Received: 4 August 2020 | Accepted: 28 March 2021 | Published online: 10 August 2021

DOI: 10.1002/agj2.20671

Agronomy Journal

ARTICLE

Agronomic Application of Genetic Resources

Genetic diversity and distinctness based on morphological and SSR markers in peanut

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Assigned to Associate Editor Henry Qu.

Funding information
the Modern Agro-industry Technology Research System, Grant/Award Number: CARS-13; the Science and Technology Program of Guangzhou, China, Grant/Award Number: 201904010190; the Special Project for Science and Technology Innovation Strategy of Guangdong Academy of Agricultural Sciences, Grant/Award Numbers: R2018PY-JX001, R2020PY-JX004; the National Natural Science Foundation of China, Grant/Award Numbers: 31771841, 31801401; the Guangdong Modern Agricultural Industry Technology System, Grant/Award Number: 2019KJ136

Abstract

The morphological and molecular diversity of 101 peanut varieties from South China were analyzed to identify distinctness among these varieties. No significant difference was observed for six morphological characteristics whereas a range of 0.25–0.51 of diversity index was observed for 11 morphological characteristics, with an average value of 0.39. Molecular characterization with 40 highly polymorphic simple sequence repeats (SSRs) generated a total of 167 alleles ranging from two to six alleles per marker with average 4.18 alleles per marker. The polymorphism information content (PIC) of these markers varied from 0.79 to 0.26 with an average value of 0.55 per marker. The diversity analysis using morphological and genotyping data grouped all the varieties into seven and six clusters, respectively, and varieties released by the same province tended to be grouped in the same cluster. Mantel testing revealed that the correlations between the similarity coefficient matrixes of the morphological characteristics and SSR markers of different varieties were weak (r = .347), implying that deployment of more SSR markers is needed for achieving distinctness among these peanut varieties. Nevertheless, the combination of morphological characteristics and SSR markers will effectively increase the accuracy of distinctiveness identification.

1 | INTRODUCTION

Peanut (*Arachis hypogaea*) belongs to the *Arachis* section, an allotetraploid ($2n = 4x = 40$, AABB). It is an important oil seed crop cultivated in more than 100 countries of the world. China and India are the two largest producers of

Abbreviations: DUS, distinctness, uniformity, and stability; PCR, polymerase chain reaction; PIC, polymorphism information content; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

peanut and both have put intensive funds and efforts in breeding improved varieties of peanut for various quality, yield, and disease-related traits. According to the Regulations of the People's Republic of China on the Protection of New Varieties of Plants (as amended on 1 Mar. 2013) (https://www.upov.int/portal/index.html.en), only new varieties of crops with distinctness, uniformity, and stability (DUS) can be authorized. By 2018, 675 peanut varieties in China have been applied for the protection of breeders' right and 116 of which were authorized successfully (http://202.127.42.47:6009/Home/BigDataIndex).

Distinctness test is the core of DUS testing in which accurate phenotyping of new varieties is the basic requirement. The current distinctness test is based on morphological features that are greatly influenced by environmental conditions along with subjective errors during data recording and storage. The majority of the breeding programs use a set of founder parents with similar pedigrees and features to combine desirable traits such as yield, quality, and disease resistance. In other words, breeders emphasize on high yield of peanut varieties excessively and often select parents with high combining ability for crossbreeding, resulting in close ties of consanguinity of varieties. The offspring heritable variation lingers in the inbreeding level (Nie et al., 2016), thus increases the difficulty of phenotype screening of approximate varieties. Due to globalization, breeding materials are exchanged across countries by public and private breeding research institutions and variety infringement events have increased gradually in recent years. Even the same genetic stock is marketed with different names and it has become very difficult to protect the infringement of new peanut varieties in the world including China, which is a major problem for farmer's community at ground level.

The current DUS testing requires detailed morphological characterization for at least two seasons to ensure the distinctive features between two varieties. This procedure is not only time consuming but also very expensive and laborious. The last decade has witnessed drastic reduction in prices of sequencing and genotyping due to advancement in sequencing technologies such genotyping-by-sequencing (GBS) (Dodia et al., 2019; Kulkarni et al., 2020), whole genome re-sequencing (WGRS) (Kumar et al. 2020 ; Pandey et al. 2017a; Agarwal et al., 2018), ddRADseq, DArT, and DArT-sEquation (Pujar et al., 2020; Vishwakarma et al., 2016) and high density single nucleotide polymorphism (SNP) array (Gangurde et al., 2020; Pandey et al., 2017b) which provided a much cheaper, time saving, and highly precise option for performing distinctiveness studies in plants including peanut. Besides, it has the advantage of not being restricted by gene expression during different developmental stages of plant growth. Molecular markers are not sensitive to environment and have high repeatability (Hayward et al., 2015). Therefore, molecular markers are providing great technical support in testing, approval, and identification of new varieties of plants (Cockram et al., 2012; Jones et al., 2013). Tommasini et al. (2003) used simple sequence repeat (SSR) markers to test the distinctness of oilseed rape varieties and suggested that SSR markers could be used as an effective supplementation of DUS test system in oilseed rape breeding. Arens et al. (2010) developed eight molecular markers on tomato (*Lycopersicon esculentum* Mill.) which were highly correlated with resistance and applied their polymorphism content to differentiate the tomato varieties. Results demonstrated that this set of markers met requirements for DUS testing of varieties and could be used as the supplementary method of resistance identification in current DUS-testing protocol. Recently, Jones and Mackay (2015) analyzed barley (*Hordeum vulgare* L.) varieties by using the high-density SNP markers and established distinctness of the variety.

Although molecular markers can play an important role for varietal identification, existing molecular markers are still unable to replace morphological characterization. Therefore, morphological markers are widely used in distinctness of candidate varieties while performing the DUS test. However, complete replacement of morphological characterization with DNA fingerprinting using molecular markers is not possible in the DUS test. Because none of the crop species have trait-linked markers available for all the DUS descriptor traits. In addition, the markers can only help in testing the distinctness among candidate varieties while dependency will still remain to test uniformity and stability during multi-location testing. Different varieties with different DNA fingerprints may display the same morphological performance, whereas different varieties with the same DNA fingerprints (e.g., mutants) may display great differences in morphology. Besides, according to requirements of regulations of The International Union for the Protection of New Varieties of Plants (UPOV), a verification problem of uniformity also exists in the application of protection of new varieties. DNA fingerprinting technology has high sensitivity and is difficult to discover varieties with high stability at all marker loci. Therefore, the standards of uniformity validation of varieties based on molecular

Core Ideas

- A range of 0.25–0.51 of diversity index was observed for 11 morphological features.
- Morphological and genotyping data grouped all the varieties into seven and six clusters.
- Deployment of more SSR markers is needed for achieving distinctness among peanut varieties.

TABLE 1 Names and sources of the peanut varieties

Variety	Breeding institution	Province
YY7, YY9, YY13, YY18, YY29, YY35, YY40, YY41, YY45, YY49, YY52, YY114, YY290, YY390, YY410, YY645, HH2, HH3	Guangdong Academy of Agricultural Sciences	Guangdong
SY21, SY52, SY71-31, SY162, SY188, SY250, SY382, SY851, SYF1, SYY1	Shantou Agricultural Science Research Institute	Guangdong
ZKH1, ZKH2, ZKH4, ZKH6, ZKH9, ZKH10, ZKH12, ZKH99	Zhongkai University of Agriculture and Engineering	Guangdong
ZY15, ZY16, ZY55, ZY58, ZY62, ZY65, ZY75, ZY82, ZY93	Zhanjiang Academy of Agricultural Sciences	Guangdong
QH052, QH511, QH551, QH557, QH627, QH701, QH726, QH2197	Quanzhou Agricultural Science Research Institute	Fujian
MH2, MH5, MH7, MH8, MH9, MH10, MH13, MH15, JH44, JH47	Fujian Agriculture and Forestry University	Fujian
LH6, LH9, LH18, LH106, LH163, LH202, LH243	Longyan Agricultural Science Research Institute	Fujian
PH1, PH3, PH21, PH23, PH25	Putian Agricultural Science Research Institute	Fujian
FH4, FH6, FH8	Fujian Academy of Agricultural Sciences	Fujian
GH026-10, GH026-7, GH24, GH26, GH35, GH56-20, GH68, GH69, GH193, GH771, GH836	Guangxi Academy of Agricultural Sciences	Guangxi
HY8, HY9, HY10, HY11, HY12, HY13, HY14, HY15	Hezhou Agricultural Science Research Institute	Guangxi
XH120, XH2008	Hunan Agricultural University	Hunan
YHS4, YHS12	Yunnan Academy of Agricultural Sciences	Yunnan

markers might be lower than those of field phenotype uniformity. However, how to determine the appropriate standards and probability of acceptance is still a difficulty to hybrid varieties. Additionally, how many individuals are needed for uniformity test, whether it requires the same sample size with that in a DUS field test and how to distinguish the allowable error of difference individuals all have to be explored in practice (Zheng et al., 2019).

Since, molecular markers alone in DUS tests have many technological bottlenecks, the combination of morphological polymorphism and molecular characterization seems to be the best approach as reported in recent DUS tests (Deng & Han, 2019). The variation between varieties during plant variety protection (PVP) can be more robust by combining molecular markers and morphological indexes to increase the coefficient of variation for closely related varieties. Other than establishing the genetic relationship among varieties, morphological and molecular markers profiles can help in protection of plant varieties.

Therefore, in this study we reported morphological and molecular characterization of peanut varieties from South China which resulted in testing the distinctness in peanut varieties successfully. It can provide technological supports to fast screening of peanut varieties and varietal purity in DUS testing.

2 | MATERIALS AND METHODS

2.1 | Plant material

Test materials (101 samples) of peanut varieties were selected from South China released during 1999–2015 (Table 1) and the seed materials were provided by the Crop Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong, China.

2.2 | Investigation of morphological characteristics

Investigation of morphological characteristics was carried out in the Wushan Test Base in the South China Agricultural University from March 2016 to August 2017. Randomized block design (RBD) was adopted for conducting field experiments. In each block, row length was set to be 3 m and plant to plant distance was 20 cm. Each entry was planted in replicated field trails. According to UPOV Guidelines for The Conduct of Tests for Distinctness, Uniformity and Stability-Peanut, 17 morphological characteristics were investigated (Table 2), including 4 quality characteristics, 2 pseudo-quality characteristics, and 11 quantitative characteristics (IBPGR

TABLE 2 Details of morphological characteristics investigated for 101 varieties

No.	Morphological characteristics	Characteristics (code)	Simpson's Diversity Index
1	Plant: growth habit	erect (1)	0
2	Plant: density	medium (2); dense (3)	0.34
3	Stem: anthocyanin coloration	absent or weak (1); medium (2); strong (3)	0.41
4	Main stem: presence of flowers	present (9)	0
5	Leaf: intensity of green color	medium (2); dark (3)	0.29
6	Leaflet: length	Medium (2); long (3)	0.33
7	Leaflet: position of broadest part	at middle (1); moderately towards apex (2); strongly towards apex (3)	0.38
8	Leaflet: shape of apex	broad pointed (2); rounded (3); retuse (4)	0.47
9	Primary branch: flowering pattern	Sequential (2)	0
10	Pod: constrictions	absent or very weak (1); weak (2); medium (3); strong (4)	0.48
11	Pod: reticulation of surface	weak (1); medium (2); strong (3)	0.46
12	Pod: number of kernels	two (1)	0
13	Kernel: main color of testa	brownish pink (2)	0
14	Kernel: presence of secondary color of testa	absent (1)	0
15	100 kernel weight	low (1); medium (2); high (3)	0.51
16	Pod: thickness of shell	thin (1); medium (2); thick (3)	0.37
17	Time of maturity	early (3); medium (5)	0.25

& ICRISAT, 1992). The quality characteristics and pseudo-quality characteristics were investigated by manual observation. For quantity characteristics, sample length was measured by straightedge or Vernier Calipers and sample weight was measured by electronic balance.

2.3 | Extraction of high quality DNA

Peanut genomic DNA was extracted from young tender peanut leaves using the new fast plant genome DNA extraction kits (article no.: DP3111, BioTeke). The DNA quality was checked on 1% agarose gel electrophoresis and quantified on UV-Vis spectrophotometers Nanodrop 2000 (Thermo Scientific). The DNA of all samples was diluted uniformly with sterile deionized water to 25 ng μl^{-1} and stored at −20 °C for future use.

2.4 | Selection of highly informative SSR markers and PCR conditions

The 40 SSR markers included 2 SSR markers from each chromosome with high polymorphism, stable amplification, and clear banding patterns were selected from the comprehensive genetic map of peanut constructed by Shirasawa et al. (2013). There are a total of 3,693 SSR markers were mapped on this genetic map. The primers of 40 selected SSR markers were synthesized at Beijing Genomics Institute, Beijing, China.

Polymerase chain reaction (PCR) was performed in a reaction mixture volume of 20 µl using standard PCR conditions {20 ng DNA, 2.0 µl 10×buffer [0.8 mol L^{-1} Tris-HCl, 0.2 mol L^{-1} $(NH_4)_2SO_4$, 0.2% (v/v) Tween 20], 2.0 µl 10× dNTPs (2.5 mmol L^{-1} each), 0.4 µl each PCR primer (10 mmol L^{-1}), 2.4 µl $MgCl_2$(25 mmol L^{-1}), one unit *Taq* polymerase (Cat. no. ET101, Tiangen, Beijing, China)}. The PCR program was set as, 1 cycle for 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 30 s at 55 °C and 45 s at 72 °C and an additional last cycle for final extension for 10 min at 72 °C on PCR machine (ETC811, EASTWIN, China). The amplified products were tested by using Fragment Analyzer full-automatic capillary electrophoresis system (AATI, FSV2-CE). Data after capillary electrophoresis was stored in ProSize 2.0 system and used for further analysis.

2.5 | Statistical analysis of morphological data

The 1-0 matrix of variety–morphological characteristics was constructed based on investigation data of morphological characteristics. The variety which occurred on the level *i* of a characteristic was recorded 1; otherwise, it was recorded 0. The diversity index of morphological characteristics was

calculated by: $D = 1 - \sum_{i=1}^{k} p_i^2$, where D is the Gini Simpson index and p_i is the percentage of material number on level i of a characteristic in total material number (Nei, 1978).

2.6 | Calculation polymorphic information content for SSR markers and phylogenetic tree construction

The 1-0 matrix of variety SSR markers was constructed based on genotyping data generated using SSR markers. If the SSR amplified band is present in one particular line then it is scored as '1′, while if the band is absent in a particular line it is scored as '0.' The polymorphic information content (PIC) of SSR markers was calculated by, $PIC = 1 - \sum_{i=1}^{k} p_i^2$, where p_i is the frequency of allele i on the loci (Anderson et al., 1993).

The NTSYS-pc2.11 software was used for construction of phylogenetic tree. The genetic similarity (GS) was calculated by the original matrixes generated individually using morphological characteristics and SSR markers by using qualitative data in the similarity module. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) algorithm was used for clustering module for cluster analysis. The dendrogram was generated using Unweighted Pair Group Method with Arithmetic mean (UPGMA). The Mantel test (Mantel, 1967) was used to confirm the correlation between similarity coefficient matrixes generated using morphological characteristics and SSR-genotyping data.

2.7 | Population structure analysis using SSR markers

Population structure analysis was conducted by using structure software v 2.3.4 (Pritchard et al., 2000) and the results were extracted using structure harvester (Earl, 2012). The structure analysis was conducted at burn-in period length and 500 and number of MCMC reps after burn-in was set to be 1,000 and number of iterations was set at 3. The phylogenetic tree was constructed using DARwin 5.0 software by using Dice algorithm (Perrier & Jacquemoud-Collet, 2006).

3 | RESULTS

3.1 | Diversity analysis of morphological characteristics

Descriptive statistics based on 17 phenotypic characteristics of 101 peanut varieties demonstrated that 6 morphological characteristics (Plant: growth habit, Main stem: presence of flowers, Primary branch: flowering pattern, Pod: number of kernels, Kernel: main color of testa, Kernel: presence of secondary color of testa) displayed no significant diversity among test varieties. The Gini Simpson index of 11 characteristics ranged from 0.25 to 0.51 with an average of 0.39. The 100-kernel weight was found to possess the highest diversity index among all morphological traits.

Although morphological characteristics of peanut varieties have certain diversity on the whole, peanut varieties released by the same breeding institute had similar phenotypes and low genetic diversity. For example, most of YY varieties had plant features such as medium density, stem: absent or weak anthocyanin coloration, leaflet: dark green color, leaflet: medium length, leaflet: at middle position of broadest part, pod: absent or weak constrictions, pod: strong reticulation of surface, 100-kernel weight: high, pod: thick shell, time of maturity: late.

In this study, 11 diversified morphological characteristics of testing varieties were selected for performing cluster analysis. The similarity coefficient is 0.76, all the testing varieties were grouped into seven clusters (Figure 1a). Cluster I comprised 10 varieties, of which 9 were from the "ZY" series and 1 was from the "YY" series. Cluster II comprised 28 varieties, 15 of which were from "YY" series, 4 were from "ZKH" series, 3 were from "HY" series, 2 each were from "MH" and "HH" series, and 1 each was from "SY" and "FH" series. Cluster III comprised 30 varieties, including 8 varieties of "QH" series, 7 varieties of "LH" series, 5 varieties each of "PH" and "MH" series, 2 varieties each of "FH" and "JH" series, and 1 variety of "HY" series. Cluster IV comprised 17 varieties, including 9 varieties of "SY" series, 4 varieties of "ZKH" series, 3 varieties of "HY" series and 1 variety of "MH" series. Cluster V comprised 12 varieties, including 11 varieties of "GH" series and 1 variety of "HY" series. Cluster VI and cluster VII comprised 2 varieties respectively, including "XH120" and "XH2008" as well as "YHS4" and "YHS12". According to clustering results of morphological features, peanut varieties from the same breeding institutes clustered together. For example, varieties of "XH" and "YHS" series from Hunan Agricultural University and Yunnan Academy of Agricultural Sciences were clustered independently in clusters VI and VII. Besides, 11 "GH" varieties from Guangxi Academy of Agricultural Sciences were clustered together and 9 "ZY" varieties from Zhanjiang Agricultural Science Research Institute clustered together in cluster I . Moreover, peanut varieties of the same province tended to cluster together. For instance, 22 of the 29 varieties in cluster II were from Guangdong Province, and 20 of the 30 varieties in cluster III were from Fujian Province.

3.2 | Diversity analysis using SSR markers

In this study, 40 uniformly distributed SSR markers (2 from each groundnut chromosome) with high polymorphism

TABLE 3 Details of SSR markers used for cluster analysis

SN	Name	Chr	Pos	Motif	No. of alleles	PIC	Amplified length
							bp
1	AHGS1647	a01	62.9	AG	4	0.52	301 (29.7%), 305 (63.3%), 307 (6.93%), 313 (26.7%)
2	AHGS1908	a01	147.0	AG	5	0.64	193 (31.7%), 195 (9.90%), 199 (49.5%), 203 (6.93%), 211 (19.8%)
3	AHGS1163	a02	41.4	AG	3	0.37	239 (77.2%), 245 (4.95%), 249 (17.8%)
4	AHGS1179	a02	67.6	AG	4	0.55	280 (21.7%), 288 (11.9%), 292 (62.4%), 296 (39.6%)
5	AHGS3647	a03	110.8	AT	3	0.45	250 (14.9%), 252 (13.9%), 260 (71.2%)
6	AHGS1838	a03	56.9	AG	4	0.52	191 (66.3%), 195 (8.91%), 201 (9.9%), 205 (14.9%)
7	AHGS1313	a04	45.2	AG	5	0.63	291 (22.8%), 295 (54.5%), 301 (10.9%), 303 (5.94%), 313 (5.94%)
8	seq15C12	a04	71.4	TAA	6	0.65	268 (1.98%), 271 (8.91%), 277 (10.9%), 280 (2.97%), 289 (22.8%), 295 (52.5%)
9	AHGS1507	a05	45.2	AG	4	0.44	248 (1.98%), 256 (70.3%), 258 (2.97%), 264 (24.8%)
10	IPAHM356	a05	71.6	GA	4	0.57	100 (33.7%), 106 (4.95%), 108 (55.4%), 118 (5.94%)
11	IPAHM509	a06	163.7	CA	3	0.35	185 (79.2%), 189 (10.9%), 195 (9.9%)
12	GM1916	a06	122.4	AGA	2	0.26	115 (84.2%), 121 (15.8%)
13	AHGS1454	a07	47.4	AG	4	0.63	208 (29.7%), 216 (51.5%), 226 (7.92%), 230 (10.9%)
14	seq5D05	a07	98.4	GA	6	0.79	274 (14.9%), 276 (23.8%), 280 (16.8%), 290 (29.7%), 292 (6.93%), 296 (7.92%)
15	seq2A05	a08	67.9	TAA	3	0.31	252 (16.8%), 261 (81.1%), 267 (1.98%)
16	seq3A08	a08	100.1	TAA	5	0.64	152 (55.4%), 158 (10.9%), 161 (20.8%), 170 (5.94%), 173 (6.95%)
17	IPAHM23	a09	83.9	CA	4	0.53	130 (12.9%), 142 (4.95%), 146 (16.8%), 148 (65.3%)
18	AHGS1543	a09	102.0	AG	3	0.37	118 (15.8%), 122 (77.2%), 130 (6.93%)
19	AHGS1368	a10	80.8	AG	4	0.55	118 (31.7%), 120 (7.92%), 126 (56.4%), 134 (3.96%)
20	AHGS1386	a10	95.0	AG	5	0.49	145 (6.93%), 149 (69.3%), 151 (11.9%), 155 (6.93%), 159 (4.95)
21	AHGS1358	b01	8.9	AG	5	0.67	258 (16.8%), 262 (49.5%), 276 (19.8%), 274 (6.93%), 278 (6.93%)
22	Ah3	b01	131.5	GA	4	0.66	188 (33.7%), 198 (12.9%), 202 (44.6%), 214 (8.91%)
23	AHGS1241	b02	76.8	AG	4	0.66	196 (29.7%), 216 (12.9%), 222 (46.5%), 228 (10.9%)
24	AHGS1940	b02	38.9	AG	4	0.56	210 (19.8%), 238 (9.90%), 240 (61.4%), 244 (8.91%)
25	seq14C11	b03	33.8	TAA	5	0.64	143 (11.9%), 149 (55.4%), 158 (13.9%), 170 (11.9%), 176 (6.93%)
26	AHGS1310	b03	45.2	AG	3	0.43	222 (15.8%), 234 (72.3%), 238 (11.9%)
27	TC11H06	b04	37.8	AG	4	0.61	205 (19.8%), 209 (14.9%), 217 (56.4%), 225 (8.91%)
28	TC4H07	b04	99.2	TC	5	0.67	226 (10.9%), 230 (50.5%), 238 (13.9%), 242 (16.8%), 248 (7.92%)
29	seq19D06	b05	35.6	AG	4	0.51	232 (8.91%), 238 (13.9%), 248 (67.3%), 256 (8.91%)
30	AHGS1342	b05	46.6	TAA	5	0.69	157 (11.9%), 163 (47.5%), 169 (12.9%), 175 (20.8%), 184 (8.91%)
31	AHGS1337	b06	78.3	AG	5	0.63	282 (11.9%), 286 (55.4%), 292 (7.92%), 300 (17.8%), 308 (8.91%)
32	PM210	b06	275.8	CT	3	0.49	194 (15.8%), 206 (67.3%), 218 (16.8%)
33	TC9H09	b07	52.9	AG	4	0.57	220 (8.91%), 226 (13.9%), 214 (61.4%), 220 (15.8%)
34	AHGS1215	b07	78.1	AG	6	0.74	189 (14.8%), 193 (17.8%), 197 (12.9%), 199 (41.6%), 207 (9.90%), 219 (2.97%)
35	Ah51	b08	30.9	AG	4	0.46	124 (6.93%), 130 (13.9%), 134 (71.3%), 138 (7.92%)
36	AHGS1945	b08	75.3	AG	4	0.58	150 (6.93%), 152 (13.8%), 164 (59.4%), 168 (19.8%)
37	TC5A06	b09	34.1	TC	5	0.65	186 (12.9%), 192 (52.5%), 198 (8.91%), 206 (17.8%), 214 (9.90%)
38	Lec1	b09	49.5	AT	4	0.51	230 (7.92%), 246 (10.9%), 248 (67.3%), 256 (13.9%)
39	AHGS2223	b10	42.7	AG	3	0.42	132 (18.8%), 144 (73.3%), 156 (7.92%)
40	AHGS1195	b10	91.1	AG	5	0.70	355 (12.9%), 359 (46.5%), 367 (11.9%), 377 (17.8%), 383 (12.9%)

Note. Chr: chromosome; Pos: position; PIC: polymorphic information count.

content and very clear banding pattern recognition were applied for genomic DNA amplification of 101 varieties. A total of 167 alleles were observed with each marker correlating to 2–6 allele's and 4.18 alleles in average per marker. The PIC of markers differed significantly around the average of 0.55 with the maximum to be 0.79 and the minimum PIC to be 0.26 (Table 3).

A cluster analysis of 101 peanut varieties based on 167 alleles generated by 40 SSR markers revealed that the similarity coefficient was 0.70, and all testing varieties could be divided into six clusters (Figure 1b). Cluster I comprised 30 varieties, including 8 varieties of the "QH" series, 7 varieties of "LH" series, 5 varieties each of "PH" and "MH" series, 2 varieties each of "FH" and "JH" series and 1 variety of "HY" series. Cluster II comprised 45 varieties, including 15 varieties of "YY" series, 9 varieties of "SY" series, 8 varieties of "ZKH" series, 4 varieties of "HY" series, 3 varieties each of "ZY" and "MH" series, 2 varieties of "HH" series and 1 variety of "FH" series. Cluster III comprised 8 varieties, including 6 varieties of "ZY" series, and 1 variety each of the "YY" and "SY" series. Cluster IV had 14 varieties, including 11 varieties of "GH" series and 3 varieties of "HY" series. Cluster V comprised two varieties, namely, "XH120" and "XH2008". Cluster VI was also comprised of two varieties, namely, "YHS4" and "YHS12". According to the clustering results, varieties from the same province tended to be clustered together. For example, 27 varieties from the Fujian Province grouped together in cluster I and 37 varieties from Guangdong Province grouped together in cluster II. Varieties from Guangdong, Guangxi, Hunan, and Yunnan grouped in clusters III, IV, V, and VI.

3.3 | Distinctness of peanut varieties

Based on comparison of the clustering results between morphological characteristics and SSR markers, the test varieties showed a consistent tendency on the whole and varieties from the same region mainly cluster together. With respect to genetic similarity between varieties, there were few differences between morphological characteristics and SSR markers. The morphological characteristics similarity coefficient among varieties ranged from 0.62 to 1.00, with an average of 0.75. The similarity coefficient of SSR markers was 0.55–1.00, with an average of 0.70. Mantel test revealed that the similarity coefficient of morphological characteristics among different varieties and the similarity coefficient of SSR markers were weakly correlated ($r = .347$) (Figure 2), indicating that distinctness of varieties identified by morphological characteristics and SSR markers was not completely consistent and similar. It can be seen from Figure 1a and 1b that both morphological characteristics and SSR markers were able to distinguish 97 varieties but both failed to distinguish 4 varieties. The "HY11" and "HY9" was not distinguished through morphology and SSR markers (similarity coefficient among varieties = 1.00). However, the "YY7" and "YY410" was distinguishable by SSR markers, but was not distinguishable with morphological characteristics. On the contrary, the "SYY1" and "SYF1" was distinguishable with morphological characteristics, but was not distinguishable with SSR markers.

Since "HY11" and "HY9" displayed no significant difference in morphology and SSR marker loci, they were inferred preliminarily as the same variety. Although "YY7" and "YY410" had similar morphology (the former one is the parent of the later one), their SSR markers displayed significant differences, which led to the detection of 11 different loci. The similarity coefficient among varieties was 0.69, which suggest that "YY7" and "YY410" were different varieties. In addition, "SYY1" and "SYF1" were varieties of the "SY212"series developed through EMS chemical induction and Co radiation induction. These two varieties displayed consistent DNA fingerprint through SSR markers based clustering. However, they possessed great differences in morphology and the similarity coefficient was 0.77. These two varieties were distinguishable with morphological characteristics. Therefore, it can be deduced from SSR markers and morphological information that "SY1" and "SYF1" are mutants of "SY212".

3.4 | Population structure and phylogeny

Population structure analysis showed that there are three major subpopulations (delta $K = 3$) in the 101 peanut genotypes (Figure 3a–c). However, from phylogenetic tree we observed few minor populations in subpopulations. Phylogenetic tree showed that there are few varieties paired together indicated narrow genetic diversity among few lines. In a subpopulation (blue) there were total 56 genotypes which was a major subpopulation. However, we observed admixture of another two subpopulations (red and green) in blue subpopulation. In a second subpopulation (green) there were 17 genotypes and admixture was observed from red subpopulation (Figure 3a). There were 28 genotypes in third population (red) with partial admixture. Therefore, because of admixture among the varieties we may not get a similar number of clusters in morphological and molecular diversity analysis.

4 | DISCUSSION

In the last two decades, large numbers of SSR markers in groundnut (*Arachis hypogaea* L.) have been developed and deployed in diversity and genetic mapping studies (Bhad et al., 2016; Bosamia et al., 2015; Koilkonda et al., 2012; Varshney et al. 2009; Wang et al., 2012; Zhang et al., 2012;

FIGURE 1 Cluster dendrograms based on the morphological traits and simple sequence repeat (SSR) marker
(a) Cluster dendrogram based on morphological characteristics; (b) Cluster dendrogram based on SSR marker

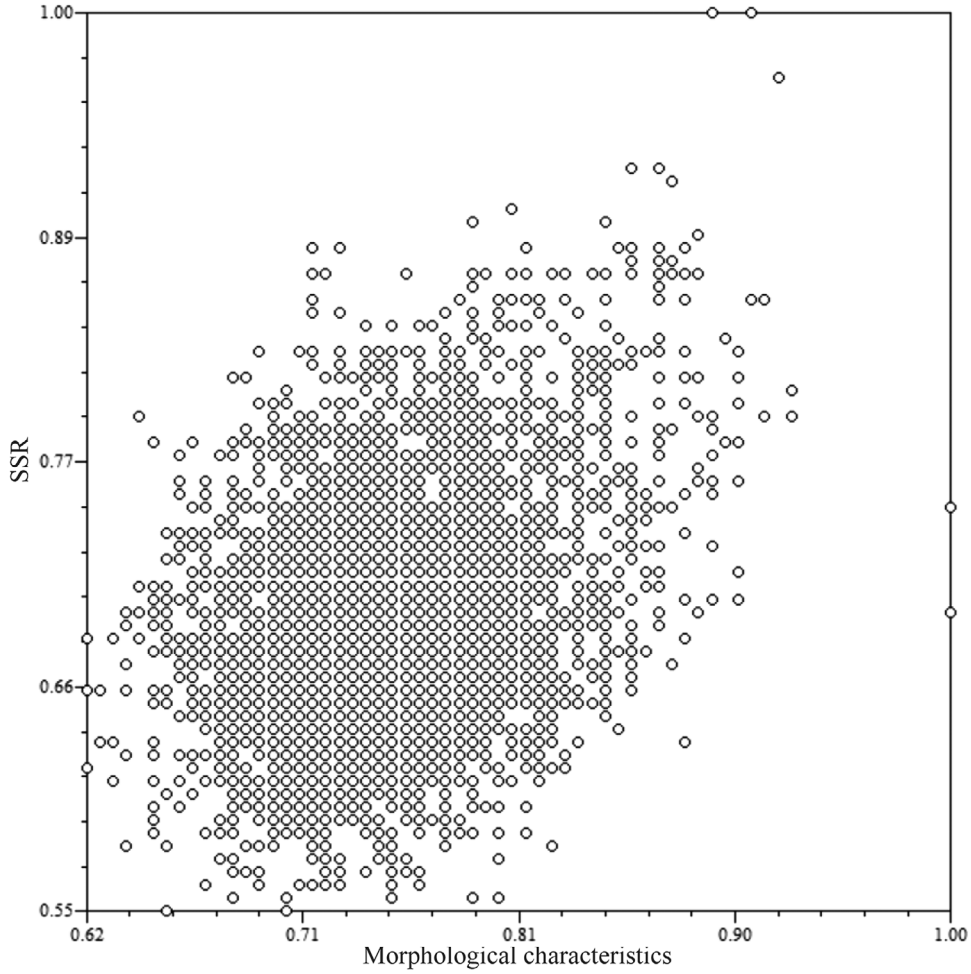

FIGURE 2 Comparison of genetic similarity coefficient matrix of morphological characteristics and simple sequence repeat (SSR) markers

Zhao et al., 2012). Prior to availability of groundnut genome sequences, SSRs were used for a variety of trait mapping studies (Gangurde et al., 2019), DNA fingerprinting for varietal protection (Gangurde et al. 2017), as well as marker-assisted breeding (Shasidhar et al., 2020). In this study, a total of 17 morphological characters were investigated on 101 varieties. Among them, 6 characters showed no genetic diversity and the remaining 11 showed average Gini Simpson index of 0.39, indicating that the cultivated varieties had a homogenization trend on phenotype. In contrast, 40 SSR markers could detect polymorphism in test varieties with an average PIC of 0.55, which is far higher than Gini Simpson index of morphological characteristics. Therefore, SSR markers demonstrated higher genetic diversity as compared to morphological characteristics. This might be because morphological characteristics were strongly sensitive to artificial selection during the breeding process, while SSR markers were highly conserved and affected by the environment but not affected by artificial breeding or selection.

According to the clustering results generated using morphological characteristics, varieties bred by the same institute tended to cluster together. This can be explained by two possible reasons. (a) Ties of consanguinity among different varieties. It can be seen from analysis of spectra of peanut varieties in South China that most varieties had at least one parent being originated from a variety of the same institute, and such varieties generally have close ties of consanguinity. (b) Different breeding institutes have different breeding programs based on the objectives. Different breeding institutes have accumulated their own breeding experiences during the long-term breeding process and they preferred specific traits based on the requirement. For instance, in semi-arid zones the pre-harvest aflatoxin contamination is a major problem and breeding to develop aflatoxin-free groundnut varieties is a top priority (Pandey et al., 2019; Soni et al., 2020). Similarly, Shantou Agricultural Science Research Institute prefers erect plants with thin stems and obvious fallen leaves at the time of maturity.

Molecular markers and morphological characteristics have inadequate correlation, which is the bottleneck for independent use of molecular markers in distinctness identification of new plant varieties. Since morphological characteristics

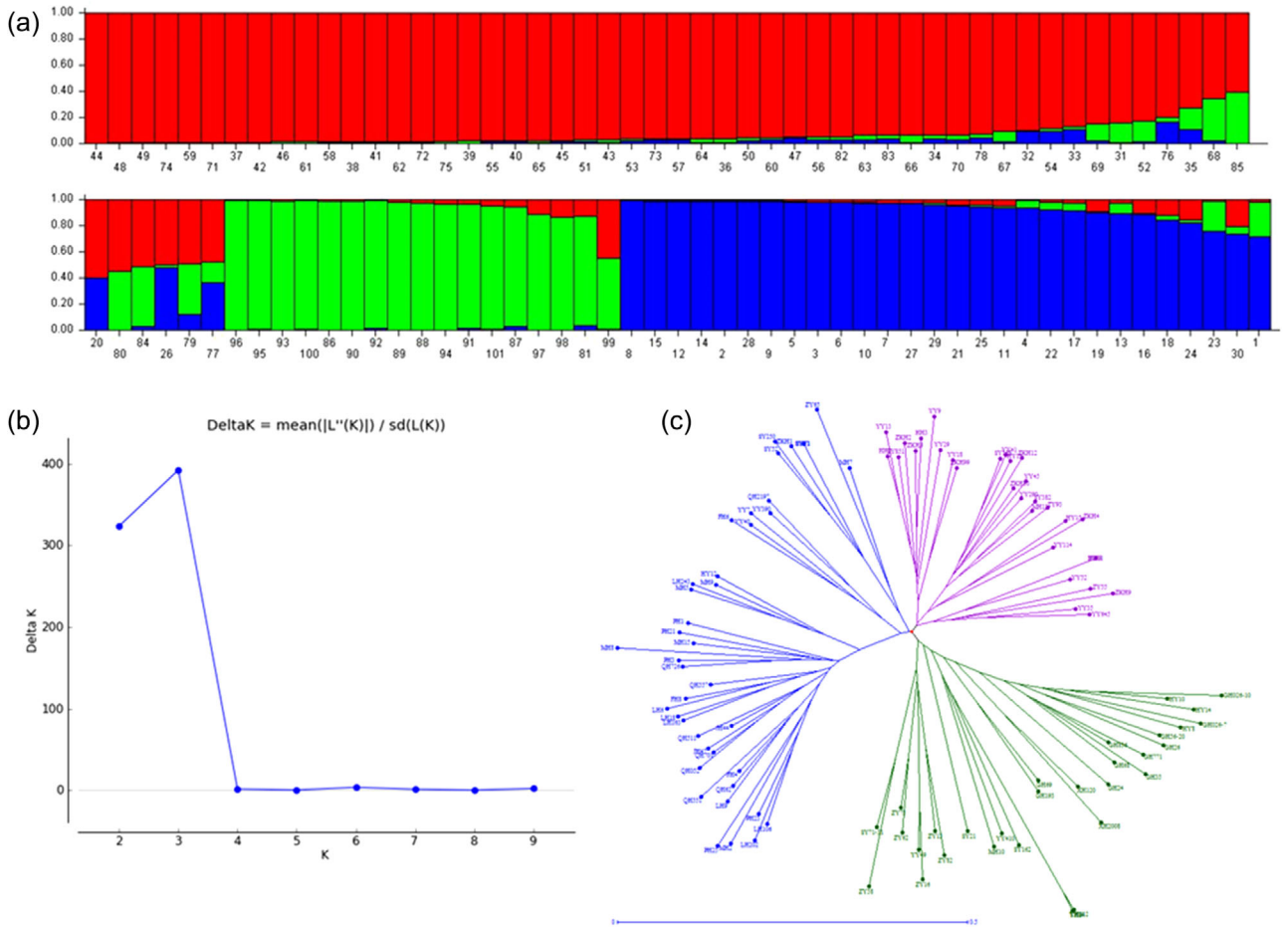

FIGURE 3 Details of population structure analysis using simple sequence repeat (SSR) markers based on 101 peanut genotypes. (a) Population structures and admixtures, (b) Graph of delta *K* (where delta $K = 3$), (c) Phylogenetic tree constructed using Dice algorithm implemented in DARwin 5.0

are mainly quantitative in nature and controlled by multiple genes. However, SSR markers are generally located in the non-coding regions of genome and are believed as the "neutral" markers and not associated with biological function. Theoretically, it is greatly difficult to search SSR markers which are highly associated with morphological characteristics. In the present study, the similarity coefficient of morphological characters was weakly correlated ($r = .347$) with similarity coefficient of SSR markers during the Mantel test, which indicated the poor correlation between SSR markers and morphological characteristics. Therefore, the SSR markers cannot be replaced with morphological characteristics and can be used independently to distinctness identification of peanut varieties. The other possibility is to increase the density of SSR markers or deploy high-density SNP arrays, or deploy low coverage sequencing such as genotyping-by-sequencing (Pandey et al., 2017a) for performing DNA fingerprinting for ultrahigh-level differentiation.

Recent studies revealed that molecular markers and morphological characteristics could supplement each other in plant classification and establishing the distinctness among varieties (Heilmann-Clausen et al., 2017; Delfini et al., 2017); variety identification (Smykal et al., 2008); and genetic diversity analysis (Ebrahimi et al., 2017; Rebaa et al., 2017), especially in seed authenticity identification, synonym and homonym identification (Wang et al., 2017; Laaribi et al., 2017). Theoretically, recombinations between parent chromosome and genetic combinations of filial generations almost have infinite possibilities. While developing a variety with the help of breeding, a breeder mainly concentrates on the traits related to yield, disease resistance, and plant types. The development of DNA markers associated with such complex traits is challenging. So far, there are abundant DNA markers located on different chromosomes which are not linked with above traits in peanut. The polymorphism generated by SSR markers can distinguish varieties which are bred by different conventional hybrid-breeding objectives. For instance, morphological characteristics cannot distinguish "YY7" and "YY410", but SSR markers can differentiate them clearly. For some mutants, especially the mutants derived from point mutations, the varieties cannot be distinguished by DNA markers. In this case these mutants can be distinguished by

SNP loci. In contrast, varieties can be distinguished easily according to phenotype mutation characteristics. In this study, "SYY1" and "SYF1" failed to distinguish from each other using SSR markers and were successfully distinguished by morphological characteristics. Therefore, if we will combine the diversity indexes generated from morphological traits and molecular markers (SSRs) it can increase the accuracy of distinctness identification between the varieties. In addition, morphological traits along with the DNA fingerprint generated using SSR markers can be used for protecting the released varieties.

5 | CONCLUSIONS

In the present study, we concluded that the genetic diversity using morphological characteristics of southern peanut regional trial varieties was lower than that of SSR markers. Varieties from the same breeding institute or the same province tended to be clustered together. There was no significant correlation between diversity indices generated using SSR markers and morphological characteristics. This indicated that the diversity indices generated from morphological traits and SSRs are independent and can both be used together to study the population diversity with more precision. However, a limited number of SSR markers cannot replace morphological characteristics in distinctness identification of varieties and therefore, a maximum number of SSR markers need to be used in performing DNA fingerprinting for establishing distinctness among varieties due to a narrow genetic base. The SNP arrays or genotyping by sequencing approaches would be useful technologies which can generate a genotyping on thousands of SNPs for distinctness. Nevertheless, the present study clearly showed that the combination of SSR markers and morphological characteristics can increase the accuracy of stabilizing distinctness of peanut varieties with accuracy.

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript. Yanbin Hong and Xiaoping Chen participated in conceiving the study and drafting the manuscript. Manish K. Pandey, Sunil S. Gangurde, and Rajeev K. Varshney participated in interpreting the results and revising the manuscript. Xuanqiang Liang and Shaoxiong Li participated in collecting the genetic materials. Hao Liu and Haiyan Liu participated in data analysis and results interpretation. Haifen Li performed analysis based on morphological characteristics while Qing Lu performed SSR analysis.

ACKNOWLEDGMENT

This research was funded by grants from the Special Project for Science and Technology Innovation Strategy of Guangdong Academy of Agricultural Sciences (R2018PY-JX001 and R2020PY-JX004); the National Natural Science Foundation of China (31771841 and 31801401);the Guangdong Modern Agricultural Industry Technology System (2019KJ136); the Science and Technology Program of Guangzhou (201904010190); the Modern Agro-industry Technology Research System(CARS-13). The founders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Hong Y, Pandey M K, Lu Q, et al . Genetic diversity and distinctness based on morphological and SSR markers in peanut. *Agronomy Journal*. (2021);*113:*4648–4660. https://doi.org/10.1002/agj2.20671