

## Jujube genotyping with cultivars from New Mexico and Kentucky in 2019

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Jujube is a nutritious fruit and grows well in the U.S. especially in the Southwestern region. Most jujube cultivars in the U.S. were imported from other countries without details. Synonyms and renaming are common. So, we conducted jujube genotyping to identify cultivars and clarify synonyms with Single Nucleotide Polymorphisms (SNP) sequences. This was the first attempt with samples from New Mexico and Kentucky in 2019. For the 2022 samplings from New Mexico, Kentucky, Missouri, and Connecticut, once the results are published, it will also be posted.

### Materials and Methods

In fall 2019, 78 jujube leaf samples from Alcalde, NM and 50 samples from McKee, KY were collected for genotyping analysis (Table 1). 5-8 fully expanded healthy jujube leaves were sampled from each tree and kept inside a 4x4" plastic bags with silica desiccant which would dry those leaves. The dried leaves were then stored in freezer until further processing. DNA extraction and genotyping analysis were performed at USDA-ARS, Sustainable Perennial Crops Laboratory, Beltsville, MD. 75 Single Nucleotide Polymorphisms (SNPs) markers were used for the genotyping.

Table 1. Jujube samples from Alcalde, NM and McKee, KY in 2019. \*Those accessions were for research purposes.

#	Cultivar	Source	Plot	Location
NM1	Abbeville	CA	OP	Alcalde, NM
NM2	Alcalde #1	China	GRP	Alcalde, NM
NM3	Ant admire	CA	GRP	Alcalde, NM
NM4	Banzao	China	GRP	Alcalde, NM
NM5	Candong #1	China	GRP	Alcalde, NM
NM6	Capri	Las Cruces, NM	GRP	Alcalde, NM
NM7	Chaoyangdapingding	China	GRP	Alcalde, NM
NM8	Chico	CA	CT	Alcalde, NM
NM9	Dabailing	China	GRP	Alcalde, NM
NM10	Daguazao	China	GRP	Alcalde, NM
NM11	Don Polenski	CA	OP	Alcalde, NM
NM12	Dragon	China	GRP	Alcalde, NM
NM13	Ed Hegard	CA	GRP	Alcalde, NM
NM14	Fitzgerald	CA	OP	Alcalde, NM
NM15	Fucuimi	GA	GRP	Alcalde, NM
NM16	Funingdazao	China	GRP	Alcalde, NM
NM17	GA866	CA	OP	Alcalde, NM
NM18	Gagazao	China	GRP	Alcalde, NM
NM19	GI-1183	CA	OP	Alcalde, NM
NM20	Globe	China	OP	Alcalde, NM
NM21	Hebei Dongzao	China	GRP	Alcalde, NM
NM22	Honeyiar	CA	GRP	Alcalde, NM
NM23	Huizao	China	GRP	Alcalde, NM
NM24	Li-2	CA	OP	Alcalde, NM
NM25	Jinchang #1	China	GRP	Alcalde, NM
NM26	Jing 39	China	GRP	Alcalde, NM
NM27	Jinkuiwang	China	GRP	Alcalde, NM
NM28	Jinsi #2	China	GRP	Alcalde, NM
NM29	jinsi #3	China	GRP	Alcalde, NM
NM30	Jinsi #4	China	GRP	Alcalde, NM
NM31	Jixinzao	China	GRP	Alcalde, NM
NM32	Junzao	China	GRP	Alcalde, NM
NM33	Kongfucui	China	GRP	Alcalde, NM
NM34	Lang	China	OP	Alcalde, NM
NM35	Local Dongzao	China	GRP	Alcalde, NM
NM36	Li	CA	OP	Alcalde, NM
NM37	linvi Li	China	GRP	Alcalde, NM
NM38	Liyuexian	China	GRP	Alcalde, NM
NM39	Mavazao	China	GRP	Alcalde, NM
NM40	Miyunxiaozao	China	GRP	Alcalde, NM
NM41	Mu	CA	OP	Alcalde, NM
NM42	Mushroom	China	GRP	Alcalde, NM
NM43	Pitless	China	GRP	Alcalde, NM
NM44	Ranch GA866*	Espanola, NM	GRP	Alcalde, NM
NM45	Redland	CA	OP	Alcalde, NM
NM46	Russian 2	CA	GRP	Alcalde, NM
NM47	Sandia	China	GRP	Alcalde, NM
NM48	Shanxi Li	CA	OP	Alcalde, NM
NM49	Sept Late	CA	CT	Alcalde, NM
NM50	Sherwood	CA	OP	Alcalde, NM

NM51	Shuimen	CA	OP	Alcalde. NM
NM52	Sihong		OP	Alcalde. NM
NM53	So	CA	OP	Alcalde. NM
NM54	<i>Sugarball*</i>	Espanola. NM	GRP	Alcalde. NM
NM55	Sugarcane	CA	OP	Alcalde. NM
NM56	<i>Suanzao1 4-24*</i>	NM	GRP	Alcalde. NM
NM57	Teapot	China	GRP	Alcalde. NM
NM58	Thornless	CA	GRP	Alcalde. NM
NM59	Topeka	CA	OP	Alcalde. NM
NM60	Tsao	CA	OP	Alcalde. NM
NM61	<i>Weeping Li*</i>	Espanola. NM	GRP	Alcalde. NM
NM62	X38	China	GRP	Alcalde. NM
NM63	Xiangzao	China	GRP	Alcalde. NM
NM64	Xingguang	China	GRP	Alcalde. NM
NM65	Yandong #3	China	GRP	Alcalde. NM
NM66	Youzao	China	GRP	Alcalde. NM
NM67	Yu	CA	GRP	Alcalde. NM
NM68	Yuanlingzao	China	GRP	Alcalde. NM
NM69	Chengwu dongzao	China	GRP	Alcalde. NM
NM70	Zanhuangdazao	China	GRP	Alcalde. NM
NM71	Zaocuiwang	China	GRP	Alcalde. NM
NM72	Zhongningvuanzao	China	GRP	Alcalde. NM
NM73	Zan Dong	China	GRP	Alcalde. NM
NM74	<i>Suanzao-2 2-24*</i>	Alcalde. NM	GRP	Alcalde. NM
NM75	Sui	CA	OP	Alcalde. NM
NM76	<i>HJS-E*</i>	Alcalde. NM	OP	Alcalde. NM
NM77	<i>HJS-W*</i>	Alcalde. NM	OP	Alcalde. NM
NM78	<i>HJS-S*</i>	Alcalde. NM	OP	Alcalde. NM
KY1	Allentown 2		Orchard Row 2	2-3
KY2	Ba Ba Hong (88 Hong)		Orchard Row	11-16
KY3	Baby Red iuiu		Farm 3	10-14
KY4	Black Sea OGW		Farm 3	8-14
KY5	Bok Jo G		Farm 3	5-15
KY6	Buluosu Zao G	China	Farm 3	5-12
KY7	Coco NWN G		Farm 3	6-1
KY8	Cool Jade iuiu		Farm 3	10-10
KY9	Da Bai (ZAO)		Farm 3	8-10
KY10	Dae Sol Jo G		Farm 3	6-10
KY11	Dong Zao	China	Farm 3	4-3
KY12	Dragon Yong Dae Chu		Farm 3	1-12
KY13	Hana Zao	China	Farm 3	9-4
KY14	Hanna Zao (Frog)	China	Farm 3	1-5
KY15	Hetian Jade Chun Kuk		Farm 3	9-13
KY16	Hui Jo		Farm 3	1-10
KY17	Huk San		Farm 3	1-16
KY18	HULU Juiube	China	Farm 3	2-1
KY19	Huping Juiube	China	Farm 3	1-7
KY20	Jin iuiuube	China	Farm 3	7-1
KY21	Juiube #1 in the world	China	Orchard Row 4	4-18
KY22	Kima Bay Laurel G 8		Farm 3	2-11
KY23	Korean #1		Orchard row #3	3-11
KY24	La Fleur		Orchard Row #7	7-4
KY25	Longevity Juiu		Farm 3	10-8
KY26	Massandra G		Farm 3	5-6
KY27	Mi AKA Gourd Juiube		Farm 3	10-7
KY28	Mi Mi Zao		Farm 3	9-16
KY29	Mu Deung Dae chu		Farm 3	7-8
KY30	NITRA JUJUBE G		Farm 3	6-13
KY31	Orange Beauty		Farm 3	1-11
KY32	Park iuiuube		Farm 3	7-4
KY33	Priest		Orchard Row 10	10-8
KY34	R1T1/R2T4/Hulu Zao		Orchard Row	13-19
KY35	R3T4		Orchard Row #6	6-10
KY36	R4T3 TVA iuiuube		Farm 3	9-7
KY37	R7T2		Orchard Row 11	11-3
KY38	Russian #4		Orchard row 10	10-23
KY39	Russian #6		Orchard Row #8	8-11
KY40	Sang Wang		Farm 3	1-4
KY41	Silverhill		Farm 3	6-11
KY42	TAE SANG WANG G		Farm 3	3-12
KY43	Te Da Su Cui Zao #3		Orchard Row	10-13
KY44	Tiger Tooth		Orchard Row #9	9-12
KY45	Utah iuiuube G		Farm 3	4-14
KY46	Wal Chul Dae chu		Farm 3	5-14
KY47	Wang Dae Chu		Farm 3	7-6
KY48	Winter Delight G		Farm 3	7-7
KY49	Xu Zao G		Farm 3	6-5
KY50	Zho Tao OLI G		Farm 3	7-3

## Results:

126 accessions were used for genotyping analysis. There were 20 synonym groups.

Table 2. 20 SNP synonym groups from Kentucky and New Mexico samples in 2019.

Synonymous Group	Sample code	Name	Synonymous Group	Sample code	Name
1a	KY11	Dong Zao	9	NM2	Alcalde #1
1a	KY16	Hui Jo	9	KY48	Winter_Delight_G
1a	KY52	Dong Zao			
1a	NM65	Yandong #3	10	KY1	Allentown 2
1a	NM73	Zan Dong	10	KY10	Dae Sol Jo G
			10	KY15	Hetian Jade Chun Kuk
1b	NM21	Hebei Dongzao	10	KY40	Sang Wang
1b	NM35	Local Dongzao	10	KY42	TAE SANG WANG G
1b	NM47	Sandia	10	KY47	Wang Dae Chu
1b	NM5	Candong #1	10	NM9	Dabailing
			10	NM10	Daguazao
2	KY19	Huping Jujube	10	NM24	Li-2
2	KY30	NITRA JUJUBE G	10	NM36	Li
2	KY39	Russian #6	10	NM37	liny Li
2	KY9	Da Baj (ZAO)	10	NM45	Redland
2	NM11	Don Polenski	10	NM48	Shanxi Li
2	NM13	Ed Hegard	10	NM61	Weeping Li
2	NM25	Jinchang #1			
2	NM32	Junzao	11	NM68	Yuanlingzao
2	NM34	Lang	11	KY17	Huk San
2	NM58	Thornless			
2	NM64	Xingguang	12	NM51	Shuimen
			12	NM75	Sui
3	NM40	Miyunxiaoza	12	KY34	RIT1/R2T4/Hulu Zao
3	NM27	Jinkuiwang			
3	NM28	Jinsi #2	13	KY36	R4T3 TVA jujube pepper jujube
3	NM29	jinsi #3	13	KY35	R3T4
3	NM43	Pitless			
			14	KY5	BOK JO G
4	NM33	Kongfucui	14	KY29	Mu Deung Dae chu
4	NM38	Liuyexian	14	KY46	Wal Chul Dae chu
5	NM55	Sugarcane	15	NM17	GA866
5	NM54	Sugarball	15	NM44	Ranch GA866
5	KY7	CoCo NWN G			
			16	KY22	Kima Bay Laurel
6	NM50	Sherwood	16	KY25	Longevity Jujube
6	NM59	Topeka	16	NM20	Globe
6	NM6	Capri			
			17	KY41	Silverhill
7	NM60	Tsao	17	KY44	Tiger Tooth
7	NM18	Gagazao			
7	NM62	X38	18	KY20	Jin jujube
7	NM39	Mayazao	18	KY51	Jin
7	KY26	Massandra G			
			19	NM15	Fucuimi
8	KY13	Hana Zao	19	KY2	Ba Ba Hong (88 Hong)
8	KY14	Hanna Zao (Frog)			
			20	NM46	Russian_2
			20	KY4	Black_Sea_OGW

Group1: Dongzao has two subgroups, Hebei Dongzao (河北冬枣) and Shandong Dongzao (山东冬枣). The KY16-Hui Jo was a kind of Shandong Dong.

Group2: Lang group with pear-shaped fruit. Junzao (骏枣) and Hupingzao (壶瓶枣) all belonged to this group.

Group3: This is the typical Jinsi group (金丝小枣), excellent drying quality, varied in fruit size.

Group4: Kongfucui and Liuyexian were very similar or synonyms.

Group5: Sugarcane and Coco were synonyms or closely related.

Group6: Sherwood and Topeka were synonyms or closely related. 108 Capri from Las Cruces was also in this group.

Group7: This group has fruit pointed at both ends. Gaga (𣎵𣎵枣), Maya(马牙枣), Massandra, Tsao and Moonlight (X38, 月光枣) belonged to this group.

Group8: Hana or Hanna were the same. The correct spelling should be Hama (蛤蟆枣, frog in Chinese).

Group9: Alcalde #1, Winter Delight (KY2019) and Qiyuexian were synonyms, early maturation with large fruit. Sometime, Autumn Beauty was also Qiyuexian. Winter Delight may or may not belong to this group.

Group10: Li group with large fruit and producing heavy crop. Allentown\_2, Dae Sol Jo, Hetain Jade, Sang Wang, Tae Sang Wang, Wang Dae Chu, Dabailing (大白铃), Daguazao (大瓜枣), Li, Li2, Linyi Li (临漪梨枣), Shanxi Li (山西梨枣), and Redland. Li, Linyi Li and Shanxi Li were Synonyms. Others were all closely related.

Group11: Yuanling (园铃枣) and Huk San belonged to this group.

Group12: Shuimen and Sui were synonyms.

Group13: R4T3 and R3T4 were synonyms or very similar.

Group14: BokJo, Mu Deung, and Wal Chu belonged to this group.

Group15: GA 866 and Ranch GA866 were identical.

Group16: Kima Bay Laurel, Longevity and Globe (灵宝大枣) were closely related.

Group17: Silver Hill and Tigertooth were synonyms or very similar.

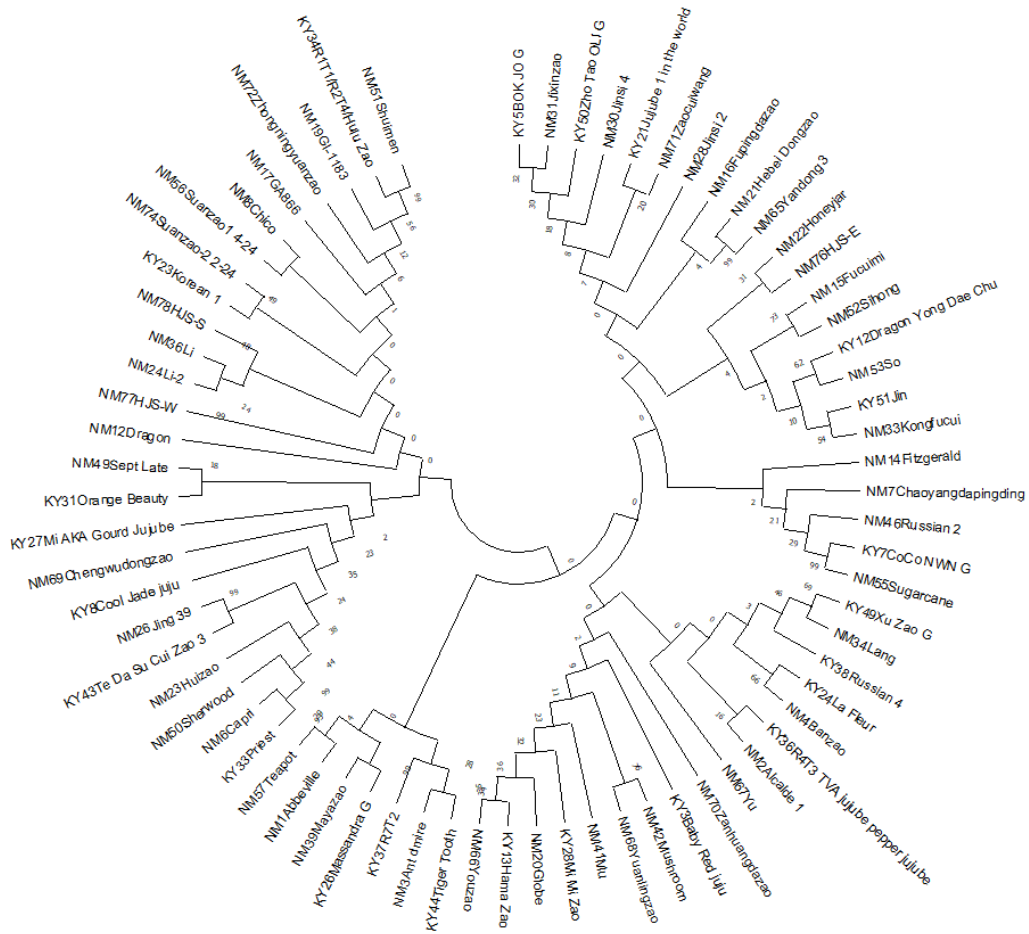
Group19: Fucumi (伏脆蜜) and Ba Ba Hong (88 红) were identical.

Group20: Russian 2 and Black Sea were synonyms.

Phylogenetic tree with all accessions:



**2019 Phylogenetic tree with no replicates without cultivar Bulusu:**



The technology of DNA fingerprinting provides a powerful tool for jujube cultivar identification because it can identify synonym cultivars and separate cultivars into genetically related clusters. However, within each identified synonymous group, morphological characters will continue to be used to complement DNA fingerprinting in case there are morphological variations caused by mutation. Researchers and growers can use the results to identify jujube cultivars, improve nursery quality, and support jujube breeding and selection.

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