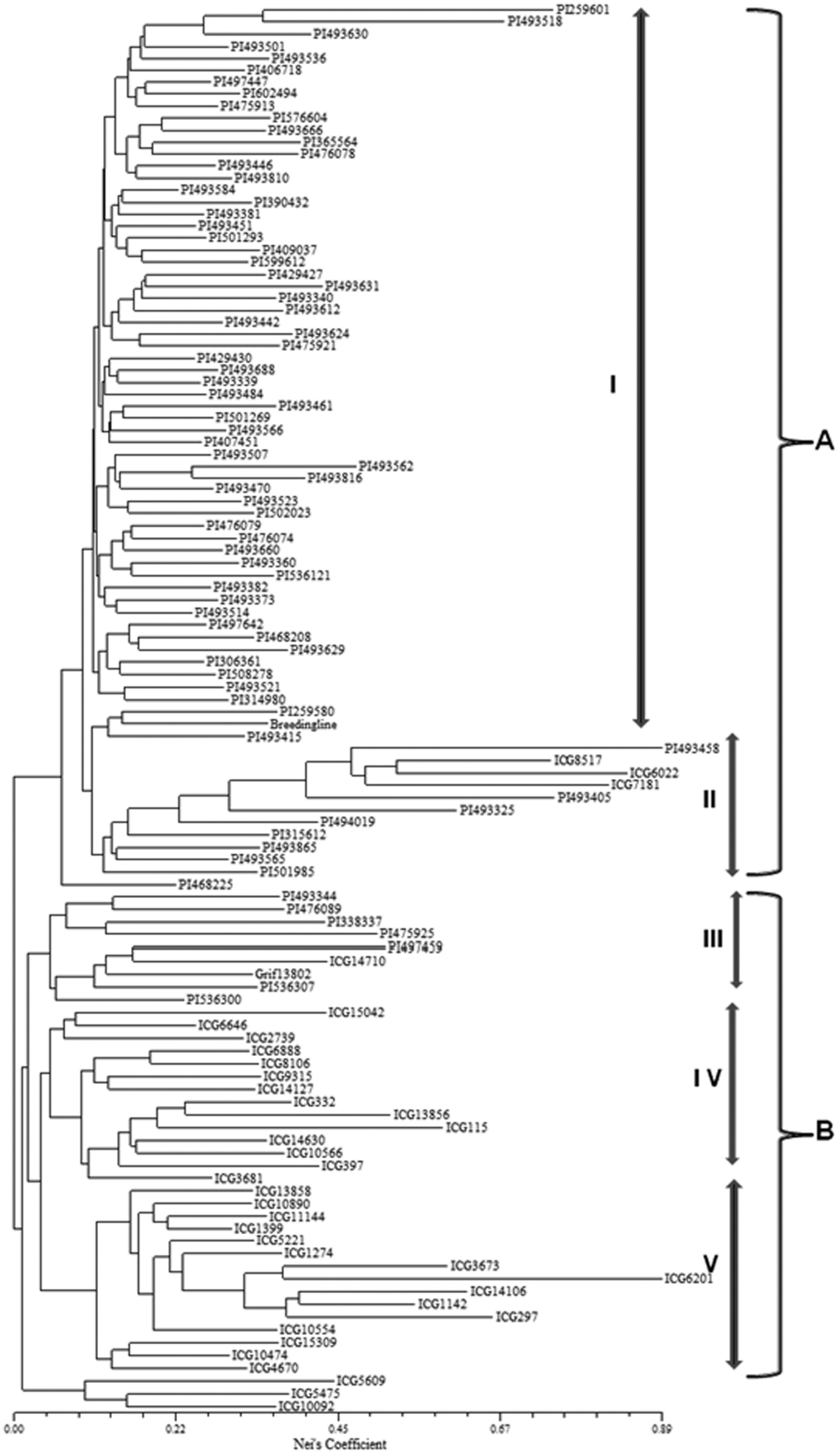

Figure 3. Neighbor-joining tree constructed using Nei's coefficient for 114 Valencia peanut genotypes from global Valencia peanut germplasm. Two main groups (A and B) with five clusters (I–V) are depicted. Source of their accessions: PI = U.S. Valencia Core; ICG = ICRISAT gene bank.

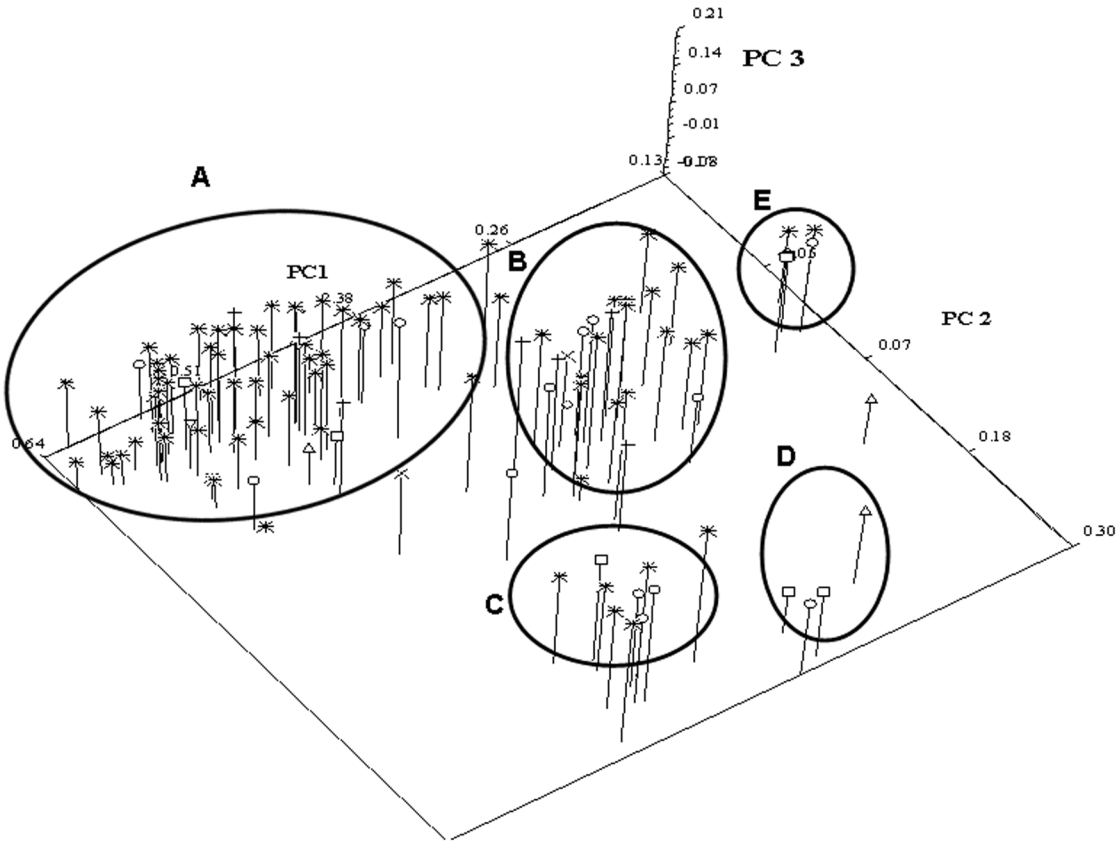

Figure 4. Principal coordinate analysis for 114 Valencia peanut accessions based on 52 simple sequence repeat markers. Two main groups, A and B, were identified. Each geographical region is identified by the following symbol: O, Africa; □, Asia; ◊, Australia; Δ, Caribbean; ▼, Central America; X, Europe; +, North America; *, South America. PC, principal component.

diversity in core or mini-core subsets of germplasm should lead to identification of genetically diverse germplasm for use in breeding and genomic applications.

Fifty-eight SSRs loci in the present study detected 683 alleles, averaging 13 alleles per locus, similar to Barkley et al. (2007). Other studies reported large variations in detecting the average number of alleles, ranging from three (Mace et al., 2007) to 31 alleles per locus (Krishna et al., 2004). The number of alleles detected per primer generally depends on the number of tandem repeat units, population size, and also on the type of electrophoresis employed to resolve the amplified fragments (Hwang et al., 2008). The moderate diversity detected by mean PIC (0.27) and gene diversity (0.335) in this set of Valencia germplasm is comparatively lower than those reported for the United States mini-core collection accessions, which represents all four market types (Kottapalli et al., 2007). This result is not surprising given the fact that we examined a single market type.

The SSR data were further used to compute the matrix based on shared alleles between accessions from different countries and the resulting NJ clustering revealed some interesting groupings (Fig. 2). Cluster 5 had common alleles representing South American countries such as Paraguay, Bolivia, Ecuador, Peru, and Brazil. In our data set, 22.8% of accessions were from Brazil. This would be expected, as Valencia genotypes are widely distributed in South America and predominantly in five centers of diversity, with Brazil being one of the important centers of diversity for Valencia peanut (Ferguson et al., 2004a; Kottapalli et al., 2007). In the entire data set a single genotype was available from Venezuela (PI 338337). Interestingly, this accession was placed in Cluster 2, which is devoid of any additional accessions from South American countries. Mostly, accessions from Asia, Africa, the Caribbean, Central America, and Europe did not share common alleles with Cluster 5, which is dominated by accessions from South America. Our findings are similar to those reported by Ferguson et al. (2004a), who concluded that for the botanical varieties of peanut, landraces from Asia and Africa were more closely related to each other than those from South America. The NJ cluster analysis and PCoA estimated the genetic diversity among the accessions and revealed the presence of two major groups. Group A (64.0% of accessions) in the NJ analysis and Group A (55.6% of accessions) in the PCoA analysis (Fig. 3 and 4), both largest clusters, comprised accessions mainly from the South American region. Group B consisted of accessions from most of the geographical regions of the world. The two major consensus groups obtained by NJ and PCoA approaches were in accordance to the consensus clustering reported by Mace et al. (2006, 2007) with multidimensional scaling plots and

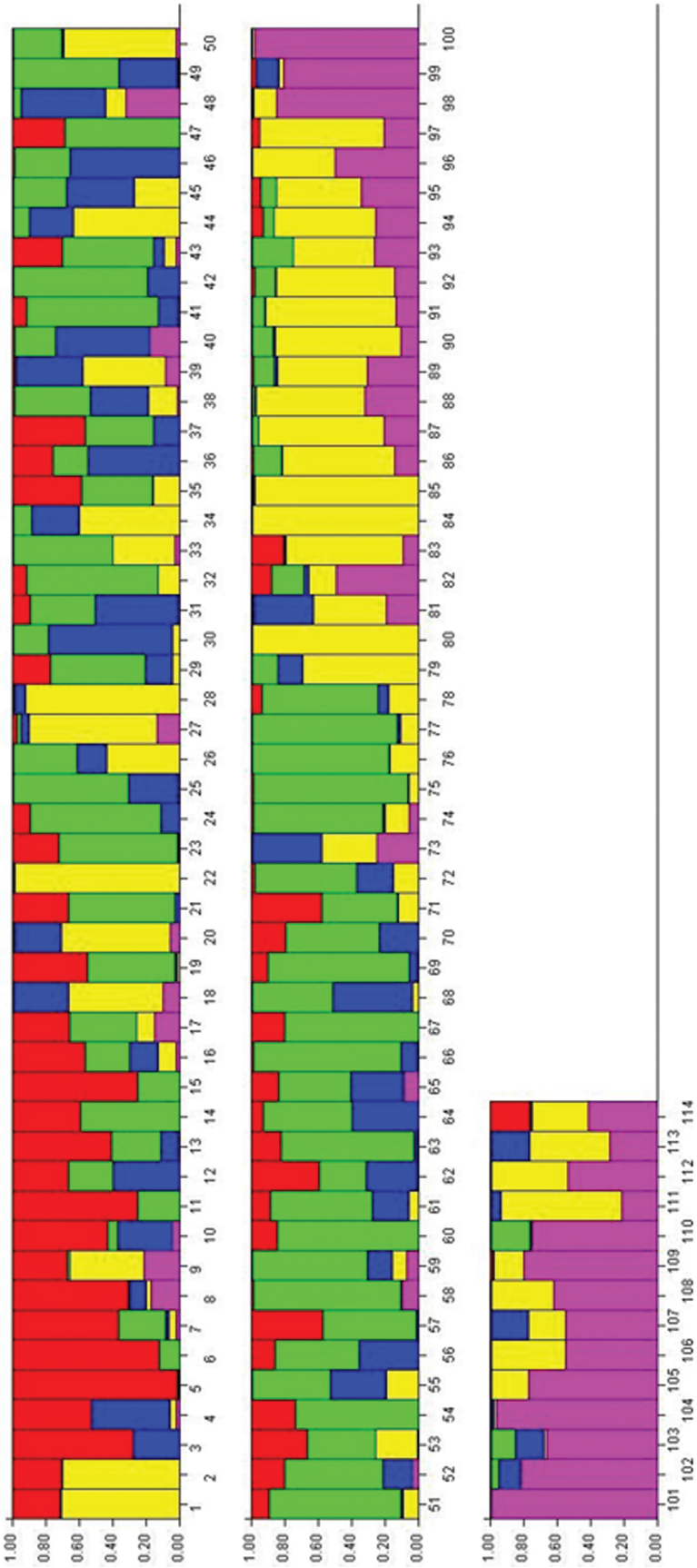

Figure 5. Graphic representation of the individual ancestry coefficients (percentage of alleles) of 114 Valencia peanut accessions, structured into five clusters, as identified by STRUCTURE software: 1—red, 2—green, 3—blue, 4—yellow, and 5—pink.

Krishna et al. (2004) with principal components analysis two-dimensional plots.

Identification of genetically homogenous groups of individuals using model-based STRUCTURE analysis was attempted. In our study, five discrete subgroups were obtained, with Subgroups 2 (30.7% of accessions) and 4 (25.2% of accessions) corresponding to the major clusters observed by tree-based on NJ clustering and multivariate PCoA clustering methods. In crop plants, STRUCTURE analysis is routinely employed to identify the presence of subgroups in core collections (Anderson et al., 2009; Abdurakhmonov et al., 2008; Hasan et al., 2008). The five *K*s structuring corresponded to the five genetic clusters revealed by PCoA plot and the five distinct clusters detected in NJ dendrograms. Together, NJ, PCoA, and STRUCTURE analysis separated the Valencia accessions into similar groups. However, a tight population STRUCTURE defined by distinct geographic locations could not be found in the Valencia germplasm studied. Furthermore, there were eight accessions from five continents that had admixtures from all five clusters. This could be due to the composition of the Valencia germplasm collected from different parts of the world at different times over the past century. The presence of admixtures in the germplasm is also not surprising as the majority of accessions included are mainly introductions from South America, the center of origin for the Valencia market type.

In summary, the 52 SSR markers detected moderate genetic diversity among the 114 Valencia peanut germplasm accessions. The NJ, PCoA clustering, and STRUCTURE analysis generated distinct groups among the Valencia genotypes. The majority of the accessions originated from Brazil and grouped into a single major cluster, emphasizing the importance of this country as a primary center of origin for the Valencia peanut market type. The molecular diversity observed in the Valencia germplasm provided useful information to aid selection of genetically diverse accessions that could be used for crop improvement. Our future efforts will focus on evaluation of the Valencia germplasm for important agronomic and seed quality traits and for resistance to biotic and abiotic stresses to identify trait-specific, genetically diverse germplasm to broaden the genetic base of Valencia peanut cultivars.

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