https://doi.org/10.17221/27/2021-CJGPB

Genetic relationships among *Cucurbita pepo* ornamental gourds based on EST-SSR markers

Weiqi Wang[1,2], Yuzi Shi[1], Ying Liu[3], Chenggang Xiang[4], Tingzhen Sun[1], Meng Zhang[1], Qin Shu[1], Xiyan Qiu[1], Kailiang Bo[1], Ying Duan[1], Changlin Wang[1]**

[1]*Key Laboratory of Biology and Genetic Improvement of Horticultural Crops of the Ministry of Agriculture and Rural Affairs, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, P.R. China*
[2]*College of Biological Sciences and Technology, Beijing Forestry University, Beijing, P.R. China*
[3]*Harbin Academy of Agricultural Sciences, Harbin, Heilongjiang, P.R. China*
[4]*Honghe University, Honghe, Yunnan, P.R. China*

**Corresponding authors: wangchanglin@caas.cn; duanying@caas.cn*

Citation: Wang W., Shi Y., Liu Y., Xiang Ch., Sun T., Zhang M., Shu Q., Qiu X., Bo K., Duan Y., Wang Ch. (2021): Genetic relationships among *Cucurbita pepo* ornamental gourds based on EST-SSR markers. Czech J. Genet. Plant Breed., 57: 125–139.

Abstract: The ornamental gourd *Cucurbita pepo* L. is a ubiquitous crop native to North America, exhibiting highly diverse fruit characteristics. Studying the genetic diversity of ornamental gourds can help identify and evaluate the curated germplasm resources, understand the phylogenetic relationships among them, and highlight ways in which the germplasm resources can be used to address gaps in the understanding. In this study, a set of 85 of 323 previously identified polymorphic expressed sequence tag-simple sequence repeat (EST-SSR) genetic markers were selected to evaluate the genetic relationships among 47 *C. pepo* accessions and one *C. foetidissima* accession. This collection consisted of accessions from the subspecies *pepo*, *texana*, and the hybrid *texana* × *pepo*. Our analyses yielded a total of 271 alleles, with an average of 3.2 alleles per genetic locus. The dendrogram construction, principal coordinate analyses, and genetic value calculation revealed several robust subclusters in the *texana* subspecies accessions. From these results, we propose five new distinct morphotypes based on our construction of a concise SSR fingerprint. Moreover, our study confirms that the fruit shape similarity among accessions is a fair reflection of genetic relatedness.

Keywords: genetic diversity; morphotypes; ssp. *texana*; SSR fingerprinting

The ornamental gourd *Cucurbita pepo* L. is native to North America and is widely cultivated there and on other continents (Trumbull 1876; Whitaker 1947; Wehner et al. 2020) for its attractive fruit. It may be the most polymorphic species in terms of its fruit characteristics (Paris 2000); some *C. pepo* varieties have highly distinct phenotypes, yielding small, exquisite fruits with various rind colours. Most of these ornamentals are not edible, but are frequently used in fall decorations; in China, these ornamental gourds were introduced in the 1990 s from Europe and North America and are now cultivated all over the country.

According to previous research on the molecular genetic polymorphisms, *Cucurbita pepo* is widely accepted to be divided into two cultivated and one wild

Supported by Beijing Innovation Consortium of Agriculture Research System (BAIC01), Beijing Forestry University Student Research Training Program (X202010022181), Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-IVFCAAS), the Central Public-interest Scientific Institution Basal Research Funding, and Special Fund for Agro-scientific Research in the Public Interest (201303112).

https://doi.org/10.17221/27/2021-CJGPB

subspecies (ssp.): ssp. *pepo*, which originated in Mexico, domesticated 8 000–10 000 years ago; ssp. *texana* (also known as ssp. *ovifera*), which originated in Texas and the nearby states in the USA; and the wild subspecies: ssp. *fraterna* (Andres 1987; Decker 1988; Wilson et al. 1992; Robinson & Decker-Walters 1997; Paris et al. 2003; Gong et al. 2012; Paris 2016; Chomicki et al. 2020). Although historically, the *C. pepo* ssp. has been classified taxonomically, commonly accepted classification systems are based on the fruit type, as this characteristic is highly diverse, making it easy to place cultivars into designated groups. The size and shape characteristics of cucurbit fruits are usually representative of the specific genotypes (Pan et al. 2020). Moreover, the fruit shape is easily observed, tightly controlled by and, therefore, representative of the genetic factors, and not subject to change as the fruit grows (Emerson 1910; Sinnott & Kaiser 1934; Sinnott 1935), making it a convenient indicator to use for classification. The fruit characteristics have previously been established as a useful indicator of the genetic relationships among cultivars (Paris 1986). Paris (1986, 2001) previously divided all known *C. pepo* cultivars into eight edible-fruited groups and two non-edible-fruited groups based on variations in the fruit shape. These are: Pumpkin, Cocozelle, Vegetable Marrow, Zucchini, Orange Gourd, Acorn, Crookneck, Straightneck, Scallop, and Ovifera Gourd; the first five groups are considered members of the ssp. *pepo* and the others are considered members of the ssp. *texana*.

According to a previous study conducted by Gong et al. (2012) on the five cultivar groups of ssp. *texana*, the average within-group genetic distances (GDs) were lower than those among the other groups in nearly all cases. The only exception was among the four Ovifera Gourd cultivars, which had a slightly higher average within-group GD (0.38) compared with the GD between the Ovifera Gourd and Scallop groups (0.37); the Ovifera Gourd cultivars also had the highest within-group GD value among all the *Cucurbita pepo* groups in the study. This research demonstrates that the classification of Ovifera Gourds by fruit characteristics alone may be insufficient, and a subdivision based on molecular markers may be useful.

Although the genomes of many cucurbit crops have been sequenced, some genetic maps have been constructed (Huang et al. 2009; Garcia-Mas et al. 2012; Yang et al. 2012; Guo et al. 2013; Qi et al. 2013), and *Cucurbita pepo* reference genome sequences have been made available in recent years (Sun et al. 2017; Montero-Pau et al. 2018), greater insights into the genetic relationships among ornamental gourds could be gleaned using expression sequence tag-simple sequence repeat (EST-SSR) markers. Therefore, in this study, we aimed to identify and use EST-SSR markers to assess the genetic diversity of ornamental gourds and improve the classification of *C. pepo* ssp. *texana.* An additional aim was to demonstrate the effective use of ornamental gourd germplasm resources to promote and guide future innovative research using these resources.

MATERIALS AND METHODS

Plant materials. In total, 47 accessions of *Cucurbita pepo* ornamental gourds and one wild species (*C. foetidissima*) accession were selected for analysis in this study. The mature fruits' appearance is shown in Figure 1. All the accessions were grown at the Institute of Vegetables and Flowers, the Chinese Academy of Agricultural Sciences (Beijing, China; 39.96°N, 116.33°E). At least twelve plants of each accession were grown to fruition to verify the identity and purity. The name, pedigree, species, morphotype, and seed source of each accession are shown in Table 1. The origin of all the accessions before the seeds were obtained is unknown; thus, the name of some approximate accessions (with similar fruit appearance and shape) from commerce are given for reference. The 47 *Cucurbita pepo* accessions include 41 high-generation, homozygous inbred lines ssp. *texana*, five randomly selected strains from recombinant inbred lines (RILs) (two from the ninth filial generation, F_9, and the other three from F_{12} resulting from a cross between *C. pepo* ssp. *texana* C38 and *Cucurbita pepo* ssp. *pepo* C39), and two ssp. *pepo*. All the *C. pepo* accessions were assigned to one of six morphotypes: Ovifera Gourd, Zucchini, Pumpkin, Scallop, Acorn, and Crookneck. Some of the accessions were assigned based on the results of our experiment rather than their appearance alone. The wild species *C. foetidissima* was selected as an outgroup. The accessions we chose to use in this study were strategically selected to be representative of as many morphotypes of ornamental gourds as possible.

DNA extraction. Twelve plants of each accession were transplanted into a plastic greenhouse in autumn. Young leaf tissue from each accession was collected from eight randomly selected seedlings

https://doi.org/10.17221/27/2021-CJGPB

at the two-leaf stage and transferred into liquid nitrogen. DNA was extracted from all the seedlings together using a slightly modified version of the cetyltrimethylammonium bromide (CTAB) method (Doyle 1991). For each of the accessions, 0.1 g of young leaf tissue from the collective frozen seedlings was suspended in 800 µL of an extraction buffer containing 20 mmol/L of ethylene diamine tetraacetic acid (EDTA), 100 mmol/L of tris-hydrochloride (tris-HCl, pH 8.0), 0.016 g of CTAB and 8 µL of β-mercaptoethanol (β-ME), and then incubated at 60 °C for 30 min. 800 µL of trichloromethane (chloroform) was used in the final extraction step and the liquid supernatant was precipitated with 800 µL of ethanol at 4 °C.

After centrifugation at a 12 396 × g relative centrifugal force (rcf) for 5 min, the DNA was washed with 1.5 mL of 70% (v/v) ethanol and 1.5 mL of pure ethanol. The DNA yield in each sample was quantified using electrophoresis on a 1.0 g/mL agarose gel stained with LabRed (Coolaber, Beijing, China). The quality of the DNA samples was determined using a NanoDrop 2000 device (Thermo Fisher Scientific, Shanghai, China).

SSR analysis. All the EST-SSR markers for the *Cucurbita pepo* used during the study were established by Xiang et al. (2018) using transcriptome data for *C. pepo* in the Cucurbit Genomics Database (http://www.icugi.org). The EST-SSR markers with polymorphic alleles (at least two different allelic variants among all the accessions) were selected for genotyping in this study. The EST-SSR markers were mapped using the Cucurbit Genomics Database (CuGenDB; http://cucurbitgenomics.org) (Montero-Pau et al. 2018; Zheng et al. 2019).

To amplify the markers, polymerase chain reaction (PCR) was carried out using commercially synthesised primers (Sangon Biotech, Shanghai, China) in a total volume of 10 µL, containing 5 µL of a 3G Taq Master Mix with red dye (Vazyme Biotech Co.,

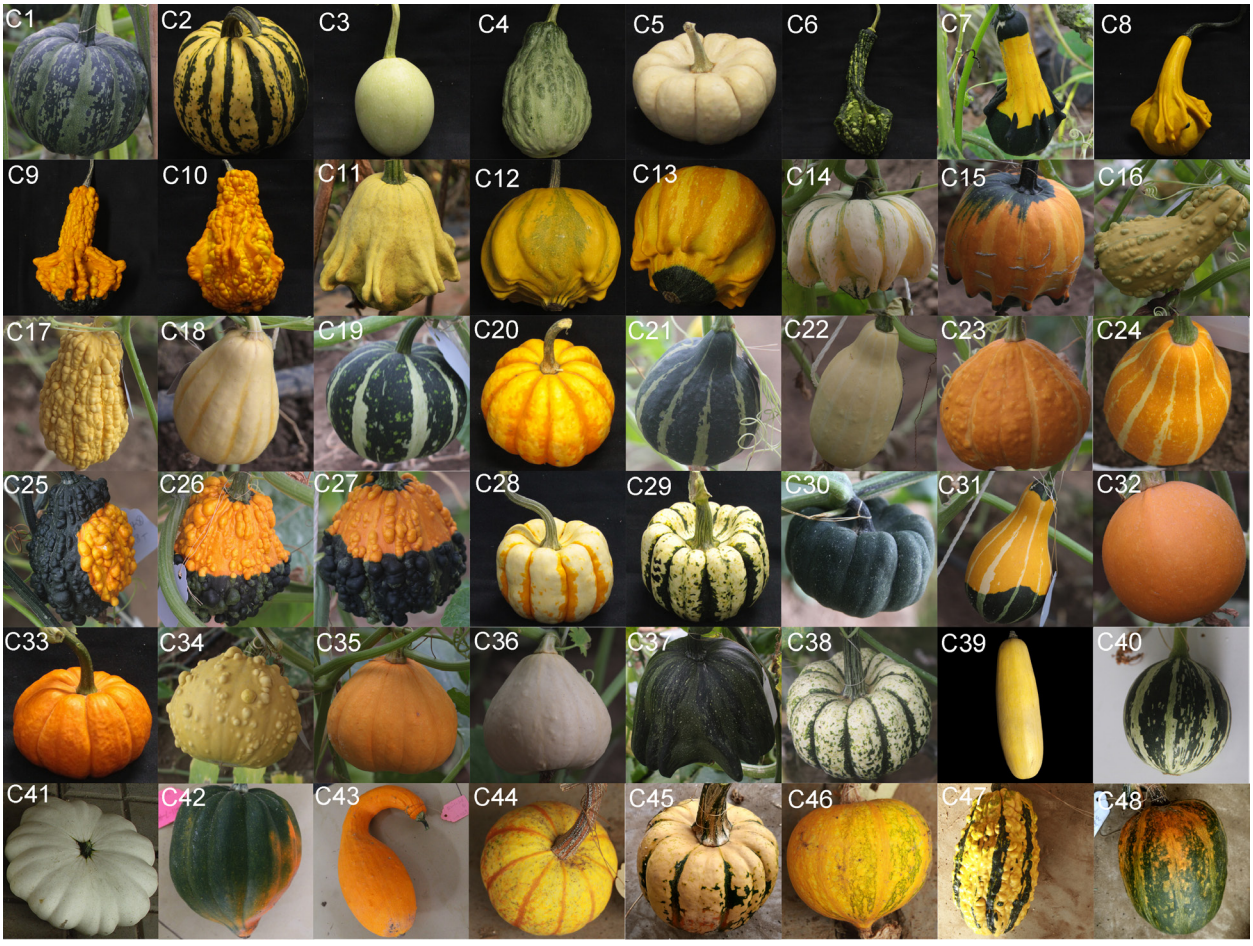

Figure 1. Fruit phenotypes of the *Cucurbita pepo* and *Cucurbita foetidissima* accessions used in this study
The code of each accession is marked in the upper left corner

https://doi.org/10.17221/27/2021-CJGPB

Table 1. *Cucurbita pepo* and *Cucurbita foetidissima* accessions used in this study

Code	Accession name	Similar commercially available accession	Accession pedigree[a]	Accession abbreviation	Growth habit	Seed source
C1	No. 5	Harlequin Squash	NO5-4-6-7-10-1-3-1-7	T-AC	vine	SD
C2	No. 6	Harlequin Squash	NO6-5-1-1-3-10-1	T-AC	vine	SD
C3	Baidan-1482	Egg Gourd	BD-1-6-7-1-5	T-OG	vine	SD
C4	Baidan-1483	Egg Gourd	BD-5-5-2-1-1	T-OG	vine	SD
C5	Baisemini	unknown	BSMN-6-7-4-6-9-3-3	T-OG	vine	NL
C6	Chouxiaoya-1485	Autumn Wings	CXY-1-7-1-11	T-OG	vine	GD
C7	Chouxiaoya-1487	Autumn Wings	CXY-1-5-2-3-6	T-OG	vine	GD
C8	Chouxiaoya-1488	Autumn Wings	CXY-1-5-2-4-5	T-OG	vine	GD
C9	Duochi-1489	Autumn Wings	DC-5-1-2-6-1	T-OG	vine	SD
C10	Duochi-1490	Autumn Wings	DC-5-2-1-3-3	T-OG	vine	SD
C11	Feidie-1491	Shenot Crown of Thorns	FD-1-10-1-6-7	T-OG	vine	SD
C12	Feidie-1492	Shenot Crown of Thorns	FD-1-10-2-5-2	T-OG	vine	SD
C13	Foshou-1493	Shenot Crown of Thorns	FS-3-6sib-9-10-2-3	T-OG	vine	SD
C14	Foshou-1494	Shenot Crown of Thorns	FS-3-6sib-9-1-12-6	T-OG	vine	SD
C15	Foshou-1495	Shenot Crown of Thorns	FS-3-6sib-9-7-1-2	T-OG	vine	SD
C16	Geda-1496	Bicolour Pear	GD-7-7-4-7-1	T-OG	vine	SD
C17	Geda-1497	Bicolour Pear	GD-5-2-3-4-8	T-OG	vine	SD
C18	Geda-1498	Bicolour Pear	GD-5-2-7-6-1	T-OG	vine	SD
C19	Guapi	Oblate Dark Striped	GP-2-2-1-5-7	T-OG	vine	SD
C20	Jinxiangyu	Sweet Dumpling	JXY-4-4-1-2-1-5-1	T-AC	vine	SH
C21	Meinan No.1-1501	Bicolour Pear	MN1-1-1-13-3-2-1-3	T-OG	vine	IZ
C22	Meinan No.1-1502	Bicolour Pear	MN1-4-7-8-1-1-3-3-1	T-OG	vine	IZ
C23	Meinan No.1-1503	Bicolour Pear	MN1-1-1-6-4-6-2-2-6	T-OG	vine	IZ
C24	Meinan No.1-1504	Bicolour Pear	MN1-4-7-8-4-1-2-1-5	T-OG	vine	IZ
C25	Meinan No.3-1505	Hoargarth	MN3-5-4-8-7-2-4-7	T-OG	vine	IZ
C26	Meinan No.3-1506	Hoargarth	MN3-5-4-11-5-3-1-4-3	T-OG	vine	IZ
C27	Meinan No.3-1507	Hoargarth	MN3-5-4-11-5-3-1-4-4	T-OG	vine	IZ
C28	Micaiguo-1508	Sweet Dumpling	MCG-6-1-1-2-2-4	T-AC	vine	SD
C29	Micaiguo-1509	Sweet Dumpling	MCG-6-3-10-2-4-3	T-AC	vine	SD
C30	Micaiguo-1510	Sweet Dumpling	MCG-6-1-3-2-4-1	T-AC	vine	SD
C31	Neimengxiaonangua	Bicolour Pear	NM-1-2-3-9-5-3	T-OG	vine	IM
C32	Yagan	Small Sugar	YG-7-1-2-5-7	P-PU	vine	SD
C33	Yichuanhong	Jack-Be-Little	YCH-3-1-3-11-1-2	T-AC	vine	SH
C34	Yunv-1514	Green Warted Pear	YN-2-7-5-2-3-1-1	T-OG	vine	SD
C35	Yunv-1515	Green Warted Pear	YN-2-7-7-2-3-2-3	T-OG	vine	SD
C36	Yunv-1516	Green Warted Pear	YN-2-7-7-7-2-4-5	T-OG	vine	SD
C37	Zhenqi	Shenot Crown of Thorns	ZQ-7-3-3-4-3-5	T-OG	vine	NL
C38	Jinganlu	Sweet Dumpling	JGL-2-7-3-11-5sib-1-12-1-2-1-2-1-2-3-4-1	T-AC	vine	IL
C39	HM-S2	unknown	HM-4-2-8-26-4-5-7-10-5-1-4-7	P-ZU	bush	CS
C40	Buffalo Gourd	Buffalo Gourd	BG	F-BG	vine	CA
C41	Feidiexihulu	White Bush Scallop	FDX	T-SC	bush	AA
C42	107	Table Queen	107	T-AC	bush	AA

https://doi.org/10.17221/27/2021-CJGPB

Table 1 to be continued

Code	Accession name	Similar commercially available accession	Accession pedigree[a]	Accession abbreviation	Growth habit	Seed source
C43	Wanjingxihulu	Early Golden Crookneck	WJ-4-1-2-3-5	T-CN	bush	SX
C44	CP-88	unknown	cp88-3-3-1-1-3-4	T × P-UD	bush	RI
C45	CP-199	unknown	cp199-3-1-4-1-5-2	T × P-UD	bush	RI
C46	CP-200	unknown	cp200-2-3-3-3-2-3-2-3-3	T × P-UD	bush	RI
C47	CP-212	unknown	cp212-3-4-4-4-2-1-1-4-3	T × P-UD	vine	RI
C48	CP-218	unknown	cp218-3-3-3-2-2-2-2-3-2	T × P-UD	vine	RI

Abbreviations for species and subspecies: F – *Cucurbita foetidissima*; P – *Cucurbita pepo* ssp. *pepo*; T – *Cucurbita pepo* ssp. *texana*; T × P – *Cucurbita pepo* ssp. *texana* (P_1) × *Cucurbita pepo* ssp. *pepo* (P_2); abbreviations for morphotype: AC – Acorn; BG – Buffalo Gourd; CN – Crookneck; OG – Ovifera Gourd; PU – Pumpkin; SC – Scallop; UD – undefined; ZU – Zucchini; abbreviations for seed source: SD – provided by Prof. Guoxiang Yin from the Yantai Academy of Agricultural Sciences, Shandong, China in 2010; NL – bought from Beijing Nongle Vegetable Research Center, Beijing, China in 2001; GD – provided by Mr. Baowei Zhang from the Guangzhou Jinshan Fresh Fruit Co., Ltd., Guangdong, China in 2010; SH – provided by Prof. Longying Chen from the Shanghai Academy of Agricultural Sciences, Shanghai, China in 2011; IZ – provided by Prof. Guanghua Zheng from the Institute of Vegetables and Flowers CAAS, Beijing, China in 1994; IM – fruit bought from Hohhot agricultural products market, Inner Mongolia, China in 2011; IL – provided by Prof. Yisheng Liu from the Institute of Vegetables and Flowers CAAS, Beijing, China in 1995; CS – provided by Mr Kun Liu from the China National Seed Group Co., Ltd., Beijing, China in 2006; CA – provided by Prof. Xiaolei Sui from China Agricultural University, Beijing, China in 2014; AA – provided by Prof. Haizhen Li from the Beijing Academy of Agriculture and Forestry Sciences, Beijing, China in 2018; SX – fruit bought from the Shenchi County Agricultural Products Market, Shanxi, China in 2016; RI – recombinant inbred lines of C38, P1 × C39, P2; [a]abbreviations/numbers without hyphens represent the original seed stock; numbers of hyphens following abbreviations/introduction numbers indicate the number of generations of self-pollination or sib-pollination (indicated by 'sib')

Nanjing, China) for polyacrylamide gel electrophoresis (PAGE), 10 ng of the DNA template from the extractions, and 0.2 μmol/L primers. The cycling programme was as follows: preheating at 94 °C for 5 min; 35 cycles of 94 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s; final extension at 72 °C for 5 min. The PCR products were resolved on 6 g/mL polyacrylamide gels with a constant voltage of 10 V/cm for 70 min and visualised using 0.1 g/mL silver staining.

Data analysis. The presence (1) or absence (0) of the polymorphic bands for each SSR marker was evaluated. A concise SSR fingerprint of all the *Cucurbita pepo* accessions was constructed using Microsoft Excel software (Ver. 2016).

POPGENE software (Ver. 1.32) (Yeh et al. 1999) was used to assess the expected heterozygosity (HE) (Nei 1978a, b) – which indicates the power of markers to discriminate between accessions – for each marker and the allele frequencies. The allele frequencies were then used to calculate the polymorphism information content (PIC) according to the formula suggested by Botsein et al. (1980) to evaluate the markers. The genetic similarity between the accessions was assessed using the Dice coefficient (Dice 1945) using the SIMQUL program within the NTSYSpc software (Ver. 2.10e) (Rohlf 2000), which produces a similarity matrix. The GD values among each accession were calculated as one minus the similarity coefficient (Nei & Li 1979). A dendrogram was constructed from the similarity matrix using the unweighted pair group method with the arithmetic mean (UPGMA; Sneath & Sokal 1973) in the SAHN program in NTSYSpc. Two- and three-dimensional principal coordinate analyses (PCoA) were conducted using the DCENTER, EIGEN, MXPLOT, and MOD3D programs in NTSYSpc. A cophenetic correlation analysis was then calculated using the COPH and MXCOMP programs in NTSYSpc to assess the correlation between the clustering and the similarity matrix. A bootstrap analysis (Felsenstein 1985) was conducted with 1 000 replications to evaluate the branch support using MEGA X software (Kumar et al. 2018; Stecheret et al. 2020).

RESULTS

Genotyping using SSR markers. Of the 323 SSR markers we assessed in this study, 238 produced

https://doi.org/10.17221/27/2021-CJGPB

Table 2. Properties of the 85 pairs of the expressed sequence tag-simple sequence repeat (EST-SSR) primers used in this study

No.	SSR marker	Allele No.	Pseudo-chromosome	HE	PIC	No.	SSR marker	Allele No.	Pseudo-chromosome	HE	PIC
1	PU006928	6	Cp4.1LG01	0.72	0.67	44	PU028216	3	Cp4.1LG08	0.62	0.55
2	PU026148	2	Cp4.1LG01	0.30	0.26	45	PU124451	4	Cp4.1LG08	0.75	0.70
3	PU124393	2	Cp4.1LG01	0.50	0.37	46	PU030875	2	Cp4.1LG08	0.04	0.04
4	PU021565	5	Cp4.1LG01	0.45	0.42	47	PU029942	2	Cp4.1LG09	0.04	0.04
5	PU022040	3	Cp4.1LG01	0.42	0.35	48	PU106768	2	Cp4.1LG10	0.04	0.04
6	PU026180	2	Cp4.1LG01	0.22	0.19	49	PU107325	4	Cp4.1LG10	0.40	0.36
7	PU100697	2	Cp4.1LG01	0.44	0.34	50	PU102156	3	Cp4.1LG10	0.29	0.27
8	PU031082	2	Cp4.1LG01	0.16	0.14	51	PU020198	2	Cp4.1LG11	0.44	0.35
9	PU020609	6	Cp4.1LG02	0.56	0.53	52	PU025208	2	Cp4.1LG11	0.46	0.36
10	PU021288	3	Cp4.1LG02	0.48	0.41	53	PU096105	2	Cp4.1LG11	0.49	0.37
11	PU025971	3	Cp4.1LG02	0.44	0.36	54	PU040365	4	Cp4.1LG12	0.56	0.46
12	PU026958	4	Cp4.1LG02	0.38	0.36	55	PU106640	3	Cp4.1LG12	0.12	0.12
13	PU029193	3	Cp4.1LG02	0.48	0.38	56	PU027013	2	Cp4.1LG12	0.04	0.04
14	PU094927	2	Cp4.1LG02	0.08	0.08	57	PU097602	2	Cp4.1LG12	0.19	0.17
15	PU005108	2	Cp4.1LG03	0.04	0.04	58	PU109408	2	Cp4.1LG12	0.49	0.37
16	PU027158	2	Cp4.1LG03	0.28	0.24	59	PU030701	3	Cp4.1LG12	0.12	0.12
17	PU020742	3	Cp4.1LG03	0.29	0.26	60	PU120790	5	Cp4.1LG13	0.66	0.59
18	PU023671	4	Cp4.1LG03	0.46	0.41	61	PU042403	4	Cp4.1LG13	0.69	0.63
19	PU025345	4	Cp4.1LG03	0.42	0.37	62	PU094255	3	Cp4.1LG13	0.60	0.52
20	PU032303	3	Cp4.1LG03	0.32	0.30	63	PU095329	2	Cp4.1LG13	0.04	0.04
21	PU033380	3	Cp4.1LG03	0.06	0.06	64	PU021410	3	Cp4.1LG13	0.58	0.50
22	PU068168	4	Cp4.1LG04	0.62	0.55	65	PU123011	2	Cp4.1LG13	0.32	0.27
23	PU132117	3	Cp4.1LG04	0.66	0.58	66	PU123519	3	Cp4.1LG14	0.58	0.52
24	PU020717	2	Cp4.1LG04	0.34	0.28	67	PU025199	3	Cp4.1LG15	0.29	0.26
25	PU035321	3	Cp4.1LG04	0.54	0.43	68	PU028034	4	Cp4.1LG15	0.51	0.42
26	PU094077	3	Cp4.1LG04	0.53	0.42	69	PU013015	3	Cp4.1LG16	0.19	0.18
27	PU108557	5	Cp4.1LG04	0.76	0.72	70	PU027166	3	Cp4.1LG16	0.45	0.40
28	PU026302	4	Cp4.1LG04	0.14	0.13	71	PU099123	3	Cp4.1LG16	0.39	0.32
29	PU022909	4	Cp4.1LG04	0.49	0.45	72	PU122491	2	Cp4.1LG16	0.15	0.14
30	PU010254	3	Cp4.1LG05	0.52	0.42	73	PU003340	5	Cp4.1LG17	0.71	0.66
31	PU106528	2	Cp4.1LG05	0.04	0.04	74	PU005292	8	Cp4.1LG17	0.74	0.71
32	PU026265	3	Cp4.1LG05	0.56	0.48	75	PU030312	3	Cp4.1LG18	0.44	0.36
33	PU021010	2	Cp4.1LG05	0.35	0.29	76	PU027784	3	Cp4.1LG18	0.26	0.25
34	PU095879	3	Cp4.1LG05	0.12	0.12	77	PU023307	3	Cp4.1LG18	0.08	0.08
35	PU027630	2	Cp4.1LG06	0.06	0.06	78	PU031266	2	Cp4.1LG18	0.20	0.18
36	PU107170	4	Cp4.1LG06	0.55	0.46	79	PU106681	4	Cp4.1LG19	0.52	0.44
37	PU109217	3	Cp4.1LG06	0.47	0.40	80	PU032419	4	Cp4.1LG19	0.55	0.48
38	PU123773	4	Cp4.1LG06	0.61	0.53	81	PU027965	5	Cp4.1LG20	0.38	0.36
39	PU029787	2	Cp4.1LG06	0.26	0.22	82	PU030458	3	Cp4.1LG20	0.51	0.40
40	PU022458	4	Cp4.1LG07	0.49	0.45	83	PU037265	6	Scaffold	0.44	0.41
41	PU009087	2	Cp4.1LG08	0.22	0.19	84	PU108345	4	Scaffold	0.45	0.38
42	PU024446	6	Cp4.1LG08	0.74	0.70	85	PU026623	2	Scaffold	0.04	0.04
43	PU033206	3	Cp4.1LG08	0.38	0.32						

HE – expected heterozygosity; PIC – polymorphism information content

https://doi.org/10.17221/27/2021-CJGPB

either nonspecific or monomorphic bands and were, therefore, excluded. The remaining 85 SSR markers (Table 2) were found to be polymorphic; a total of 271 alleles were amplified among the 48 accessions. The number of SSR loci per pseudochromosome ranged from one to eight with an average of 4.1 (Table 2).

The number of alleles per locus ranged from two to eight with an average of 3.2. Of all the loci, 28 (32.9%) had two alleles, and only one locus had eight alleles.

Nei's HE values ranged from 0.04 to 0.76 with an average of 0.39 across all the markers. The PIC values ranged from 0.04 to 0.72 with an average of 0.34. Among the 85 SSR markers, PU108557 had the highest PIC value (0.72) and HE value (0.76); 27 SSR markers showed a high power to discriminate between the accessions, with HE values of 0.50 and above (Table 2).

Phylogenetic relationships between accessions. The cophenetic correlation coefficient linking the similarity and cophenetic matrices was very high at 0.90 (Figure S1 in the Electronic Supplementary Material (ESM)), suggesting a good fit between the two, and high reliability of the clustering result.

The phylogenetic clustering of the similarity matrix based on the SSR analysis, using the wild species (*Cucurbita foetidissima*) as an outgroup, generated a dendrogram with two major clusters (Figure 2): one cluster of 45 accessions (cluster I), supported by a bootstrap value of 68, and one of only two accessions (cluster II) with a bootstrap value of 95, representing ssp. *pepo*. The larger cluster is divided into seven subclusters with good support as indicated by the bootstrap values (Figure 2). Among these subclusters, the most outlying and robust subcluster (Ig) is formed from five interspecific hybrids

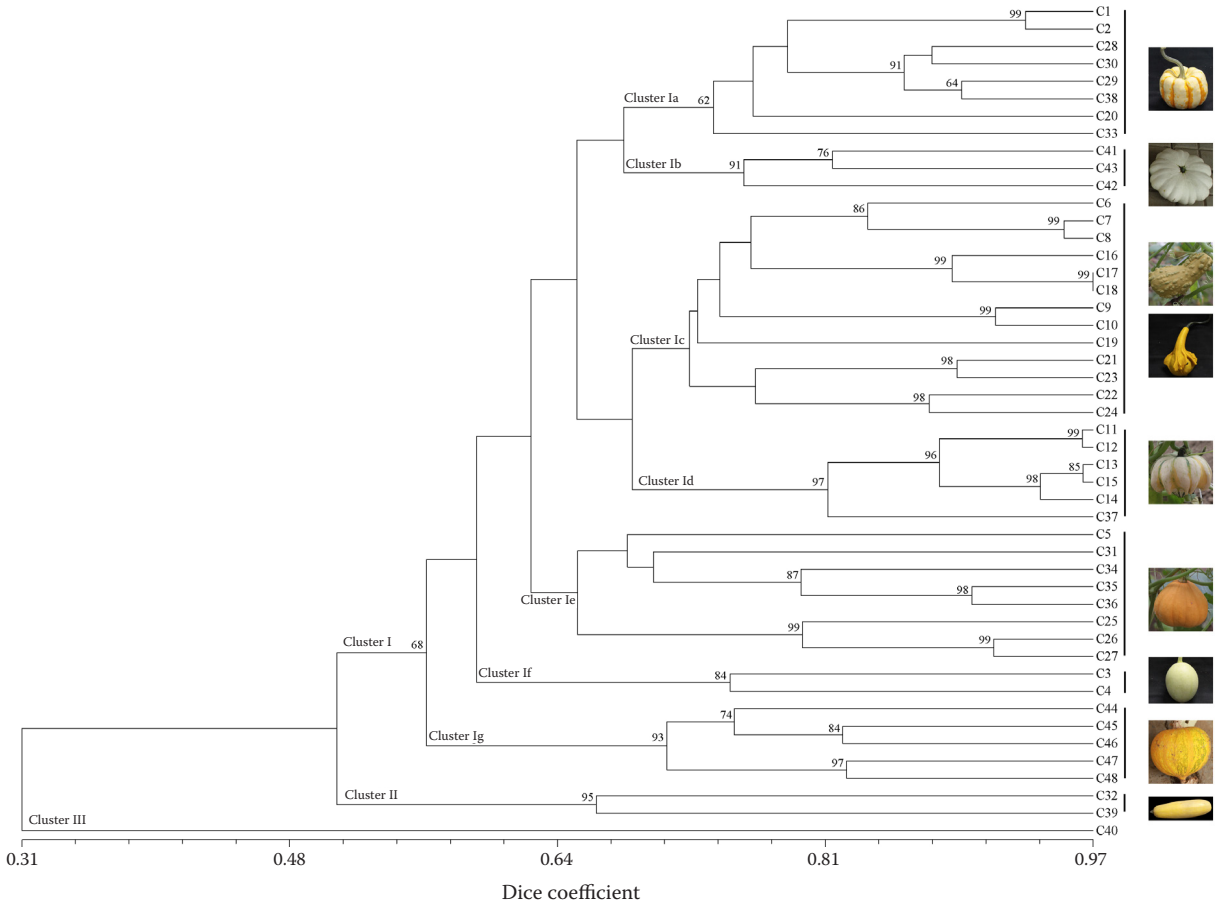

Figure 2. Dendrogram using 85 simple sequence repeat markers based on the Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of 47 *Cucurbita pepo* accessions and one *Cucurbita foetidissima* wild species accession outgroup

Only bootstrap values > 50 are placed on the nodes

https://doi.org/10.17221/27/2021-CJGPB

(*C. pepo* ssp. *texana* C38 × *C. pepo* ssp. *pepo* C39) with a high bootstrap value of 93, indicating a close genetic relationship among these five accessions. Eight edible, medium-sized Acorn squashes in this study formed another cluster (Ia) with a bootstrap value of 62. Three non-Ovifera Gourd accessions – C41 (Scallop), C42 (Acorn), and C43 (Crookneck) – were grouped into cluster Ib, which is sister to cluster Ia, supported by a high bootstrap value of 91. The remaining 27 accessions were grouped into three

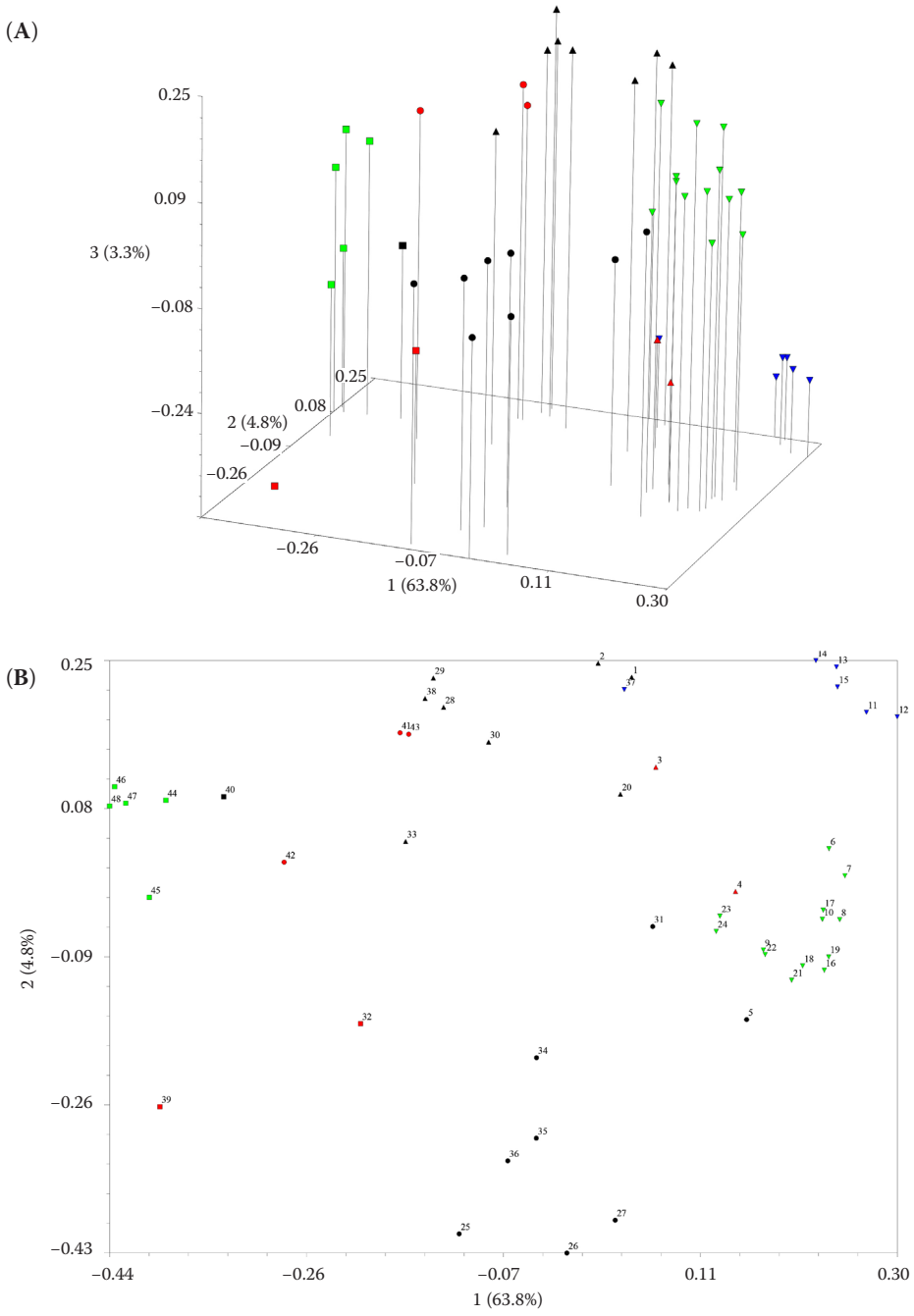

Figure 3. Principal components analysis (PCoA) output diagrams showing the relationships among the 48 accessions used in this study: a three-dimensional PCoA diagram (A), a two-dimensional PCoA diagram (B)
The number above each plotted data point indicates the corresponding accession code; the plot symbols used represent the different clusters or subclusters as follows: cluster Ia – black triangle; cluster Ib – red circle; cluster Ic – green triangle; cluster Id – blue triangle; cluster Ie – black circle; cluster If – red triangle; cluster Ig – green square; cluster II – red square; cluster III – black square

https://doi.org/10.17221/27/2021-CJGPB

distinct subclusters (clusters Ic, Id, and Ie) based on the bootstrap values (Figure 2), supported by similarity in the morphological characters among these accessions (Figure 1).

Principal coordinate analysis. The two- and three-dimensional PCoA diagrams shown in in Figure 3 were used to explore the genetic relationships among 48 accessions. Dimensions 1 and 2 in the two diagrams are identical; Figures 3A and B are alternate views of the same diagram, with 3B as a two-dimensional, top-down view of the three-dimensional view in 3A.

The genetic differences shown in the PCoA are consistent with the dendrogram (Figure 2). Cluster III is in the top far left of Figure 3B, appearing close to C44 and C47; however, the different view in Figure 3A reveals a large distance between this cluster and C44 and C47. The two ssp. *pepo* gourds in cluster II are isolated from all the other ssp. *texana* accessions, supporting a relatively distant genetic relationship between ssp. *pepo* and ssp. *texana*. Located in the top left corner (Figure 3A), the five interspecific hybrid gourds (C44, C45, C46, C47, C48) occupy a middle position between their parents (C38 and C39) as expected. The scatter plots in Figure 3 reveal that clusters Ia and Ib are close together, despite having formed tight sub-clusters according to the dendrogram (Figure 2). This may be because they are edible squashes from the Acorn, Scallop, and Crookneck cultivar groups, rather than the Ovifera Gourd cultivar group. Cluster Ic, containing six accessions (C11, C12, C13, C14, C15, and C37), is in the extreme top right corner of Figure 3B and is marked by negative vectors in Figure 3A. Cluster Id is also located in the top right corner of Figure 3B near Ic, this cluster is marked by positive vectors in Figure 3A. Notably, compared with the other accessions in cluster Id, C37 is dissimilar in colour although it shares the same fruit shape; the PCoA shows that this accession is more loosely associated with the others (Figure 3B). The eight accessions in cluster Ie are all located in the centre near the bottom of the two-dimensional PCoA diagram; cluster If is located next to C20, C23, and C24 in Figure 3B, while the distance between cluster If and the three accessions is large in Figure 3A. The two accessions in cluster If are distinctly separate from the other accessions in both PCoA diagrams (Figure 3).

Genetic distances between accessions. The genetic distance (GD) values between the individual accessions ranged from 0.03 (between C17 and C18, which were, therefore, most similar to one another in genetic composition) to 0.77 (between C40 and C26, and C40 and C27), with a mean of 0.38 (Table S1 in the ESM). The highest GD value (0.77) supports a considerable genetic difference between the outgroup *Cucurbita foetidissima* and *C. pepo* (ssp. *texana*), although they share some similar phenotypic traits.

The average GD values among the three major clusters were high (Table 3). All the subclusters in cluster I had relatively low average GD values between the individuals (≤ 0.50), reflecting the fact that they all belong to ssp. *texana*. The average GD values between the individuals within the same cluster or subcluster are even lower (≤ 0.36), supporting greater genetic similarity at lower phylogenetic levels in the dendrogram (Figure 2). More importantly, the average GD values between the accessions within the same clusters or subclusters are lower than the average

Table 3. Genetic distance values within and between the different *Cucurbita pepo* and *Cucurbita foetidissima* groupings based on the SSR markers

		Cluster									
		Ia	Ib	Ic	Id	Ie	If	Ig	I	II	III
Cluster	Ia	0.21									
	Ib	0.32	0.23								
	Ic	0.34	0.38	0.25							
	Id	0.34	0.37	0.32	0.13						
	Ie	0.38	0.41	0.36	0.39	0.30					
	If	0.41	0.49	0.40	0.35	0.46	0.26				
	Ig	0.39	0.34	0.46	0.46	0.47	0.50	0.26			
	I	–	–	–	–	–	–	–	0.36		
	II	0.49	0.53	0.52	0.51	0.47	0.47	0.45	0.50	0.34	
	III	0.66	0.63	0.70	0.70	0.73	0.71	0.64	0.69	0.70	–

https://doi.org/10.17221/27/2021-CJGPB

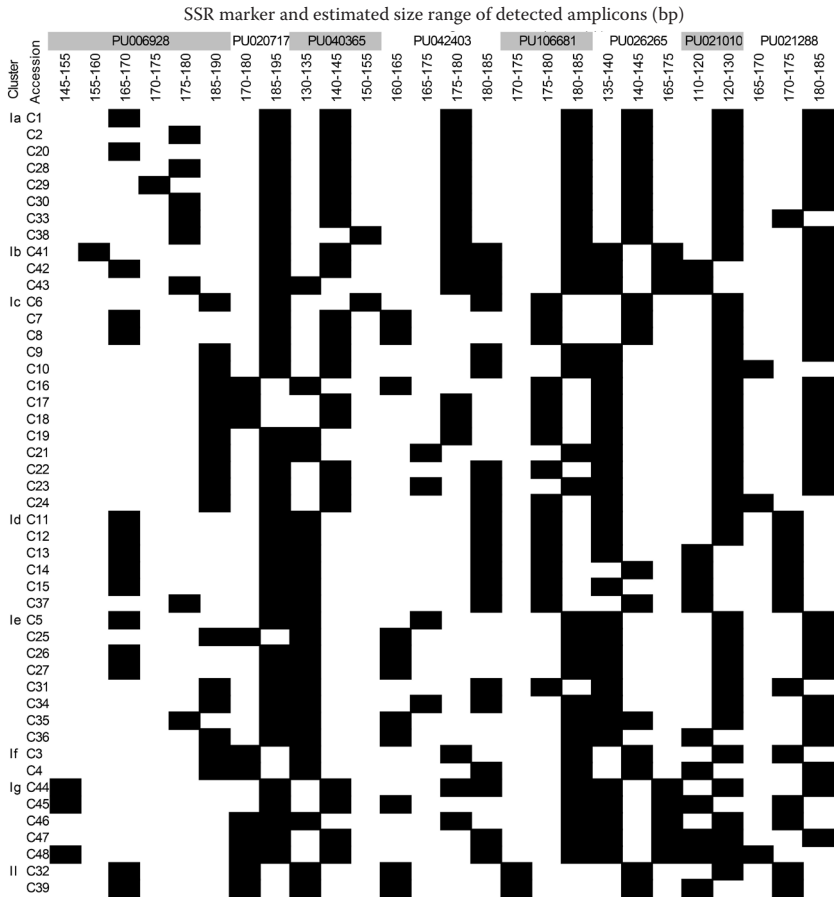

Figure 4. A concise simple sequence repeat (SSR) fingerprint for 47 *Cucurbita pepo* accessions, using a binary system indicating the presence (black) or absence (white) of an allele

GD values between the clusters or subclusters in all the cases, supporting the robustness of each group. These results reveal that the cluster designations, as shown in our dendrogram, are valid. Squashes in cluster Ia, the fruits of which are all edible, have the lowest among-group average GD values from cluster Ib (0.32), which contain other edible squashes, also supporting the validity of the clusters.

SSR fingerprinting. Overall, eight of the 85 SSR markers with high polymorphism were selected to construct a concise SSR fingerprint (Figure 4). In this analysis, specific cluster information for any individual ornamental gourd accession that belongs to the clusters and subclusters defined in the present work can be easily determined using this fingerprint.

Several distinct alleles that are specific to certain clusters are shown in the fingerprint. For example, an allele of 145–155 base pairs (bp) in PU006928 only appeared in accessions from cluster Ig (C44, C45, and C48), and an allele of 170–175 bp in PU106681 only appeared in cluster II (C32 and C39).

Interestingly, all the accessions in cluster Ia had the same allele at five loci, indicating that these alleles have a relatively strong power to discriminate between this cluster and others. Similarly, seven alleles (including one distinct allele that is not included in the other clusters) appeared in both C32 and C39; only one marker, PU021010, distinguishes between C32 and C39, indicating close similarity between these individuals.

DISCUSSION

Analysing genetic relationships among *C. pepo* subspecies using EST-SSR markers. So far, many studies on quantitative trait locus (QTL) mapping and assessing the genetic relationships within *Cucurbita pepo* as well as among species of the *Cucurbita* genus have been conducted using SSR markers (Katzir et al. 2000; Paris et al. 2004; Gong et al. 2012, 2013; Kong et al. 2014; Ntuli et al. 2015; Kaźmińska et al. 2017; Xiang et al. 2018) or other

https://doi.org/10.17221/27/2021-CJGPB

molecular markers (Sanjur et al. 2002; Ferriol et al. 2003; Zheng et al. 2013; Radwan 2014; Xanthopoulou et al. 2015; Castellanos-Morales et al. 2019). SSR markers are developed as codominant markers based on the variable microsatellite DNA regions and display a reliable result in genetic diversity studies. They are more suitable for both the diversity analysis and the fingerprinting (Varshney et al. 2007; Singh et al. 2013). The most widely used SSR markers in the *Cucurbita* genus were developed by Blanca et al. (2011) and Gong et al. (2008). In the present study, 323 EST-SSR markers were screened, of which 85 of these were selected to determine the genetic relationships among the accessions. High-quality reference genome sequences of the Zucchini type (*C. pepo* ssp. *pepo*) accession MU-CU-16 are available (Montero-Pau et al. 2018); 82 of the EST-SSR markers used in this study are mapped on this genome, with an even coverage of all twenty pseudochromosomes (Table 2).

We found that the average number of alleles per locus was 3.2, similar to the figure calculated in the previous work by Blanca et al. (2011) using 30 EST-SSR markers in ten *Cucurbita* genotypes. The study by Gong et al. (2012) revealed 134 SSR and four sequence characterised amplified region (SCAR) markers with a total of 418 alleles across 104 accessions, and an average number of alleles per locus of 3.0. Nearly 70% of the EST-SSR marker loci in this study had only two or three alleles across the accessions (Table 2), although the reason behind this low polymorphic potential in *C. pepo* is unknown. However, Gong et al. (2012) proposed that these loci may be in the genes with a particular functional role in which the allelic variations are likely to negatively affect the function, and a large proportion of the SSR markers used in their studies may be located within the sequences of the expressed genes. In general, our analyses showed that *C. pepo* displayed a relatively abundant allele resource, and the number of alleles seems relatively similar across the different subspecies.

EST-SSR markers were used in this study because their PCR products vary in length (as compared with, for example, single-nucleotide polymorphisms), and the diversity and fingerprinting analyses we used depend on polymorphism length (Varshney et al. 2007; Singh et al. 2013). Furthermore, our results indicate that concise SSR fingerprinting may represent a quick and efficient way to identify which group the unclassified *Cucurbita pepo* gourds belong to, using only eight SSR markers.

A prior study by Gong et al. (2012) revealed the genetic relationships among 30 *Cucurbita pepo* ssp. *texana* accessions; however, only four belonged to the Ovifera Gourd cultivar group. In addition, proven by Xanthopoulou et al. (2019), accessions belonging to ssp. *ovifera* (ssp. *texana*) presented about twice as many single nucleotide polymorphisms (SNPs) than that of ssp. *pepo*, indicating the higher genetic variability of the ssp. *texana* accessions. In our study, a broader variety of *C. pepo* ssp. *texana* accessions with different characteristics were used to investigate the genetic relationships, contributing to a more precise understanding of the relationships within this subspecies.

A possible new subspecies and morphotype for accession C32. In previous breeding works, C32 (also called Yagan) is classified as an ornamental gourd accession, which may have derived from ssp. *texana,* based on the shared fruit characteristics (round shape, orange rind colour, small size; Table S2 in the ESM and Figure 1). However, the phenotype of C32 is also similar to gourds from the previously established Pumpkin morphotype in ssp. *pepo*, although this group generally has large fruit compared with other ssp.; for example, culinary forms of ssp. *pepo* generally have larger leaves, fruits, and seeds than those of their counterparts in ssp. *texana* (Paris et al. 2012).

Based on this study, however, C32 formed a tight cluster with the known ssp. *pepo* accession C39 (also known as HM-S2) (Figure 2) and was positioned near this accession in the PCoA diagram (Figure 3). The lowest GD value from C32 was with C39 at 0.34; this value was also generally low in the context of other accessions. Notably, this value was slightly lower than the maximum within-group GD value of cluster I, which contained ssp. *texana* and the interspecific hybrids (GD, 0.36; Table 3 and Table S1 in the ESM). Furthermore, some unique alleles that only showed up in C32 and C39 (such as the one sized 170 to 175 bp in PU106681, Figure 4), which may belong to the ssp. *pepo* signature bands, also indicate a relatively close relationship between them. Despite the different skin colours, (orange and light yellow), shapes (round and uniformly cylindrical), and modes of growth (vine and bush), C32 and C39 have several common traits, including a glossy fruit skin lustre, almost absent fruit warts, and no fruit skin flecking (Table 1 and Figure 1). Thus, C32 appears to belong to the Pumpkin morphotype.

Analysing relationships between subspecies through the five interspecific hybrids. Because

https://doi.org/10.17221/27/2021-CJGPB

ornamental gourds are not the primary 'cash crop', few investigations into their breeding have been carried out, and the primary method of reproduction in ornamental gourds is open pollination isolation. Reciprocal hybridisation is likely where different types of ornamental gourds are cultivated simultaneously side-by-side in near proximity; eventually, new cultivars can be produced by continual segregation. In this study, five randomly selected interspecific hybrids from recombinant inbred lines were included for observation.

Our PCoA diagrams show that the five hybrids are loosely associated with and occupy a central position between their parents (Figure 3). Although the traits of these hybrids vary (Figure 1) and certain unique alleles showed up only in some of these five hybrids (Figure 4), all five form a very robust subcluster with a relatively low GD value (0.26), indicating a close relationship. Our results suggest that other existing morphotypes (such as Acorn) are unlikely to be derived from crosses between ssp. *pepo* and *texana*, consistent with the findings by Gong et al. (2012).

Relationships between C41, C42, C43, and Cluster Ia. Although C41, C42, and C43 belong to different cultivar groups (as defined in previous studies), our results, shown in Figure 2 and Figure 3, indicate a relatively close relationship among these accessions, which are all edible squash; this phenotypic feature may also explain why they have the lowest average GD values with accessions in cluster Ia, which consists of eight edible squashes (Table 3). Moreover, three of the five alleles in Figure 4 that showed up in all the accessions in cluster Ia also exist in C41, C42, and C43. Given that these squashes are all edible, these alleles may help to distinguish the edible varieties when combined together.

Compared with C42 (Acorn), C41 (Scallop) demonstrates a closer genetic relationship and lower GD value with C43 (Crookneck), consistent with the result reported by Gong et al. (2012). At the outset, the seeds of the accessions comprising cluster Ia were thought to be of ornamental gourds; however, when grown and subjected to the molecular marker analysis, they turned out to be squash of the Acorn morphotype and their identity as squash is confirmed by their close affinity with the accessions of cluster Ib. This is in accordance with the finding that their commercially available look-alikes Sweet Dumpling and Jack-Be-Little belong to the Acorn morphotype (Gong et al. 2012).

Five newly-proposed morphotypes in *C. pepo* ssp. *texana*. Based on our results and as best illustrated by the dendrogram (Figure 2), the Ovifera Gourd accessions are divided into five distinct morphotypes:

The first two morphotypes, Autumn Wings (C6–C10) and Thin Warty Pear (C16–C19 and C21–C24), contain accessions from cluster Ic. This subcluster mainly contains two types of gourds: gourds with wings and thin pyriform gourds with warts (Figure 1).

The third proposed morphotype, Crown of Thorns, is derived from cluster Id, and consists of only one type of gourd. This morphotype is one of the most unique of the *C. pepo* gourds, and its members were previously categorised as 'other gourds', along with Spoon gourds, according to Paris (2001).

The last two proposed morphotypes, Warty Pear and Egg, are derived from clusters Ie and If, respectively. All gourds in cluster Ie (warty, pyriform, and flatter in shape compared to the Thin Warty Pear) are assigned to the Warty Pear morphotype, and the two egg-shaped gourds in cluster If are assigned to the Egg morphotype.

According to our results, the differences in the fruit shapes between *Cucurbita pepo* ssp. *texana* accessions are highly consistent with their genetic relationships. In other words, gourds with the same shape or pattern tend to have close genetic relationships with one another and the cluster in the same group. Although our five proposed new morphotypes have taken as many factors into account as possible, a few accessions have some dissimilarity in appearance from the majority of the accessions in their assigned morphotype group; concise SSR fingerprinting (Figure 4) was constructed to help with clustering. However, the purpose of this work was to assign classifications according to both the phenotype and genetics to improve the robustness of the classifications, and some within-group deviation from the phenotypic similarity is, therefore, unavoidable.

CONCLUSION

We explored 85 highly-polymorphic EST-SSR markers in order to define a highly efficient way to assess the genetic diversity among ornamental gourds. In addition, we demonstrated the genetic relationships among the accessions used in our study through a dendrogram, a PCoA diagram, genetic distance analyses, and SSR fingerprinting. Based on our results, we propose designating five new morphotypes

https://doi.org/10.17221/27/2021-CJGPB

of *Cucurbita pepo* ssp. *texana* based on both the genetic and phenotypic similarity; this study is, to our knowledge, the first to do so.

Through the SSR analysis, we were able to provide various perspectives on the genetic relationships through various methods, which allowed us to demonstrate that our classification system was well supported by a variety of analyses. Our study demonstrates the advantages and feasibility of using SSR markers to determine the genetic relationships in *Cucurbita pepo*, and presents new visualisations of these relationships and new subclusters in *C. pepo* ssp. *texana*.

Previously, the molecular marker-assisted identification of *Cucurbita pepo* subspecies has been shown to be reliable. Our work incorporates this method to extend the classification of ornamental gourds proposed by previous works (Paris et al. 2003; Gong et al. 2012, 2013). Our results also corroborate the earlier assertion that the fruit shape largely reflects the genetic relationships (Paris 1986).

Most notably, ours is the most comprehensive analysis of the genetic relationships among ornamental gourd accessions that has been undertaken to date, the largest study (containing the most Ovifera Gourd accessions) that has been undertaken to analyse their genetic relationships, and the first work to investigate the interspecific hybrids of ssp. *pepo* and *texana*. Furthermore, our newly-proposed morphotype classification for them may help guide further attempts at achieving a better appreciation and understanding of the genetic relationships within and among the subspecies of *Cucurbita pepo*.

Acknowledgement: We thank Prof. Haizhen Li in the National Engineering Research Center for Vegetables, Beijing Academy of Agriculture and Forestry Sciences for providing accessions C41 and C42 used in the study.

REFERENCES

Andres T.C. (1987): *Cucurbita fraterna*, the closest wild relative and progenitor of *C. pepo*. Cucurbit Genetics Cooperative Report, 10: 69–71.

Blanca J., Cañizares J., Roig C., Ziarsolo P., Nuez F., Picó B. (2011): Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (Cucurbitaceae). BMC Genomics, 12: 104.

Botstein D., White R.L., Skolnick M., Davis R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314.

Castellanos-Morales G., Ruiz-Mondragón K.Y., Hernández-Rosales H.S., Sánchez-de la Vega G., Gámez N., Aguirre-Planter E., Montes-Hernández S., Lira-Saade R., Eguiarte, L.E. (2019): Tracing back the origin of pumpkins (*Cucurbita pepo* ssp. *pepo* L.) in Mexico. Proceedings of the Royal Society B, 286: 20191440.

Chomicki G., Schaefer H., Renner S.S. (2020): Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. New Phytologist, 226: 1240–1255.

Decker D.S. (1988): Origin(s), evolution, and systematics of *Cucurbita pepo* (Cucurbitaceae). Economic Botany, 42: 4–15.

Dice L.R. (1945): Measures of the amount of ecologic association between species. Ecology, 26: 297–302.

Doyle J. (1991): DNA protocols for plants. In: Hewitt G.M., Johnston A.W.B., Young J.P.W. (eds.): Molecular Techniques in Taxonomy. 1st Ed. Berlin, Springer: 283–293.

Emerson R.A. (1910): The inheritance of sizes and shapes in plants. The American Naturalist, 44: 739–746.

Felsenstein J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783–791.

Ferriol M., Pico B., Nuez F. (2003): Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theoretical and Applied Genetics, 107: 271–282.

Garcia-Mas J., Benjak A., Sanseverino W., Bourgeois M., Mir G., González V.M., Hénaff E., Câmara F., Cozzuto L., Lowy E., Alioto T. (2012): The genome of melon (*Cucumis melo* L.). Proceedings of the National Academy of Sciences, 109: 11872–11877.

Gong L., Stift G., Kofler R., Pachner M., Lelley T. (2008): Microsatellites for the genus Cucurbita and an SSR-based genetic linkage map of *Cucurbita pepo* L. Theoretical and Applied Genetics, 117: 37–48.

Gong L., Paris H.S., Nee M.H., Stift G., Pachner M., Vollmann J., Lelley T. (2012): Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. Theoretical and Applied Genetics, 124: 875–891.

Gong L., Paris H.S., Stift G., Pachner M., Vollmann J., Lelley T. (2013): Genetic relationships and evolution in *Cucurbita* as viewed with simple sequence repeat polymorphisms: the centrality of *C. okeechobeensis*. Genetic Resources and Crop Evolution, 60: 1531–1546.

Guo S., Zhang J., Sun H., Salse J., Lucas W.J., Zhang H., Zheng Y., Mao L., Ren Y., Wang Z., Min J. (2013): The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nature Genetics, 45: 51–58.

Huang S., Li R., Zhang Z., Li L., Gu X., Fan W., Lucas W.J., Wang X., Xie B., Ni P., Ren Y. (2009): The genome of

https://doi.org/10.17221/27/2021-CJGPB

the cucumber, *Cucumis sativus* L. Nature Genetics, 41: 1275–1281.

Katzir N., Tadmor Y., Tzuri G., Leshzeshen E., Mozes-Daube N., Danin-Poleg Y., Paris H.S. (2000): Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. In: Katzir N., Paris H.S. (eds.): VII Eucarpia Meeting on Cucurbit Genetics and Breeding 510. 1st Ed. Ma'ale Ha Hamisha, ISHS: 433–440.

Kaźmińska K., Sobieszekzmi K., Targońska-Karasek M., Korzeniewska A., Niemirowicz-Szczytt K., Bartoszewski G. (2017): Genetic diversity assessment of a winter squash and pumpkin (*Cucurbita maxima* Duchesne) germplasm collection based on genomic *Cucurbita*-conserved SSR markers. Scientia Horticulturae, 219: 37–44.

Kong Q., Chen J., Liu Y., Ma Y., Liu P., Wu S., Huang Y., Bie Z. (2014): Genetic diversity of *Cucurbita* rootstock germplasm as assessed using simple sequence repeat markers. Scientia Horticulturae, 175: 150–155.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018): MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35: 1547–1549.

Montero-Pau J., Blanca J., Bombarely A., Ziarsolo P., Esteras C., Martí-Gómez C., Ferriol M., Gómez P., Jamilena M., Mueller L., Picó B. (2018): De novo assembly of the zucchini genome reveals a whole-genome duplication associated with the origin of the Cucurbita genus. Plant Biotechnology Journal, 16: 1161–1171.

Nei M. (1978a): The theory of genetic distance and evolution of human races. Japanese Journal of Human Genetics, 23: 341–369.

Nei M. (1978b): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583–590.

Nei M., Li W.H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76: 5269–5273.

Ntuli N.R., Tongoona P.B., Zobolo A.M. (2015): Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. Scientia Horticulturae, 189: 192–200.

Pan Y., Wang Y., McGregor C., Liu S., Luan F., Gao M., Weng Y. (2020): Genetic architecture of fruit size and shape variation in cucurbits: A comparative perspective. Theoretical and Applied Genetics, 133: 1–21.

Paris H.S. (1986): A proposed subspecific classification for *Cucurbita pepo*. Phytologia, 61: 133–138.

Paris H.S. (2000): Paintings (1769–1774) by A.N. Duchesne and the history of *Cucurbita pepo*. Annals of Botany, 85: 815–830.

Paris H.S. (2001): History of the cultivar-groups of Cucurbita pepo. Horticultural Reviews, 25: 71–170.

Paris H.S. (2016): Germplasm enhancement of *Cucurbita pepo* (pumpkin, squash, gourd: Cucurbitaceae): progress and challenges. Euphytica, 208: 415–438.

Paris H.S., Yonash N., Portnoy V., Mozes-Daube N., Tzuri G., Katzir N. (2003): Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. Theoretical and Applied Genetics, 106: 971–978.

Paris H.S., Hanan A., Baumkoler F., Lebeda A. (2004): Assortment of five gene loci in *Cucurbita pepo*. In: Lebeda A., Paris H.S. (eds.): Proceedings of Cucurbitaceae. 1st Ed. Olomouc, Palacky University in Olomouc: 389–394.

Paris H.S., Lebeda A., Křistkova E., Andres T.C., Nee M.H. (2012): Parallel evolution under domestication and phenotypic differentiation of the cultivated subspecies of *Cucurbita pepo* (Cucurbitaceae). Economic Botany, 66: 71–90.

Qi J., Liu X., Shen D., Miao H., Xie B., Li X., Zeng P., Wang S., Shang Y., Gu X., Du Y. (2013): A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. Nature Genetics, 45: 1510.

Radwan S.A.A. (2014): Molecular discrimination and genetic relationships between some cultivars of *Cucurbita pepo* ssp. *pepo* using random amplification of polymorphic DNA (RAPD) analysis. African Journal of Biotechnology, 13: 1202–1209.

Robinson R.W., Decker-Walters D.S. (1997): Cucurbits. Wallingford, Cab International.

Rohlf F.J. (2000): NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.10e. New York, Exeter Publications.

Sanjur O.I., Piperno D.R., Andres T.C., Wessel-Beaver L. (2002): Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from a mitochondrial gene: Implications for crop plant evolution and areas of origin. Proceedings of the National Academy of Sciences, 99: 535–450.

Singh N., Choudhury D.R., Singh A.K., Kumar S., Srinivasan K., Tyagi R.K., Singh N.K., Singh R. (2013): Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. PLoS ONE, 8: e84136.

Sinnott E.W. (1935): Evidence for the existence of genes controlling shape. Genetics, 20: 12–21.

Sinnott E.W., Kaiser S. (1934): Two types of genetic control over the development of shape. Bulletin of the Torrey Botanical Club, 61: 1–7.

Sneath P.H., Sokal R.R. (1973): Numerical Taxonomy. The Principles and Practice of Numerical Classification. San Francisco, W.H. Freeman and Company.

https://doi.org/10.17221/27/2021-CJGPB

Stecher G., Tamura K., Kumar S. (2020): Molecular evolutionary genetics analysis (MEGA) for macOS. Molecular Biology and Evolution, 37: 1237–1239.

Sun H., Wu S., Zhang G., Jiao C., Guo S., Ren Y., Zhang J., Zhang H., Gong G., Jia Z., Zhang F. (2017): Karyotype stability and unbiased fractionation in the paleo-allotetraploid Cucurbita genomes. Molecular Plant, 10: 1293–1306.

Trumbull J.H. (1876): Vegetables cultivated by the American Indians. I. Bulletin of the Torrey Botanical Club, 613: 69–71.

Varshney R.K., Chabane K., Hendre P.S., Aggarwal R.K., Graner A. (2007): Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. Plant Science, 173: 638–649.

Wehner T.C., Naegele R.P., Myers J.R., Narinder P.S., Crosby K. (2020): Cucurbits. 2nd Ed. Wallingford, CABI: 20–21.

Whitaker T.W. (1947): American origin of the cultivated cucurbits. Annals of the Missouri Botanical Garden, 34: 101–111.

Wilson H.D., Doebley J., Duvall M. (1992): Chloroplast DNA diversity among wild and cultivated members of Cucurbita (Cucurbitaceae). Theoretical and Applied Genetics, 84: 859–865.

Xanthopoulou A., Ganopoulos I., Kalivas A., Nianiou-Obeidat I., Ralli P., Moysiadis T., Tsaftaris A., Madesis P. (2015): Comparative analysis of genetic diversity in Greek Genebank collection of summer squash ('Cucurbita pepo') landraces using start codon targeted (SCoT) polymorphism and ISSR markers. Australian Journal of Crop Science, 9: 14–21.

Xanthopoulou A., Montero-Pau J., Mellidou I., Kissoudis C., Blanca J., Picó B., Tsaballa A., Tsaliki E., Dalakouras A., Paris H.S., Ganopoulou M. (2019): Whole-genome resequencing of *Cucurbita pepo* morphotypes to discover genomic variants associated with morphology and horticulturally valuable traits. Horticulture Research, 6: 1–7.

Xiang C., Duan Y., Li H., Ma W., Huang S., Sui X., Zhang Z., Wang C. (2018): A high-density EST-SSR-based genetic map and QTL analysis of dwarf trait in *Cucurbita pepo* L. International Journal of Molecular Sciences, 19: 3140.

Yang L., Koo D.H., Li Y., Zhang X., Luan F., Havey M.J., Jiang J., Weng Y. (2012): Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. The Plant Journal, 71: 895–906.

Yeh F.C., Boyle T., Rongcai Y., Ye Z., Xian J. (1999): POPGENE Version 1.31. A Microsoft window-based freeware for population genetic analysis. Available at https://sites.ualberta.ca/~fyeh/popgene.pdf/.

Zheng Y.H., Alverson A.J., Wang Q.F., Palmer J.D. (2013): Chloroplast phylogeny of *Cucurbita*: evolution of the domesticated and wild species. Journal of Systematics and Evolution, 51: 326–334.

Zheng Y., Wu S., Bai Y., Sun H., Jiao C., Guo S., Zhao K., Blanca J., Zhang Z., Huang S., Xu Y., Weng Y., Mazourek M. K., Reddy U., Ando K., McCreight J.D., Schaffer A.A., Burger J., Tadmor Y., Katzir N., Tang X., Liu Y., Giovannoni J.J., Ling K.S., Wechter W.P., Levi A., Garcia-Mas J., Grumet R., Fei Z. (2019): Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops. Nucleic Acids Research, 47: 1128–1136.

Received: March 28, 2021
Accepted: June 23, 2021
Published online: July 29, 2021