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Collection and evaluation of thirty-seven pomegranate germplasm resources

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Abstract

Pomegranates (*Punica granatum* L.) are gaining popularity among consumers because of their high antioxidant activity and multiple medical benefits. China is rich in pomegranate genetic resources, but how to use them effectively is a problem worthy of deep consideration. In this article, thirty-seven pomegranate varieties from seven provinces in China were collected and analyzed for twelve phenotypic traits and twelve biochemical indicators (seeds and juices). The fruit and aril fresh weight ranged between 210.5 and 576.5 g and 121.0 to 327.5 g, respectively, and the edible rate (42.58–64.80%), seed weight (1.80–3.41 g), seed number (249.1–838.9), fruit height (10.51–15.48 mm), fruit diameter (11.46–17.50 mm), skin thickness (2.14–6.98 mm), and shape index (0.82–0.96) varied among the different genotypes. The pomegranate juice total phenolic content ranged from 40.91 to 132.47 µg/mL, and the total flavonoid content (14.08–137.72 µg/mL), vitamin C content (12.80–66.63 µg/mL), pH (3.10–4.34), total soluble solids (13.13–17.50°Brix), and titratable acidity (0.26–2.71%) also varied; the pomegranate seed total phenolic content ranged from 0.62 to 1.78 mg/g, and the total flavonoid content (0.39–0.99 mg/g), vitamin C content (7.55–13.90 mg/g), DPPH radical scavenging capacity (85.98–98.24%), and ABTS scavenging ability (28.72–51%) were also measured. The coefficients of variation of the studied traits ranged from 5.62 to 54.02%, and the phenotypic traits' Shannon–Weaver diversity indexes ranged from 0.67 to 1.53. Cluster analysis divided the 37 varieties into three categories, providing a reference for improved variety breeding. In addition, genotypic and environmental effects mainly affected the pomegranate flavor and antioxidant activity, respectively.

Keywords: Pomegranate, Genetic diversity, Environment, Cluster analysis, Principal component analysis

Introduction

Pomegranate (*Punica granatum* L.) is an ancient and widely cultivated fruit native to Iran, Afghanistan, and other parts of Central Asia. Besides its nutritional value, more studies established the medicinal effects of pomegranate including antibacterial, anti-inflammatory, antiviral, and benefits on cardiovascular health and obesity [1]. During the Western Han dynasty, Chinese envoy Zhang Qian introduced pomegranates into China [2]. After long-term natural hybridization, gene mutation and the implementation of varied breeding and propagation (e.g., seeding, ramets, grafting, etc.) methods, a wide collection of pomegranate varieties has been produced. Pomegranate is one of the essential fruits in China and is widely distributed and cultivated in the provinces of Henan, Shandong, Sichuan, Anhui, Shaanxi, Yunnan, and Xinjiang in China. Because of the differences in the aspects of geographical distribution, climatic environment, and genetic variation, pomegranate varieties have distinct local characteristics in different parts of China. Therefore, the recognition and measurement of such diversity and its nature and magnitude are crucial to a breeding program.

Pomegranate variety identification is based on the external and internal characteristics of the fruit. Martinez-Nicolas et al. [3] established that pomegranate fruit and seed size have a relatively strong relationship with the juice's pH. However, the differences in pomegranate leaf and flower characteristics between varieties is not significant, which indicates a certain relationship between the phenotype

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and physiological status in pomegranate. Zaouay et al. [4] studied 38 Tunisian pomegranate varieties, analyzing the effects of clones, age, and their interactions on fruit quality, and demonstrated that genetics (variety) contributed the most to fruit size and skin color and thickness variation and concluded that aril and juice yields were affected by age. Li et al. [5] studied the relationship between antioxidant capacities and the planting environment of 9 pomegranate juice varieties in China; however, the studied varieties did not present a comprehensive germplasm collection. At present, pomegranate diversity studies have mainly focused on tree shape, leaves, and fruit juice [6, 7]. However, few writers have focused on extensive research into pomegranate seeds. In China, pomegranate seeds are discarded as waste, which pollutes the environment and wastes resources. Some scholars have used pomegranate seeds to develop new applications, and the addition of pomegranate seed powder to bread was shown to significantly improve its antioxidant activity [8]. Lucci et al. [9] proposed the use of a pomegranate seed ethanol extract as a nutraceutical and functional food ingredient to utilize its antihormone-dependent antioxidant and antiproliferative effects against human prostate cancer and breast cancer cells. These studies show the potential use of these fruit by-products as natural antioxidants.

The environment greatly influences crop growth state and tolerance to stress. Plants grow better in a suitable environment and grow slowly or even die in harsh environments. However, the environmental impact is varied, and for pomegranates, we still do not know which aspects of the pomegranate quality are affected by the environment. In the interaction between genotype and phenotype, it is worth exploring which pomegranate parameter is mainly controlled by genotype and which parameter is closely related to the environment.

The present paper is an attempt to investigate the genetic diversity and nutrition (seed and juice) of thirty-seven pomegranate varieties from seven Chinese provinces and to propose a conceptual image and solution to the following problems: (1) analyzing phenotypic traits to determine the genetic relationship between different pomegranate cultivars; (2) screening pomegranate varieties to identify those suitable for fresh consumption, juice processing, acid extraction, and high-antioxidant seeds; and (3) revealing the effect of different environments on pomegranate phenotypic traits and nutrition. This work provides the first comprehensive assessment of pomegranate phenotypic traits and nutrition, and the relationship between them and with different environmental conditions, which has great value for breeders and processing factories.

Materials and methods

Collection of the pomegranate varieties

In 2018, thirty-seven commercial pomegranate cultivars were collected from eight cities in seven Chinese provinces (Yunnan (YN), Anhui (AH), Sichuan (SC), Henan (HN), Shandong (SD), Xinjiang (XJ), and Shaanxi (SN)) (Tables 1, 2). Climate and elevation information for the different regions were obtained from weather, elevation, and satellite positioning websites (http://haiba.ugoto.cn/, https://baike.baidu.com, http://www.gpsspg.com/maps.htm) (Table 1). From each pomegranate variety, 10 fresh fruits were randomly collected for further analyses. After determining the phenotypic traits, the seeds were cleaned, dried and used for nutrient measurements.

Characteristics of the fruit

Fruit fresh weight (FFW; g), fresh aril weight (FAW), 100-seed grain weight (SW), and skin fruit weight (SFW; g) were determined. Seed number (SN) was based on the average number of seeds from 10 fruits. Fruit height (FH; mm), fruit diameter (FD at the equator; mm), and skin thickness (ST; mm) were recorded using a digital caliper at 0.01 mm accuracy. Skin color (SC) was assessed according to a 4-point grading scale (1 = yellow-greenish; 2 = pink yellowish; 3 = red-pink; and 4 = dark-red to purple) Juice color (JC) was determined according to

Table 1 Environmental information in different regions

No.	Location		Temperature (°C)	Precipitation (mm)	Longitude	Latitude	Altitude (m)
	Province	City					
1	Yunnan	Jianshui	19.5	805.0	102° 49′ 32.13″	23° 38′ 15.92″	1517
2	Sichuan	Panzhihua	20.0	982.6	101° 48′ 35.35″	26° 07′ 3.78″	1438
3	Sichuan	Huili	23.0	1211.7	102° 14′ 35.12″	26° 39′ 31.67″	1737
4	Shandong	Zaozhuang	13.9	815.8	117° 22′ 18.82″	34° 45′ 56.41″	76
5	Xinjiang	Kashgar	11.7	61.5	75° 59′ 12.43″	39° 28′ 12.64″	1279
6	Henan	Xingyang	14.3	650.0	113° 23′ 46.25″	34° 62′ 52.95″	116
7	Anhui	Huaiyuan	15.4	289.0	116° 40′ 53.92″	30° 28′ 6.09″	17
8	Shaanxi	Lintong	13.5	507.7	109° 13′ 8.11″	34° 22′ 55.64″	443

Table 2 Origin and abbreviation of all pomegranate varieties

Variety	Location	Abbreviation	Variety	Location	Abbreviation
Tianlvzi	Yunnan (Jianshui)	YN-TLZ	Huaibeiyihao	Anhui (Huaiyuan)	AH-HBYH
Guangyan	Yunnan (Jianshui)	YN-GY	Huaibeierhao	Anhui (Huaiyuan)	AH-HBEH
Hongmanao	Yunnan (Jianshui)	YN-HMN	Qipiruanzi	Anhui (Huaiyuan)	AH-QPRZ
Hongzhenzhu	Yunnan (Jianshui)	YN-HZZ	Baiyushizi	Anhui (Huaiyuan)	AH-BYSZ
Ruanzi	Yunnan (Jianshui)	YN-RZ	Hongyushizi	Anhui (Huaiyuan)	AH-HYSZ
Zimei	Sichuan (Panzhihua)	SC-ZM	Fenyushizi	Anhui (Huaiyuan)	AH-FYSZ
Qipiruanzi	Sichuan (Huili)	SC-QPRZ	Hongmanao	Anhui (Huaiyuan)	AH-HMN
Taishansanbai	Shandong (Zaozhuang)	SD-TSSB	Dabenzi	Anhui (Huaiyuan)	AH-DBZ
Dahongpao	Shandong (Zaozhuang)	SD-DHP	Erbenzi	Anhui (Huaiyuan)	AH-EBZ
Daqingpi	Shandong (Zaozhuang)	SD-DQP	Dabawa	Shaanxi (Lintong)	SN-DBW
Damaya	Shandong (Zaozhuang)	SD-DMY	Dahongtian	Shaanxi (Lintong)	SN-DHT
Qiuyan	Shandong (Zaozhuang)	SD-QY	Sanbaitian	Shaanxi (Lintong)	SN-SBT
Qinli	Shandong (Zaozhuang)	SD-QL	Sanbaisuan	Shaanxi (Lintong)	SN-SBS
Zipitian	Shandong (Zaozhuang)	SD-ZPT	Linxuanyihao	Shaanxi (Lintong)	SN-LXYH
Kashitian	Xinjiang (Kashga)	XJ-KST	Linxuanerhao	Shaanxi (Lintong)	SN-LXEH
Hetian	Xinjiang (Kashga)	XJ-HT	Yichuanlin	Shaanxi (Lintong)	SN-YCL
Ruanzi	Henan (Xingyang)	HN-RZ	Dazishiliu	Shaanxi (Lintong)	SN-DZSL
Yudazi	Henan (Xingyang)	HN-YDZ	Jingpitian	Shaanxi (Lintong)	SN-JPT
			Bairuanzi	Shaanxi (Lintong)	SN-BRZ

a 5-point grading scale (1 = light pink; 2 = pink; 3 = red-pink; 4 = red; and 5 = reddish-purple). The edible rate (ER) and shape index (SI) were calculated as FAW/FFW and FH/FD, respectively [10].

Total phenolic (TP) content determination

Total phenolic (TP) content was determined following the Folin-phenol method [11]: (1) first, 0.8 g of pomegranate seed powder was dissolved in 8 mL of 60% ethanol and sonicated for 30 min, and the mixture was then centrifuged at 12,000 rpm for 15 min; (2) then, 50 μL of the supernatant was mixed with 250 μL of Folin-Ciocalteu reagent and 750 μL of 20% sodium carbonate (Na_2CO_3), and 3 mL pure H_2O, and (3) after adequate reaction of the solution (2 h), the absorbance was read at 760 nm with a spectrophotometer (BECKMAN DU-800®).

Total flavonoid (TF) content determination

Total flavonoid (TF) content was determined using the method of Viuda-Martos et al. [12] as follows: (1) first, 0.2 g of pomegranate seeds were dissolved in 4 mL of 60% ethanol and thoroughly ground to obtain an extract; (2) the phenolic extract was centrifuged at 12,000 rpm for 15 min; (3) then, 1 mL of supernatant was mixed with 0.3 mL of 5% $NaNO_2$ and 0.3 mL of a 10% $AlCl_3$ solution; (4) after 5 min, 2 mL of 1 M NaOH was added, the total volume was brought to 10 mL with ddH_2O, and (5) the absorbance was measured at 510 nm with a spectrophotometer (BECKMAN DU-800®).

Vitamin C (VC) content determination

The VC content was determined using the method of Kampfenkel et al. [13] with slight modifications as follows: (1) first, 1 g of pomegranate seed powder was mixed with 5 mL of a trichloroacetic acid solution and then sonicated for 30 min; (2) after centrifugation at 12,000 rpm for 15 min, the supernatant was filtered through a filter to obtain an extract, and (3) 1 mL pomegranate juice or pomegranate seed powder extract solution was mixed with 1 mL of a 0.5% trichloroacetic acid solution, 1 mL of ethanol, and 0.5 mL of a 0.4% phosphoric acid solution; (4) after 5 min, 1 mL of a 5% 2,2′-bipyridine solution and 0.5 mL iron trichloride were added, the mixture was incubated in a water bath at 37 °C for 30 min, and (5) the absorbance was measured at a wavelength of 525 nm with a spectrophotometer (BECKMAN DU-800®).

DPPH radical scavenging capacity estimation

To make the DPPH solution, 3.98 mg of DPPH was accurately weighed and mixed well with 100 mL of 80% ethanol and kept in the dark at 4 °C in a refrigerator. The sample extract was mixed with 2 mL of DPPH solution and allowed to stand for 30 min. The absorbance at 517 nm was measured for triplicate samples. The following formula was used:

$$K = \left(1 - \frac{A_i - A_j}{A_c}\right) \times 100\% \tag{1}$$

where K is the sample's clearance rate of DPPH free radicals, Ai is the absorbance of 2 mL of DPPH solution + 2 mL of sample extract, Aj is the absorbance of 2 mL of sample extract + 2 mL of an ethanol solution, and Ac is the absorbance of 2 mL of DPPH solution + 2 mL of an ethanol solution [14].

ABTS scavenging ability

The ABTS radical cation is generated by a reaction of 7 mM ABTS and 2.45 mM potassium persulfate with 12 h of incubation at room temperature in the dark. The ABTS + solution was diluted with phosphate buffer saline (PBS) at pH 7.4 to an absorbance of 0.70 ± 0.02 at 734 nm before analysis. The ABTS solution (3.9 mL) was added to 0.1 mL of the tested sample and mixed thoroughly. The mixture was incubated in a water bath at 37 °C for 30 min, and the absorbance was read at 760 nm by BECKMAN DU-800. We used water as a control. The experiment was repeated three times, and the result was calculated using the following formula:

$$K = \left(\frac{A_{control} - A_{test}}{A_{control}} \right) \times 100\% \qquad (2)$$

where A control is distilled water (0.1 mL) mixed the ABTS solution (3.9 mL) and A test is the tested sample (0.1 mL) mixed the ABTS solution (3.9 mL) [15].

Total soluble solids (TSS), pH, and titratable acidity (TA)

Total soluble solids (°Brix) and pH were determined on juice samples using a handheld refractometer and digital pH meter, respectively. Titratable acidity (TA) was measured colorimetrically by titration with 0.1 N NaOH using the pH indicator phenolphthalein[16].

The maturity index (MI)

The fruit maturity index (MI) was determined as TSS/TA according to Martinez et al. [17]. The classification of MI values for sweet = 31–98, sour–sweet to sweet = 25–30, sour–sweet: 17–24, sour–sweet to sour = 9–16 and sour = 5–8.

Analysis of phenotypic diversity

The mean (x) and standard deviation (δ) of quantitative traits were calculated using the Python3.7 language (Numpy library and Pandas library). Assuming all data metrics follow the normal distribution probability, the quantitative characterization of all materials was divided into 5 levels based on the mean and standard deviation data. The standard normal distribution coefficients (X-1.2816δ), (X-0.5244δ), (X-0.5244δ), (X-1.2816δ) were divided into 5 levels, and the probability of occurrence of levels 1 to 5 was 10%, 20%, 40%, 20%, and 10%, respectively. Nonnumerical characteristics were represented by assignment. The diversity of a characteristic was determined using the Shannon–Weaver diversity index (H') as follows:

$$H' = -\sum_{i=1}^{n} (P_i \times \ln P_i)\ (i = 1, 2, 3 \ldots) \qquad (3)$$

where H' is the diversity index and Pi is the effective percentage of the distribution frequency for the Nth rank of a trait [18].

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA), and sample means were compared by Tukey's test. $P < 0.05$ was considered significant in all cases. Python scripts were used for the Pearson Correlation Analysis and Cluster analysis.

Results and discussion

Phenotypic diversity of the pomegranate varieties

The Shannon–Weaver diversity index (SHDI) was calculated to compare the phenotypic diversity among characters and regions [19]. The larger the SHDI value, the richer the diversity of the community. As shown in Table 3, the coefficient of variation (VCo) between twelve quantitative traits of the 37 local pomegranate varieties ranged from 5.62 to 54.02%, and the SHDI varied from 0.67 to 1.53, which was higher than that reported by Polyzos et al. [20] in a study on thirty-four garlic genotypes of greek garlic (0.37 to 0.99). This variation also indicates rich diversity within the Chinese pomegranate germplasm resources. Through analyzing the fruit picking date, all of the early-maturing varieties (YN-TLZ, YN-GY, YN-HMN, YN-HZZ, YN-RZ, and SC-QPRZ) originated from the Yunnan and Sichuan provinces, while the late-maturing varieties (AH-EBZ, AH-DBZ, and SN-DBW) came from Anhui and Shaanxi provinces. Yunnan and Sichuan are located in areas with a high average temperature, abundant precipitation and high altitude (> 1000 m), while Anhui and Shaanxi are located in low-altitude areas. Accordingly, the ripening time of pomegranate planted in higher altitude regions is remarkably earlier than that for the fruits of the lower altitude regions, and the maturing time of pomegranates is closely associated with the climate and altitude of cultivated areas.

The fresh fruit weight (FFW) of the 37 varieties ranged between 210.5 and 576.5 g, with 86.5% of the fruit weighing more than 500 g (SC-QPRZ: 576.5 g, SD-DHP: 568.0 g, and SD-DQP: 558.5 g) and with four varieties weighing less than 300 g (AN-HBEH: 255.0 g, SD-QY: 295.0 g, YN-GY: 291.5 g, and SD-ZPT: 210.5 g) (Table 3). Pomegranate aril yield is one of the most important industrial production parameters [21]. Fresh aril weight (FAW) varied between 121.0 and 327.5 g, with the SD-DQP, SC-QPRZ and SD-DHP varieties suitable for fruit juice processing. Skin fruit

Table 3 Phenotypic parameters of thirty-seven pomegranate varieties

Variety	Weight and number						Size and thickness				Color		Picking date
	FFW	FAW	SFW	ER	SW	SN	FH	FD	ST	SI	SC	JC	
YN-TLZ	326.5 ± 36.21	212.5 ± 17.83	114.0 ± 26.12	64.80	2.18 ± 0.03	315.2 ± 44.40	11.92 ± 1.10	13.71 ± 0.87	2.52 ± 0.30	0.87	2	3	9/5
YN-GY	291.5 ± 92.62	138.0 ± 17.19	153.5 ± 93.90	47.40	2.59 ± 0.02	307.7 ± 53.21	10.70 ± 1.00	12.98 ± 10.82	2.14 ± 0.29	0.82	2	3	9/5
YN-HMN	357.0 ± 32.16	199.5 ± 32.87	157.5 ± 45.78	56.00	2.19 ± 0.02	253.5 ± 52.88	12.40 ± 0.81	14.39 ± 0.67	3.71 ± 0.19	0.86	3	4	9/5
YN-HZZ	431.0 ± 61.77	227.0 ± 21.11	204.0 ± 62.75	52.70	2.77 ± 0.01	465.6 ± 42.24	13.35 ± 0.78	15.36 ± 10.78	3.67 ± 0.33	0.87	3	4	9/5
YN-RZ	462.0 ± 67.59	277.0 ± 46.62	185.0 ± 57.59	60.00	1.80 ± 0.03	472.0 ± 77.18	13.66 ± 1.19	15.15 ± 1.06	3.45 ± 0.61	0.88	3	4	9/5
SC-ZM	318.5 ± 41.77	180.0 ± 26.25	138.5 ± 55.58	56.40	2.99 ± 0.06	537.2 ± 59.64	12.05 ± 0.73	14.06 ± 0.60	2.55 ± 0.26	0.86	4	5	9/10
SC-QPRZ	576.5 ± 78.18	299.3 ± 69.86	277.2 ± 92.92	51.80	3.12 ± 0.06	508.9 ± 135.56	14.46 ± 0.99	17.07 ± 21.23	3.54 ± 0.30	0.85	2	3	9/5
SD-TSSB	318.0 ± 55.94	180.5 ± 35.47	137.5 ± 75.25	56.60	2.39 ± 0.08	495.3 ± 86.61	12.44 ± 1.16	14.42 ± 1.37	3.79 ± 0.47	0.89	1	1	9/15
SD-DHP	568.0 ± 133.40	297.1 ± 80.42	270.9 ± 68.92	52.29	2.75 ± 0.05	807.6 ± 185.16	15.48 ± 1.63	17.50 ± 1.58	4.18 ± 0.86	0.88	4	3	9/25
SD-DQP	558.5 ± 75.32	327.5 ± 47.27	231.0 ± 92.43	58.78	2.86 ± 0.06	838.9 ± 118.33	14.78 ± 0.65	15.87 ± 0.74	3.88 ± 0.50	0.93	3	2	9/25
SD-DMY	465.0 ± 67.04	288.0 ± 40.19	177.0 ± 65.48	61.94	3.01 ± 0.02	547.4 ± 63.44	13.73 ± 0.95	15.96 ± 1.10	3.32 ± 0.20	0.86	3	2	9/25
SD-QY	295.0 ± 47.84	182.0 ± 23.48	113.0 ± 52.50	61.69	2.83 ± 0.04	282.0 ± 36.62	12.62 ± 0.72	13.68 ± 0.93	4.36 ± 0.86	0.92	2	2	9/25
SD-QL	382.6 ± 31.11	217.5 ± 26.59	165.1 ± 38.20	56.66	2.84 ± 0.03	566.6 ± 62.01	13.16 ± 0.61	14.91 ± 0.44	4.93 ± 0.68	0.88	3	4	9/25
SD-ZPT	210.5 ± 76.62	121.0 ± 26.22	89.5 ± 22.17	58.60	2.61 ± 0.01	283.5 ± 65.56	10.51 ± 0.57	11.46 ± 0.56	3.30 ± 0.72	0.92	4	4	9.25
XJ-KST	330.5 ± 52.89	177.5 ± 35.14	153.0 ± 69.45	53.47	2.66 ± 0.05	506.5 ± 69.53	12.23 ± 0.46	13.28 ± 0.60	3.78 ± 0.58	0.92	3	5	9/20
XJ-HT	417.0 ± 33.85	211.0 ± 41.35	206.0 ± 55.02	50.60	2.76 ± 0.05	514.7 ± 96.20	12.10 ± 0.66	13.26 ± 0.70	4.83 ± 1.10	0.91	3	5	9/30
HN-RZ	424.0 ± 15.24	267.5 ± 20.03	156.5 ± 20.69	63.21	1.80 ± 0.06	639.1 ± 53.86	13.20 ± 0.68	14.47 ± 1.04	2.82 ± 0.40	0.87	2	5	9/30
HN-YDZ	402.0 ± 35.06	239.5 ± 30.95	162.5 ± 36.31	59.45	3.16 ± 0.10	350.4 ± 26.31	13.67 ± 0.39	15.04 ± 1.10	4.86 ± 0.92	0.91	2	2	10/10
AH-HBYH	300.5 ± 71.20	164.5 ± 37.75	136.0 ± 83.53	55.00	2.71 ± 0.03	347.5 ± 115.47	13.19 ± 0.90	13.90 ± 0.89	4.24 ± 0.31	0.95	2	3	9/10
AH-HBEH	255.0 ± 38.01	139.0 ± 39.07	116.0 ± 51.58	54.51	2.62 ± 0.03	302.9 ± 47.12	12.10 ± 0.68	12.63 ± 0.97	3.59 ± 0.41	0.96	2	3	9/10
AH-QPRZ	302.0 ± 28.01	174.5 ± 36.62	127.5 ± 52.40	56.95	2.79 ± 0.05	357.3 ± 91.53	12.06 ± 1.48	13.61 ± 1.16	4.70 ± 0.56	0.89	2	3	9/10
AH-BYSZ	408.0 ± 56.58	169.5 ± 30.68	238.5 ± 44.10	46.44	3.20 ± 0.03	249.1 ± 90.12	13.68 ± 0.58	15.64 ± 0.81	6.98 ± 0.69	0.86	1	1	9/15
AH-HYSZ	336.5 ± 39.51	190.0 ± 22.73	146.5 ± 34.16	56.38	3.17 ± 0.02	291.8 ± 62.18	12.80 ± 1.11	14.66 ± 1.23	4.22 ± 0.39	0.87	2	2	9/15
AH-FYSZ	415.0 ± 77.24	218.5 ± 29.35	196.5 ± 79.93	52.53	2.91 ± 0.01	358.4 ± 46.08	14.37 ± 1.74	16.06 ± 1.81	5.54 ± 0.81	0.89	2	2	9/15
AH-HMN	446.0 ± 32.64	222.5 ± 19.19	223.5 ± 37.79	56.02	2.72 ± 0.04	424.6 ± 61.32	14.00 ± 1.51	15.81 ± 1.58	6.71 ± 1.12	0.87	2	4	9/25
AH-DBZ	458.5 ± 37.64	253.5 ± 26.46	205.0 ± 32.74	55.34	2.80 ± 0.01	568.5 ± 48.10	14.03 ± 0.63	15.93 ± 0.68	5.36 ± 0.64	0.88	2	3	10/15
AH-EBZ	380.0 ± 20.14	235.5 ± 14.23	144.5 ± 19.21	61.84	2.74 ± 0.01	479.1 ± 43.94	13.05 ± 1.01	14.47 ± 0.98	3.96 ± 0.89	0.90	2	2	10/15
SN-DBW	396.0 ± 36.35	212.5 ± 26.06	183.5 ± 32.24	53.54	3.39 ± 0.04	590.4 ± 55.15	13.79 ± 0.67	15.46 ± 0.88	3.99 ± 0.38	0.89	2	4	10/15
SN-DHT	370.5 ± 19.18	157.5 ± 13.12	213.0 ± 20.71	42.58	3.16 ± 0.04	389.3 ± 56.98	13.24 ± 0.58	14.25 ± 0.76	6.43 ± 0.51	0.93	4	5	10/10

Table 3 (continued)

Variety	Weight and number						Size and thickness				Color		Picking date
	FFW	FAW	SFW	ER	SW	SN	FH	FD	ST	SI	SC	JC	
SN-SBT	432.5 ± 56.04	236.0 ± 45.63	196.5 ± 40.96	54.63	2.78 ± 0.02	499.0 ± 77.00	13.39 ± 0.42	15.55 ± 0.77	5.93 ± 0.44	0.84	1	4	9/25
SN-SBS	417.5 ± 78.29	220.0 ± 48.59	197.5 ± 34.34	54.63	2.65 ± 0.03	472.8 ± 100.81	13.85 ± 1.11	14.68 ± 0.90	5.67 ± 0.67	0.95	1	4	10/10
SN-LXYH	345.5 ± 37.23	187.5 ± 12.53	158.0 ± 26.58	52.76	2.92 ± 0.01	466.0 ± 62.28	12.60 ± 1.11	13.94 ± 0.93	3.64 ± 0.42	0.86	1	4	9/20
SN-LXEH	360.5 ± 8.64	205.3 ± 12.23	155.7 ± 10.87	54.49	2.90 ± 0.01	518.8 ± 43.50	13.40 ± 0.74	14.28 ± 1.01	4.04 ± 0.51	0.89	1	4	9/20
SN-YCL	338.5 ± 14.15	188.0 ± 21.63	150.5 ± 25.22	55.46	2.65 ± 0.02	387.9 ± 60.35	12.88 ± 0.57	14.87 ± 1.21	3.95 ± 0.32	0.87	3	5	10/10
SN-DZSL	356.0 ± 32.47	199.5 ± 29.20	156.5 ± 79.90	56.18	3.41 ± 0.01	299.0 ± 68.98	12.98 ± 0.75	14.90 ± 1.09	5.26 ± 1.01	0.87	1	3	10/10
SN-JPT	396.5 ± 43.78	208.5 ± 21.86	188.0 ± 28.30	52.39	2.67 ± 0.01	539.8 ± 67.68	13.80 ± 0.54	15.62 ± 0.57	4.83 ± 0.82	0.88	2	1	10/10
SN-BRZ	389.5 ± 4.13	210.0 ± 27.29	179.5 ± 28.23	53.85	2.93 ± 0.02	544.9 ± 80.11	12.95 ± 0.93	14.84 ± 10.73	3.50 ± 0.39	0.92	1	1	10/10
VCo (%)	24.57	26.71	37.69	19.20	10.07	34.59	10.29	10.33	29.81	5.62	54.02	37.83	
SHDI	1.39	1.39	1.40	1.39	0.67	1.44	1.38	1.38	1.46	1.44	1.37	1.53	

Data are expressed as the mean ± standard deviation (n = 10). 'VCo' is the abbreviation of coefficient of variation, and 'SHDI' is the abbreviation of the Shannon–Weaver diversity index. Abbreviations for the phenotypic parameters are outlined in "Materials and methods" section weight (SFW) varied from 89.5 to 277.2 g, with SC-QPRZ, SD-DHP, AH-BYSZ, SD-DHP, and AH-HMN as top varieties and YN-TLZ, SD-QY, AH-HBEH, AH-QPRZ, and SD-ZPT as the bottom. This pattern largely overlapped with the FFW variety ranking. Remarkably, the fruit weight of the same variety planted in different areas (Sichuan and Anhui) was obviously different. The FFW and SFW values of QPRZ in Sichuan Province were much higher than those of the same variety grown in Anhui province. Based on the FAW and FFW ratio, the YN-TLZ showed the highest edible rate (ER) (64.83%), while SN-DHT had the lowest edible rate (42.58%).

Ordinarily, the harder the seed, the greater the impact of taste. The main reason why soft-seeded pomegranate is popular in the market is that its taste and flavor are better than those of other varieties. The seed number (SN) was between 253.5 and 838.9 for all varieties. An increase in seed number will affect the taste of pomegranate, but it helps improve the nutritional value of the pomegranate seeds. The fruit height (FH) and diameter (FD) reflect the size of the pomegranate, and their ratio is defined as the shape index (SI), which can be effectively used to evaluate the shape of the fruit. The fruit SI of all varieties except for that of SN-SBT and YN-GY were above 0.85, and among those, AH-HBYH and AH-HBEH had the highest, reaching 0.95 and 0.96, respectively. Skin thickness (ST) significantly differed among the varieties and ranged between 2.14 and 6.98 mm. More significantly, all varieties with the thinnest skin, smallest seed number, shape index, and edible rate originated from Yunnan Province (Table 3).

Skin color is a critical quality attribute in pomegranate marketing. The attractive, red color is an important parameter for commercial quality classification, which influences consumer behaviors [22]. The color for peels and arils of 37 pomegranate varieties from China varied from white to deep red. Most of them had pink yellowish peels and red-pink aril. The SD-ZPT, SD-DHP, SC-ZM, and SN-DHT varieties exhibited a beautiful red color and a very attractive appearance.

Correlation analysis of phenotypic parameters

We can compose some conclusions through correlation analysis of the phenotypic parameters of pomegranate (Fig. 1). Fresh fruit weight (FFW), fresh aril weight

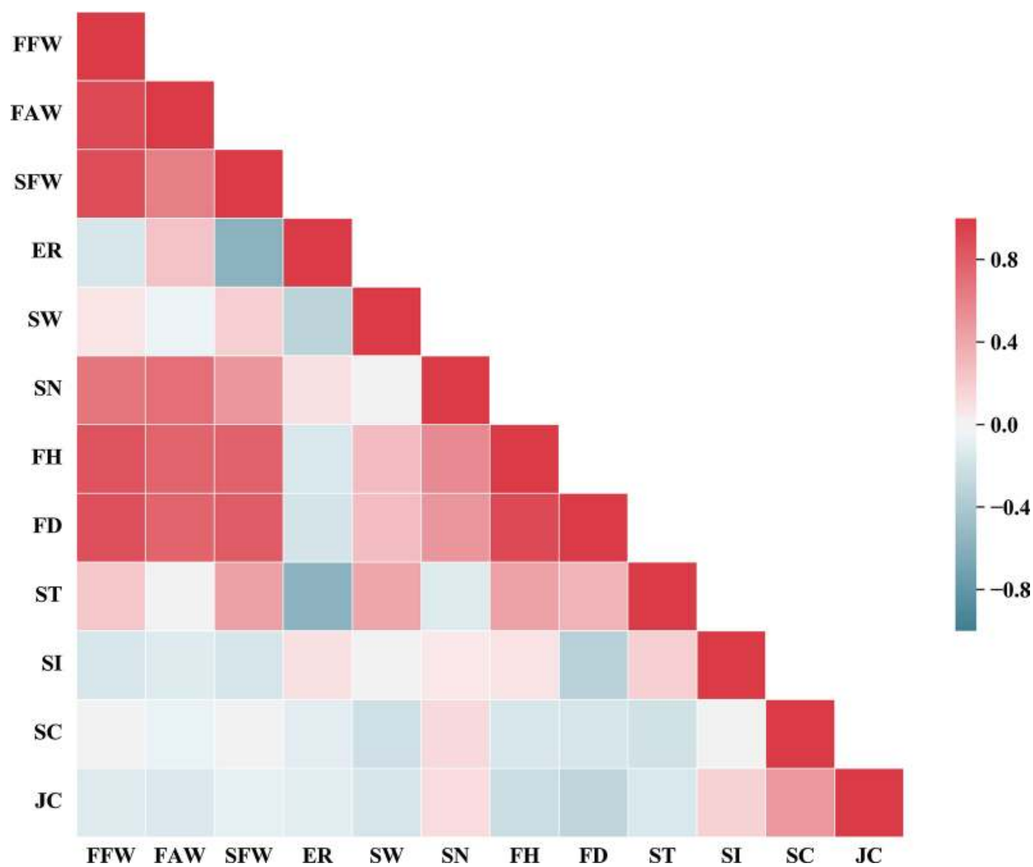

Fig. 1 Pearson's correlation plot based on the correlation of phenotypic traits. Red and green represent positive and negative correlation, respectively. The darker the color, the larger the correlation coefficient value

(FAW), skin fruit weight (SFW), fruit height (FH), and fruit diameter (FD) were significantly positively correlated with each other. A significantly negative correlation was found between edible rate (ER) and skin thickness (ST). Skin color (SC) and juice color (JC) were weakly positively correlated.

Genetic relationship of different pomegranate varieties

To effectively compare the difference in pomegranate cultivars, all phenotypic characteristics were normalized before conducting the cluster analysis (Fig. 2). Cluster analysis indicated that the pomegranate varieties were not simply affected by geographical location. In addition, the genetic distance of some varieties from different provinces (such as XJ-HT, SN-DHT and SD-QL) was close (Fig. 2). The 37 pomegranate varieties were divided into three subcategories according to genetic distance (Fig. 2). The first category featured the bright color of the peel and aril, a large number of seeds, and a moderate skin thickness (XJ-KST, XJ-HT, SN-DHT, SN-YCL, SD-QL, YN-HZZ, YN-HMN, SC-ZM, YN-RZ, HN-RZ, YN-TLZ, YN-GY, and SD-ZPT). The features of the second category were medium fruit size, light skin but brightly colored aril, and heavy seed weight (HN-YDZ, AH-HBYH, SN-SBS, SN-BRZ,

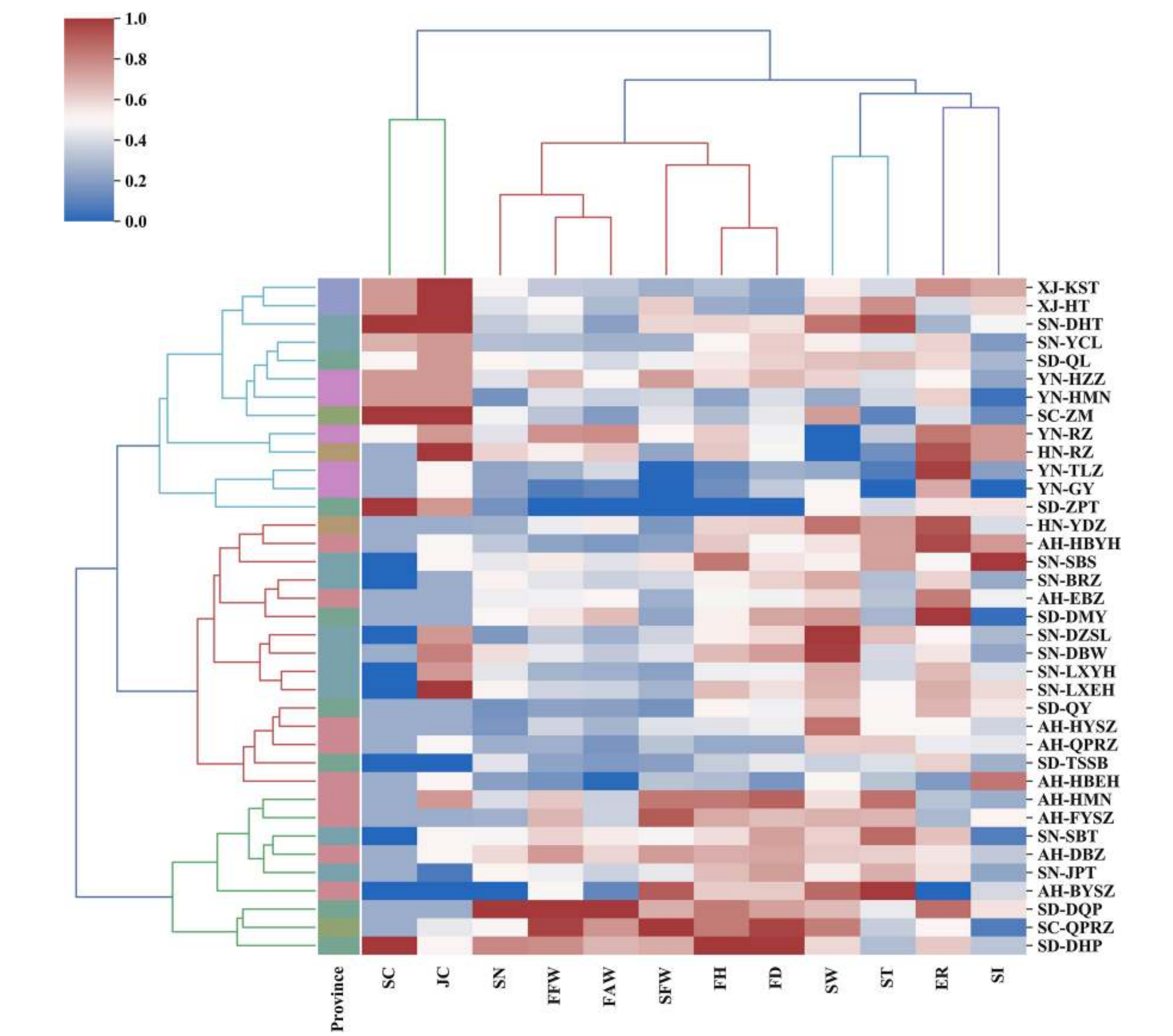

Fig. 2 Cluster analysis of 37 Chinese pomegranate varieties based on phenotypic traits. Each small square reflects the phenotypic characteristics of pomegranate varieties. The color represents the normalized value, with red representing the larger value and blue representing the lower value. Each row represents the normalized content of different phenotypic characteristics from one variety. Each column represents the difference in the normalized results of different varieties in a single specific phenotype. Seven colors distinguish the seven provinces

AH-EBZ, SD-DMY, SN-DZSL, SN-DBW, SN-LXYH, SN-LXEH, SD-QY, AH-HYSZ, AH-QPRZ, SD-TSSB, and AH-HBEH). The third category was characterized by large fruits and a thick fruit skin (AH-HMN, AH-FYSZ, SN-SBT, AH-DBZ, SN-JPT, AH-BYSZ, SD-DQP, SC-QPRZ, and SD-DHP). The fact that AH-HMN and YN-HMN were not in the same group was due to the significant differences in fruit size and skin thickness.

Additionally, the phenotypic indicators of pomegranate could be classified into three categories through cluster analysis (Fig. 2). The skin color (SC) and juice color (JC) showed a close relationship. Seed number (SN), fresh fruit weight (FFW), fresh aril weight (FAW), skin fruit weight (SFW), fruit height (FH), and fruit diameter (FD) were clustered into one group, reflecting fruit weight and size. Generally, the size of fruits was proportional to the weight, i.e., larger fruits were generally heavier. Edible rate (ER), shape index (SI), 100-seed weight (SW) and skin thickness (ST) were grouped together, which is in agreement with the correlation analysis of the phenotypic parameters.

Nutrition and flavor analysis of pomegranate juice

The total phenolic (TP) content in the pomegranate juice ranged from 40.91 to 132.47 μg/mL (Table 4), which was lower than that reported in a previous study (2380–9300 mg/L) of eight Iranian cultivars [23]. Total flavonoid (TF) content values ranged from 14.08 to 137.72 μg/mL. The results show that the highest pomegranate juice total flavonoid content was that of SN-JPT, followed by that of SN-LXYH, SN-YCL, SN-DHT, and SN-SBT. In addition, all the above mentioned varieties were planted in Shaanxi Province. The pomegranate juice VC content ranged from 12.80 to 66.63 μg/mL, with the top values observed for XJ-KST, SD-QL, AH-HBYH, SN-JPT and YN-RZ (Table 4). The results were lower than those reported for five Pakistani (10.5 to 12.6 mg/100 mL) [24] and Spanish (80 to 190 μg/mL) [25] cultivars.

Nutrition analysis showed the difference in the same variety (RZ, HMN or QPRX) from different growing areas. The RZ cultivar grown in Yunnan Province had a higher TP and VC content but a lower TF content than those of the RZ cultivar grown in Henan Province. Similarly, the TP and VC contents in YN-HMN were higher than those in AH-HMN, but the TF content was significantly lower. Differences in soluble solids content between YN-RZ and HN-RZ were not significant, but QPRZ and HMN showed significant differences when planted in different areas, which showed that the geographical locations and climatic conditions are important factors affecting the antioxidant activity and soluble solids content.

The AH-HBEH and SC-ZM pomegranate juice exhibited the highest (4.34) and lowest (3.10) pH, respectively, with a similar range as that of Spanish pomegranate (pH = 2.56–4.31) [26] and a maximum value higher than that of Moroccan pomegranate (pH = 2.76–4.03) [27]. Among the 37 pomegranate cultivars, the total soluble solids (TSS) in the pomegranate juice of SC-ZM (17.50°Brix) and YN-TLZ (13.13°Brix) exhibited the highest and lowest values, respectively. The range was lower than that reported by Fernandes et al. (14.87–18.04°Brix) [26] and Ferrara et al. (14.7–18°Brix) [28].

The titratable acidity (TA, expressed as citric acid percentage) and the maturity index (MI, ration of TSS toTA) are critical for the juice flavor and palatability of pomegranates and can be used for classification. The MI values for the tested genotypes were in the range of 6.46–57.81, which can be further grouped as sour (SC-ZM), sour–sweet to sour (YN-HMN, AH-HMN, and YN-HZZ), sour–sweet (SN-SBS), sour–sweet to sweet (XJ-KST, HN-YDZ, SD-DMY, XJ-HT, SD-DQP, and SD-ZPT) and sweet (all other varieties) (Table 4). In addition, pomegranate cultivars are also classified by TA as sweet, sour–sweet, and sour [26, 28]. Sweet pomegranate varieties have an acidity lower than 0.9% and are mainly destined for fresh consumption. Sour–sweet cultivars have an acidity between 1 and 2% and are used for soft drink production. Sour varieties have an acidity higher than 2% and are used in the food industry for acid extraction [29]. According to the above classification criteria, the AH-HMN, YN-HZZ, and YN-HMN varieties can be used for juice production, and SC-ZM is ideal for industry acid extraction criteria; the others are suitable for fresh consumption. Each person has a different definition of sweetness and acidity, which depends on their diet preferences and habits. Therefore, the result of MI classification is different from that of TA classification, and the MI classification is more detailed than TA classification.

Nutrition and antioxidant activity analysis of pomegranate seeds

Pomegranate seeds are rich in oil and have antioxidant activity that differs greatly between varieties [30]. In the present study, the seed TP content ranged from 0.57 to 1.78 mg/g (Table 5). The total phenolic content in HN-RZ was the highest, while SN-DBW exhibited the lowest total phenolic content. The TF content in pomegranate seeds ranged from 0.39 to 1 mg/g (Table 5). The VC content in the seeds ranged from 7.55 to 13.90 mg/g, with the highest and lowest values observed for SN-BRZ and AH-HYSZ seeds, respectively. Our results showed that lower TP and VC contents were not entirely consistent with those of previous studies [30], which could be attributed to discrepancies in experimental methods, reference materials, and pomegranate varieties. This research used the same experimental methods to compare multiple pomegranate varieties, which can mitigate experimental errors and increase the reliability of the data. Two in vitro assays (DPPH and ABTS) were used to evaluate the potential antioxidant activity of the pomegranate seeds. The DPPH and ABTS scavenging ability of the 37 Chinese pomegranate juices varied from 83.39 to 98.24% and from 28.72 to 51%, respectively. Among them, YN-RZ showed the strongest antioxidant ability.

Correlation analysis of pomegranate juice and seeds

Figure 3 shows the correlations among the characters studied. Significant correlations were found between TSS and TA, MI and TA, and pH and TA. Therefore, pH can also be used as a reference index for pomegranate juice flavor. Furthermore, the above indicators can reflect pomegranate juice taste and provide references for customers and factories. Additionally, TP and TF were positively correlated with each other (Fig. 3), which is consistent with published data [30]. With respect to the antioxidant activity, there is a strong positive relationship between TP and DPPH, as well as between TP and ATBS.

Principal component analysis (PCA)

In this study, we collected multiple data from 37 pomegranate varieties for analysis. If each indicator was analyzed separately, the results would likely be isolated rather than integrated. Additionally, blindly reducing indicators can lose a great deal of information and is prone to producing erroneous conclusions. The advantages and uses of different pomegranate varieties are distinct. Therefore, it is essential to find a reasonable method for reducing the loss of information contained in the original indicator while reducing the indicators that need to be analyzed to achieve a comprehensive analysis of the collected data. Principal component analysis (PCA) is one such method of dimensionality reduction. An Eigen value provides a measure of the significance of the factor; thus, the factors with the highest eigenvalues are the most significant, and eigenvalues ≥ 1 are considered significant [31].

The 24 indexes of the 37 varieties were subjected to principal component analysis in this study. The eigenvalues of the top six principal components were all greater than 1. Among them, the contribution values of the first and second principal components were 18.9% and 15.1%, respectively, and the cumulative contribution rate was 30% (Fig. 4).

As shown in Fig. 4, FFW, FAW, and FD were the maximum positive values of the PCA1 eigenvector, and these indicators reflect the size of the fruit's appearance, which is one of the key indicators that attracts consumer purchasing. Higher PCA1 values indicated larger fruit, and SD-DHP, SC-QPRZ, SD-DHP, YN-RZ, and YN-HZZ were representative varieties. In the PCA1 eigenvector, the indexes with the most significant negative values were TSS/TA (MI) and pH, which can reflect the fruit flavor. The representative varieties in the negative direction of PCA1 were AN-HYSZ, AH-HBEH, YN-GY, SD-TSSB, and YN-TLZ. These pomegranate varieties are sweet and have a low acid content. The most distinctive features of PC2 positive

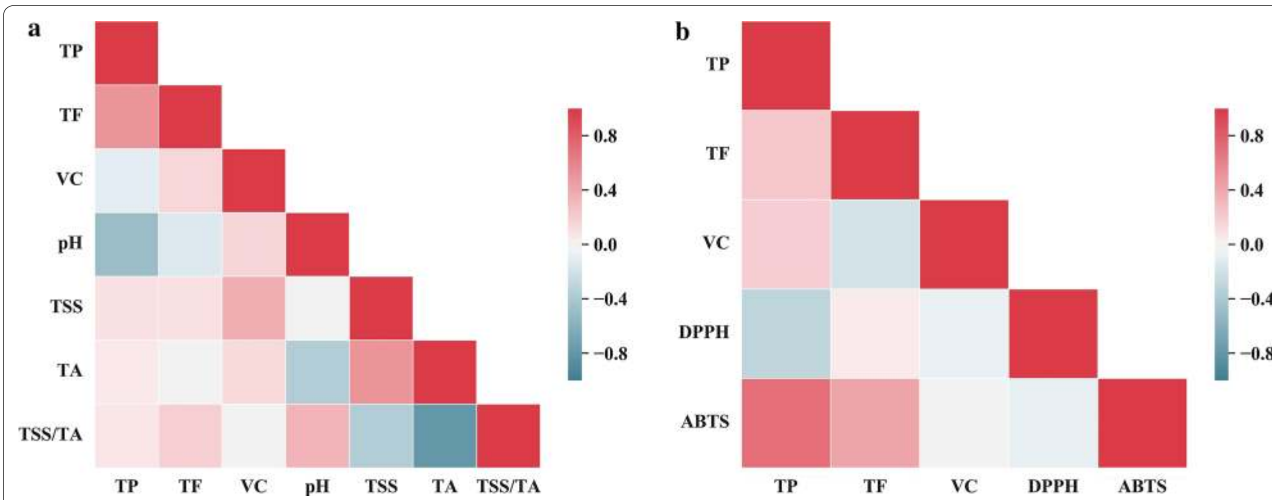

Fig. 3 Pearson's correlation plot based on the correlation of physico-chemical traits of pomegranate juice (**a**) and seed (**b**). Red and green represent positive and negative correlation, respectively. The darker the color, the larger the correlation coefficient value

Table 4 Juice nutrition and flavor analysis of thirty-seven pomegranate cultivars grown in China

Variety	TP (μg/mL)	TF (μg/mL)	VC (μg/mL)	pH	TSS (∘Brix)	TA (%)	TSS/TA	MI
YN-TLZ	75.29 ± 4.28	14.08 ± 0.39	31.60 ± 1.36	3.64 ± 0.02	13.13 ± 0.06	0.36 ± 0.05	36.47 ± 3.59	Sweet
YN-GY	77.48 ± 2.55	26.97 ± 0.64	41.58 ± 1.99	3.94 ± 0.04	15.03 ± 0.06	0.26 ± 0.03	57.81 ± 8.21	Sweet
YN-HMN	88.05 ± 0.72	16.79 ± 0.23	49.10 ± 0.29	3.14 ± 0.06	15.83 ± 0.06	1.14 ± 0.10	13.89 ± 0.94	So-Sw to So
YN-HZZ	64.97 ± 2.79	30.92 ± 0.28	33.11 ± 1.16	3.92 ± 0.02	16.40 ± 0.00	1.34 ± 0.06	12.23 ± 0.186	So-Sw to So
YN-RZ	90.28 ± 2.40	43.68 ± 0.52	53.11 ± 1.54	3.97 ± 0.06	15.93 ± 0.06	0.38 ± 0.06	41.92 ± 0.03	Sweet
SC-ZM	119.66 ± 0.58	81.56 ± 0.57	52.09 ± 2.65	3.10 ± 0.04	17.50 ± 0.00	2.71 ± 0.06	6.46 ± 0.147	Sour
SC-QPRZ	114.25 ± 1.27	19.94 ± 0.44	51.31 ± 1.54	3.58 ± 0.02	15.60 ± 0.00	0.48 ± 0.02	32.5 ± 04.47	Sweet
SD-TSSB	131.39 ± 1.33	34.58 ± 1.97	18.80 ± 1.19	3.80 ± 0.01	14.73 ± 0.21	0.33 ± 0.02	44.64 ± 01.91	Sweet
SD-DHP	104.38 ± 3.23	39.98 ± 0.70	50.53 ± 1.51	3.24 ± 0.01	15.93 ± 0.06	0.48 ± 0.02	33.19 ± 1.61	Sweet
SD-DQP	111.93 ± 1.33	38.94 ± 0.38	39.64 ± 0.56	3.51 ± 0.06	17.23 ± 0.06	0.67 ± 0.04	25.72 ± 1.40	So-Sw to Sw
SD-DMY	110.89 ± 2.39	40.43 ± 0.24	42.50 ± 0.53	3.48 ± 0.01	15.93 ± 0.12	0.57 ± 0.01	27.95 ± 0.50	So-Sw to Sw
SD-QY	106.42 ± 2.44	35.21 ± 0.94	12.80 ± 1.98	3.32 ± 0.02	15.00 ± 0.10	0.37 ± 0.02	40.54 ± 1.97	Sweet
SD-QL	90.96 ± 1.80	61.50 ± 0.24	60.94 ± 4.06	3.61 ± 0.04	15.10 ± 0.00	0.35 ± 0.02	43.14 ± 1.95	Sweet
SD-ZPT	114.12 ± 2.81	52.29 ± 5.44	16.69 ± 0.31	3.69 ± 0.04	14.93 ± 0.10	0.60 ± 0.02	24.88 ± 0.93	So-Sw to Sw
XJ-KST	71.67 ± 0.27	38.5 ± 0.52	66.63 ± 0.82	3.71 ± 0.06	15.13 ± 0.06	0.54 ± 0.03	28.01 ± 1.66	So-Sw to Sw
XJ-HT	67.92 ± 0.69	49.53 ± 0.20	51.11 ± 1.61	4.25 ± 0.02	16.20 ± 0.00	0.60 ± 0.02	27.00 ± 0.90	So-Sw to Sw
HN-RZ	80.09 ± 0.41	58.12 ± 2.67	23.61 ± 1.38	3.97 ± 0.07	15.87 ± 0.06	0.36 ± 0.03	44.08 ± 3.87	Sweet
HN-YDZ	82.97 ± 1.10	46.25 ± 1.70	19.40 ± 0.97	3.53 ± 0.02	15.33 ± 0.15	0.55 ± 0.03	27.87 ± 1.09	So-Sw to Sw
AH-HBYH	85.76 ± 1.99	37.92 ± 0.14	60.36 ± 4.06	3.94 ± 0.02	16.07 ± 0.06	0.30 ± 0.02	53.57 ± 1.27	Sweet
AH-HBEH	40.91 ± 1.91	19.25 ± 0.10	49.49 ± 1.54	4.34 ± 0.04	15.83 ± 0.06	0.43 ± 0.02	36.81 ± 1.81	Sweet
AH-QPRZ	90.26 ± 3.09	51.31 ± 1.54	43.10 ± 1.62	3.62 ± 0.03	16.27 ± 0.06	0.41 ± 0.02	39.68 ± 1.39	Sweet
AH-BYSZ	96.43 ± 2.17	37.92 ± 0.14	14.09 ± 0.85	3.42 ± 0.06	14.10 ± 0.10	0.38 ± 0.02	37.11 ± 1.70	Sweet
AH-HYSZ	73.42 ± 1.38	36.03 ± 0.43	15.50 ± 0.38	3.85 ± 0.02	15.00 ± 0.00	0.47 ± 0.02	31.91 ± 1.06	Sweet
AH-FYSZ	76.29 ± 1.11	38.99 ± 0.50	19.91 ± 0.38	3.56 ± 0.02	14.60 ± 0.25	0.37 ± 0.01	39.46 ± 1.82	Sweet
AH-HMN	80.56 ± 1.57	48.75 ± 0.13	26.28 ± 2.67	3.14 ± 0.05	15.10 ± 0.00	1.22 ± 0.02	12.38 ± 0.17	So-Sw to So
AH-DBZ	92.70 ± 2.32	58.89 ± 0.51	47.30 ± 1.54	3.38 ± 0.02	15.10 ± 0.02	0.31 ± 0.00	48.71 ± 1.74	Sweet
AH-EBZ	86.96 ± 2.10	52.99 ± 0.14	27.10 ± 1.51	3.83 ± 0.02	15.10 ± 0.10	0.45 ± 0.02	33.56 ± 1.40	Sweet
SN-DBW	102.36 ± 1.73	73.35 ± 0.43	30.08 ± 0.68	3.55 ± 0.01	15.09 ± 0.03	0.35 ± 0.01	43.11 ± 1.35	Sweet
SN-DHT	79.52 ± 3.58	102.96 ± 1.66	50.99 ± 0.56	3.78 ± 0.00	15.37 ± 0.03	0.41 ± 0.02	37.49 ± 1.12	Sweet
SN-SBT	123.66 ± 2.38	99.66 ± 0.73	28.54 ± 4.10	3.32 ± 0.02	15.17 ± 0.06	0.40 ± 0.02	37.92 ± 0.40	Sweet
SN-SBS	98.16 ± 1.85	55.96 ± 1.31	23.17 ± 1.62	3.21 ± 0.02	15.50 ± 0.00	0.67 ± 0.01	23.13 ± 2.10	Sour sweet
SN-LXYH	132.47 ± 1.68	125.88 ± 0.10	50.68 ± 2.10	3.69 ± 0.01	15.80 ± 0.00	0.37 ± 0.02	42.70 ± 2.06	Sweet
SN-LXEH	73.60 ± 2.33	14.93 ± 0.07	31.02 ± 1.66	3.74 ± 0.01	15.77 ± 0.06	0.42 ± 0.02	37.55 ± 1.44	Sweet
SN-YCL	126.74 ± 1.60	105.26 ± 2.51	31.04 ± 3.15	3.43 ± 0.01	16.00 ± 0.00	0.38 ± 0.01	42.11 ± 1.28	Sweet
SN-DZSL	81.00 ± 2.50	45.50 ± 1.78	21.37 ± 2.01	3.70 ± 0.02	16.07 ± 0.06	0.39 ± 0.01	41.21 ± 1.37	Sweet
SN-JPT	103.40 ± 2.70	137.72 ± 1.04	58.55 ± 0.57	3.68 ± 0.02	15.07 ± 0.12	0.31 ± 0.02	48.61 ± 2.40	Sweet
SN-BRZ	121.39 ± 0.75	94.40 ± 1.00	22.44 ± 1.54	3.76 ± 0.02	15.03 ± 0.21	0.37 ± 0.02	40.62 ± 2.25	Sweet

Data were expressed as mean ± standard deviation (n = 3)

So Sour, *Sw* Sweet

values were ER, STP, and ABTS, which can fully reflect the nutritional status of the pomegranate seeds. The higher the positive value of the pomegranate variety in PCA2, the stronger its seed antioxidant capacity. The main negative contributors to PCA2 were STH, PW, and TA, whose absolute values of the eigenvector were more than 2. These values mainly reflected the comprehensive information (size, flavor, and weight) of fruit. Representative varieties of the PCA2 negative axis area were AH-HMN, SN-SBS, and AN-BYSZ.

The effect of environmental factors on phenotypic and antioxidant physicochemical traits

We analyzed the correlation of environmental factors with twelve phenotypes and the physicochemical traits of three pomegranate varieties from different regions

Table 5 Seed nutrition and antioxidant activity analysis of thirty-seven pomegranate cultivars grown in China

Variety	TP (mg/g)	TF (mg/g)	VC (mg/g)	DPPH(%)	ABTS (%)
YN-TLZ	1.20 ± 0.07	0.99 ± 0.02	9.44 ± 1.23	90.59 ± 2.43	42.05 ± 1.08
YN-GY	0.86 ± 0.01	0.88 ± 0.02	8.63 ± 0.40	90.54 ± 1.98	28.72 ± 0.64
YN-HMN	1.24 ± 0.06	0.56 ± 0.04	10.11 ± 0.47	97.81 ± 1.24	40.00 ± 1.38
YN-HZZ	0.96 ± 0.03	0.55 ± 0.02	12.80 ± 0.84	92.40 ± 2.24	38.57 ± 0.88
YN-RZ	1.76 ± 0.02	0.62 ± 0.02	11.18 ± 1.23	98.24 ± 3.99	51.00 ± 6.17
SC-ZM	0.69 ± 0.02	0.74 ± 0.00	12.80 ± 2.03	93.03 ± 0.84	37.52 ± 1.13
SC-QPRZ	0.81 ± 0.02	0.57 ± 0.01	9.71 ± 1.17	89.53 ± 0.54	39.33 ± 0.91
SD-TSSB	1.09 ± 0.04	0.48 ± 0.01	10.78 ± 0.62	95.09 ± 0.47	43.38 ± 1.30
SD-DHP	1.18 ± 0.06	0.67 ± 0.01	10.78 ± 2.07	96.53 ± 1.92	36.95 ± 0.99
SD-DQP	0.80 ± 0.10	0.98 ± 0.03	10.78 ± 0.47	91.56 ± 1.98	42.04 ± 1.38
SD-DMY	1.20 ± 0.03	0.99 ± 0.03	8.63 ± 0.70	94.28 ± 3.68	40.24 ± 1.12
SD-QY	0.89 ± 0.02	0.57 ± 0.01	12.93 ± 2.29	88.03 ± 1.83	36.52 ± 0.78
SD-QL	1.00 ± 0.02	0.61 ± 0.01	12.47 ± 1.24	93.91 ± 0.35	38.66 ± 0.60
SD-ZPT	0.78 ± 0.05	0.47 ± 0.01	10.85 ± 0.58	88.46 ± 1.72	34.57 ± 0.20
XJ-KST	1.05 ± 0.03	0.65 ± 0.03	10.51 ± 1.53	91.25 ± 0.84	38.50 ± 0.47
XJ-HT	0.78 ± 0.01	0.79 ± 0.03	9.30 ± 0.23	83.37 ± 2.18	39.05 ± 2.62
HN-RZ	1.78 ± 0.03	0.86 ± 0.03	10.78 ± 0.93	95.47 ± 1.67	46.67 ± 0.44
HN-YDZ	0.97 ± 0.03	0.59 ± 0.01	9.70 ± 0.84	86.65 ± 0.98	36.14 ± 0.40
AH-HBYH	0.86 ± 0.02	0.45 ± 0.01	8.09 ± 1.02	90.03 ± 0.65	34.62 ± 1.13
AH-HBEH	0.67 ± 0.07	0.41 ± 0.01	9.57 ± 0.62	94.55 ± 0.39	36.14 ± 1.04
AH-QPRZ	0.81 ± 0.02	0.54 ± 0.01	9.57 ± 0.84	95.62 ± 0.72	38.14 ± 0.62
AH-BYSZ	0.93 ± 0.01	0.39 ± 0.01	8.09 ± 1.02	94.02 ± 0.36	41.04 ± 0.36
AH-HYSZ	0.95 ± 0.02	0.66 ± 0.01	7.55 ± 0.62	94.82 ± 0.33	40.52 ± 1.30
AH-FYSZ	0.88 ± 0.02	0.76 ± 0.02	9.44 ± 1.45	93.31 ± 1.52	39.38 ± 1.52
AH-HMN	0.59 ± 0.01	0.72 ± 0.01	10.11 ± 1.30	92.09 ± 1.66	36.52 ± 1.38
AH-DBZ	1.03 ± 0.02	0.54 ± 0.03	9.17 ± 0.23	93.91 ± 0.35	36.00 ± 0.53
AH-EBZ	0.92 ± 0.03	0.61 ± 0.02	9.97 ± 1.02	94.97 ± 0.27	39.90 ± 1.10
SN-DBW	0.57 ± 0.01	0.72 ± 0.01	7.82 ± 0.42	87.45 ± 1.74	36.66 ± 1.80
SN-DHT	0.69 ± 0.04	0.52 ± 0.01	11.32 ± 1.53	91.72 ± 0.60	37.19 ± 1.20
SN-SBT	0.98 ± 0.03	0.52 ± 0.02	9.44 ± 0.68	89.26 ± 0.54	38.61 ± 0.59
SN-SBS	0.66 ± 0.02	0.40 ± 0.02	9.44 ± 1.76	93.69 ± 0.15	36.57 ± 1.52
SN-LXYH	0.77 ± 0.02	0.56 ± 0.00	9.30 ± 0.62	92.74 ± 0.59	35.24 ± 0.54
SN-LXEH	0.62 ± 0.02	0.57 ± 0.03	10.38 ± 1.53	94.71 ± 0.74	37.10 ± 1.14
SN-YCL	0.77 ± 0.03	0.57 ± 0.01	8.63 ± 0.81	95.25 ± 0.60	38.14 ± 0.53
SN-DZSL	1.09 ± 0.01	0.65 ± 0.01	10.91 ± 2.62	95.41 ± 0.15	38.04 ± 1.15
SN-JPT	0.77 ± 0.01	0.66 ± 0.04	10.14 ± 0.26	95.46 ± 1.67	37.14 ± 0.82
SN-BRZ	1.22 ± 0.02	0.40 ± 0.01	13.90 ± 0.42	97.44 ± 0.45	35.90 ± 0.89

Data were expressed as mean ± standard deviation (n = 3)

(Table 6). Since the number of phenotypic samples was ten and the number of repetitions of physiochemical experiments was three, we evaluated their correlation with the environment separately. The environment influences the weight and size of the pomegranate, which also affects the coloration of the fruit (Table 6). The higher the rainfall and altitude, the brighter color of the fruit skin, while too much temperature and precipitation will cause the juice to be lighter. In addition, the contents of total phenols, flavonoids, and VC in the pomegranate were strongly correlated with temperature, precipitation and altitude. In addition, the TSS and TA of the pomegranate did not differ significantly in different environments. Pomegranate flavor was mainly affected by genotype, which is supported by earlier work [32]. This research shows an important effect of altitude on the pomegranate VC content (Table 5). This finding was also reported by Mphahlele et al. [33]. In addition, he proposed that the VC content of pomegranate at middle altitudes was apparently higher than that at other altitudes.

Our study provided a detailed report on the phenotypic diversity of 37 Chinese pomegranate varieties grown in seven provinces. The phenotypic diversity of the pomegranate coefficient variation was between 5.62 and 54.02%, and the Shannon–weaver diversity index was between 0.67 and 1.53. The cluster analysis clearly showed phenotypic differences and genetic relationships among the studied cultivars, which laid the foundation for cross-breeding and selection breeding. The determination of pomegranate flavor can be used as a reference for selecting varieties for fresh consumption use or industrial acid treatment. Additionally, this paper comprehensively assesses the antioxidant activities of different pomegranate cultivar juices and seeds to provide a reference for the production of functional beverages and the development of pomegranate seed products.

The phenotype of pomegranate is the ultimate manifestation of both the environment and the genotype, and the different pomegranate varieties showed differences associated with climate change. After observing and comparing pomegranate varieties in different regions, we found that genetics mainly influenced pomegranate flavor. Furthermore, the effect of different cultivation environments on the fruit size was diverse. Most noteworthy, the environment had a significant impact on the antioxidant

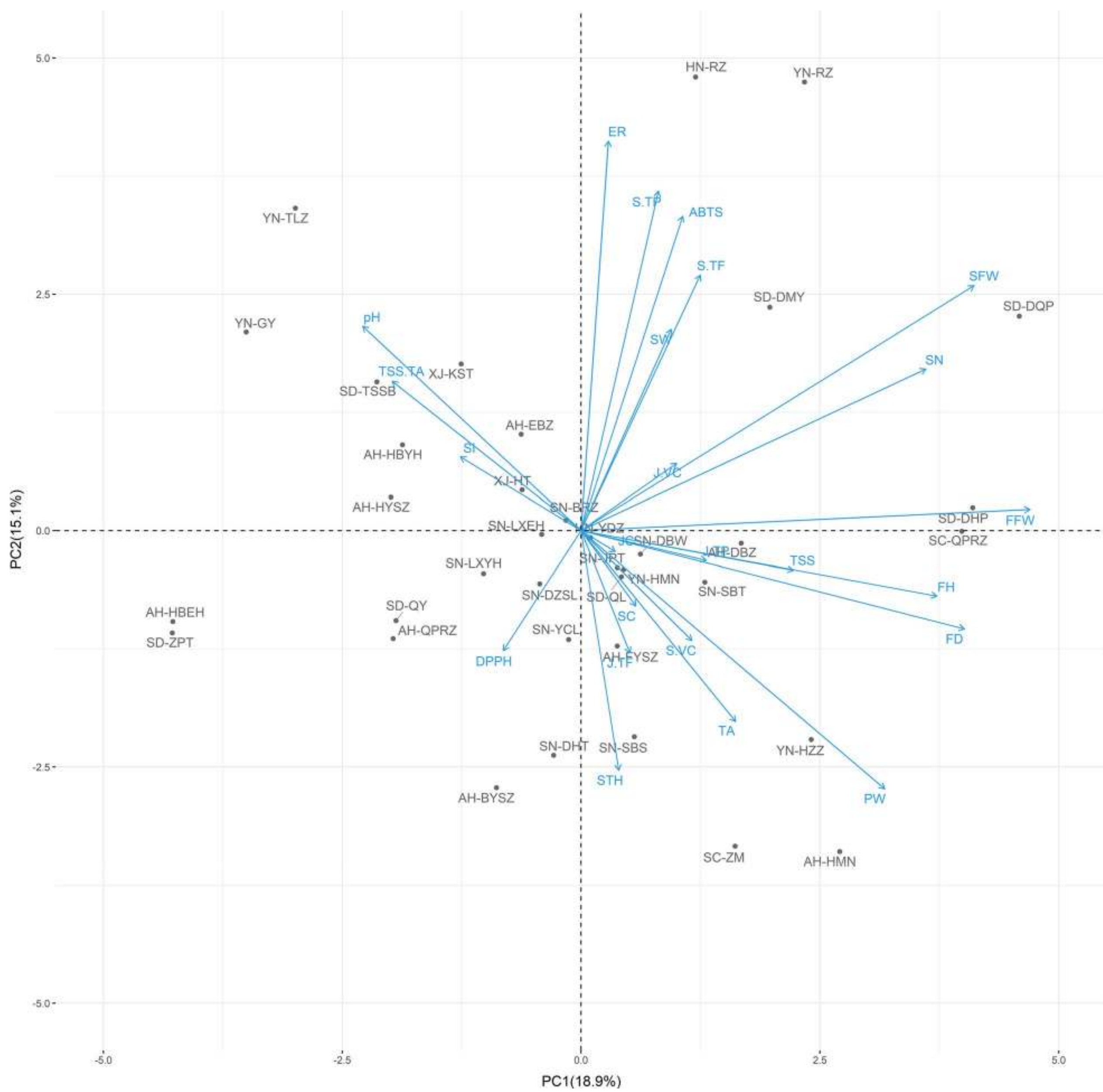

Fig. 4 Principal Component Analysis of 37 pomegranate varieties and 24 characters. The longer the arrow, the greater the contribution

capacity of the pomegranate juice and seeds. Moreover, the genotype × environment interaction effects showed a variable influence on the pomegranate varieties. More detailed research is needed to determine how environmental factors affect the nutritional changes of pomegranate and their mechanisms of action.

Table 6 Correlation between phenotypic traits, physicochemical traits and environmental factors

	Temperature	Precipitation	Altitude
Phenotypic traits			
FFW	0.581*	0.573*	0.434*
FAW	0.426*	0.421*	0.344*
SFW	0.433*	0.425*	0.302*
ER	− 0.118	− 0.114	− 0.061
SW	0.239*	0.223	0.002
SN	− 0.06	− 0.069	− 0.183
FH	0.266*	0.26*	0.175
FD	0.436*	0.455*	0.333*
ST	− 0.389*	− 0.392*	− 0.42*
SI	− 0.302*	− 0.299*	− 0.25*
SC	0.216	0.234*	0.456*
JC	− 0.295*	− 0.286*	− 0.159
Physico-chemical traits			
J.TP	0.85*	0.749*	0.664*
J.TF	− 0.875*	− 0.706*	− 0.83*
J.VC	0.815*	0.585*	0.829*
pH	− 0.068	0.223	0.026
TSS	− 0.063	0.004	0.044
TA	0.01	− 0.228	0.055
TSS/TA	− 0.211	0.074	− 0.255
S.TP	− 0.521*	0.285	0.664
S.TF	− 0.638*	− 0.799*	− 0.794*
S.VC	− 0.454	− 0.272	− 0.466*
DPPH	0.539*	0.419	0.552*
ABTS	− 0.501*	− 0.257	− 0.481*

The numbers were expressed as r value of Pearson correlation coefficients ($n = 10$ for the test of phenotypic traits; $n = 3$ for the test of physicochemical traits). *$p < 0.05$. S.TP, S.TF, S.VC represent the total phenolic, flavonoid, and VC of the seeds, respectively. J.TP, J.TF, J.VC represent the total phenolic, flavonoid, and VC of the juice, respectively

Abbreviations
ABTS: (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; TA: Titratable acidity; TP: Total phenolic; TF: Total flavonoid; TSS: Total soluble solids; VC: Vitamin C.

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Research design
In this article, thirty-seven pomegranate varieties from seven provinces in China were collected and analyzed for twelve phenotypic traits and twelve biochemical indicators (seeds and juices). We determined the physiological and biochemical indicators of pomegranate and provided a reference for the juice factory. Cluster analysis was used to determine the genetic relationship of different varieties of pomegranate. The effect of the environment on pomegranate quality was explained by Pearson correlation analysis. Finally, we use principal component analysis to clarify the specific uses of different pomegranates.

Authors' contributions
YP conceived, designed and performed the experiments; YP wrote and revised the manuscript; GW and FC interpreted data and revised manuscript; FF designed the experiments, wrote and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data is available in the main text.

Competing interests
The authors declare no conflict of interests.

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References
1. Eleonora T, Lorenzo F, Carmela F (2015) Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxid Med Cell Longev 2015:1–19
2. Jones RA (2009) Centaurs on the silk road: recent discoveries of Hellenistic textiles in western China. Silk Road 6:23–32
3. Martinez JJ, Melgarejo P, Hernandez F, Salazar DM, Martinez R (2006) Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. Sci Hortic 110:241–246. https://doi.org/10.1016/j.scienta.2006.07.018
4. Zaouay F, Mena P, Garcia-Viguera C (2012) Antioxidant activity and physico-chemical properties of Tunisian grown pomegranate (Punica granatum L.) cultivars. Ind Crops Prod 40:81–89. https://doi.org/10.1016/j.indcrop.2012.02.045
5. Li X, Wasila H, Liu L, Yuan T, Gao Z, Zhao B, Ahmad I (2015) Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. Food Chem 175:575–584. https://doi.org/10.1016/j.foodchem.2014.12.003
6. Khadivi-Khub A, Kameli M, Moshfeghi N, Ebrahimi A (2015) Phenotypic characterization and relatedness among some Iranian pomegranate (*Punica granatum* L.) accessions. Trees 29:893–901. https://doi.org/10.1007/s00468-015-1172-9
7. Zamani Z, Adabi M, Khadivi-Khub A (2013) Comparative analysis of genetic structure and variability in wild and cultivated pomegranates as revealed by morphological variables and molecular markers. Plant Syst Evol 299:1967–1980. https://doi.org/10.1007/s00606-013-0851-5
8. Bourekoua H, Rozyło R, Gawlik-Ziki U, Benatallah L, Zidoune MN, Dziki D (2018) Pomegranate seed powder as a functional component of gluten—free bread (Physical, sensorial and antioxidant evaluation). Int J Food Sci Technol 53:1906–1913. https://doi.org/10.1111/ijfs.13777
9. Lucci P, Pacetti D, Loizzo MR, Frega NG (2015) Punica granatum, cv. Dente di Cavallo seed ethanolic extract: antioxidant and antiproliferative activities. Food Chem 167:475–483. https://doi.org/10.1016/j.foodchem.2014.06.123
10. Pablo MS, Juan JM, Legua P, Rafael M, Melgarejo P (2015) Quality, antioxidant activity and total phenols of six spanish pomegranates clones. Sci Hortic 182:65–72. https://doi.org/10.1016/j.scienta.2014.11.020
11. Parry J, Su L, Moore J, Cheng Z, Luther M, Rao JN, Wang JY, Yu LL (2006) Chemical compositions, antioxidant capacities, and antiproliferative activities of selected fruit seed flours. J Agric Food Chem 54:3773–3778. https://doi.org/10.1021/jf060325k
12. Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Sendra E, Sayas-Barbera E, Perez-Alvarez JA (2011) Antioxidant properties of pomegranate

(*Punica granatum* L.) bagasses obtained as co-product in the juice extraction. Food Res Inter 44:1217–1223. https://doi.org/10.1016/j.foodres.2010.10.057
13. Kampfenkel K, Vanmontagu M, Inze D (1995) Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Analytical Biochem 225:165–167. https://doi.org/10.1006/abio.1995.1127
14. Yang WM, Liu JK, Hu L, Dong ZJ, Wu WL, Chen YH (2004) Antioxidant properties of natural p-terphenyl derivatives from the mushroom, *Thelephora ganbajun*. Zeitschrift fur Naturforschung C 59:359–362. https://doi.org/10.1515/znc-2004-5-612
15. Fang Z, Zhang Y, Lu Y, Ma G, Chen J, Liu D, Ye X (2009) Phenolic compounds and antioxidant capacities of bayberry juices. Food Chem 113:884–888. https://doi.org/10.1016/j.foodchem.2008.07.102
16. Borochov-Neori H, Judeinstein S, Harari M, Bar-Yaakov I, Patil BS, Holland LS (2011) Climate effects on anthocyanin accumulation and composition in the pomegranate (*Punica granatum* L.) fruit arils. J Agric Food Chem 59:5325–5334. https://doi.org/10.1021/jf2003688
17. Martinez-Nicolas JJ, Pablo M, Pilar L, Francisco GS, Francisca H (2016) Genetic diversity of pomegranate germplasm collection from Spain determined by fruit, seed, leaf and flower characteristics. Peer J 4:e2214
18. Dahlberg MD, Odum EP (1970) Annual cycles of species occurrence, abundance, and diversity in Georgia estuarine fish populations. Am Midl Nat 1970:382–392. https://doi.org/10.2307/2423951
19. Polyzos N, Papasotiropoulos V, Lamari FN, Petropoulos SA, Bebeli PJ (2019) Phenotypic characterization and quality traits of Greek garlic (*Allium sativum* L.) germplasm cultivated at two different locations. Genetic Resourc Crop Evol 66:1–19. https://doi.org/10.1007/s10722-019-00831-4
20. Hari D, Upadhyaya PJ, Bramel RO, Sube S (2002) Geographical patterns of diversity for morphological and agronomic traits in the groundnut germplasm collection. Euphytica 128:191–204
21. Maestre J, Melgarejo P, Tomas-Barberan FA, Garcia-Viguera C (2000) New food products derived from pomegranate. Prod Process Market Pomegranate Med Reg 2000:243–245
22. Zaouay F, Mars M (2014) Phenotypic variation and estimation of genetic parameters to improve fruit quality in Tunisian pomegranate (*Punica granatum* L.) accessions. J Horticultural Sci Biotech 89:221–228. https://doi.org/10.1080/14620316.2014.11513072
23. Mousavinejad G, Emam-Djomeh Z, Rezaei K, Khodaparast MH (2009) Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem 115(4):1274–1278. https://doi.org/10.1016/j.foodchem.2009.01.0
24. Akhtar N, Zafar H, Ahmad N (2019) Nutritional quality evaluation of different varieties of pomegranate under climatic conditions of Faisalabad. Eurasian J Soil Sci. 8:184–188. https://doi.org/10.18393/ejss.552543
25. Mena P, Garcia-Viguera C, Navarro-Rico J, Moreno DA, Bartual J, Saura D, Marti N (2011) Phytochemical characterisation for industrial use of pomegranate (Punica granatum L.) cultivars grown in Spain. J Sci Food Agric 91:1893–1906. https://doi.org/10.1002/jsfa.4411
26. Ferrara G, Cavoski I, Pacifico A, Tedone L, Mondelli D (2011) Morpho-pomological and chemical characterization of pomegranate *(Punica granatum* L.) genotypes in Apulia region, Southeastern Italy. Scientia Hortic 130:599–606. https://doi.org/10.1016/j.scienta.2011.08.016
27. Legua P, Melgarejo P, Abdelmajid H, Martinez JJ, Martínez R, Ilham H, Hafida H, Hernandez H (2012) Total phenols and antioxidant capacity in 10 Moroccan pomegranate varieties. J Food Sci 77:C115–C120. https://doi.org/10.1111/j.1750-3841.2011.02516.x
28. Fernandes L, Pereira JA, Lopez-Cortes I, Salazar DM, Gonzalez-Alvarez J, Ramalhosa E (2017) Physicochemical composition and antioxidant activity of several pomegranate (*Punica granatum* L.) cultivars grown in Spain. European Food Res Technol 243:1–16
29. Depalma L, Novello V (1995) Il melograno: attualità di una coltura antica. Rivista Di Frutticoltura E Di Ortofloricoltura 57:45–49
30. Peng Y (2019) Comparative analysis of the biological components of pomegranate seed from different cultivars. Inter J Food Properties 22:784–794. https://doi.org/10.1080/10942912.2019.1609028
31. Fawole OA, Opara UL (2013) Seasonal variation in chemical composition, aroma volatiles and antioxidant capacity of pomegranate during fruit development. Afr J Biotechnol 12:4006–4019
32. Schwartz E, Tzulker R, Glazer I, Bar-Yaakov I, Wiesman Z, Tripler E, Bar-Ilan I, Fromm H, Borochov-Neori H, Holland D (2009) Environmental conditions affect the color, taste, and antioxidant capacity of 11 pomegranate accessions' fruits. J Agric Food Chem 57:9197–9209. https://doi.org/10.1021/jf901466c
33. Mphahlele RR, Stander MA, AFWole OA, Opara UL (2014) Effect of fruit maturity and growing location on the postharvest contents of flavonoids, phenolic acids, vitamin C and antioxidant activity of pomegranate juice (cv. Wonderful). Sci Hortic 179:36–45. https://doi.org/10.1016/j.scienta.2014.09.007

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