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RESEARCH ARTICLE

Relationship of European persimmon (*Diospyros kaki* Thunb.) cultivars to Asian cultivars, characterized using AFLPs

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Abstract Sixty one persimmon (*Diospyros kaki* Thunb.) selections, including 17 Italian, 11 Spanish, 13 Japanese, six Korean, five Chinese, one Israeli, and eight of unknown origin, were evaluated for genetic differences by AFLP analysis. Relationships among cultivars were evaluated by UPGMA clustering, Neighbor Joining, and MultiDimensional Scaling. While similarities among groups were generally less than 0.60, both UPGMA and Neighbor Joining separated European and Asian cultivars. Spanish and Italian cultivars were not separated by any of the analyses, suggesting that they share a common gene pool, while Japanese, Chinese and Korean cultivars formed distinct clusters. Diversity within groups was greater than diversity between groups. Most cultivars were quite polymorphic (only 0.60–0.80 similarity between cultivars). In addition, the presence of several Japanese cultivars in the European group and a group of European cultivars nested between Chinese and Korean groups suggest that similar, but different progenitors were used in the development of the present European cultivars. 'Kaki Tipo' selections from different sources were clearly different by AFLP analysis, indicating that they are separate cultivars.

Keywords AFLP · Cluster analysis · Dendrogram · *Diospyros kaki* · Molecular markers

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Introduction

Persimmons (*Diospyros kaki* Thunb.) have been grown in Japan for several hundred years. Persimmons were cultivated in 10th century in Japan and persimmon cultivar names appeared in 17th century Japanese literature (Kikuchi 1948).

Persimmons are believed to have originated in China and were an important food source in China, Korea, and Japan from prehistoric times. Currently, it is one of the most important fruit crops in Asian countries. In 2005, persimmon production was 1,837,000 t in China, 250,000 t in Korea, and 230,000 t in Japan (FAO 2006). Persimmons were not commonly grown in European countries until the 20th century, although the related species (*D. lotus* L.) was probably present in the Roman Empire (Bellini and Giordani 2005). Cultivated persimmons (*D. kaki* or *D. lotus*) were mentioned once in 17th century in European literature (Bellini and Giordani 2005). Persimmon trees were imported to France in 1860 and to Italy in 1870 (Bellini and Giordani 2005). In Italy, the first persimmon tree was planted in the Boboli Garden in Florence. The Fratelli Ingegnoli in Milan had distributed persimmon trees throughout Italy by 1884 (Bellini and Giordani 2005). Persimmon appeared in Spain by the end of 19th century.

Italy is the main producer of persimmon in Europe. The first production orchards were planted in the Campania Region (Salerno) at the beginning of the 20th century. The main Italian cultivar is 'Kaki Tipo', a pollination variant non-astringent (PVNA) type (Yonemori et al. 2000).

The present study was undertaken to investigate (1) the relationship of Italian and Spanish cultivar groups, (2) the general level of diversity among European cultivars, and (3) the origin of 'Kaki Tipo', an important European cultivar.

Materials and methods

Plant materials

Persimmon germplasm collections were performed under the GENRES 29 Project on ''Conservation of minor fruit tree species'' during 1996–1999. Many persimmon cultivars were gathered, primarily in Italy, under this EC Project (Bellini and Giordani 2005). Cultivars of Spanish, Israel, and Asian origin were collected. The Israeli cultivar, 'Triumph' is a pollination variant astringent (PVA) type, and is widely cultivated in Israel for domestic and export use (Yonemori et al. 2000). Leaves of 17 Italian, 11 Spanish, 13 Japanese, 6 Korean, 5 Chinese, and one Israeli selections were obtained from collections in Italy and Japan (Table 1). All samples were lyophilized. Eight selections of unknown origin were also included in the analysis. Samples were collected from four locations in Italy: (1) Istituto Professionale Agricoltura e Ambiente in Faenza (Ravenna Province), (2) the IVALSA-CNR Institute in Scandicci (Firenze Province), (3) the Montepaldi Experimental Farm of the University of Florence in San Casciano Val di Pesa (Firenze Province), and (4) Istituto Sperimentale per la Frutticoltura of Caserta-C.R.A. (Caserta Province). Separate selections of 'Kaki Tipo' were collected from six locations, including local persimmon orchards, to determine if this cultivar is a clone or a group of cultivars with a common name. Samples were collected from two locations in Japan: (1) persimmon germplasm collections in Kyoto University of Kyoto, and (2) Department of Grape and Persimmon Research, National Institute of Fruit Tree Science, of Hiroshima. *Diospyros lotus* L. was used as the outgroup taxa. Countries of origin and collection sites for the 61 selections and *D. lotus* are shown in Table 1.

AFLP analysis

DNA extraction: 50 ng/μl total genomic DNA/sample were isolated from freeze-dried leaves with Nucleon Phytopure Plant DNA extraction kit (Amersham Biosciences) or by the CTAB method of Doyle and Doyle (1987) when needed.

Digestion and ligation: 5.5 μl of reaction mixture (1X T4 ligase buffer, 0.5 unit of T4 ligase, 50 μM of NaCl, 0.01% of BSA, 2.5 unit of *Eco*RI, 0.5 unit of *Mse*I, 0.5 μl *Eco*RI adaptor, 0.5 μl *Mse*I adaptor) and 5 ng of genomic DNA were incubated at 25°C overnight. The mixture was diluted 1:15 with TE buffer at the completion of incubation.

Pre-selective amplification: 20 μl of reaction mixture (1X PCR buffer, 0.2 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.6 pmol of each pre-selective primer {*Mse*I+C [GAT GAG TCC TGA GTA AC] and *Eco*RI+A [GAC TGC GTA CCA ATT CA]}, 1U of DNA polymerase), and 4 μl of the diluted digestion-ligation mixture were subjected

Table 1 61 European and Asian persimmon accessions used for UPGMA, Neighbor Joining, and Multidimensional scaling analyses

Cultivar	Origin	Sampling location	Group[a]	Astringent type[b]	Notes
Bikengyushinshi	China	Hiroshima, Japan		PCA	
Kokushinshi	China	Hiroshima, Japan		PCA	
Koukyakushi	China	Hiroshima, Japan		PCA	
Kyokuseisuishi	China	Hiroshima, Japan		PCA	
Mabanshi	China	Hiroshima, Japan		PCA	
Triumph	Israel	Scandicci, Italy		PVA	
Brazzale	Italy	Faenza, Italy	2	PVNA	
Castellani	Italy	Faenza, Italy	2	PVNA	
Cardinale Maglione	Italy	Faenza, Italy	1	PCA	
Cioccolatino	Italy	Scandicci, Italy	2	PVNA	
Costata	Italy	Faenza, Italy	1	PCA	Synonym of 'Cardinale Maglione'
Kaki Tipo 1	Italy	Faenza, Italy	2	PVNA	'Kaki Tipo' with typical fruit shape
Kaki Tipo 2	Italy	Faenza, Italy	2	PVNA	'Kaki Tipo' from old tree
Kaki Tipo 3	Italy	Acerra 7 (Acerra road side), Italy	2	PVNA	'Kaki Tipo' with typical fruit shape
Kaki Tipo 4	Italy	Acerra 6 (Acerra in Via de Gasperi), Italy	2	PVNA	'Kaki Tipo' with typical fruit shape
Kaki Tipo 5	Italy	Roadside near Baiano between Avellino and Napoli, Italy	2	PVNA	Probable progeny or mutation of 'Kaki Tipo'
Kaki Tipo 6	Italy	Roadside between Ravello and Valico di Chiunzi, Italy	2	PVNA	Probable progeny or mutation of 'Kaki Tipo'
Lampadina	Italy	Parolise, Italy	2	PVNA	
Mercatelli	Italy	Faenza, Italy	2	PVNA	
Moro	Italy	Faenza, Italy	2	PVNA	
Rispoli	Italy	Faenza, Italy	2	PVNA	
Thiene	Italy	Faenza, Italy	2	PVNA	
Vainiglia	Italy	Parolise , Italy	2	PVNA	
Bruniquel	Italy?	Faenza, Italy	2	PVNA	Questionable origin
Farmacista Honorati	Italy?	Faenza, Italy	?	PCA	Questionable origin
Fennio	Italy?	Faenza, Italy	2	PCA	Questionable origin
Mandarino	Italy?	Faenza, Italy	2	PVNA	Questionable origin
Amahyakume	Japan	Kyoto, Japan		PVNA	
Fuyu	Japan	Scandicci, Italy		PCNA	
Gosho	Japan	Hiroshima, Japan		PCNA	
Hanagosho	Japan	Kyoto, Japan		PCNA	
Jiro	Japan	Kyoto, Japan		PCNA	
Koshuhyakume	Japan	Kyoto, Japan		PVA	
Kurokuma	Japan	Faenza, Italy		PVNA	
Monpei	Japan	Kyoto, Japan		PVA	
Saijo	Japan	Kyoto, Japan		PCA	
Shogatsu	Japan	Kyoto, Japan		PVNA	
Yamatogosho	Japan	Hiroshima, Japan		PCNA	
Yokono	Japan	Kyoto, Japan		PCA	
Zenjimaru	Japan	Kyoto, Japan		PVNA	
Akoumankaki	Japan?	Faenza, Italy		PVNA	Questionable origin. Not found in Japan
Amankaki 1	Japan?	Montepaldi, Italy		PVNA	Questionable origin. Not found in Japan

Table 1 continued

Cultivar	Origin	Sampling location	Group[a]	Astringent type[b]	Notes
Amankaki 2	Japan?	Parolise, Italy		PVNA	Questionable origin. Not found in Japan
Hirotakaki	Japan?	Scandicci, Italy		PVNA	Questionable origin. Not found in Japan
Banshi	Korea	Hiroshima, Japan		PCA	
Houkikoushushi	Korea	Hiroshima, Japan		PCA	
Kouraisuishi	Korea	Hiroshima, Japan		PCA	
Koushushi	Korea	Hiroshima, Japan		PCA	
Seidoushi	Korea	Hiroshima, Japan		PCA	
Suishi	Korea	Hiroshima, Japan		PCA	
Anheca	Spain	Montepaldi, Italy	2	PCA	
Betera 1	Spain	Montepaldi, Italy	2	PVA	
Betera 2	Spain	Montepaldi, Italy	2	PVA	
Betera 3	Spain	Montepaldi, Italy	2	PVA	
Castanti 13	Spain	Montepaldi, Italy	2	PVA	
Enguera 1	Spain	Montepaldi, Italy	1	PCA	
La Selva	Spain	Montepaldi, Italy	2	PVA	
Picudo	Spain	Montepaldi, Italy	1	PCA	
Rojo Brillante	Spain	Montepaldi, Italy	2	PVNA?	
Tomatero	Spain	Montepaldi, Italy	2	PCA	
Xato	Spain	Montepaldi, Italy	?	PVA	
D. lotus		Campania Region, Italy			Used as outgroup species

[a] European cultivar groups from Neighbor Joining analysis.

[b] Astringent type: PCNA is pollination constant non-astringent type, PCA is pollination constant astringent type, PVNA is pollination variant non-astringent type, and PVA is pollination variant astringent type (Yonemori et al. 2000).

to 20 cycles of 94°C for 30 s, 56°C for 25 s and 72°C for 60 s. After pre-selective PCR, amplification was verified by agarose gel electrophoresis, reaction mixtures were diluted 8 × or 15 × with Tris–EDTA (TE) buffer (pH 8.0), depending on product concentration.

Amplification: Aliquots were amplified using six combinations of Label + *Eco*RI + 3 and *Mse*I + 3 primers: FAM-E-ACA + M-CTA, HEX-E-ACG + M-CAG, NED-E-ACC + M-CTA, FAM-E-ACT + M-CAG, HEX-E-AGG + M-CAG, or NED-E-AGC + M-CTA. 10 µl of reaction mixture (0.5U of DNA polymerase, 3.3 pmol of each primer, 0.2 mM of each dNTP, 2.5 mM of $MgCl_2$, 1X PCR buffer) were added to 1.5 µl aliquots of the pre-amp mixture, and subjected to 94°C for 10 min; 10 cycles of 94°C for 20 s, 66°C for 30 s, reduced by 1°C per cycle, and 72°C for 2 min; 20 cycles of 94°C for 20 s, 56°C for 30 s and 72°C for 2 min; followed by final extension at 60°C for 30 min. Size standards (GS500ROX, Applied Biosystems) were added and the amplified products separated on an ABI 3100 sequencer.

Data analysis

About 470 AFLP bands were scored as present (1) or absent (0) by Genotyper software (Applied Biosystems), and manually verified. Of 390 polymorphic putative loci, 281 were informative for parsimony analysis. Data were evaluated using the index of Nei and Li (1979). Neighbor Joining analyses with outgroup rooting (*Diospyros lotus* as the outgroup) were performed with both NTSYS 2.0 (Rohlf 1998) and PAUP v.4.0 b10 (Swofford 1998). UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis and a Multidimensional Scaling analysis (MDS) were also performed on the data set with NTSYS 2.0.

Results and discussion

Neighbor Joining analysis provided the most logical arrangement of cultivar relationships. UPGMA clustering from NTSYS 2.0 gave cultivar relationships that were generally similar to

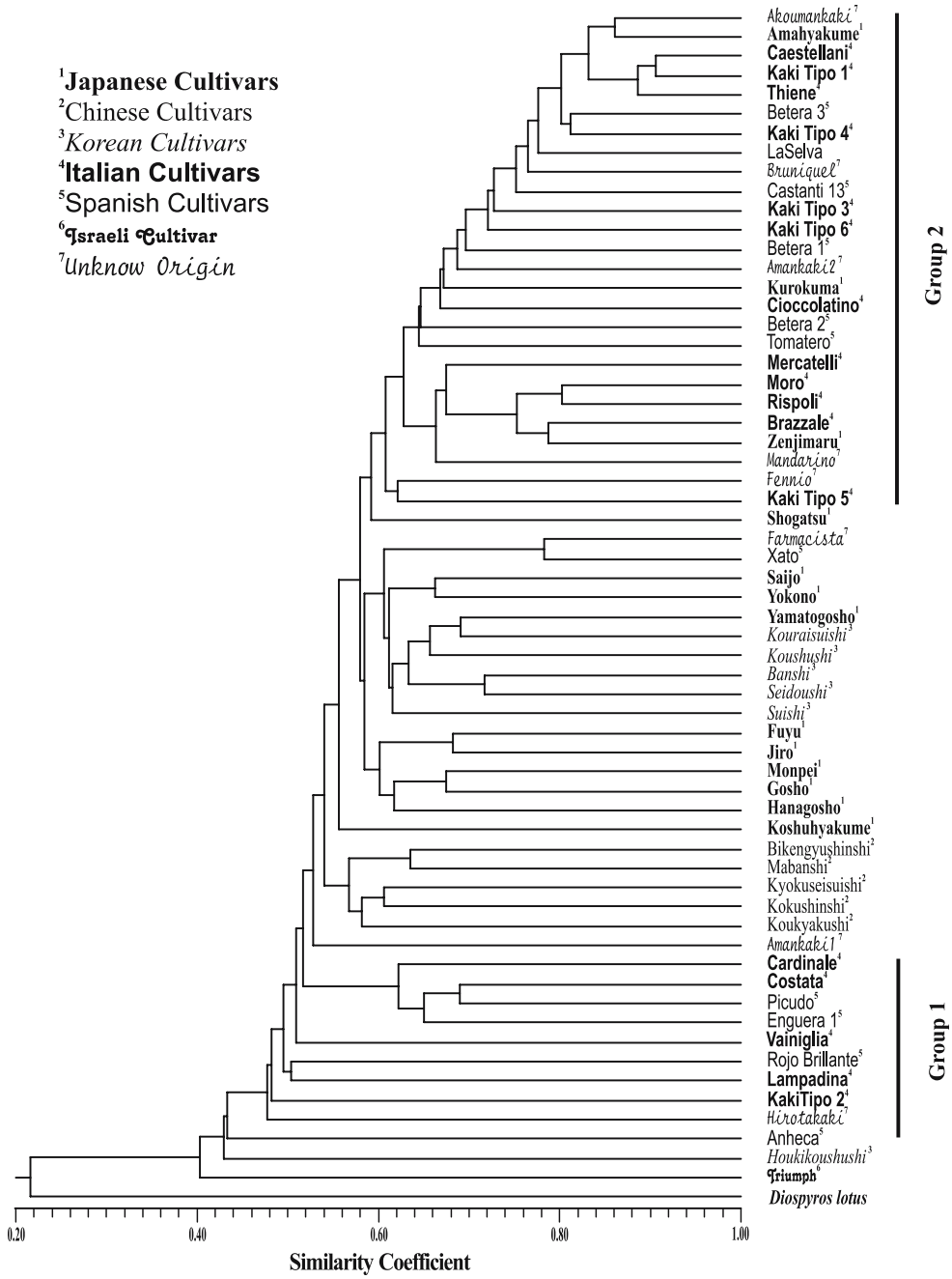

Fig. 1 UPGMA cluster analysis for 61 persimmon cultivars and *D. lotus*. Cultivar origins are indicated by differences in fonts and superscripts

those derived from Neighbor Joining (Figs. 1 and 2). The Neighbor Joining analyses from NTSYS (not shown) and PAUP were similar but not identical. Nei and Li's similarity indices were used for both. The Multidimensional Scaling analysis (a method similar to principal components analysis) showed a central cluster of Italian-Spanish cultivars (Group 2) with two groups of less related genotypes, one composed of primarily Italian-Spanish cultivars (Group 1)

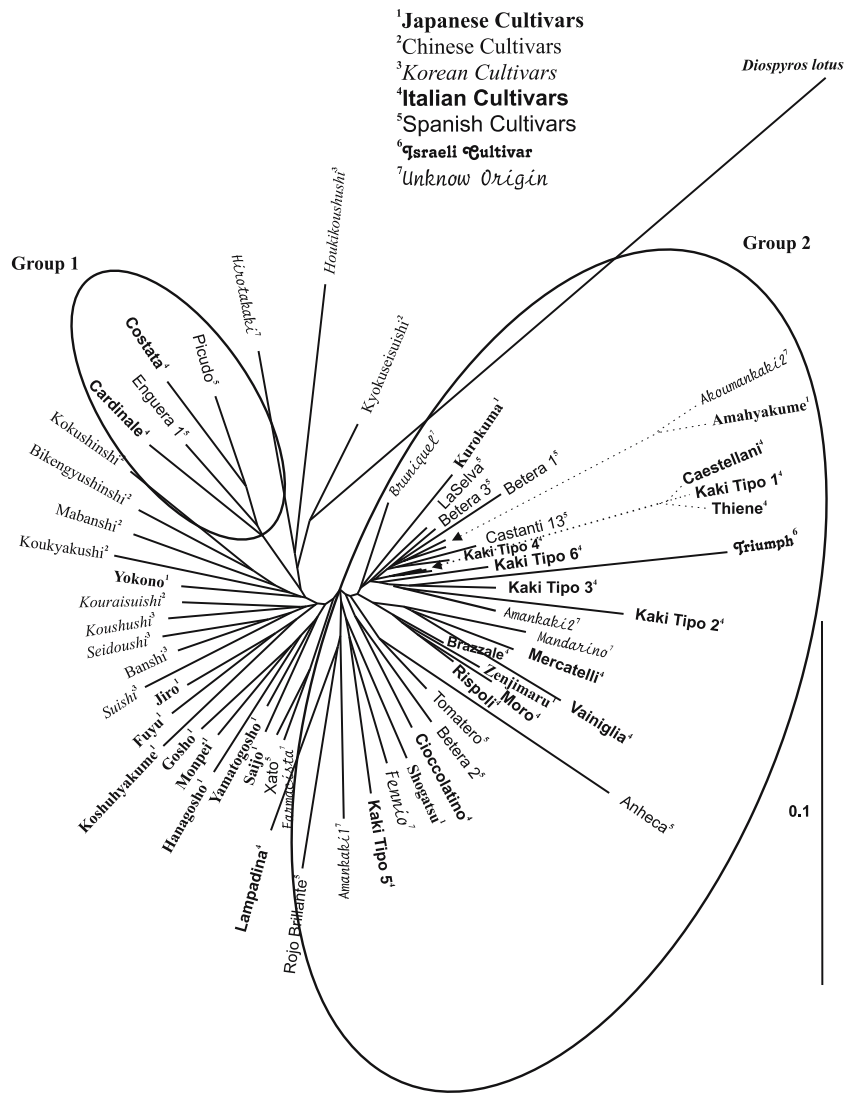

Fig. 2 Phylogram from Neighbor Joining analysis from PAUP v4.0 b10 for 61 persimmon cultivars and *D. lotus*. Cultivar origins are indicated by differences in fonts and superscripts

and the other representing the Asian group of cultivars. This can be seen most clearly in the two dimensional inset for the MDS figure (Fig. 3). The first two dimensions (X and Y axis) are shown as if looking at the three dimensional figure from above. The Asian cultivars are well separated from each other, suggesting considerable genetic diversification, but are separate from the Group 1 European cultivars, which are also well dispersed but which do not overlap with the Asian group. Cultivars historically classified as Korean, Chinese, and Japanese were separately but jointly clustered (Figs. 1 and 2), suggesting that historical records for most of these materials are correct and that the North Asian group of cultivars share a common gene pool.

Differences in computational methodology often produce different trees. This is likely to occur when many OTUs (operational taxonomic units) share different sets of scored characters and when one set of characters is favored by a particular analytical method. Neighbor Joining produces results similar to parsimony analysis in general and is considered to provide a more accurate representation of OTU relationships than UPGMA analysis (Kim et al. 1993). One of the characteristics of the present data set is the high level of unclassified variation (not

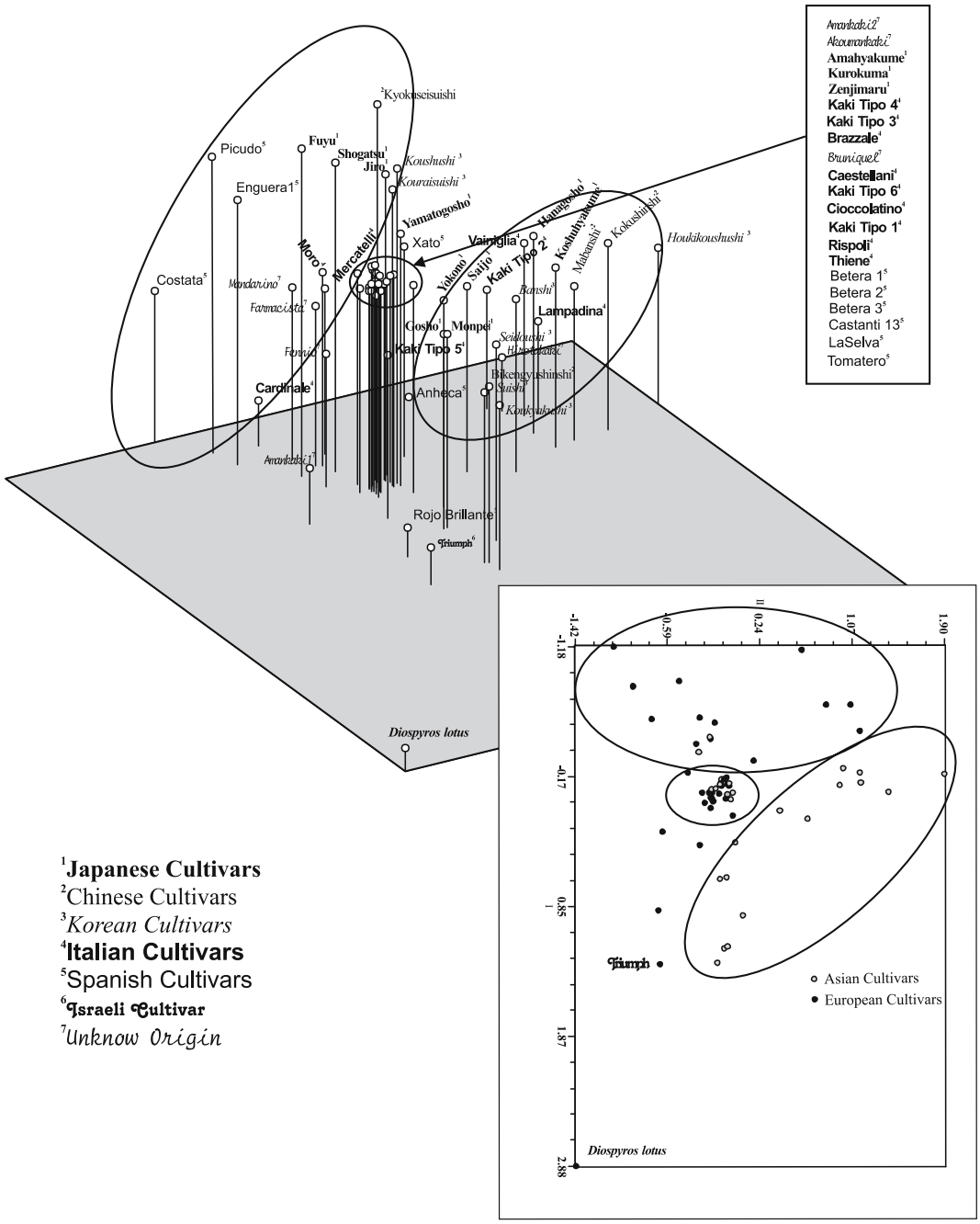

Fig. 3 Multidimensional scaling analysis from NTSYS 2.0. A three dimensional figure is shown, representing the 1st three coordinates. The first 2 dimensions are also shown in the lower right inset figure, showing the relative placement of the Asian cultivars to the European cultivars

useful for grouping) found among the persimmon cultivars.

Both Neighbor Joining and UPGMA analyses suggest that European cultivars may be divided into two distinct groups based on DNA polymorphisms. As noted previously, several cultivars have marker states that may place them in different groups depending on the method used

to create the associations. Although they were not delineated by Italian or Spanish identity, there were two groups of European cultivars in both UPGMA and Neighbor Joining analyses. In the Neighbor Joining tree, Group 1 includes 'Cardinale', 'Costata', 'Picudo', and 'Enguera' (Fig. 2). Group 1 cultivars are all pollination constant astringent (PCA) type cultivars (Table 1). The remaining cultivars are found in Group 2. UPGMA and Neighbor Joining differ in their placement of 'Vainiglia', 'Rojo Brilliante', 'Lampadina', 'Anheca', and 'Kaki Tipo 2' among European cultivars. UPGMA analysis places these cultivars in Group 1 while Neighbor Joining analysis places them in Group 2. Except for 'Anheca', these cultivars are not PCA types, typical of Group 1. It is probably appropriate to think of the cultivars within Groups 1 and 2 as members in common gene pools.

Much of the preceding discussion has been focused on the placement of cultivars into relationship groups. However, Figs. 1 and 2 clearly show that most of the observed polymorphisms were at the cultivar level and that differences among cultivar groups are much less significant than differences among individual cultivars. This result may be due to a high level of selection from a diverse germplasm base and/or the large number of characters scored. Badenes et al. (2003) found larger differences among cultivar groups than among associated cultivars. This could be the result of fewer scored polymorphisms (28) used in that study.

The RAPD dendrogram of Bellini et al. (2003), derived from 142 markers, showed a within vs. between cultivar variation distribution similar to that in the present study.

Group 2 European cultivars share common polymorphisms with three Japanese cultivars, 'Amahyakume', 'Kurokuma', and 'Zenjimaru'. The morphological characteristics between Group 2 Italian cultivar 'Kaki Tipo' and Japanese cultivar 'Amahyakume', and between Italian cultivar 'Moro' and Japanese cultivar 'Zenjimaru' are very similar. Group 2 cultivars, including 'Kaki Tipo' may either have been developed from common Japanese progenitors or that the Japanese cultivars found with Group 2 were used in the development of this group of cultivars. Group 2 may represent cultivars developed after trading relationships were developed between Europe and Japan and more organized plant improvement efforts were initiated.

'Mandarino', 'Mercatelli', and 'Moro' also appear to be associated with the Group 1 cultivars in the MDS analysis (Fig. 3), but were found with the Group 2 cultivars in the UPGMA and Neighbor Joining analyses. This may be a result of similarity among a subset of OTUs as seen in the MDS analysis where similarities in the third dimension (Z axis) brought 'Mandarino', 'Mercatelli', and 'Moro' closer to the other Group 2 cultivars. 'Farmacista Honorati' and 'Xato' do not appear to be closely associated with either group, but are associated with a group of Japanese and Korean cultivars. The Israeli cultivar 'Triumph' had a unique AFLP genetic profile compared to the other cultivars. The origin of this cultivar is likely to be different than for other European cultivars.

The origin of the important Italian cultivar 'Kaki Tipo' is of particular interest to horticultural scientists. The 6 'Kaki Tipo' selections that were tested are distinctly genetically different and should not be classified as a single cultivar. The level of diversity among these selections is similar to that for the entire European cultivar group, suggesting that these cultivars do not share a common origin beyond that of the European group in general. However, the different 'Kaki Tipo' selections share some morphological characters. The name 'Kaki Tipo' was probably applied to persimmon selections for marketing purposes rather than to uniquely delineate these cultivars. Italian cultivars are thought to be introduced directly from Japan or indirectly though North American in 19th century (Bellini and Giordani 2005). Cultivars that had Japanese names originally were probably given Italian names. New cultivars may have been developed by hybridization and selection soon after the initial introduction of persimmon to Europe.

The origin of 'Kaki Tipo' in Italy may be associated with the Japanese cultivar 'Amankaki'. Two 'Amankaki' selections were collected at different locations in Italy, and one of them ('Amankaki 2') was placed in the same group as

several of the 'Kaki Tipo' selections in NJ trees (Fig. 2). In Japan, there is no 'Amankaki'. ''Ama'' means ''non-astringent'' in Japanese, so that 'Amankaki' was probably the name for non-astringent types of persimmon, not a cultivar name (Bellini et al. 2003). Since persimmon was not a common fruit in European countries and because many Chinese or Japanese words were used for cultivar names, it is reasonable to expect that nomenclature confusion could occur when persimmon was introduced into European countries.

Conclusions

Italian and Spanish persimmon cultivars share a common gene pool. Within that common gene pool is a subset of cultivars that are somewhat different and more diverse than most of the selections that were tested. 'Kaki Tipo' is a group of distinct and relatively diverse cultivars, not a single cultivar. The diversity within 'Kaki Tipo' also suggests that they may have been selected or developed from different parents. The placement of several Japanese cultivars within the European cultivar group suggests that European cultivars were developed from Japanese germplasm relatively recently, as suggested in European literature. Differences among cultivars are much greater than differences among cultivar groups.

References

Badenes M, Garces A, Romero C, Romero M, Clave J, Rovira M, Llacer G (2003) Genetic diversity of introduced and local Spanish persimmon cultivars revealed by RAPD markers. Genet Resour Crop Evol 50:579–585

Bellini E, Bellini C, Giordani E, Perria R, Paffetti D (2003) Genetic and morphological relationships between possible Italian and ancestral cultivars of persimmon. Acta Hort 601:192–197

Bellini E, Giordani E (2005) Germplasm and breeding of persimmon in Europe. In: Park YM, Kang SM (eds) Proceedings of 3rd International Symposium on Persimmon. Acta Hort 685:65–75

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin No. 19. The Phytochemical Section of the Botanical Society of America, Irvine, California, pp 11–15

FAO (2006) FAOSTAT Database. http://faostat.fao.org/

Kikuchi A (1948) Pomology, Part 1. Yokendo, Tokyo Japan, pp 347–400

Kim J, Rohlf FJ, Sokal RR (1993) The accuracy of phylogenetic estimation using the neighbor-joining method. Evolution 47:471–486

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Soc USA 76:5269–5273

Rohlf FJ (1998) NTSYS-PC Numerical taxonomy and multivariate analysis system, Version 2.0. Exeter Publications Setauket, New York

Swofford DL (1998) PAUP*: Phylogenetic analysis using parsimony. Version 4.0 beta10a. Sinauer Associates, Sunderland, MA

Yonemori K, Sugiura A, Yamada M (2000) Persimmon genetics and breeding. In: Janick J (ed) Plant breeding reviews, vol. 19, pp 191–225