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Genetic Diversity and Population Structure of Jujube Cultivars in the United States Revealed by Single Nucleotide Polymorphism Markers

Dikshya Sapkota
Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003, USA

Dapeng Zhang, Sunchung Park, and Lyndel W. Meinhardt
Sustainable Perennial Crops Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA

Dennis N. Lozada
Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003, USA; and Chile Pepper Institute, New Mexico State University, Las Cruces, NM 88003, USA

Robert Steiner
Department of Economics and International Business, New Mexico State University, Las Cruces, NM 88003, USA

Shengrui Yao
Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003, USA; and Sustainable Agriculture Sciences Center, New Mexico State University, Alcalde, NM 87511, USA

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Abstract. The nutritional and medicinal significance of jujube (*Ziziphus jujuba*) has led to persistent efforts in genomics to accelerate the utilization of its germplasm resources. However, the absence of accurate genetic identity of existing germplasm limits these studies. In the United States, different names were frequently given to the same jujube cultivars because the pedigrees of the imported germplasm are unclear. The present study selected a panel of 147 single nucleotide polymorphism (SNP) markers distributed across the jujube genome to examine genetic identity, genetic diversity, and population structure in 177 jujube cultivars sampled from different locations in the United States. SNP profile multilocus matching reported a total of 23 synonymous groups including 116 samples that were identical to at least one other sample. This led to the detection of 74 unique genotypes for subsequent diversity analysis. Model-based genetic structure analysis divided the distinctive genotypes into three major groups, with some admixtures among the groups. The genetic differentiation among these groups was further validated by analysis of molecular variance (F_{st} = 0.199, P value < 0.001), principal coordinate analysis, and clustering analysis. Morphological traits were studied in some of the genetically identical commercial cultivar groups, (i.e., Li, Lang, and Jinsi). Results demonstrated significant morphological differences within genetically identical cultivars in the Jinsi group, indicating phenotypic variation resulting from mutations in these clones.

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S.Y. is the corresponding author. E-mail: yaos@nmsu.edu.

Jujube (*Z. jujuba*) is a plant of the Rhamnaceae family that originated in China (Liu and Wang 2009). Jujubes are currently gaining popularity in the United States, southern Europe, northern Africa, Australia, and other nations, in addition to being produced on a large scale in China and South Korea (Crawford et al. 2011; Liu et al. 2020). Because of their tolerance to drought and salinity, lower irrigation and fertilization requirement, and potential use in food production, jujubes are of interest to both researchers and growers (Liu 2006; Liu et al. 2020). Furthermore, jujube is becoming more popular because of its high levels of vitamin C, cyclic adenosine monophosphate (cAMP), phenolic compounds, flavonoids, and polysaccharides; its deep red natural colorant properties that have potential uses in food and beverages; and medicinal properties, which include but are not limited to antioxidant, anticancer, anti-insomniac, antimicrobial, neuroprotective, cardioprotective, and hepatoprotective activities (Alsayari and Wahab 2021; Ivanisova et al. 2017; Khadivi and Beigi 2022; Liu et al. 2022; Sapkota et al. 2023b; Yao et al. 2023).

Jujube cultivars have a wide variety of traits such as tree size, tree shape, fruit size, fruit shape, taste, and color (Liu and Wang 2009; Ma et al. 2011). The jujube fruit comes in a variety of shapes, ranging from spherical, oval, ovate, and oblong to pear-shaped (Liu and Wang 2009). Jujube fruits vary from thumb sized to golf ball sized depending on cultivar. It has whitish to yellowish flesh and thin, edible skin. When the fruit is mature, it develops a dark red color (Khadivi and Beigi 2022; Krška and Mishra 2008). The fruit continues to wrinkle after turning completely red, remaining edible. Jujube fruit is generally consumed fresh or dried, but it may also be processed into confectionery recipes for bread, cakes, compotes, and sweets (Krška and Mishra 2008; Liu and Zhao 2009).

Jujube cultivars were first introduced in the United States in 1908 by Frank N. Meyer of the US Department of Agriculture, who projected their appropriateness for the semiarid south and southwest (Meyer 1911, 1916; Sapkota et al. 2023a; Yao 2013). Cultivated jujube (*Z. jujuba*) and sour jujube (*Ziziphus spinosa*) are the two species found in the United States (Sapkota et al. 2023a). Currently, there are ∼100 jujube cultivars in the United States, but none has been formally released with detailed information (Yao 2013). Most of them were imported from China or other countries; several cultivars are from the Chico Plant Introduction Station, Chico, CA, USA; and quite a few were named or renamed by home gardeners and growers in different states using a person's name or city/town names like Don Polenski, Redlands #4, Sherwood, Abbeville, and so on (Yao 2013). Synonyms are common and growers are confused about cultivar selection. It is therefore critical to identify/classify and verify the relationship of existing jujube cultivars in the United States.

DNA profiling has been an invaluable tool for managing genetic resources, studying population genetics, and improving crops (Nybom et al. 2014; Sucher et al. 2012; Weising et al. 2005). For the management of jujube germplasm, a variety of molecular markers have been used, including amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), and SNPs (Fu et al. 2016; Gao et al. 2013; Liu et al. 2016; Ma et al. 2011; Song et al. 2021; Wen et al. 2008; Xiao et al. 2015; Xu et al. 2016). SNPs are the most prevalent marker type and are appropriate for research on a broad genomic scale in breeding programs (Akpertey et al. 2021; Rafalski 2002). In comparison with earlier DNA marker systems, SNP markers have several advantages, such as an abundance of markers, quick processing of large populations, a selection of genotyping systems to suit different needs, high-throughput, and straightforward allele calling and database storage due to the biallelic nature of SNP markers (Thomson 2014). The routine application of SNP markers, however, calls for flexible and cost-effective genotyping systems capable of handling a small number of SNPs across large breeding populations. These platforms include Fluidigm's Dynamic Arrays, Douglas Scientific's Array Tape, and Laboratory of the Government Chemist (LGC, Middlesex, UK) automated systems for running kompetitive allele-specific PCR (KASP) markers (Thomson 2014). Although genotyping based on a low-density SNP panel is precise and reliable for plant genotype identification, it usually cannot adequately distinguish between similar plants with variations caused by mutations. Morphological characterization is one of the initial steps in understanding phenotypic diversity of plant genetic resources, which complements molecular characterization in plant germplasm management.

The objective of this study was to use DNA marker genotyping to identify most existing jujube cultivars in the United States, assess their genetic diversity and population structure, and study morphological characteristics to assist identification/classification of closely related cultivars.

Materials and Methods

Plant materials. Jujube leaf samples were collected from the New Mexico State University (NMSU) collection at the Sustainable Agriculture Science Center at Alcalde, NM, USA. Additional samples were collected from three jujube growers/enthusiasts including Cliff England of the England Orchard and Nursery at McKee, KY, USA; Michael Nave at Republic, MO, USA; Bob Vance at New Canaan, CT, USA; and seven anonymous samples from NMSU, Las Cruces, NM, USA. Detailed information on cultivar names, sampling locations, and the year of sampling is provided (Table 1, Supplemental Table 1). From each jujube plant, 5 to 10 fully expanded young leaves were collected into labeled 10×10 cm^2 plastic bags with silica desiccant, which facilitated the drying of samples within 24 h. The samples were stored in a freezer until further processing. A total of 8 to 10 leaf disks were collected using the BioArk Leaf kit (Biosearch Technologies, Hoddesdon, Hertfordshire, UK). The prepared BioArk Leaf kits were then submitted to LGC Genomics for DNA extraction and subsequent genotyping.

The 159 SNPs, previously validated by Song et al. (2021) along with their corresponding flanking sequences were submitted to LGC for a KASPar assay design. The KASP chemistry (Biosearch Technologies) was used for genotyping. The KASPar™ Genotyping System is a competitive allele-specific dual fluorescence resonance energy transfer (FRET)-based technology for SNP genotyping (Cuppen 2007). The genotyping was conducted following an in-house protocol of LGC. The resulting genotypic data were returned as .csv files and analyzed using an SNP marker analysis software (SNP Viewer version 1.99; Biosearch Technologies).

Morphological characterization. Fruit samples were collected at the full maturity stage on 31 Aug and 17 Sep 2022 from jujube cultivar trials at the NMSU Leyendecker Plant Science

Table 1. Jujube (*Ziziphus jujuba*) leaf sampling locations in different states of the United States.

Location	Number of samples	GPS coordinates	Elevation, m
Alcalde, NM, USA	94	36°5′17″N, 106°3′25″W	1,741
Las Cruces, NM, USA	7	32°18′52″N, 106°46′44″W	1,191
McKee, KY, USA	14	37°25′49″N, 83°59′37″W	314
Republic, MO, USA	28	37°7′18″N, 93°28′17″W	399
New Canaan, CT, USA	34	41°8′48.48″N, 73°29′41.64″W	105
Total	177		

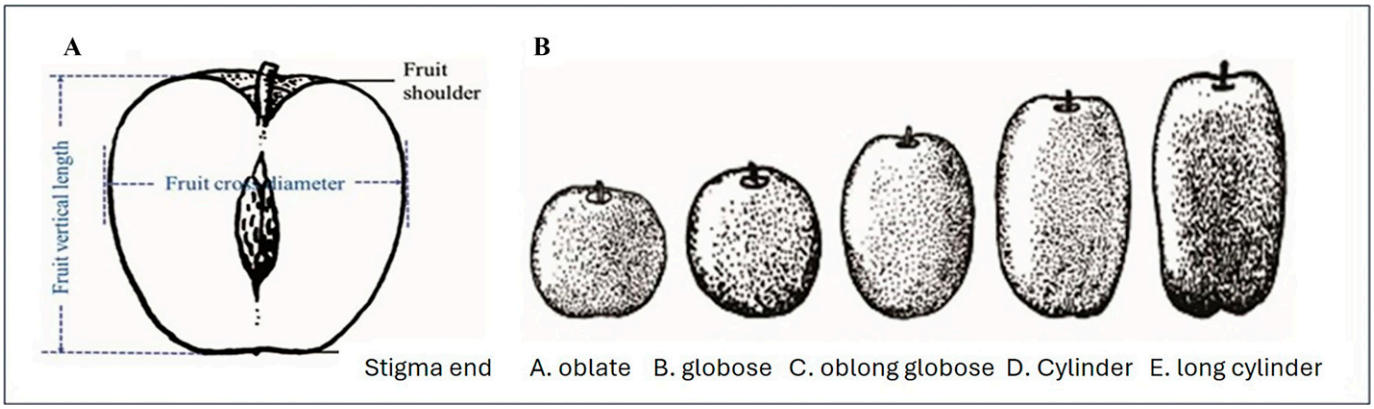

Fig. 1. (**A**) Diagram of the vertical length and cross-diameter measurement methods used for jujube (*Ziziphus jujuba*) fruits, and (**B**) jujube fruit shape (Liu and Wang 2009).

Research Center in Las Cruces, NM, USA (lat. 32°12′08.9″N, long. 106°44′41.4″W; elevation, 1176 m), and at the NMSU Agricultural Science Center at Los Lunas, NM, USA (lat. 34°46′04.7″N, long. 106°45′45.7″W; elevation, 1478 m), respectively (Yao et al. 2019, 2020). Jujube trees at Los Lunas and Leyendecker Centers were planted in 2015 and 2017, respectively. Ripened fruits at the full red maturity stage were sampled from a total of 12 cultivars: Daguazao, Dabailing, Redlands #4, Li, Shanxi Li, Lang, Junzao, Xingguang, Jinsi 2, Jinsi 3, JKW, and Pitless, with two to four replications (trees) from Leyendecker. In addition, 16 cultivars were sampled at Los Lunas: Daguazao, Li, Linyi Li, Weeping Li, Redlands #4, Lang, Don Polenski, Junzao, Jinchang 1, Xingguang, Jinsi 2, Jinsi 3, JKW, Jinsi 4, and Pitless, with two to three replications.

The following properties were measured for morphological analysis. Photographs were taken to assist in the study of morphological characteristics.

Fruit length and cross diameter. For each set of replicates, a random selection of 20 fruits was made, and their dimensions (length and diameter) were measured using an electronic digital caliper (Neiko Tools, Wenzhou, China). The vertical length of the jujube fruit was measured as the maximum distance from the stigma end to the shoulder of the jujube fruit (Liu and Wang 2009), and the cross diameter was measured as the length of the widest section of jujube fruit as shown in Fig. 1A.

Fruit shape. Fruit shape was determined using the fruit shape index, which was measured as the ratio of the fruit length to the fruit cross diameter. The measurements were taken from the fruits sampled for length and diameter. Jujube fruits were categorized into different shapes based on their fruit index values as follows: fruits with a fruit shape index value of <0.90 were classified as oblate; 0.90 to 1.10, as globose or nearly globose; 1.10 to 1.30, as oblong globose; 1.30 to 1.60, as cylindrical; and >1.60, as long and cylindrical (Liu and Wang 2009) (Fig. 1B). Visual observations and photographs were used to assist in identifying any other shapes of jujube fruits.

Fresh fruit weight, dry weight, and moisture content. The average fresh fruit weight of each cultivar replicate was measured using a standard laboratory scale (Mettler Toledo, Columbus, OH, USA).

For each replicate, 10 g of fresh fruit flesh wedges was measured and then placed in an oven for drying at 80 °C for 6 h (Zhu and Xiao 2018). Moisture content was then calculated based on the fresh and dry fruit weights (Reeb et al. 1999).

$$\text{Moisture content (\%)} = \frac{\text{Fresh fruit weight (10 grams)} - \text{Dry fruit weight} \times 100}{\text{Fresh fruit weight}}$$

Stone length, cross diameter, and weight. Ten stones were chosen at random from each cultivar replication to be measured for fruit length and diameter with an electronic digital caliper. The vertical length of the jujube stone was measured as the maximum distance from the top to the shoulder, and the cross diameter was measured as the length of the widest section of the jujube stone. For stone weight, the average fresh stone weight (10 stones) of each cultivar replicate was measured using a standard laboratory scale.

Tree dimensions and presence of thorns on the current shoot. The height, and canopy cover of trees were measured using an extended ruler. The trunk circumference was measured 20 cm above the ground using a measuring tape. The presence or absence of thorns on the current year's shoots was recorded based on observations.

Jujube flower blooming type. Jujube blooming times were observed in early June to mid-June 2022 for all the cultivars to identify their blooming type. Flower blooming was monitored hourly from 0600 to 1700 HR. Cultivars whose sepal splitting times were from sunrise to 1300 HR were designated as morning blooming types and the ones that opened their sepals between 1300 to 1600 HR were considered afternoon blooming types, as described by Yao et al. (2015).

Total soluble solids (%). Immediately after the fruits were harvested, five fruits from each cultivar replication were cut into small pieces. One wedge from each fruit was taken and placed in a garlic press secured with aluminum foil at the base to extract the juice. Soluble solids content (%) was measured by a digital refractometer (Atago digital pocket refractometer; Atago, Bellevue, WA, USA).

Morphological analysis. For statistical analysis of the morphological traits, SAS version 9.4 (SAS Institute, Cary, NC, USA) software was used. All the parameters and cultivar groups were analyzed separately. Analysis of variance was performed using PROC GLM over the means of cultivars within cultivar groups. The level of significance was set at $P = 0.05$. When there was a significant difference in the means, Tukey's honestly significant difference post hoc test was used to further separate the means.

Genetic diversity analysis and population structure. Pairwise multilocus matching across all jujube cultivars was performed to identify duplicate samples using the SNP data. Jujube samples that perfectly matched across all genotyped SNP loci were referred to as duplicates/same cultivar or clones. The computation was performed using the multilocus matching algorithm of the program GenAlEx 6.5 (Peakall and Smouse 2006). The number of locus mismatches for pairwise comparison between jujube samples was computed using the "bitwise.dist" function from the R package "poppr" (Kamvar et al. 2015), where a missing locus was considered as a match. The distribution of mismatch frequencies was visualized using the R package "hist" (R Core Team 2023). The statistical rigor of the jujube SNP panel for genotype identification was determined using the probability of identity of siblings (PID-sib) (Waits et al. 2001). PID-sib is the likelihood that two sibling individuals picked at random from a population would have the same multilocus genotype across all SNP loci. The maximum range of PID values that can occur in a population is represented by the overall PID-sib, which provides the bare minimum number of loci required to identify every individual, including relatives. The redundant samples were eliminated following duplicate detection, and only one genotype from each duplicate group was retained and used in the subsequent diversity study. Using the program GenAlEx 6.5, summary statistics comprising minor allele frequency, observed heterozygosity, expected heterozygosity, and Shannon's information index were calculated (Peakall and Smouse 2006).

The SNP data were further evaluated using the model-based Bayesian clustering method software to determine the population structure (STRUCTURE version 2.3.4, Stanford University, Stanford, CA, USA) (Pritchard et al. 2000). This algorithm aims to identify genetically different subpopulations in the sample based on allele frequency using an admixture model. The *K* value for the genetic clustering was set from 1 to 10. For each given number of clusters (*K*), 10 independent runs, each with 100,000 iterations following a burn-in of 200,000, were performed. All cultivars were considered to have unknown origins. Using an online genetic clustering software (STRUCTURE HARVESTER, University of California, Santa Cruz, CA, USA), the Delta *K* value was used to determine the optimal number of clusters that define the population (Earl and vonHoldt 2012; Evanno et al. 2005).

Analysis of molecular variance. Analysis of molecular variance (AMOVA) is a statistical method used to partition the genetic variance observed in a population or set of populations using molecular marker data. AMOVA was performed in GenAlEx 6.5 program to verify the genetic differentiation between the clusters inferred from the genetic structure analysis. A distance-based multivariate analysis was performed. Pairwise genetic distances were calculated using the "distance" function in the GenAlEx 6.5 program (Peakall and Smouse 2006). Principal coordinate analysis (PCoA) was also performed in the same computer program. To further illustrate the genetic relationships among the cultivars as a complementary method, cluster analysis based on the neighbor-joining method was used and a phylogenetic tree was generated using MEGA 11 software with 1000 bootstrap replicates (Tamura et al. 2021).

Results

Genotyping results and SNP markers. Of 159 SNPs used to genotype the jujube samples, a total of 147 genome-wide SNP markers were retained for analysis. Data filtering was done to remove SNPs with minor allele frequency <0.05. The markers that make up the core set were chosen based on their information index, linkage disequilibrium (LD) values, and their distribution throughout the 12 chromosomes. The PID-sib, obtained from the 177 samples included in this study, predicted the likelihood of two unrelated samples having the same genotype at all 147 SNP loci to be 1.4×10^{-20} (Fig. 2). This means that the chance of two separate jujube genotypes in the population having the same genotype was almost 0% based on 147 SNPs. Accordingly, these markers have adequate statistical power to accurately authenticate our jujube samples. The accuracy of the method was depicted by completely identical DNA profiles of the duplicated samples of cultivar Chico, Teapot, and Fupingdazao, which were sampled from New Mexico both in 2019 and 2022.

Fig. 2. Sibling probability of identity (PI-sib) based on 147 single nucleotide polymorphism (SNP) markers and 177 jujube (*Ziziphus jujuba*) cultivars in the United States. The chance that two sibling individuals randomly selected from this collection have identical multilocus genotypes was close to zero after 147 SNP loci were used.

Cultivar identification. Multilocus matching of the SNP fingerprints revealed duplicates in our jujube germplasm collection. A total of 116 accessions were classified into 23 synonymous groups of the 177 analyzed germplasm accessions (Table 2). There were 2 to 24 accessions in each synonymous group. Cultivars Weeping Li, Allentown, and Express Gee had matching SNP profiles at all but one locus and thus, were almost identical to the first synonymous group in Table 2. The samples that differed by one SNP locus were also considered synonymous, as this difference was attributed to genotyping errors (Supplemental Fig. 1) (Akpertey et al. 2021; Waits and Paetkau 2005; Zhang et al. 2006). We did not report any samples that differed by only a few loci (more than one). The cultivars Thornless and Cangdong were similar to the cultivars in synonymous group 2 and 6, respectively differing by only one SNP locus. For synonymous group 10, two other anonymous cultivars were similar, differing only at a single locus. Several samples from Connecticut were originally from Missouri or Kentucky. Thus, cultivars Ant Admire, Xu Zao, Buluosu, Goose Egg, Orange beauty, and Tigerooth were sampled twice from two different locations.

Mislabeling was also reported in our samples. For example, 'Sihong' was mislabeled as 'Porterville' in Connecticut and Missouri. In Connecticut, 'Autumn Beauty' was mislabeled as 'Mango Dong' and 'Sugarcane' was mislabeled as 'Winter Delight'. We also reported the identity of anonymous samples. Two samples from Milton Hall, NMSU, were found identical to cultivar Li, and a sample near Neale Hall, NMSU, was identical to cultivar Mu. One cultivar was retained for diversity analysis from each synonymous group, multiyear sampled group, and multilocation sampled group. In the end, there were 74 jujube cultivars having distinct SNP fingerprints.

Genetic relationships among jujube cultivars. Population stratification based on the Delta *K* value as provided by genetic structure analysis divided the 74 jujube cultivars into three groups (Fig. 3A and B). The first and the second group had 12 core members each, and the third group had two core members, and the

Table 2. List of 23 jujube (*Ziziphus jujuba*) synonymous groups, including 116 cultivars, identified by single nucleotide polymorphism markers in the United States. The cultivars in bold in the table were retained for subsequent diversity analysis.

Synonymous group	Cultivar	Synonymous group	Cultivar
1	19NM10-Daguazao	8	19NM54-Sugarball
1	19NM24-Li2	8	**19NM55-Sugarcane**
1	**19NM36-Li**	8	22CT7-Coco
1	19NM37-Linyi-Li	8	22MO10-Coco
1	19NM45-Redlands #4	8	22CT30-WinterDelight (Mislabeled)
1	19NM48-Shanxi-Li		
1	19NM9-Dabailing	9	**19NM1-Abbeville**
1	21NM3-MiltonHall1	9	22NM11-LCRanchN
1	21NM4-MiltonHall2	9	22NM12-LCRanchS
1	22CT11-EMpressGee	9	22NM13–3ClarkRdE
1	22CT24-TaeSangWang	9	22NM14–3ClarkRdW
1	22CT32-YazooLi	9	22NM15–3Clarknearditch
1	22CT33-HetianJade	9	22NM16-BearpawRanch
1	22CT9-DaeSolJo	9	22NM17-GIlaSouthend
1	22KY5-DaeSolJo	9	22NM19-QsBistro
1	22KY8-HunanEgg	9	22NM1-Abbeville (Resampled)
1	22MO12-DaesolJo		
1	22MO22-HunanEgg	10	**19NM51-Shuimen**
1	22MO34-YazooLi	10	19NM75-Sui
1	22MO8-BigMelon	10	22CT21-R1T4
1	22NM6-Li2 (Resampled)	10	21NM22-McAurthur1
1	19NM61-Weeping-Li	10	22CT22-R4T3
1	22CT1-Allentown		
1	22MO15-ExpressGee	11	**19NM41-Mu**
		11	21NM25-NealeHall
2	19NM11-Don-Polenski		
2	19NM13-Ed-Hegard	12	**19NM20-Globe**
2	19NM25-Jinchang	12	22CT12-Kima
2	19NM32-Junzao		
2	**19NM34-Lang**	13	**19NM52-Sihong**
2	19NM64-Xingguang	13	22MO35-Porterville2 (Mislabeled)
2	19NM58-Thornless	13	22CT19-Portville (Mislabeled)
3	**19NM39-Maya**	14	**19NM69-Mango Dong**
3	19NM62-X38	14	22MO33-WinterDelight
3	19NM18-Gaga		
3	19NM60-Tsao	15	**19NM2-Alcalde1**
3	22CT16-Moonlight	15	22CT3-Autumn-Beauty
3	22CT34-Tsao	15	22KY14-Autumn Beauty
3	22KY9-Massandra	15	22CT13-MangoDong (Mislabeled)
3	22MO24-Massandra	15	22MO3-AutumnBeauty
4	19NM27-JKW	16	**19NM46-Russian2**
4	19NM40-Miyunxiaozao	16	22CT8-Confetti
4	**19NM28-Jinsi2**	16	22MO11-Confetti/Yalta2
4	19NM29-Jinsi3		
4	19NM43-Pitless	17	**19NM23-Huizao**
4	22CT10-ElkGrove	17	22KY12-YingLuoZao
4	22MO14-ElkGrove		
		18	**19NM26-Jing39**
5	**19NM50-Sherwood**	18	22KY2-TeDaSuCuiZao
5	22MO31-TexasSawmill		
5	19NM59-Topeka	19	**19NM15-Fucuimi**
5	19NM6-Capri	19	22KY4-CuiWangZao
5	22CT20-Priest		
5	22CT26-TexasSawmill	20	**19NM31-Jixinzao**
5	22CT28-VegasBooty	20	22MO1-Huizao

(*Continued on next page*)

Table 2. (*Continued*)

Synonymous group	Cultivar	Synonymous group	Cultivar
5	22MO21-Sherwood		
5	22NM18-701MtView	21	**22KY11-SeptRed**
5	22NM3-Capri (Resampled)	21	22MO25-Nanjing
5	22NM9-Topeka (Resampled)		
		22	19NM71-Zaocuiwang
6	**19NM21-Hebei-Dong**	22	**22MO29-ShandongPear**
6	19NM65-Yandong		
6	19NM35-Local-Dongzao	23	**22KY7-GooseEgg**
6	19NM47-Sandia	23	22MO17-Gooseegg
6	19NM5-Cangdong	23	22MO5-Bang
7	19NM38-Liuyuexan		
7	**19NM33-KFC**		

remaining 48 cultivars were classified as admixed genotypes because they could not meet high assignment coefficient value ($Q > 0.70$). The first group had cultivars Russian 2, September Red, Mango Dong, Mushroom, Banzao, Youzao, Yuanlingzao, Xiangzao, Church point, Halina, Baby Red, and McCurdy. Cultivars Li, Lang, Alcalde #1, Xuzhou, Chico, and Teapot, and so on were in the second group. The third group comprised cultivar Fitzgerald and a seedling with large fruit from Connecticut. The admixture group comprised cultivars like Maya, Jinsi 2, Sherwood, Mu, Globe, Sihong, Huizao, and so on.

The three germplasm groups, as inferred from the genetic structure analysis, were subjected to AMOVA. Results showed that the within-group and among-group variances were 80% and 20%, respectively, and both were highly significant (Fig. 3C). The pairwise F_{st} among the three groups was 0.199 (P value $<$ 0.001) based on a permutation test. Information index values ranged from 0.000 to 0.693 with a mean value of 0.469. The mean observed heterozygosity was 0.327, ranging from 0.000 to 0.715, whereas the average expected heterozygosity was 0.301, ranging from 0.000 to 0.500.

The 26 jujube cultivars of three groups (12, 12, and two cultivars from the first, second, and third groups, respectively) obtained from the structure analysis were further analyzed by PCoA (Fig. 4). The first three axes accounted for a total of 67.73% variation with 32.64%, 24.57%, and 10.52% variation explained by the first, second, and third axes respectively. We observed an apparent pattern of clustering among 26 cultivars (Fig. 4). The first, second, and third clusters as shown by red-, green-, and blue-colored dots in PCoA demonstrated a consistent pattern resembling the first, second, and third group assigned by the genetic structure analysis.

A phylogenetic tree was constructed to further illustrate the relationship among all the jujube cultivars. The phylogenetic tree showed results that were consistent with the results from

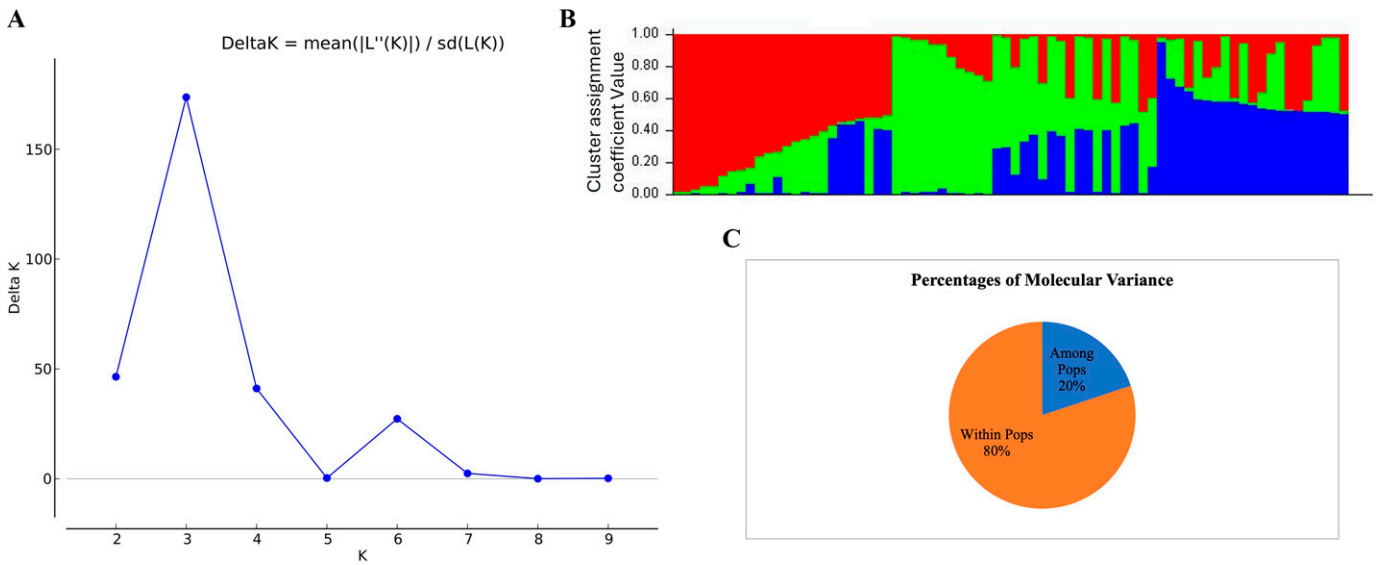

Fig. 3. (**A**) Plot of Delta *K* (filled circles, blue line) calculated as the mean of the second-order rate of change in likelihood of *K* divided by the standard deviation of the likelihood of *K*, m(|L″(K)|/s[L(K)]. Red line shows the most rational value of *K* (most probable number of populations or genetic groups) based on the Evanno method. (**B**) Inferred clusters in the 74 jujube (*Ziziphus jujuba*) cultivars using a genetic clustering software (STRUCTURE HARVESTER, University of California, Santa Cruz, CA, USA). Each vertical line represents an individual multilocus genotype. Each color represents the cluster from which the genotype or partial genotype was produced, suggesting its most likely ancestry. Red, green, and blue colors represent the first, second, and third cluster, respectively. Individuals with multiple colors indicate admixed genotypes with contributions from different clusters. (**C**) Analysis of molecular variance of the core members in the three jujube cultivar groups designated by a genetic structure analysis software (STRUCTURE, Stanford University, Stanford, CA, USA).

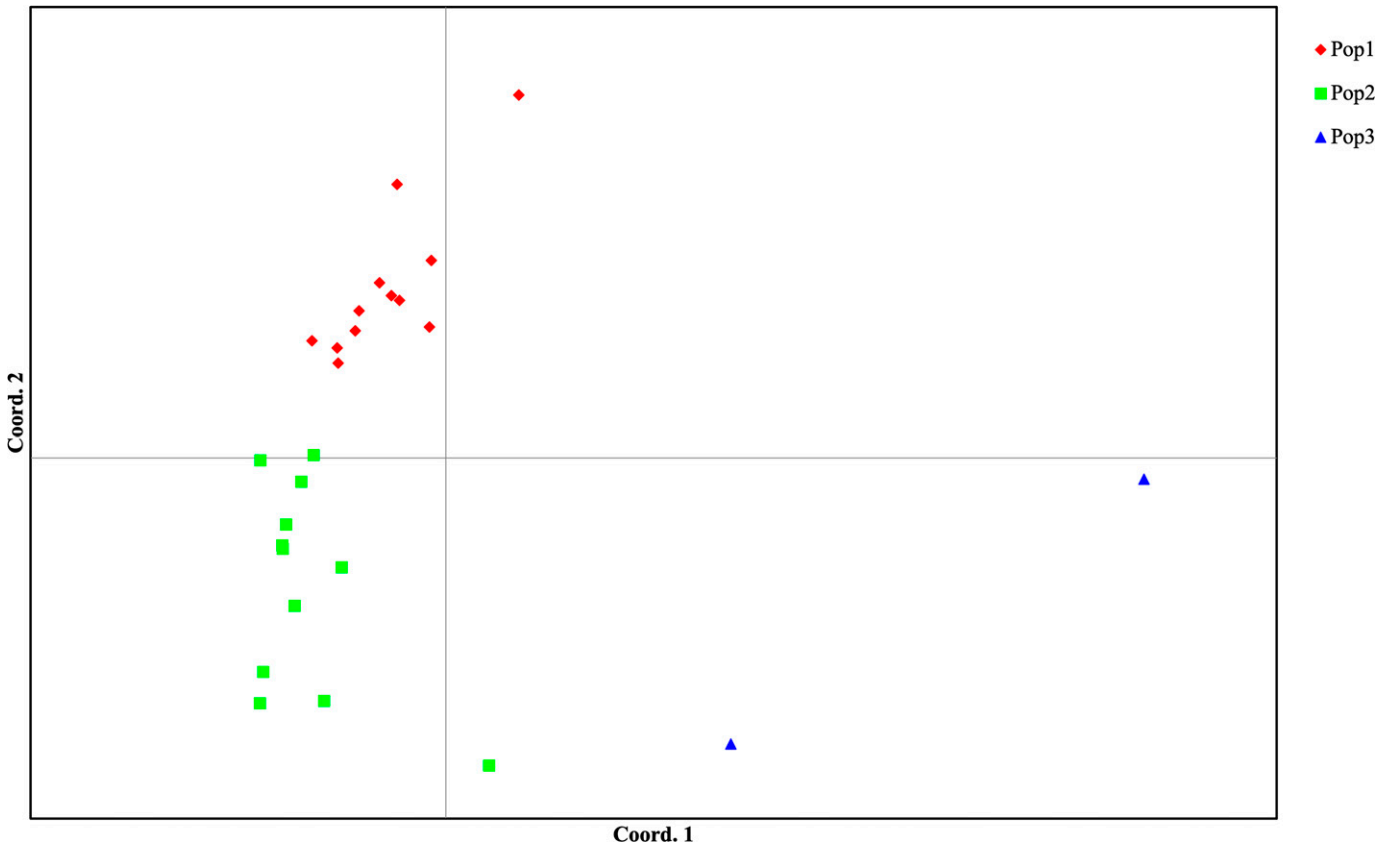

Fig. 4. Principal coordinate analysis (PCoA) plot of 26 jujube (*Ziziphus jujuba*) cultivars from the three distinctive groups inferred from the genetic structure analysis. The plane of the first three main PCoA axes accounted for 67.73% of total variation.

pairwise multilocus analysis, genetic structure analysis, AMOVA, and PCoA. It partitioned 74 unique jujube cultivars into two main clusters. The first cluster (shown in red color in Fig. 5) aligned with the first group inferred by the structure analysis, whereas the second cluster (shown in green color in Fig. 5) aligned with the second group assigned from the genetic structure analysis. Cultivar Dongzao, KFC, Sherwood, Jinsi, etc. were in the first cluster, and cultivars Li, Lang, Maya, Abbeville, etc. were in the second cluster. The third group reported by STRUCTURE (shown in blue color in Fig. 5) was included in the first cluster in the tree.

Quantitative characteristics analysis within synonymous cultivar groups. Based on the genotyping results, the 116 jujube germplasm accessions can be classified into 23 synonymous groups. The cultivars (or germplasm accessions) that had identical or near identical SNP profiles were the members of the same cultivar group. The cultivars/germplasm accessions that share identical or near identical SNP genotypes were further compared for their morphological characteristics. Three cultivar groups Li, Lang, and Jinsi were studied (Fig. 6).

Analysis within the 'Li' group. At Leyendecker, the five cultivars of the Li group were found to be significantly different from each other in all the traits except for total soluble solids (TSS), canopy width, and trunk circumference (Fig. 7). Based on the fruit and stone dimensions, as well as fruit and stone weight, 'Daguazao' was observed to be the smallest. 'Li', 'Shanxi Li', and 'Redlands #4' were similar for most of the attributes and were the largest ones. 'Redlands #4' had the smallest trees among all.

At Los Lunas, the cultivars within the Li group were found statistically significant only for stone traits, TSS, and tree height (P value < 0.001). Based on the result, the 'Daguazao' was observed to have smaller stones with lower stone weight, and lower tree height; however, it had significantly higher TSS. The tree dimensions were similar for all cultivars.

Analysis within the 'Lang' group. At Leyendecker, all the fruit, stone attributes, tree height, canopy width, and trunk circumference as well as TSS were found to be statistically nonsignificant among three cultivars within the Lang group.

At Los Lunas, the cultivars within the Lang group were observed to be statistically significant in terms of fruit and stone dimension traits, fruit fresh weight and stone weight, TSS, and trunk circumference (Fig. 8). However, the cultivars were similar in terms of fruit moisture percent, tree height, and canopy width. 'Jinchang 1' had significantly greater average fresh fruit weight, dry fruit weight, stone weight, and fruit dimensions followed by 'Lang'.

Analysis within the 'Jinsi' group. All the morphological parameters and TSS were found to be statistically significant among the cultivars within the Jinsi group sampled from Leyendecker except for the fruit moisture content and canopy width. Even though the cultivars were genetically identical, we observed distinct morphological differences in fruit traits. Based on fruit dimensions and fresh fruit weight, 'Jinsi 2' and 'Pitless' were the smallest, whereas 'Jinsi 3' and 'JKW' were the largest (Figs. 6C and 9). Pitless is a cultivar with incomplete stones, and thus no stone attributes were measured for this cultivar (Fig. 6C).

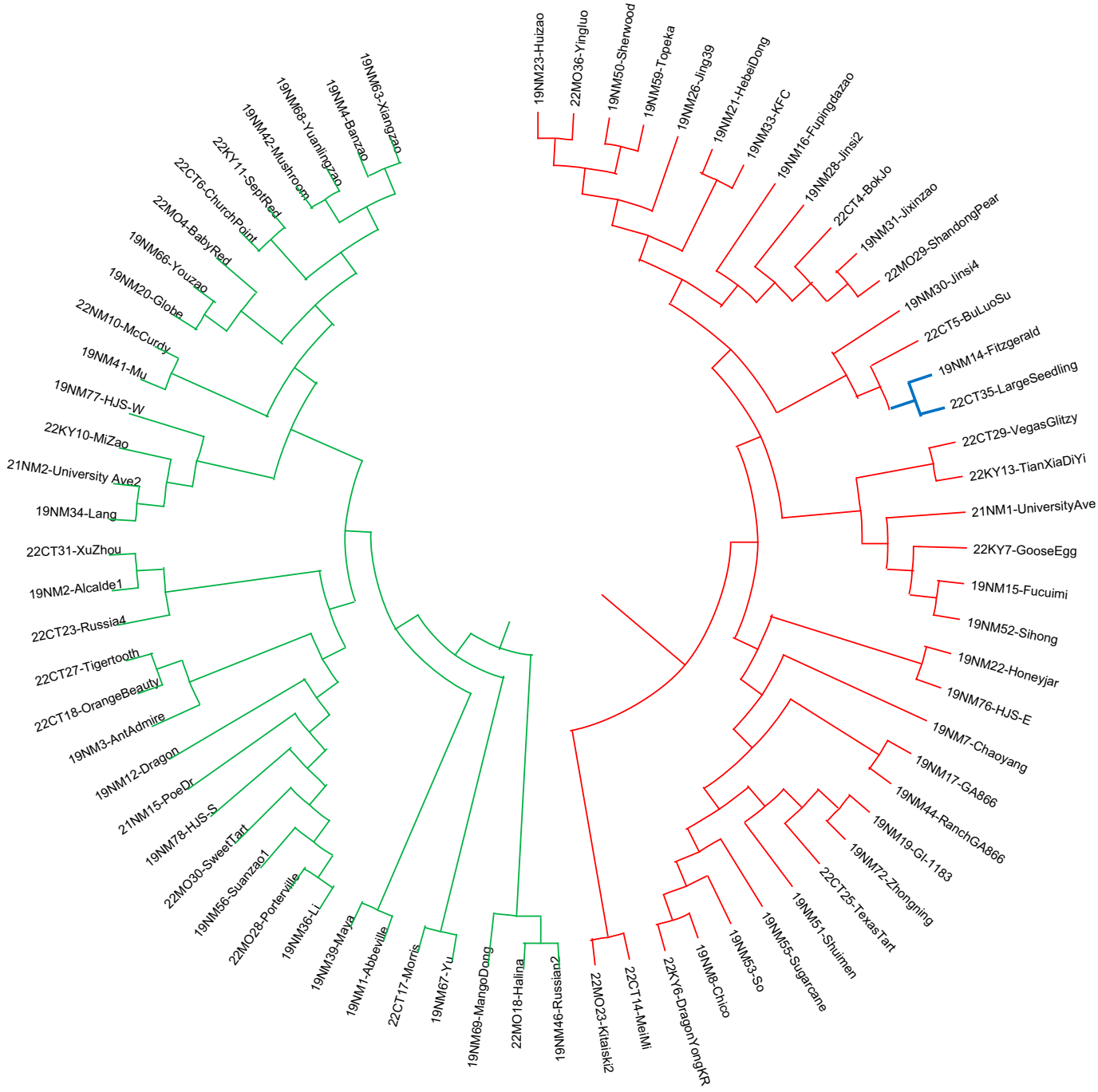

Fig. 5. Relationship shown among 74 unique jujube (*Ziziphus jujuba*) cultivars/selections in the United States by a neighbor-joining phylogenetic tree. The cluster shown in red color resembled the first group and the first cluster of structure and principal coordinate analyses (PCoA), respectively, and the cluster shown in green color resembled the second group and the second cluster of structure and PCoA analyses, respectively.

'Jinsi 2' had the highest average TSS, whereas 'Pitless' had the lowest. The trees of 'JKW' were the strongest and tallest and had larger trunk circumference followed by 'Jinsi 3', 'Jinsi 2', and 'Pitless'.

There were five cultivars in the Jinsi group at Los Lunas. The results in Fig. 10 show that except for TSS, all other measured parameters of jujube fruits, stones, and trees were statistically significant for all the cultivars in this group. We found that 'JKW' was the largest in terms of fruit dimensions, fresh weight, moisture content, and tree height, as well as stone dimensions and stone weight followed by 'Jinsi 3'. The morphological characteristics of 'Jinsi 4' were distinct from others in this group, as the tree was smaller both in terms of height and canopy and had small fruits. Regardless of the location, 'Jinsi 2' and 'Pitless' were the smallest and 'JKW' was the largest.

Qualitative characteristics. Qualitative morphological characteristics like fruit shape, presence/absence of thorns, and blooming type showed considerable similarities among different cultivars within the same cultivar group (Table 3). The cultivars within the Li and Jinsi groups had globose- and oblong globose–shaped fruits, respectively. The cultivars in Lang group

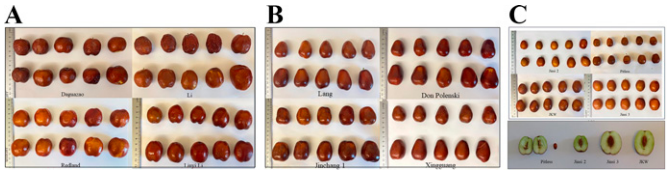

Fig. 6. (**A**–**C**) Fruits of jujube (*Ziziphus jujuba*) cultivars in the Li, Lang, and Jinsi groups, respectively. The bottom picture in **C** shows an incomplete pit in cultivar Pitless.

had cylindrical/pear-shaped fruits except for the cultivar Jinchang 1, which had oblong globose–shaped fruits. Cultivars in the Li and Jinsi groups were similar in terms of presence of thorns as well as flower blooming type.

Discussion

SNP GENOTYPING FOR CULTIVAR IDENTIFICATION. SNP genotyping allows for accurate multilocus genotype matching at a lower cost, making it an effective DNA fingerprinting technique. Using high-throughput genotyping, this method enables quick processing and analysis of multiple samples while producing reliable and consistent results. SNP markers have been extensively used in various plant species to evaluate genetic diversity and population structure in wheat (*Triticum aestivum*) (Kumar et al. 2020), maize (*Zea mays*) (Boakyewaa et al. 2019), cowpea (*Vigna unguiculata*) (Xiong et al. 2016), rice (*Oryza sativa*) (Aesomnuk et al. 2021), grapes (*Vitis* sp.) (Bianchi et al. 2020), chile peppers (*Capsicum annuum*) (Lozada et al. 2021), and melons (*Cucumis melo*) (Esteras et al. 2013), among others.

Numerous studies have reported different rates of duplication in plant germplasm collections, such as in soybean (*Glycine max*) (Kuroda et al. 2009), lychee (*Litchi chinensis*) (Sun et al. 2012), grape (Emanuelli et al. 2013), melon (Hu et al. 2015), tea (*Camellia sinensis*) (Fang et al. 2016), and coffee (*Coffea canephora*) (Akpertey et al. 2021).

In the present study, we found that 116 cultivars of 177 (~65%) were duplicates (Table 1). This finding aligns with previous results reported by Song et al. (2021) and Sapkota et al. (2023a) in Chinese jujube germplasm collections. This higher redundancy could be attributed to mislabeling errors, the presence of synonymous cultivars, and closely related commercial cultivars. As reported by Sedlacek et al. (2016), a threshold of mismatches must be determined for practical implementation because genotyping errors can occur. The mismatch distribution of 177 Chinese jujube cultivars revealed that at least 20 loci could distinguish between two cultivars (i.e., a pair) based on their SNP profiles (Supplemental Fig. 1). About 80 loci were considered dissimilar or mismatches in 300 accession pairs. More than 300 pairs of jujube cultivars had zero mismatches, which can be considered as identical or duplicates. This observation supports our conclusion that there is a high incidence of duplication and/or mislabeling, and genotyping errors in the jujube population.

Somatic mutations are known to be common in jujube and they can have an impact on phenotypic attributes (Song et al. 2021), and thus genetically identical cultivars, as revealed by SNP genotyping, may not be phenotypically identical. As a result, it is important to proceed with caution when drawing conclusions. Similar issues were encountered by other plant species such as banana (*Musa* sp.) (Irish et al. 2014), apple (*Malus* sp.) (Jiang et al. 2019), and pineapple (*Ananas comosus*) (Collins 1936; Zhou et al. 2015) during genotyping. To supplement the findings of DNA fingerprinting, it is still critical to compare phenotypic traits across members within each synonymous group. Phenotypic variation caused by mutation may serve as an alternative for distinguishing cultivars. The combination of genetic and phenotypic data could provide a more comprehensive and robust approach to authenticate and differentiate jujube cultivars.

GENETIC RELATIONSHIPS AMONG THE JUJUBE CULTIVARS. The Delta *K* calculated using Evanno's method (Evanno et al. 2005) indicated the presence of three genetic groups in our samples. The core members of the three groups (assigned at $Q > 0.70$) displayed significant differentiation ($F_{st} = 0.199$; $P < 0.001$). The high percentage of admixture cultivars revealed by the genetic structure analyses suggested that there might be population mixing or hybridization, recent or ongoing gene flow, complex population structures, and a history of domestication or breeding events during evolution (Chen et al. 2017). This divergence was further supported by AMOVA and phylogenetic tree results. AMOVA showed significant differences in both within-group and among-group variances (80% and 20%, respectively). The

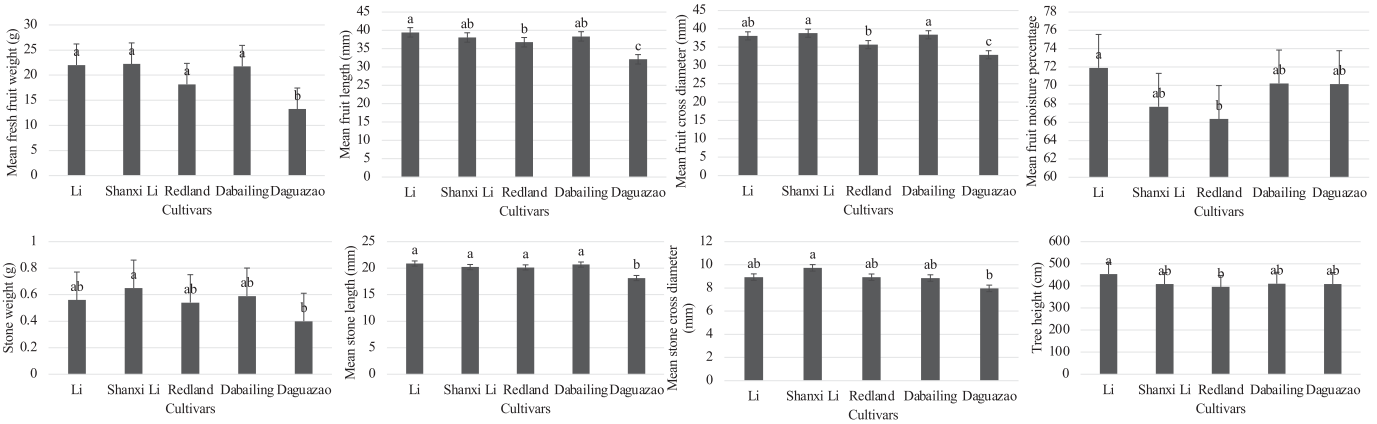

Fig. 7. From top left to bottom right, mean fresh fruit weight (g), fruit length (mm), fruit cross diameter (mm), fruit moisture percentage, stone weight (g), stone length (mm), stone cross diameter (mm), and tree height (cm) of different jujube (*Ziziphus jujuba*) cultivars within the Li group harvested from Leyendecker, NM, USA, in 2022. Different letters denote a significant difference at $P < 0.05$.

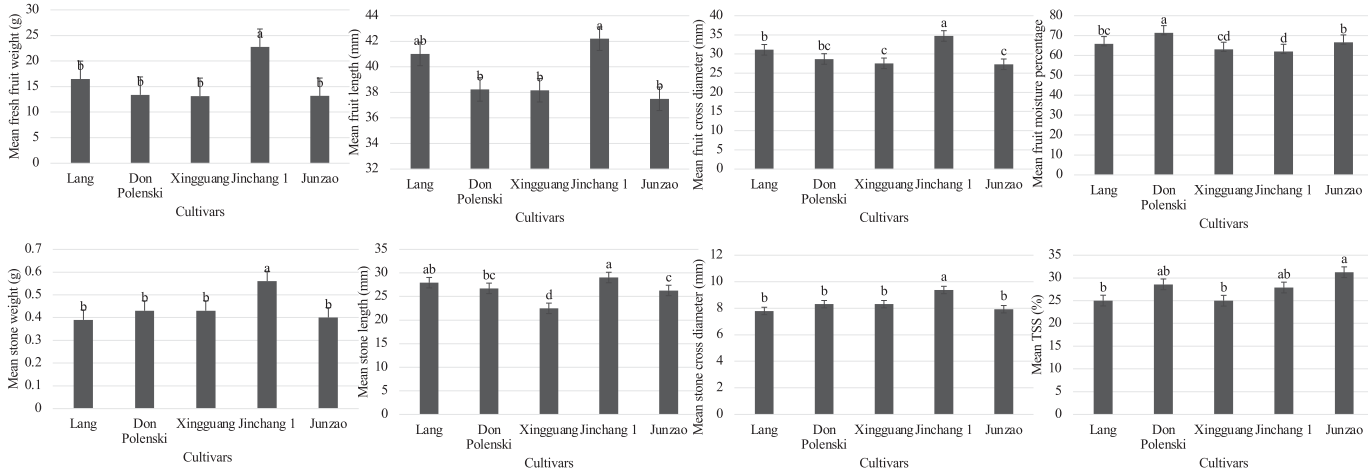

Fig. 8. From top left to bottom right, mean fresh fruit weight, fruit length, fruit cross diameter, fruit moisture percentage, stone weight, stone length, stone cross diameter, and total soluble solids content (%) of different jujube (*Ziziphus jujuba*) cultivars within the Lang group harvested from Los Lunas, NM, USA, in 2022. Different letters denote a significant difference at $P < 0.05$.

high within-group variance suggested that most of the genetic variation is observed within the individual groups or populations, indicating a high-level genetic diversity within each population, possibly influenced by factors such as genetic drift. On the other hand, the low among-group variance suggested limited genetic differentiation between these groups. This implies that although there are significant genetic differences among the groups, the differentiation between them is not as pronounced as the diversity within each group. Factors like limited gene flow between groups and geographic isolation may contribute to this pattern. It is worth noting that different studies have reported varying substructures in *Z. jujuba*, including two (Chen et al. 2017; Fu et al. 2016; Song et al. 2021) and three (Xu et al. 2016; Wang et al. 2014) substructures. The Delta *K* plot (Fig. 3A) also indicated secondary peaks at $K = 6$, suggesting that these jujube cultivars might potentially be classified into six genetic groups. This could be because of some groups being underrepresented in our collection, leading them to not be classified as distinct genetic clusters by the genetic structure analysis (Kalinowski et al. 2007). The importance of SNP markers in detecting genetic structure in breeding programs is imperative. Genetic analysis based on SNP markers provides more accurate genetic information compared with phenotypic data, which can be influenced by environmental factors. Genotypic data can also serve to complement and/or validate results obtained from phenotypic studies.

Morphological relatedness among synonymous cultivar groups. Within the Li group, cultivars Li, Shanxi Li, Linyi Li, Weeping Li, Redlands #4, and Dabailing exhibited identical morphological attributes. The cultivar Weeping Li from Espanola, NM, USA, lacks formal release information. 'Weeping Li' was named based on its tree appearance. 'Li' was imported to the United States in 1914 from Shanxi Province, China by Frank N. Meyer, Department of Agriculture, PI 38249. 'Shanxi Li' was imported in the 1990s by Roger Meyer in California. It is originally from Shanxi Province of China. Linyi is a county in Shanxi Province where most of 'Shanxi Li' is produced. Thus, these four

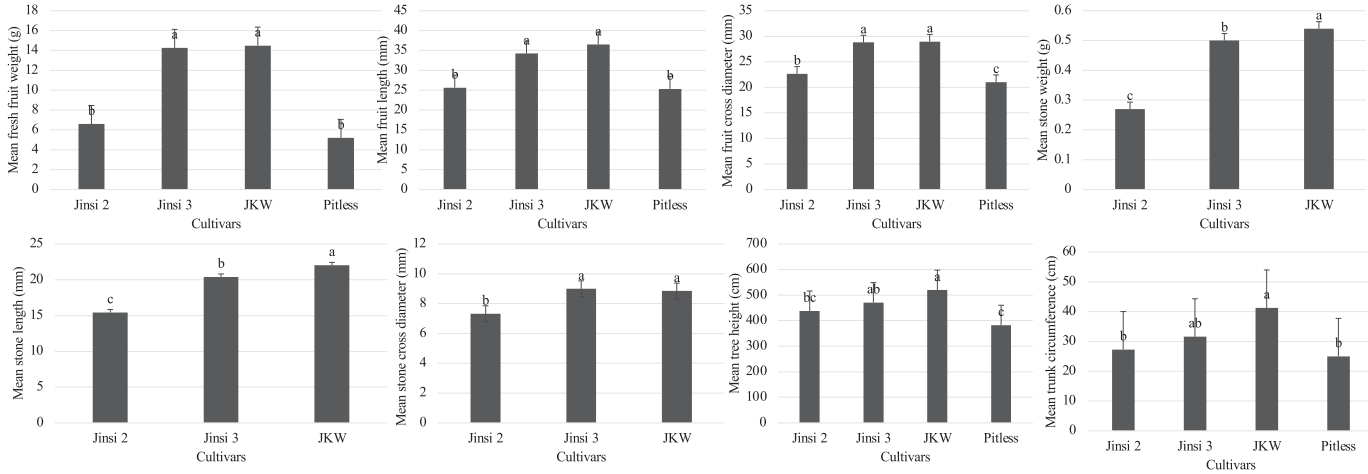

Fig. 9. From top left to bottom right, mean fresh fruit weight, fruit length, fruit cross diameter, stone weight, stone length, stone cross diameter, tree height, and trunk circumference of different jujube (*Ziziphus jujuba*) cultivars within the Jinsi group harvested from Leyendecker, NM, USA, in 2022. Different letters denote a significant difference at $P < 0.05$.

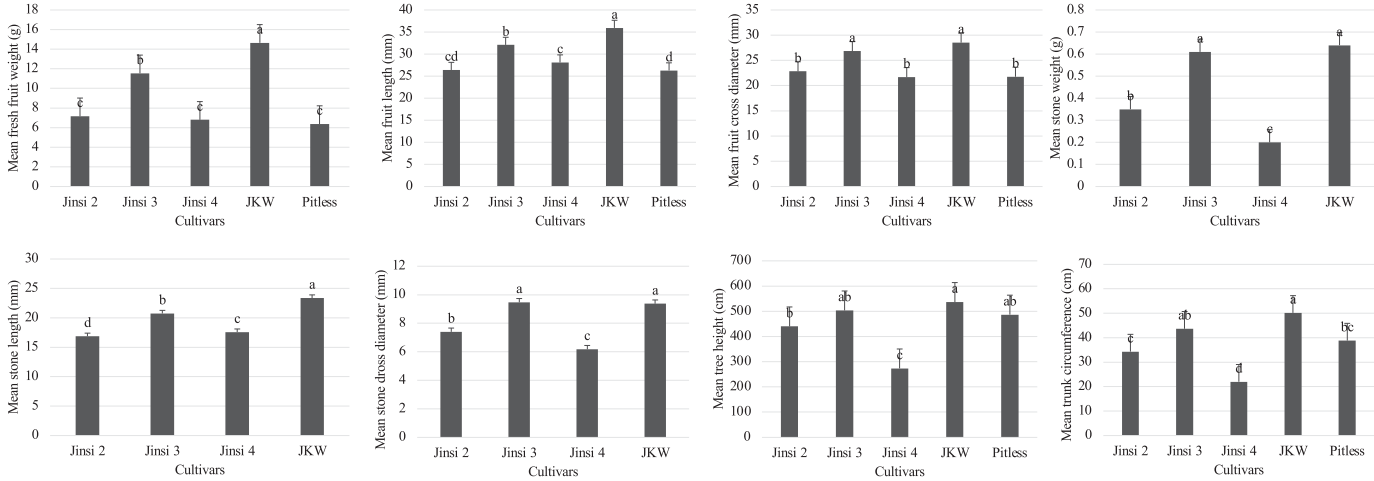

Fig. 10. From top left to bottom right, mean fresh fruit weight, fruit length, fruit cross diameter, stone weight, stone length, stone cross diameter, tree height, and trunk circumference of different jujube (*Ziziphus jujuba*) cultivars within the Jinsi group harvested from Los Lunas, NM, USA, in 2022. Different letters denote a significant difference at $P < 0.05$.

cultivars might be synonymous with different origins. Based on our genotyping results, 'Linyi Li', and 'Shanxi Li' are synonymous, which is consistent with our phenotypic results; however, the fruits of cultivar Daguazao within the Li group were relatively smaller in size. 'Daguazao' was selected in Donge County, Shandong Province, China. Its origin remains unknown, but 'Daguazao' means "big melon" in Chinese. The trees of 'Daguazao' are very similar to other cultivars in the Li group and are highly productive. The smaller fruit size of 'Daguazao' might be because of crop load or somatic mutations, a phenomenon frequently observed in jujubes.

The cultivars in the Lang group have pear-shaped fruits. The current Chinese 'Lang' with columnar-shaped fruits is different from American pear-shaped 'Lang' (Liu and Wang 2009). Cultivars Lang, Don Polenski, and Xingguang were almost the same, whereas Jinchang 1 had larger and wider fruits and Junzao had smaller fruits. All cultivars in the Lang group might be synonymous with slight mutation. 'Jinchang 1' is a selection from 'Hupingzao', which means bottle-shaped fruits (Liu and Wang 2009). 'Junzao' and 'Hupingzao' originated and were cultivated in nearby counties in Shanxi Province, China (Guo and Shan 2010; Liu and Wang 2009).

Significant morphological differences were observed within the cultivars in the Jinsi group. 'Jinsi 2' and 'Pitless' had smaller fruits and stones with lower fruit weight. 'Jinsi 3' and 'JKW' had larger fruits and complete stones, and higher fruit weight. 'Jinsi 4' had small to medium-sized fruits that did not match any other cultivars in this group. 'Jinsi 4' had the smallest tree size within this group followed by 'Jinsi 2'. 'JKW' had the strongest and largest trees. 'Pitless' has an incomplete pit with exposed seeds, whereas all other cultivars in this group have complete pit. 'Jinsi 4' was selected by the Shandong Institute of Pomology (Tai'an, Shandong Province, China) (Guo and Shan 2010). 'Jinsi 4' is an open pollinated seedling of 'Jinsi 2' and was named as a member of the 'Jinsi' group. However, it did not belong to this group based on both genotypic and phenotypic results. Therefore, the present study confirmed that the cultivar Pitless was a mutant of Jinsixiaozhao. 'Jinsi 4' was selected from open

Table 3. Qualitative characteristics of jujube (*Ziziphus jujuba*) cultivars within Li, Lang, and Jinsi cultivar groups.

Cultivar group	Cultivar	Fruit shape	Thorn presence/absence	Blooming type
Li	Daguazao	Globose	Present	Afternoon
	Dabailing	Globose	Present	Afternoon
	Li	Globose	Present	Afternoon
	Linyi Li	Globose	Present	Afternoon
	Redlands #4	Globose	Present	Afternoon
	Shanxi Li	Globose	Present	Afternoon
	Weeping Li	Globose	Present	Afternoon
Lang	Lang	Cylindrical/Pear shaped	Absent	Morning
	Junzao	Cylindrical/Pear shaped	Absent	Morning
	Xingguang	Cylindrical/Pear shaped	Absent	Morning
	Don Polenski	Cylindrical/Pear shaped	Absent	Morning
	Jinchang 1	Oblong globose	Absent	Morning
Jinsi	Jinsi 2	Oblong globose	Present	Afternoon
	Jinsi 3	Oblong globose	Present	Afternoon
	Jinsi 4	Oblong globose	Present	Afternoon
	JKW	Oblong globose	Present	Afternoon
	Pitless	Oblong globose	Present	Afternoon

pollinated progenies of 'Jinsixiaozhao', but the exact male parent remains to be identified.

Morphological descriptors have been commonly used in jujube germplasm management (Liu et al. 2020). Based on an evaluation of 25 morphological descriptors, Kim et al. (2019) reported that the presence or absence of spikes and fruit shape were two descriptors with good stability for jujube cultivar differentiation. In the present study, we also found that jujube fruit shape plays a crucial role in cultivar and/or genotype identification and classification. The 'Li' group has large globose-shaped fruits, whereas the cultivars in the Lang group have pear-shaped fruits. Similarly, cultivars in the Maya and the Sherwood group have football-shaped and cylindrical-shaped fruits, respectively. These observations suggest that the gene(s) associated with fruit shape are genetically highly conserved. Even though the cultivars were distributed to several states, provinces, or nations, their genetic background remained the same, and thus were identical. Nonetheless, further selection of morphological descriptors with high heritability is necessary for jujube germplasm characterization.

Conclusions

We genotyped and authenticated jujube cultivars in the United States using 147 SNP markers via KASP genotyping. Our results identified 23 synonymous cultivar groups, including 116 accessions. Furthermore, we assessed genetic diversity and population structure in the jujube cultivars with unique genotypes, leading to the classification of jujube cultivars in the United States into distinct cultivar groups. The present study showed that SNP genotyping is a powerful tool for jujube cultivar identification. However, it is important to note that jujube is prone to mutations, which can impact its phenotypic characteristics. Therefore, morphological characterization and phenotypic evaluation remain essential to complement the SNP-based molecular analysis. The current findings provided essential information about the genetic identity of jujube cultivars in the United States, which will guide jujube growers, nurseries, and researchers for their cultivar identification and selections.

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