Japan. J. Breed. 37 : 377～388 (1987)

Genetic Analysis of Leaf Isozymes in Citrus[1)]

Masashi HIRAI[2)] and Ichiro KAJIURA[3)]

Okitsu Branch, Fruit Tree Research Station,
Ministry of Agriculture, Forestry and Fisheries,
Okitsu, Shizuoka, 〒 424-02

Leaf isozymes of four enzymes in citrus were analyzed using polyacrylamide gel electrophoresis to demonstrate the phylogenic relationship of the cultivars. Two loci for the superoxide dismutase(SOD) (EC 1.15.1.1), one locus for the peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.2), respectively were examined. In addition to the two loci of glutamate oxal-acetate transaminase(GOT) (EC 2.6.1.1), reported in the former study, a new locus was detected in the present report.

These experiments suggest that the mandarin cultivars originated from three genetic sources ; Indian, Chinese, and Japanese ones. Most of the Chinese mandarins including 'Kinokuni' showed identical genotypes, while the 'Ponkan' and 'Dancy tangerine' differed from the Chinese cultivars in their genotypes at the *Px* locus, and they were assumed to have originated from India as suggested by T. Tanaka. On the other hand, the Japanese mandarins were considered to be genetically affected by the Chinese mandarins. But some of the Japanese mandarins including 'Yatsushiro' and 'Koji' exhibited genetic effects from Tachibana, the wild type of the mandarin in Japan.

The genotypes of the sweet orange cultivars were homogeneous and similar to those of the Chinese mandarins. Sour orange was heterozygous at several loci, and was assumed to be a hybrid between mandarin and pummelo. Among the pummelo cultivars, some heterogeneity was detected at some loci.

KEY WORDS : *Citrus, Poncirus, Fortunella,* mandarin, phylogeny.

Introduction

The taxonomy of *Citrus* and its related genera is complex. SWINGLE (1967) recognized 16 species, but T. TANAKA (1969) proposed 159 species. The difficulty in the analysis of the taxonomy of the genus *Citrus* may be partly ascribed to the presence of a wide crossability, and partly to asexual propagation associated with the embryogenesis in nucellar tissues. Once commercially valuable hybrids occur, wide mutant variations can be selected within a single clone during a long period of cultivation as shown in the case of satsuma mandarin (IWAMASA 1976).

BARRET and RHODES (1976), POTVIN *et al.* (1983) and HANDA and OOGAKI (1985) have described three basic species ; *C. grandis, C. reticulata* and *C. medica.* Studies on the cytoplasmic genes also have supported this theory (HANDA et al. 1986, GREEN *et al.* 1986), though the number of cultivars used was limited.

TORRES *et al.* (1978) examined the leaf isozymes of a large number of citrus cultivars and their relatives. They found that the well recognized species exhibited specific combinations of alleles, and they also observed the allelic heterogeneity of the mandarin group. In a previous study (HIRAI *et al.* 1986 b), we observed genetical differences

Received February 7, 1987.

1) Contribution B-135, Fruit Tree Res. Stn.,

2) Present address : *Department of Vegetable Breeding, National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie,* 〒514-23

3) Present address : *Division of General Administration, National Institute of Agrobiological Resoureces, Yatabe, Ibaraki,* 〒305

between the Chinese mandarins and the Japanese wild mandarin, Tachibana, and also detected a close affinity between Chinese mandarins and sweet oranges. Since this study dealt only with GOT and MDH for three loci, a more extensitive analysis should be performed in future.

Here we analyzed additional four enzymes controlled by six genes, and discussed the phylogenic relationships of citrus cultivars.

Materials and methods

Citrus species and the cultivars grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Okitsu, Shizuoka, were used as materials. But in some cases, sample leaves were collected at several prefectural fruit tree stations or citrus experimental stations in Japan.

For the peroxidase and polyphenol oxidase, 0.2 g of expanded current leaves were homogenized with 2 m*l* of the buffer used for the extraction of GOT (HIRAI *et al.* 1986 a). The homogenate was centrifuged briefly, and the supernatant was used as enzyme sample.

For the analysis of GOT in the particulate fraction, 1 g of leaves was homogenized with the medium containing 0.1 M Tris-HCl buffer, pH 8.0, and 0.5 M mannitol. The homogenate was centrifuged briefly at 45 *g* for 5 min., then the supernatant was centrifuged at 7,000 *g* for 10 min. Resultant residue was washed with the above medium, then mixed with 0.5 m*l* of the medium containing 0.02 M Tris-HCl, pH 8.0, and 0.3% Triton X-100. The solubilized fraction was collected by centrifugation at 15,600 *g* for 10 sec., desalted as described previously (HIRAI, *et al.* 1986 a), then used for the electrophoresis.

Polyacrylamide gel electrophoresis was carried out as described previously (HIRAI *et al.* 1986 a). For the peroxidase, the gel was incubated with the medium containing 1 mM 5-amino-2-naphthol (dissolved in N, N-dimethylformamide at a final concentration of 5%), 0.06% H_2O_2, and 0.05 M sodium acetate buffer, pH 5.5. The staining for superoxide dismutase and GOT was performed as formerly described by VALLEJOS (1983) and HIRAI *et al.* (1986 a), respectively. For the polyphenol oxidase, the gel was stained by the method of VALLEJOS (1983) with a slight modification, where pyrogallol was used as substrate instead of catechol.

Results

The band intensity of peroxidase increased with the leaf age. Moreover, the banding pattern changed depending on the leaf age in some cases. The changes in the pattern in citrus were classified into three types as follows : The first type was observed in pummelos : Expanded current year leaf of pummelos showed a two-band pattern, but old leaf showed three bands, of which the most intense band appeared in the faster migrating zone. Second type : In the relatives of pummelo, young leaf showed a three-band pattern, and more than five bands were seen in old leaf. Third type : The other citrus cultivars showed changes in the band intensity only. However, the changes in the pattern of the zymogram were limited in the above three cases, and

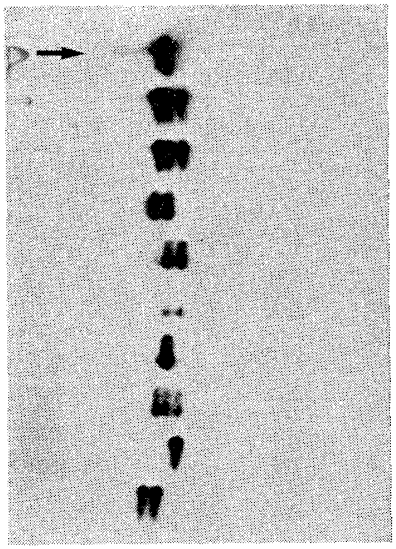

Fig. 1. Isozyme pattern of peroxidase in citrus leaves. Arrow indicates the electrophoretic direction. The genotypes are indicated in the parentheses.

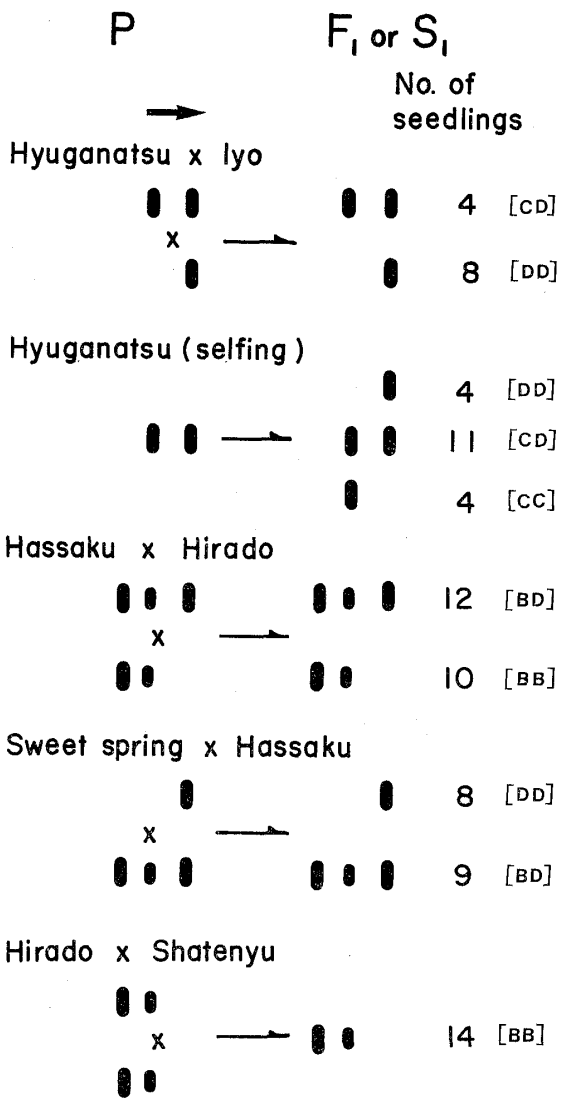

Fig. 2. Diagram of segregation pattern for peroxidase. Arrow above the diagram indicates the electrophoretic direction. The proposed genotypes of the progenies are indicated in the parentheses.

no other change in the banding pattern was observed. Expanded current year leaf was then used for the genetic analysis of the enzyme.

Since a one- or two-band pattern was observed except for the pummelo-relatives, the monomeric nature of the peroxidase was suggested (Fig. 1). The segregation of the banding pattern was also examined using the selfed and hybrid progenies of several monoembryonic cultivars(Fig. 2). The F_1 between 'Hyuganatsu' and 'Iyo' segregated into two patterns. The selfed seedlings of 'Hyuganatsu', which showed a two-band pattern segregated into a one- or two- band pattern. The results also suggested a monomeric nature for the peroxidase. The F_1 between 'Hassaku' and 'Hirado' pummelo segregated into two types : a two-band pattern as pummelo and a three-band pattern as 'Hassaku'. The backcrossed hybrid between 'Hassaku' and 'Sweet Spring' (one band) segregated also into two types : a three-band pattern as 'Hassaku' and one band as 'Sweet Spring'. All the inbred seedlings of pummelo showed a two-band pattern as their parents (Fig. 2). The above results suggest that the pummelos are homozygous for the peroxidase gene, while the three-band pattern is assumed to represent a heterozygous combination of the alleles from pummelo and the other citrus species. In other words, the faster band of pummelo, and the intermediate band of the three bands in the pummelo-relatives were assumed to be the so-called ghost bands. The proposed genotypes of the citrus examined are shown in Fig. 1 and also listed in Table 1.

Table 1. Genotype of citrus species and cultivars

Common name [Latin name or Chinese name]	Genotype				
	Got-3	Px	Ppo	Sod-2	Sod-3
Papedas					
Purrut [*Citrus hystrix*]	C-	CC	AA	?	AC
Khasi papeda [*C. latipes*]	AC	BB or BC	BB	AA	AA
Kabuyao [*C. macroptera*]	C-	CC	AB	?	AA
Ichang papeda [*C. ichangensis*]	A-	AC or AA	AA	BB	AB
Citrons					
Etrog, Fingered [*C. medica*]	A-	CC	AA	AA	EE or OO
Lemons, Limes and their relatives					
Eureka lemon, Lisbon lemon [*C. limon*]	AC	CD	AA	AB	AE
Villafranca lemon [*C. limon*]	?	CD	AA	BB	AE
Grant lemon	A-	CD	AA	AA	AE
Rough lemon	AE	CC	AA	AA	AC
Mexican lime	AE	CC	AA	AA	AA
Tahiti lime	A-	CC	AA	?	?
Sweet lime	AC	CC	AA	AA	AC
Hime lemon, Otaheito	AE	CC	AA	AB	?
Lumie	AE	CD	AA	AB	?
Karna katta	AC	?	AA	AA	AC
Pummelos and their relatives					
Pummelos [*C. grandis*]	C-	BB	AB	AA	AA
Hirado	CD	BB	AA	AA	AA
Egami, Ban peiyu					
Mato	D-	BB	AA	AA	AA
Mato peiyu	C-	BB	AA	AB	AA
Mato anyu	C-	BB	AB	BB	AA
Sekitou yu, So yu	CD	BB	AA	AB	AA
Shaten yu	CD	BB	AA	AB	AA
Kao Pan	D-	BB	AA	AB	AA
Kao Phuang	D-	BB	AA	AA	AA
Pummelo-like miscellaneous					
Anseikan	C-	BD	AB	AA	AA
Kawachibankan	CD	BD	AA	AB	AA
Iwaikan	DE	BD	AA	?	?
Hassaku	DO	BD	AA	AA	AA
Natsumikan (Natsudaidai)	DE	BD	AA	AA	AA
Sanbokan	E-	BD	AA	AB	AA
Naruto	DE	BD	AA	AA	AA
Kinukawa	E-	BD	AA	AB	AA
Otachibana	CD	BD	AA	AA	AA
Yamamikan	DE	BD	AA	AA	AA
Tengu	E-	BD	AA	AA	AA
Grapefruits					
Marsh, Red Blush, Star Ruby, Triumph	CD	BD	AA	AA	AA
Sour oranges					
Shuto (Kabusu), Choushuto, Zadaidai, Sour orange (Standard), Fuiridaidai	CE	BD	AA	AB	AA

Table 1. (Continued)

Common name [Latin name or Chinese name]	Genotype				
	Got-3	Px	Ppo	Sod-2	Sod-3
Sweet oranges					
Valencia, Hamlin, Trovita, Washington Navel, Maltese blood, Ruby blood	DE	DD	AA	AA	AA
Kanton, Taikoutentou [Dahongtiancheng], Inshikan [Yinzigan], Fukuhara	DE	DD	AA	AA	AA
Mandarins					
Originated in China					
Kishu (Kinokuni) [Mi-chü(shin-chieh)], Sunki, Ponki, Kobenimikan [Chu-sha-chü], Yuhikitsu [Yu-pi-chieh], Jimikan [Pen-ti-tsao], Soukitsu [Tsao-chieh], Tankan [Tsiao-kan],	E-	DD	AA	AA	AA
Mankitsu [Man-kieh]	E-	DD	AA	?	?
Shikaikan [Szu-ui-kom]	E-	CD	AA	AA	AA
Genshokan	E-	CD	AA	AA	AA
Originated in Japan					
Tachibana, Korai-tachibana	E-	CC	AA	AA	AA
Shiikuwasha	E-	AD or CD	AA	AA	AA
Keraji	E-	DD	AA	AA	AA
Oto	E-	CD	AA	AA	AA
Tarogayo	?	CD	AA	AA	AA
Girimikan	E-	CD	AA	AB	AA
Fukuremikan	E-	CD	AA	AA	AA
Koji	?	CC	AA	AB	AA
Satsuma	E-	DD	AA	AA	AA
Kawabata	E-	CD	?	AB	AA
Yatsushiro	E-	CD	AA	AA	AA
Ujukitsu	DE	DD	AA	AA	AA
Originated in other areas					
Ponkan, Kodakithuli	E-	CC	AA	AA	AA
Dancy tangerine	E-	CD	AA	AA	AA
King	E-	DD	AA	AB	AA
Amblycarpa	E-	DD	AA	?	?
Mediterranean (Willow-leafed), Clementine Cleopatra	E-	DD	AA	AA	AA
Yuzu and its relatives					
Yuzu	?	AC	AA	AB	AA
Ichang lemon	?	BB or AB	AA	AB	AA
Sudachi	E-	CC	AA	AB	AA
Kabosu	?	AD	AA	AB	AA
Hanayu (Tokoyu)	E-	CC	AA	AB	AA
Mochiyu	OO	CD	AA	AA	AA
Yuko	OO	CD	AA	AB	AA
Miscellaneous					
Iyo	EO	DD	AA	AB	AA
Kikudaidai	E-	DE	AB	AB	AA
Hyuganatsu	OO	CD	AA	AA	AA
Shishiyuzu (Jagatarayu)	?	AC	AA	?	?

Table 1. (Continued)

Common name [Latin name or Chinese name]	Genotype				
	Got-3	Px	Ppo	Sod-2	Sod-3
Nanshodaidai	?	AD	AA	AB	AA
Shunkokan	OO	DD	AB	AA	AA
Binkitsu	?	CD	AA	AB	AA
Bergamot	AC	BB or BC	AA	AB	AA
Kumquats and their relatives					
Oval kumquat [*Fortunella margarita*]	B-	FF	AA	BB	AD
Round kumquat [*Fortunella japonica*]	B-	FF	AA	BB	AA
Hongkong kumquat [*Fortunella hindsii*]	B-	FF	AA	BB	AC
Meiwa kumquat [*Fortunella crassifolia*]	B-	FF	AA	BB	AA
Changshou Kumquat	BE	FD	AA	BB	AA
Calamondin	E-	DD	AA	AB	AC
Trifoliate orange [*Poncirus trifoliata*]	OO	PP	AA	AA	AA

Allele "O" indicates null. Symbol "?" indicates that the genotype was not determined
Altanative name is shown in parentheses.

When the gel was stained for polyphenol oxidase, bands appeared in two regions. The banding pattern in the slower migrating region was identical with that of peroxidase, and the band intensity increased by the addition of hydrogen peroxide. Thus, another region, *i. e.*, the faster migrating bands were considered to correspond to polyphenol oxidase. Most of the citrus examined exhibited one band of polyphenol oxidase identical with each other. But *C. latipes* had another band, while some pummelos and their relatives showed two bands (Fig. 3). The F_1 seedlings obtained from the crossing between 'Hassaku' (one band) and 'Hirado' pummelo (two bands) showed a segregation into the one-band pattern (15 seedlings) and the two-band pattern (18 seedlings). These results indicate the monomeric nature of the enzyme.

The major activities of GOT in the whole homogenate were detected in two regions, *i. e.*, GOT-1 and GOT-2 (HIRAI *et al.* 1986 b). These major activities were not found in the precipitated fraction. The faint bands observed in the whole homogenate in the GOT-2 region were intensified in the particulate fraction. The electrophoretic pattern of GOT in the precipitated fraction showed one or three bands, suggesting the dimeric nature of the enzyme (Fig. 4). The gene specifying this enzyme was named *Got-3*. Trifoliate orange and some citrus cultivars did not show any activity in this fraction, and are assumed to be a null form at this locus. Thus several biotypes showing one band must be heterozygous for the null allele at *Got-3*. In Yuzu and some cultivars, a two-band pattern was observed, though the genotypes in this pattern are still unknown.

The SOD banding pattern, which was somewhat complicated (Fig. 5), was ascribed to at least two genes. One of them, which controls the fastest band group, was named *Sod-2*, because TORRES *et al.* (1985) had already reported the gene controlling SOD. Most of the citrus showed clear narrow bands. Kumquats and some other citrus showed another clear band, while, 'Iyo', 'Shatenyu' and several other citrus exhibited broad bands. Hybrids between 'Iyo' and 'Shatenyu' segregated into two types ; clear-band

type and broad-band one. This finding indicates that these two citrus were heterozygous at this locus (Fig. 5).

The other set of the bands was controlled by *Sod-3*. Although most of the citrus examined showed a single band(*AA*), Hongkong kumquat, 'Calamondin' and 'Sweet lime' showed triplet bands. A seedling of Ichang papeda showed another type of triplet bands. 'Eureka' and some other lemons showed only two bands, but it is suggested that the third band overlapped the band of *Sod-2*. Some of the selfed seedlings of 'Grant' lemon showed two bands, while the other did not exhibit any activity in this region. These findings support the above assumption. The genotype of the lemons was designated as *AE* for the *Sod-3* gene (Table 1).

Discussion

Origin of mandarins

In the revious report (HIRAI *et al.* 1986 b), we described genetic differences between mandarins originating from Japan and those from mainland China and other areas. These findings were confirmed in the present study. Chinese mandarins showed the *DD* genotype at the *Px* locus, except for 'Szu-ui-kom'. While Tachibana, a Japanese wild mandarin, was homozygous for *Px-C*. This allele was also detected in several mandarins originating from Japan including 'Yatsushiro' and 'Keraji'. Moreover, most of them harbour the alleles common to Tachibana at loci *Got-2* and *Mdh-1* (HIRAI *et al.* 1986 b). One of the oldest cultivars in Japan 'Koji' has been assumed to arise from a spontaneous cross between Tachibana and other mandarins (Iwamasa 1976). Although our previous study did not reveal the affinity of this cultivar to Tachibana, *Px-C* detected here may suggest a genetic effect from Tachibana. On the other hand, the genetic effect from the wild mandarin was not found in 'Satsuma' mandarin, as shown previously. The 'Satsuma' had the same genotype as that of Chinese mandarins.

Among the mandarins, the 'Ponkan' and 'Dancy tangerine' are assumed to originate from India (T. TANAKA 1954). These mandarins had the *Px-C* allele, but most of the Chinese mandarins were homozygous for *Px-D*. In addition, 'Kodakithuli', a small-fruited mandarin recently introduced from India, showed the *CC* genotype for the *Px* gene and is different from the Chinese mandarins. It is noteworthy that 'Szu-ui-kom', a Chinese mandarin (T. TANAKA 1954), also had *Px-C* allele. This cultivar is morphologically similar to 'Ponkan' and is widely distributed in tropical Asia (T. TANAKA 1954). HANDA *et al.* (1986) reported that 'Ponkan' exhibited a different genotype from that of 'Sunki' a Chinese mandarin, and also from 'Satsuma' mandarin based on the electrophoretic analysis of ribulose 1, 5-bisphosphate carboxylase/oxygenase.

Although 'Ponkan' and Tachibana showed the same genotype for the *Px* gene, it is difficult to demonstrate that 'Ponkan' had affected Tachibana, because 'Ponkan' was introduced to Japan in the late 19 th century. (Y. TANAKA 1948). It is, however, possible to consider that the wild ancestor of 'Ponkan' showed a genetic continuity with the Japanese wild mandarin, Tachibana, because many types of common or similar species of higher plants were observed in the Himalaya range and in Japan.

'Kunenbo' and its relative, 'King', which originated from tropical Asia (T. TANAKA

1954), are another distinct group of mandarins. The present results show a genetical similarity to the Chinese mandarins although the extremely thick peel and large leaf of these cultivars suggest the effect from other citrus species. the *Sod-2-B* allele detected here supports this hypothesis.

From the above findings, the genetic sources of the mandarins can be classified into three groups. The first, the Chinese source, is typically shown in 'Kinokuni'. The wild ancestor of the Chinese mandarin has not been determined. A wild mandarin

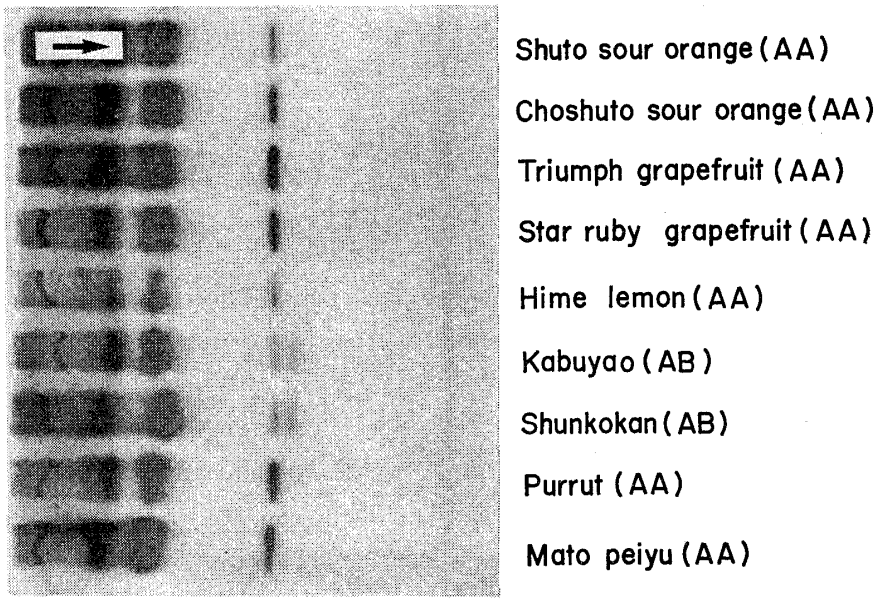

Fig. 3. Isozyme pattern of polyphenol oxidase in citrus leaves. Arrow indicates the electrophoretic direction. The genotypes are indicated in the parentheses.

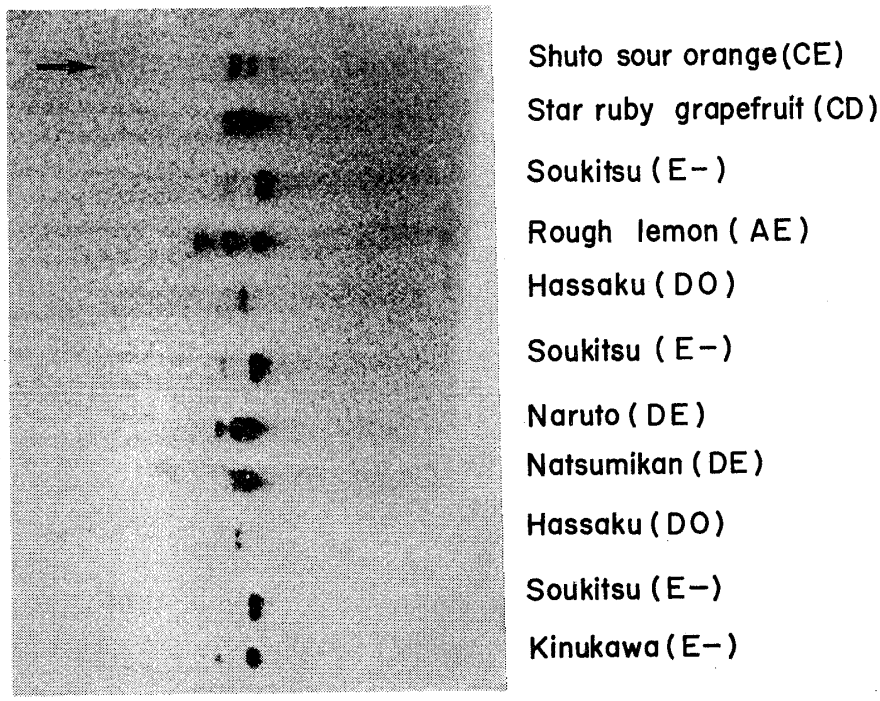

Fig. 4. Isozyme pattern of GOT in the precipitate fraction. Arrow indicates the electrophoretic direction. The genotypes at *Got-3* are indicated in the parentheses.

recently found in China must be a candidate of the ancestor (LIN and ZHEN 1985). The second source, the Japanese one, is typically shown in the wild mandarin, Tachibana. The third source must be Indian, and 'Ponkan' may be the representative of this group. T. TANAKA (1928) already reported *C. indica* as a wild mandarin in this area.

Heterogeneity of the pummelo genotype

Pummelos originting from Thailand, and those distributed in Taiwan, mainland China, and Japan were examined. The pummelo group showed a substantial heterogeneity in genotypes for the *Ppo*, *Got-3* and *Sod-2* genes. The *Ppo-B* allele was found only in pummelo, its relatives, and *C. latipes*. The *Got-3-D* allele was observed in pummelo and also in sweet oranges. Based on the isozyme analysis, the citrus cultivars having the *Got-3-D* allele are considered to be hybrid offsprings of pummelo spontaneously crossed with some other citrus (HIRAI *et al.* 1986 b). Therefore, the *Ppo-B* and

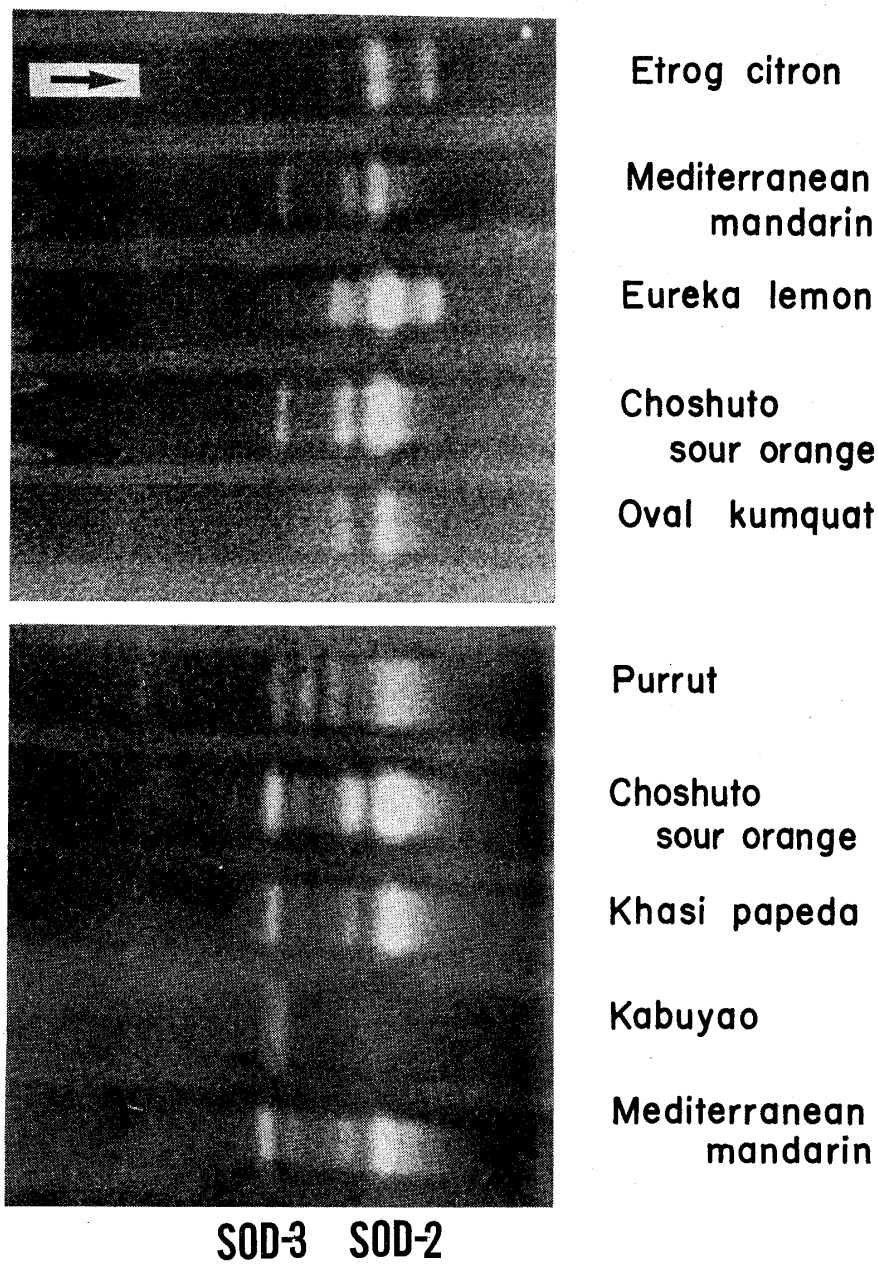

Fig. 5. Isozyme pattern of SOD in citrus leaves. Arrow indicates the electrophoretic direction.

Got-3-D alleles are considered to have occurred originally in the pummelo group. A similar assumption can be made for *Sod-2-B*.

Most of the pummelo cultivars examined here showed typical morphological characters of pummelo, *e.g.*, the large wing of the leaf was observed in most of the cultivars. On the contrary, 'Hirado' exhibited wingless leaves, and some genetic effect from other citrus cultivars cannot be ruled out. Although the pummelo group is well characterized, a wide variation in the fruit shape and pigmentation was recorded (Y. TANAKA 1946). It is reasonable that there is a genetypic heterogeneity due to monoembryony. However the relationship between genotype and the origin of the pummelo cultivars has not yet been clarified. The role of *C. latipes* in the origin of pummelo may be considered due to the similarity of the genotypes base on the current isozyme analysis.

Origin and development of sour and sweet orange cultivars

It has been suggested that the sour orange is a hybrid of pummelo and mandarin (SCORA 1975). This assumption is supported by the genotype of sour orange, *i.e.*, *BD* at *Px*, *CE* at *Got-3*, *AB* at *Sod-2*, which was observed in this study, and *DG* at *Mdh-1* as reported previously (HIRAI *et al.* 1986 b). Isozyme analysis by TORRES *et al.* (1978) also support such an assumption.

Moreover, SCORA (1975) pointed out the possibility that sweet orange had occurred as a hybrid of mandarin and pummelo. The genotype of sweet orange is very similar to that of the Chinese mandarin. Moreover, the presence of the *Got-3-D* allele in sweet orange suggests the genetic effect from pummelo. From the examination of 26 cultivars of sweet orange, genetic variation at the heterozygous locus *Got-3* could not be revealed. The uniformity of the genotype of the sweet orange cultivars for other genes was also reported by TORRES *et al.* (1978). These findings suggest that the wide variations in sweet orange cultivars have been developed through mutation. Such mechanism may also be proposed for the development of the cultivars in 'Satsuma' mandarin (IWAMASA 1976).

Role of papeda in the development of citrus cultivars

Non-edible citrus papedas were classified into one subgenus different from that of edible citrus (T. TANAKA 1954, SWINGLE 1967). The analysis of four of the six known papeda species was not able to identify the specific and common allele in the subgenus *Papeda*. Moreover, *C. latipes* showed a substantial similarity to pummelo. In addition, *C. ichangensis* is assumed to have affected Yuzu, Ichang lemon and some other citrus grown in east Asia. These findings suggest that papedas have not been isolated genetically from other species in the subgenus *Citrus*. If species and cultivars in the subgenus *Citrus* evolved from papedas, the evolutionary pathway may be polyphyletical.

Acknowledgements

We thank Dr. M. IWAMASA of Saga University, Dr. T. ENDO of the National Institute of Genetics, for the critical reading of the manuscript, the members of the 1st laboratory of citrus breeding at Okitsu Branch for offering plant materials, and Mmes. M. IKEDA and S. YAMANASHI for their technical assistance.

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カンキツ類のアイソザイム遺伝解析

平井　正志・梶浦　一郎
（果樹試験場・興津支場　清水市　〒 424-02）

カンキツ類の種・品種の類縁関係を明らかにするため，葉粗抽出物中のペルオキシダーゼ，ポリフェノールオキシダーゼ，スーパーオキシドディスムターゼおよび顆粒画分のグルタミン酸オギザル酢酸トランスアミナーゼのアイソザイムをポリアクリルアミドゲル電気泳動で分析した．この結果，*Px*, *Ppo*, *Sod*-2, *Sod*-3 および *Got*-3 の5遺伝子座が明らかになり，前報で分析した3座の分析の結果とあわせて以下の点が明らかになった．

キシュウ，ソウキツ，スンキなど中国産のマンダリンはシカイカンを除き全て同一の遺伝子型を示し *Px* は *DD* であった．この中国産マンダリンと日本のタチバナの間には前報の *Mdh-1* や *Got*-2 における対立遺伝子の差のほかに新たに *Px* 座においても差がみられた．この *Px* の分析からコウジもヤツシロなどと同様にタチバナの遺伝的影響を受けていると考えられた．しかし日本で生じたとされるウンシュウミカンの遺伝子型は中国産マンダリンと全く同じであり，タチバナの遺伝的関与を見い出すことはできなかった．一方インド原産と考えられているポンカンでは *Px* が *CC*, ダンシータンジェリンでは *CD* であり，中国産のものと異なっていた．この結果は Ribulose-1, 5-bisphosphate carboxylase/oxygenase の分析でポンカンとウンシュウや中国産のスンキの間に差異を見いだした Handa らの報告と一致する．以上の結果からマンダリン類の遺伝子には中国，インド，日本の3つの起源のものがあると推定された．

スィートオレンジの遺伝子型は中国産のマンダリンに極めて良く似ており，この両者が近縁であることを推定させる．スィートオレンジでは分析した全ての品種で，ヘテロである *Got*-3 座を含む全ての座において同じ遺伝子型を示した．この結果から，スィートオレンジの品種群が枝変わりや個体レベルでの突然変異によって生じたものと推定される．ダイダイではほとんどの座においてブンタンと中国産マンダリンとの双方からの遺伝子を持ちヘテロになっており両者の F_1 である可能性が強い．単ぱい性であるブンタン類ではいくつかの座において品種間で変異が見られた．パペダ亜属については知られている6種のうち4種を分析したが，この亜属を特徴づける遺伝子型や対立遺伝子を見い出すことはできなかった．また *C. latipes* の遺伝子型はブンタンのそれとよく似ており，ブンタン類の発生にこのパペダが関与した可能性を示唆させる．