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Isozyme Identification of Japanese Persimmons (*Diospyros kaki* L.): Comparisons of Cultivars in California and Japan[1]

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Abstract

There has been considerable confusion concerning the identity of oriental persimmon cultivars (*Diospyros kaki* L.) in California. Most scion material used for propagation in recent years came from collections at the University of California South Coast Field Station (SCFS), the Wolfskill Experimental Orchards (WEO) at the University of California, Davis, or the United States Department of Agriculture (USDA) Plant Introduction Gardens at Chico, California. To verify cultivar identification, glucose phosphate isomerase (GPI) and PGM isozymes patterns were used to compare the cultivars at the SCFS and WEO with cultivars at Kyoto University, Kyoto, Japan. Pollination status and astringency were also used for evaluation of the SCFS cultivars. Several cultivars could not be compared since they are not present at Kyoto. However, among 108 trees in the UC collections the identity of 31 trees was verified and 13 mistakes were detected. Extensive isozyme variability was observed between cultivars. Isozyme analysis in persimmon was more difficult than for other species that we have studied (*Prunus, Vitis,* and *Juglans*) because of the large number of bands for GPI and PGM and the polyploid nature of *D. kaki.*

Introduction

Japanese persimmons have been grown in California since the 1870s (5). Many were imported from Japan and grown at the USDA Plant Introduction Gardens; others were collected from local orchards established by Japanese immigrants for their own use. Importation of oriental persimmons continued until 1919 when a quarantine was imposed by the U.S. government. The collection located at SCFS was repropagated from the one established earlier on the Westwood campus of the University of California, Los Angeles. The material for the collection at WEO probably came directly from the Chico Plant Introduction Gardens. These materials have formed the basis for a developing fresh fruit and shipping industry. There has been a growing awareness that nomenclature problems exist among these cultivars (4, 5). Errors in nomenclature were brought to our attention by both commercial and amateur growers. Identification of cultivars by their true name is important because the California State Department of Food and Agriculture has been approached to initiate a state marketing order for 'Fuyu' persimmons. The need for correct identification of these cultivars has become even more critical as plans were developed to include them in the National Clonal Germplasm Repository, at Davis, CA. Furthermore, mislabelled cultivars grown in California are being sent to other countries, spreading the nomenclature problem worldwide.

Traditional identification has made use of leaf and fruit characters. The most reliable fruit characters are astringency, flesh color with or without pollination, and to a lesser extent fruit shape. During the past 3 years the potential for characterizing persimmon cultivars with isoenzymes has been demonstrated (1, 7).

During the summer of 1987, we had an opportunity to compare the cultivars at the South Coast and Wolfskill orchards with cultivars of the same name at Kyoto, Japan. Both isozyme

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and fruit characters were used to compare these cultivars. A description of commercially important cultivars and some of the errors in California nomenclature are provided in Ryugo, et al. (4). The present paper provides a complete characterization of the South Coast Field Station and Wolfskill Experimental Orchard materials as well as the correct names. This information should be especially useful to the scientists and growers that used these materials for research or propagation.

Materials and Methods

Six to eight fruit from each tree at SCFS were collected in September and evaluated for the criteria of Hume (2). Cultivars were identified as being a) pollination constant and astringent (PCA), b) pollination constant and nonastringent (PCNA), c) pollination variant and astringent (PVA), or d) pollination variant and nonastringent (PVNA). Flesh of the fruit does not turn color after pollination and seed formation if the cultivar is pollination constant.

Two enzyme systems were used in this study, glucose phosphate isomerase (GPI) and phosphoglucomutase (PGM). The procedures are described by Tao and Sugiura (7). Mature leaves were collected at the Wolfskill Experimental Orchards (WEO) on 29 May and at the South Coast Field Station (SCFS) on 18 June and stored at 0° to 4°C until used. The procedures described in this paper are somewhat different than those used for isozyme analysis of most plants, and mature leaves provide better band resolution using these methods than younger leaves. 20 cm^2 of leaf tissue were macerated with a Polytron blender in 10 ml of 50 mM trismaleate (pH 8.5) buffer containing 20% glycerol, 5% soluble polyvinylpyrrolidone (PVP-40), 0.5% Triton X-100, and 10 mM 2-mercaptoethanol. Electrophoresis was carried out in 12% horizontal starch slab gels. Discontinuous tris-citrate/lithiumborate (pH 8.3) and continuous histidine (pH 6.5) gel buffers were used for GPI and PGM, respectively. The gels were electrophoresed at 200 volts (GPI) or 300 volt (PGM) for 3 hrs and sliced horizontally into three 2 mm slabs. The middle slab was retained and stained.

GPI gels were stained for 1 hr with 100 ml of pH 7.5, 0.1M tris-HCl buffer with 1 ml 1M $MgCl_2 \cdot 6H_2O$, 80 mg fructose-6-phosphate (disodium salt), 20 mg MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), 20 mg NADP (nicotinamide adenine dinucleotide phosphate), 4 mg PMS (phenazine methosulphate), and 20 units of glucose-6-phosphate dehydrogenase. PGM gels were stained for 1 hr with 100 ml of pH 7.5, 0.1M tris-HCl buffer with 1 ml 1M $MgCl_2 \cdot 6H_2O$, 150 mg glucose-1-phosphate (disodium salt), 20 mg MTT, 15 mg NADP, 4 mg PMS, and 20 units of glucose-6-phosphate dehydrogenase. Banding patterns were recorded on graph paper and compared to the staining patterns for cultivars in the Kyoto, Japan collections (7). Identical procedures were used to test materials in California and Japan. Additional enzyme staining systems including AAT (aspartate amino transferase), MDH (malate dehydrogenase) and GDH (glutamate dehydrogenase) were tested but did not provide consistently clear results.

Results and Discussion

Twenty-one isozyme patterns were obtained after staining for GPI (Figure 1). These patterns correspond to the 24 patterns reported by Tao and Sugiura (7) as follows.

1 = new pattern	12 = new pattern
2 = A	13 = N
3 = B	14 = U
4 = C	15 = new pattern
5 = D	16 = T
6 = new pattern	17 = S
7 = new pattern	18 = Q
8 = O	19 = R
9 = M	20 = K
10 = new pattern	21 = L
11 = G	

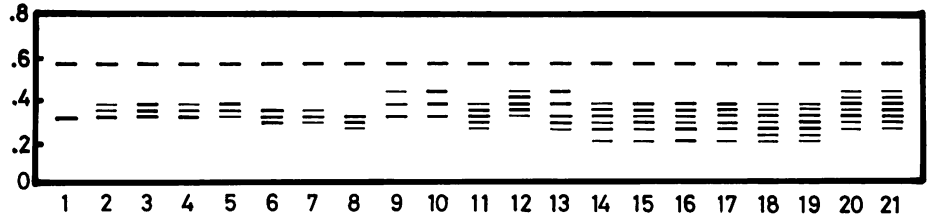

Figure 1. GPI banding patterns observed in persimmon trees of UC collections with relative mobilities. Anode is at the top.

Nine of the patterns (E, F, H, I, J, O, P, V, W) found by Tao and Sugiura (7) were not present for the cultivars in the California collections. Six new patterns not described by Tao and Sugiura (7) were observed.

Figure 2 shows the 24 patterns that were observed after staining for PGM. Examples of GPI and PGM patterns are shown in Figure 3. GPI and PGM patterns with correct names for cultivars in the SCFS and WEO collections are shown in Tables 1 and 2, respectively. Fruit and pollination characteristics for cultivars at the SCFS are shown in Table 1. Pollination status of some cultivars at WEO, as determined from past observations, are given in Table 2. Since environmental conditions at WEO are different than those at SCFS or in Japan, these results may not be typical of cultivar performance elsewhere. Several cultivar lists in the USDA Plant Inventory have associated data on astringency collected in Japan (8, 9, 12, 13).

Isozyme analysis is more difficult for persimmon than for many other plant species because of the large number of bands for GPI and PGM and the polyploid nature of *D. kaki*. However, the many possible band patterns provide an opportunity for relatively unique identification. Of the 48 genotypes surveyed at SCFS, 35 have unique combinations of GPI and PGM patterns. Seven pattern combinations were represented twice and only one pattern combination occurred four times.

Sixteen cultivars were represented in both the California and Japanese collections. Of the 108 trees in the University of California collections (many cultivars were replicated several times), only 43 could be compared to Japanese cultivars. The others had unique isozyme patterns or nomenclature. Three named *D. virginiana* L. cultivars are also present at WEO (Table 2).

Two types of 'Fuyu' were identified in the California collection. They were not easily distinguished by fruit characters. However, the 'Fuyu' that bears male flowers (R2-T14 at SCFS and R22-T9 at WEO) is not 'Fuyu' as determined by isozyme analysis. This 'California Fuyu' is probably the Japanese

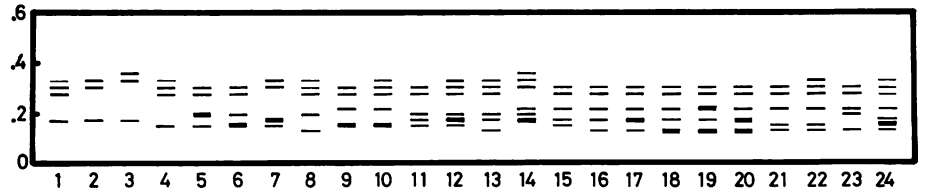

Figure 2. PGM banding patterns observed in persimmon trees of UC collections with relative mobilities. Anode is at the top.

Table 1. Plant locations, California and Japanese names, fruit characters, and isozyme pattern numbers for persimmons at South Coast Field Station.

California name	Row-Tree	Fruit type[6]	Japanese name	GPI[4]	PGM[5]
Akadango (PI 85699)	R1-T20	PC	NI	11	21
Blackman Hachiya	R2-T8	PCA	Hachiya	2	17
Chien Ting	R2-T5	PVNA	NI	18	19
C18166	R2-T22		NI	8	7
C26902	R2-T20		NI	15	5
Dai Dai Maru	R1-T6	PVNA	NI	19	5
D. olerifera L. (PI 92995)	R1-T19			3	6
Eboshi (PI 91496)	R2-T21	PC	Eboshi	14	11
Fresno Hachiya	R1-T10	PCA	Hachiya	2	17
Fuji	R2-T7	PCA	Fuji[7]	2	17
Fuyu (PI 72662)	R1-T15	PCNA	Fuyu	20	10
Fuyu (C26491)	R2-T14	PCNA	not Fuyu[8]	7	9
Gosho	R2-T15	PCNA	Gosho	8	17
Hachiya	R1-T9	PCA	Hachiya[9]	2	17
Hana Fuyu (PI 83709)	R2-T17&18	PCNA	probably Mikado	11	18
Hiragaki	R1-T16	PCA	Hiragaki	4	20
Honan Red	R1-T14	PCA	NI	3	1
Hyakume	R2-T6	PVNA	Ama-Hyakume	17	23
Jiro (C24276)	R2-T13	PCNA	Jiro	20	4
Jiro	R2-T24	PVNA	*not* Jiro	14	16
Kawabata (PI 71946)	R1-T1	PVNA	probably *not* Kawabata	8	17
Kishimoto	R1-T3	PCA	NI[10]	5	1
Kurokuma	R1-T18	PVNA	NI	14	16
Maru	R1-T2	PVNA	Zenji Maru[11]	11	16
Mazugata	R1-T4	PVNA	NI	3	17
Midzushima (PI 42562)	R2-T23	PVNA	Midzushima	21	9
Mishirazu	R2-T12	PV	probably *not* Mishirazu	7	9
Nui Nai	R2-T11	PCA	NI	9	20
O-Gosho	R2-T16	PCNA	*not* O-Gosho[12]	8	17
Otani	R2-T4	PC	NI	11	19
Otera	R2-T9	PC	NI	3	20
PI 29102	R2-T1		NI	11	11
PI 32871 (Kubo)	R1-T21	PC	NI	7	15
PI 83784	R2-T25		NI	8	9
Saijo	R2-T10	PCA	Saijo	4	13
San Pedro	R1-T22		NI	4	9
Sinensis	R1-T23	PVNA	NI	4	16
Tamopan	R1-T13	PCA	Tamopan	3	1
Tanenashi	R1-T12	PCA	NI	4	16
Tishihtzu (PI 58971)	R2-T2	PCA	NI	10	13
Tribble	R1-T7	PVNA	NI	11	16
Tsuru[13]	R1-T11	PCA	NI	14	9
Wase Jisha (PI 91513)	R2-T27		probably *not* Wase Jisha[14]	16	8
Yamagaki	R2-T3	PVNA	NI	3	24
Yeddo Ichi	R1-T5	PVNA	NI	19	5
Yemon	R1-T8	PVA	probably *not* Yemon	4	9
Yen Shih Tzu(PI 37951)	R2-T19		NI	4	2
20th Century (PI 72960)	R2-T26		*not* 20th Century[15]	16	8

Table 1. (Continued).

[4]Glucose phosphate isomerase
[5]Phosphoglucomutase
[6]PCA = pollination constant, astringent
PVA = pollination variant, astringent
PCNA = pollination constant, nonastrigent
PVNA = pollination variant, nonastringent
[7]syn. 'Hachiya'
[8]probably 'Oku-Gosho'; syn. 'California Fuyu' [Ryugo, et al. (4)]
[9]syn. 'Koshu-Hyakume'
[10]not identified
[11]syn. in U.S., 'Maru'
[12]probably 'Gosho'
[13]syn. 'Tsuru-no-ko'
[14]same as R2-T26
[15]probably the same as R2-T27

Table 2. Plant locations, California and Japanese names, fruit characters, and isozyme pattern numbers for persimmons at Wolfskill Experimental Orchard.

California name	Row-Tree	Fruit type[18]	Japanese name	GPI[16]	PGM[17]
Chien Ting	R11&12-T7	PV	NI[19]	18	19
Dai Dai Maru	R19-T10	PV	NI	19	5
Early Golden (*D. virginiana* L.)	R11-T10			11	2
Fen Nio	R24-T10		NI	4	5
Fuji	R23&24-T9	PC	Fuji	2	17
Fuyu	R21-T9	PV	Jiro	20	4
Fuyu	R22-T9	PC	not Fuyu[20]	7	9
Gosho Lotus (PI 78502)	R19&20-T9		Gosho[21]	8	17
Hachiya	R11&12-T9	PC	Hachiya	2	17
Hiragaki	R18-T10		Hiragaki	4	20
Honan Red	R21&22-T8	PC	NI	3	1
Hyakume	R13-T8	PV	Ama-Hyakume	17	23
Jiro (PI 83790)	R21&22-T7	PV	*not* Jiro[22]	14	16
Jumbu	R15&16-T9	PC	probably Mikado	11	18
Kishimoto	R23&24-T8	PC	NI	5	1
Maru	R13&14-T9	PV	Zenji Maru	11	16
Miller (*D. virginiana* L.)	R16-T10			7	2
Mishirazu	R15-T7		probably *not* Mishirazu	11	18
Mishirazu	R16-T7		probably *not* Mishirazu	7	9
Nui Nai	R19&20-T8	PC	NI	9	20
Okame	R21-T10	PC	NI	19	5
Otera (PI 37177)	R17-T10		*not* Otera[23]	1	2
PI 32886	R13&14-T7	PC	NI	12	12
PI 59343	R23-T10		NI	4	22
PI 59344	R15-T10		*not* PI 59344[24]	6	2
Ruby (*D. virginiana* L.)	R12-T10			13	14
Saijo	R13&14-T10, R12-T8	PC	Saijo	4	13
Tamopan	R19&20-T7	PC	Tamopan	3	1
Tishihtzu (PI 58971)	R17&18-T8		NI	10	13
Tribble	R17-T7		NI	11	16
Tsuru	R23&24-T7	PC	NI	14	9
Yeddo	R22-T10		NI	19	5

[16]glucose phosphate isomerase
[17]phosphoglucomutase
[18]PC = pollination constant
PV = pollination variant
[19]not identified
[20]probably 'Oku-Gosho,' syn. 'California Fuyu'
[21]PI 78502 is listed as 'Gosho' in the PI records.
[22]syn. 'California Maru' [Ryugo, et al. (4)]
[23]*D. virginiana* L. rootstock
[24]*D. virginiana* L. rootstock

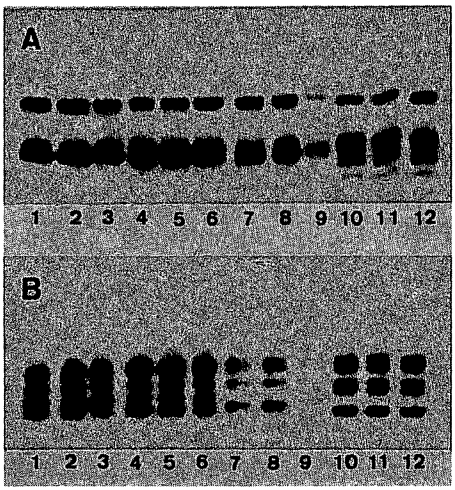

Figure 3. Example of isozyme patterns for GPI (A) and PGM (B). 1-3, 'Hachiya' (R11-T9 in WEO); 4-6, 'Maru' (R13-T9 in WEO); 7-9, 'Cal-Fuyu' (R22-T9 in WEO); 10-12, 'Hyakume' (R13-T8 in WEO).

cultivar 'Oku-Gosho.' The 'Fuyu' tree R21-T9 at WEO is 'Jiro.' Tree R1-T15 at SCFS is the true 'Fuyu.'

'Gosho Lotus' (R19-T9 and R20-T9 at WEO) is 'Gosho.'

'Hachiya' is the same cultivar in Japan and California. 'Fuji' trees (at SCFS and WEO) are also 'Hachiya' trees. 'Fresno Hachiya' and 'Blackman Hachiya' (SCFS) also have isozyme patterns identical to 'Hachiya' and are probably synonymous. Both 'Hachiya' and 'Fresno Hachiya' are pollination constant although some discoloration may occur around the seeds.

'Hana Fuyu' at SCFS is the same as 'Jumbu' at WEO. Neither of these names is used in Japan. This cultivar is probably the same as the Japanese cultivar 'Mikado.' 'Hanan Fuyu' was imported in 1929 from the Narusada fruit farm in Fusan, Korea (12).

California 'Hayakume' is the same as 'Ama-Hyakume' in Japan.

'Jiro' at R2-T13 (SCFS) is correctly identified. However, trees at R2-T24 (SCFS), R21-T7 (WEO), and R22-R7 (WEO) are not 'Jiro.' These trees are pollination variant while 'Jiro' is pollination constant.

'Kawabata' in California is not the same 'Kawabata' in Japan.

'Maru' is called 'Zenji Maru' in Japan.

Two types of 'Mishirazu' are present in the Kyoto collection, 'Aizu-Mishirazu' and 'Sakushu-Mishirazu.' Neither of these selections have isozyme patterns consistent with the California cultivar. The California 'Mishirazu' is incorrectly named.

'O-Gosho' (Japan) is not the same as the California 'O-Gosho.' The California selection is probably mislabelled. This is also true for 'Wase Jisha' and 'Yemon.'

The 'Otera' trees are not the same at SCFS and WEO. The tree at WEO appears to be a *D. virginiana* from leaf, bark, and fruit morphology. Thus, it is likely that an error during propagation resulted in a rootstock tree at WEO.

'20th Century,' described as a cultivar with large, flat, sweet fruits, was introduced in 1927 from the Yokohama Nursery Co. in Japan (11). It was reported to be nearly identical to 'Fuyu' (6). However, tree R2-T26 at SCFS ('20th Century') is identical to R2-T27 labelled (probably incorrectly) 'Wase Jisha.' Thus, the cultivar '20th Century' may no longer exist or the California 'Wase Jisha' could be '20th Century.' Neither tree is comparable to 'Fuyu.' There is no '20th Century' Japanese persimmon cultivar.

Cultivars 'Eboshi,' 'Hiragaki,' 'Midzushima,' 'Saijo,' and 'Tamopan' are correctly named and are the same in California and Japan.

A number of California cultivars could not be checked against Japanese cultivars since corresponding selections were not available at Kyoto. Thirteen errors were detected in the California collections. However, correct names could not be assigned to many of these selections. Persimmon cultivars in other parts of the United States (3)

are also likely to be misidentified if they were originally propagated from California sources. It is also worth noting that these problems are not limited to the U.S. and that terminology for persimmon cultivars in Japan is not always clear or consistent.

Nomenclature problems have arisen in several ways. Growers, nurserymen, and marketers have assigned new or generic names to cultivars to promote sales of plants or fruit. This can take the form of name simplification or in some cases the assignment of entirely new names. Mistakes may have also occurred during propagation through mislabelling. The occurrence of sports is probably rare. Labelling or renaming problems can be confounded by multiple introductions of the same cultivar. 'Fuyu' has been introduced at least four times with six different PI numbers (8, 9, 10, 12). Many other cultivars have been introduced under several PI numbers. While the present study has not answered all of identity problems, it shows that some confusion exists and provides correct identities for many of the cultivars available in California.

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