Jujube Germplasm in New Mexico and the Southwestern corner of Texas

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Introduction

- Jujube (*Ziziphus jujuba* Mill, 2n = 2x = 24), also called Chinese date or red date, is native to China.
- In ancient times, farmers selected and cultivated sour jujubes with big fruit and good flavor, and they gradually became the cultivated modern jujube species (*Z. jujuba*) (Yao, 2013).
- Robert Chisholm first brought jujube (*Ziziphus jujuba* Mill.) seedlings to the United States from Europe and planted them in Beaufort, NC, in 1837.
- The jujube cultivars in the United States now include several groups: Frank Meyer's direct imports from China, U.S. cultivars from the USDA Chico jujube program, selections from across the United States, and recent imports from China or other jujube growing countries (Yao, 2013).
- Synonyms are quite common for jujube cultivars in the United States, due to which growers are confused with cultivar selections. Nurseries randomly rename even the imported cultivars. Many cultivars from different states are named after towns or people.

Research Objectives

- Genotyping jujube cultivars and germplasms.
- Identifying the genetic relationships between the cultivars/germplasms genotyped.

Materials and Methods

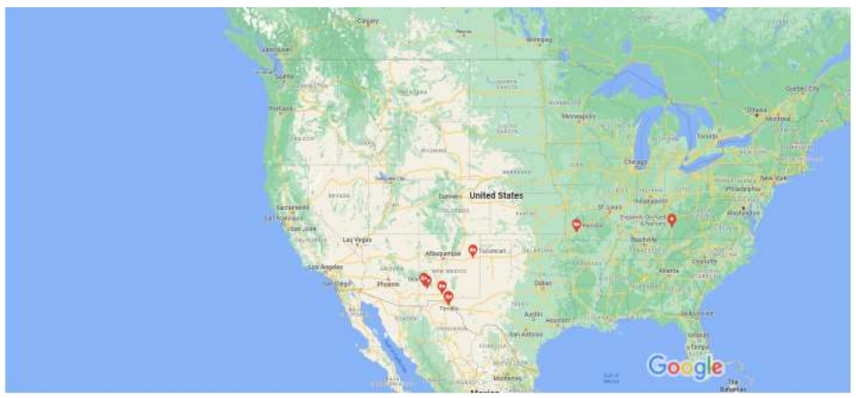
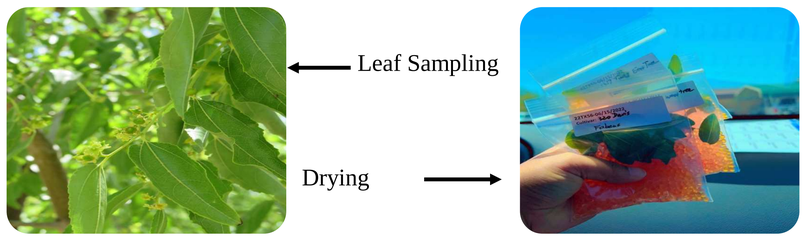


Figure 1. Jujube genotyping sampling sites: Las Cruces, Tucumcari, Gila, and Silver City in NM, Tornillo and Fabens in TX, England's Orchard and Nursery in McKay, KY, and Michael Nave's collection in Republic, MO.

Biological Material Collection
- Leaves from a total of 187 jujube accessions were used.



DNA Extraction
- The DNeasy Plant Mini kit (Qiagen Inc., Valencia, CA, USA), was used to extract DNA from the dried jujube leaves.
- A TissueLyser II (Qiagen Inc.) was used to disrupt the dry leaf tissue samples with high-speed shaking (30 Hz for 1 min) using Lysing Matrix A (MP Biomedicals. Solon, OH, USA) as described in Song et al., 2021.
- A NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to determine DNA concentration by absorbance at 260 nm and to estimate DNA purity at ratios of 260:280 and 260:230.

Genotyping
- Song et al. (2021) developed a large panel of single nucleotide polymorphism (SNP) markers and validated 288 SNPs by genotyping 114 accessions of Chinese jujube germplasm.
- The validation resulted in the designation of a set of 192 polymorphic SNP markers from which a subset of 94 SNPs with high information index was selected for nano fluidic array genotyping of our jujube samples.

Data Analysis
- Pairwise multilocus matching across all samples using GenAlEx 6.5 to identify duplicate accessions (Peakall, 2006).
- After eliminating redundant samples, samples with unique SNP profiles were further analyzed using the model-based Bayesian clustering algorithm using the STRUCTURE v2.3.4 software to determine the population structure (Pritchard et al., 2000).
- The number of genetic clusters (K –value) was set from 1 to 20. Ten independent runs, each with 100,000 iterations after a burn-in of 200,000 iterations were evaluated for each fixed number of clusters (K value).
- The Delta K value was used to determine the most rational number of clusters using the online program STRUCTURE HARVESTER (Earl & vonHoldt, 2012).
- Cluster analysis based on the Neighbor-Joining method was employed and a phylogenetic tree was generated using MEGA 11 software with 1000 bootstrap replicates (Tamura et al., 2021).

Results and Conclusions

Cultivar Identification
- SNP profile multilocus matching revealed a significant rate of duplication in these jujube samples.
- 48 cultivars (or approximately 26%) of the 187 examined cultivars can be categorized into 14 synonymous groups. Each synonymous group contained two to eight cultivars as shown in Table 1.

Population structure
- Population stratification of 153 accessions computed by STRUCTURE HARVESTER based on delta K value revealed two clusters as the most probable value of K. We observed a clear delta K peak at K=2 as shown in Figure 2 and 3.

Cluster Analysis
- A neighbor-joining phylogenetic tree is shown in Figure 4.
- 70 samples from Fabens/Tornillo, TX were grouped into two big groups: wild jujube group (*Z. spinosa*) consisting of 46 accessions and cultivar group (*Z. jujuba*) consisting of 24 accessions.

Table 1. List of 48 accessions of 14 synonymous groups identified by SNP markers. Accessions in bold were retained for subsequent analysis.

Synonymous Group	Accession	Synonymous Group	Accession
1	**22TX9**	8	**21NM6**
1	22TX3	8	21NM20
1	22TX2	8	21NM11
1	22TX18		
1	22TX16	9	22TX66
1	22TX14	9	22TX67
1	22TX13	9	**22TX68**
1	22MO31 (Texas Sawmill)		
		10	22TX11
2	**22TX61**	10	22TX22
2	22TX60	10	22TX30
2	22TX44	10	22TX38
2	22TX21	10	22TX50
		10	22TX51
3	**22TX59 (Mount Lebanon)**	10	22TX54
3	22TX10	10	**22TX58**
4	**22NM35-TUC16**	11	**22TX52**
4	22NM30-TUC11	11	22TX35
4	22NM29-TUC10		
4	22NM28-TUC9	12	22TX12
4	22NM23-TUC4	12	**22TX20**
5	**21NM5 (Fupingdazao)**	13	22TX23
5	21NM13	13	**22TX31**
6	**22TX77**	14	22NM52-TUC33
6	22TX78	14	22NM53-TUC34
		14	**22NM54-TUC35**
7	**21NM19**		

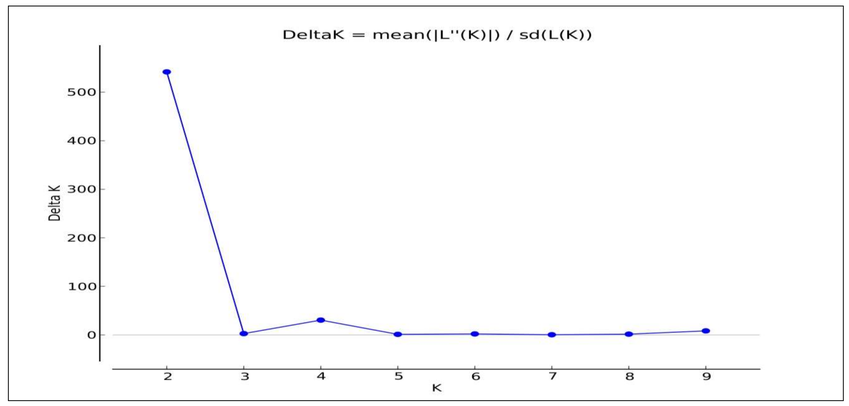


Figure 2. Plot of ΔK (filled circles, solid line) calculated as the mean of the second-order rate of change in likelihood of K divided by the standard deviation of the likelihood of K.

- The wild group has two subgroups: a small, round fruit group with 35 accessions and a medium-sized fruit group with 11 accessions.
- The 35 samples sequenced from Tucumcari were divided into three groups: wild jujube group (20 accessions), cultivar group 1 (7 accessions), and cultivar group 2 (8 accessions).
- Cultivars from Kentucky and Missouri grouped with some accessions from Texas, Las Cruces and Tucumcari.

Figure 3. Inferred clusters in the 153 Chinese jujube accessions using STRUCTURE in the overall analyzed jujube accessions. Each vertical line corresponds to a distinct multilocus genotype. Individuals with multiple colors have admixed genotypes from multiple clusters. Each color indicates the most likely ancestry of the cluster from which the genotype or partial genotype was derived.

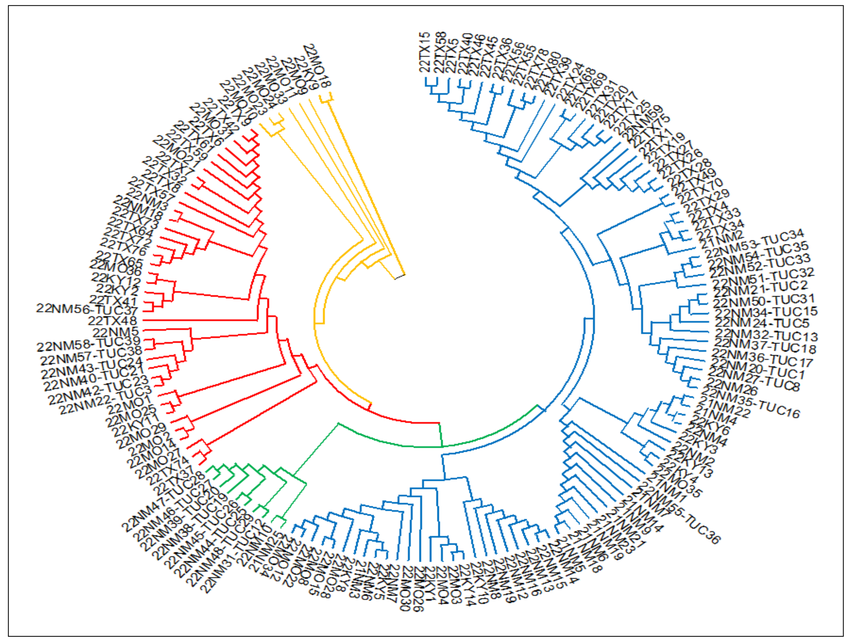


Figure 4. Phylogenetic tree of 153 Jujube accessions based on neighbor-joining method using MEGA 11.

Conclusion

This jujube genotyping study with cultivars/germplasm form various locations in NM, the southwest corner of TX and cultivars from KY and MO identified synonyms in cultivars and revealed their relationship to each other. Genotyping using SNP markers and the nanofluidic array can be valuable in gene bank management. The results can be used to assist growers/home gardeners, and nurseries in their cultivar selection and identification, and guide researchers in jujube breeding parent selections.