日本におけるタチバナ(Citrus tachibana(Mak.)Tanaka)の分布とアイソザイム分析

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A Survey and Isozyme Analysis of Wild Mandarin, Tachibana (*Citrus tachibana* (Mak.) Tanaka) Growing in Japan

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Summary

Tachibana (*Citrus tachibana* (Mak.) Tanaka) trees growing in the wild and cultivated in Japan were surveyed and analyzed for their isozyme genes. Many trees were observed in south-western areas from Izu Peninsula, Shizuoka Prefecture to Ishinami, Kushima, Miyazaki Prefecture. Although most of them are cultivated, some are suggested to be wild. Isozyme analysis of the tachibana population clearly demonstrated a difference between the tachibana and mandarin cultivars introduced from China. Almost all tachibana trees examined had *CC* genotype at *Px* (peroxidase) locus, while most of the Chinese mandarins are reported to have *DD* at *Px*. In addition, an allele *A* at *Got* (glutamate oxalacetate transaminase)-*2* was unique to the tachibana population. A rare allele *T* at *Got-3*, which has not been recorded in any other citrus, was found in some individuals of the tachibana population. A heterogeneity for isozyme genes was also observed in the tachibana population. These findings indicate that tachibana has been genetically isolated from other citrus in the Asian Continent, and is native to Japan. Several natural hybrids between tachibana and cultivated Chinese mandarins were found in the tachibana-growing areas.

Introduction

Although the genus *Citrus* contains many cultivars and they have been extensively cultivated in the world, its taxonomy is complicated by the two systems proposed by Tanaka (19) and Swingle (14). Recently, Barrett and Rhodes proposed citron (*C. medica*), pummelo (*C. grandis*) and mandarin (*C. reticulata*) as basic species in *Citrus*, based on a numerical taxonomic study (3), and this hypothesis has been supported by recent workers (4, 13). However, it is still uncertain whether these basic species themselves were wild species, or had already been modified from wild plants by human activities.

Among the three species discussed above, mandarins showed substantial heterogeneity in isozyme genotypes (21). Our previous studies based on isozyme analysis also revealed a heterogeneity in this group, suggesting three genetic sources of mandarin cultivars: Chinese, Indian and Japanese (5, 7). Although the presence of wild mandarins has been reported from Japan (17), China (10, 12) and India (16), only a brief study on peroxidase isozymes of Chinese wild mandarins was reported (13). In this report, some differences in isozyme patterns between the wild mandarins and cultivated mandarins were demonstrated. Since no genetic study on these isozymes was reported, the relationship between these wild mandarins and the mandarin cultivars has, therefore, remained unclear.

Tachibana (*Citrus tachibana* (Mak.) Tanaka), a wild mandarin reported to be native to southwest Japan and Taiwan (17), is considered to have

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played an important role in the origin of some of the mandarin cultivars in Japan (5, 7). Therefore, a genetic survey of the tachibana in Japan may give a suitable example to clarify relationships between citrus cultivars and their original wild species. In our previous papers, analysis of the isozymes was performed on a single tree of tachibana.

In the present paper, we tested many tachibana trees grown in the wild and cultivated in Japan and also obtained genetic information from isozyme analysis.

Materials and Methods

Several reports have already been published on the occurrence of tachibana trees in Japan (1, 2, 8, 11, 18, 20). Additional information was received from prefectural fruit tree experiment stations and local botanists. Based on this information, more than one hundred adult tachibana trees growing from Shizuoka to Miyazaki Prefectures were surveyed in the present study. In the case of cultivated trees, only large trees (more than 10 cm in diameter) were surveyed, because modern transport of citrus scions may confuse the original distribution of the tachibana trees. The samples examined are listed in Table 1, and their locations are illustrated in Fig. 1.

Isozyme analyses for peroxidase (EC. 1. 11. 1. 7), glutamate oxalacetate transaminase (EC. 2. 6. 1. 1), and malate dehydrogenase (EC. 1. 1. 1. 37) were carried out on individual trees as described (5). Average weight of fruit was determined from 10 to 27 mature and fully colored fruits harvested from a single tree.

Results and Discussion

Distribution of tachibana trees

Most of the tachibana trees surveyed are planted in yards of houses or shrines. In some cases, however, wild trees were observed. A population of more than 30 trees of tachibana was observed on a hill slope at 150～200 m above sea level at Kamiya, Heda, Shizuoka. The slope was covered with mixed forest, in which *Castonopsis cuspidata* and *Quercus acutissima* dominate. Since the forest has been utilized only for collecting materials for charcoal, these tachibana trees are inferred to be natural. In Aichi Prefecture naturally growing trees could not be found in spite of the record of their occurrence in literature (8). Cultivated tachibana trees were found in a temple at the top of Atsumi Peninsula.

Three tachibana trees grew on a hill in Toshijima Island in Mie Prefecture, while a very old tree (110 cm in circumference) had grown until 1960 in a village near the hill. It is, therefore, uncertain whether these three trees are truly wild or escaped. In the southern part of Mie, tachibana trees are found in yards of shrines or houses, and some of them have probably been transplanted from neighboring hillsides. A population of tachibana has been observed in the forest of Toyoura Shrine, Kiinagashima, and it is possibly wild. These findings suggest that tachibana is distribut-

Fig. 1. Location of tachibana surveyed in the present study.

Table 1. Isozyme analysis of tachibana populations and their tree and fruit dimensions.

Location	No. of trees	Max. diameter of trunk (cm)	Max. tree height (cm)	Isozyme genotype			Tree status[z]	Av. wt. of fruit (g)
				Got-2	Got-3	Px		
Kamiya Heda, Shizuoka	23	11	10	MA	E-	CC	N	7.0-7.5 (n=3)[y]
Ida Shrine, Heda, Shizuoka	11	-	-	MA	E-	CC	C	
Okitsu, Shimizu, Shizuoka	1	-	-	MA	E-	CC	C	8.2 (n=1)
Reisenji-temple, Atsumi, Aichi	4	11	2.5	AA	E-	CC	C	9.9-10.7 (n=2)
Toshijima Is., Toba, Mie	3	19	7	AA	E-	CC	N or E	
Iseji, Nansei, Mie	1	11	3	AA	E-	CC	C	
Toyoura Shrine, Kiinagashima, Mie	9[x]	15	8	AA	E-	CC	N?	
Kumano, Mie	6	30	8	AA	E-	CC	C	9.2-12.8 (n=2)
Mizujiri, Toyo, Kochi	1	13	6	AA	E-	CC	N or E	
Muroto, Kochi	24	46	8	AA	E-	CC	C & N	16.2-16.4 (n=2)
Kitagawa, Kochi	5	30	7	AA	E-	CC	C & N?	
Nangoku, Kochi	1	12	6	AA	E-	CC	C	
Tosashimizu, Kochi	11	38	8	AA	E-	CC(7)[w]	C	14.6-16.4 (n=2)
				MA	ET	CC(4)		
Ootsuki, Kochi	2	-	-	AA	E-	CC	C	14.0 (n=1)
Nagasu, Misho, Ehime	1	-	-	MA	ET	CC	N or E	8.4 (n=1)
Ishinami, Kushima, Miyazaki (protected area)	5[u]	11	6	AA	E-	CC(2)	N	
				MA	E-	CC(1)		
Ishinami, Kushima, Miyazaki	2	-	-	AA	E-	CC(1)	C	
				MA	ET	AD(1)		
Unakita, Kushima, Miyazaki	1	22	5	AA	E-	CC	C	
Nienami, Nango, Miyazaki	2	22	5	AA	E-	CC	N	

[z] N: naturally grown, C: cultivated, E: escaped.

[y] n: number of tree examined.

[x] Nine large trees over 4 m in tree height were found and analyzed by isozymes. Besides them, more than 40 young seedlings were also counted.

[w] The number in parentheses indicates number of plants having that genotype.

[u] In addition to 5 trees, 94 young seedlings less than 50 cm in tree height were also found (15). Isozyme analysis was done on 3 trees.

ed naturally in this area. In this study, no detailed survey was carried out in Wakayama Prefecture because of lack of information.

In the eastern Kochi, many cultivated tachibana trees were observed in backyards of local farmers' houses along Sakihama River, Muroto. Nearby, two trees grown on a hill slope covered with evergreen forest at Tachiiwa, Muroto were considered to be natural. In the western Kochi and adjacent southern Ehime, a number of trees growing in yards at Tosashimizu, Ootsuki and Misho were surveyed. In addition, many trees have been recorded in the central and western Kochi (11). A population at Ashizuri Cape has been recorded as native (20). Therefore, tachibana seems to be able to grow in wild in this area.

At Ishinami, Kushima, in Miyazaki Prefecture, more than 200 tachibana trees had been recorded to be mingled in a pine (*Pinus thunbergii*) forest, and have been protected as a precious natural vegetation (2). Unfortunately, the vegetation had been drastically changed by the sudden death of the pines infected by nematode, and then many tachibana trees have been lost. Only three trees were found in the protected area in our preliminary survey. After that, totally 99 trees were found in a detailed survey in 1989. Most of them were, however, still young and only 13 trees were higher than 2m(15). In addition, naturally grown trees were observed in Sub-tropical Branch, Miyazaki Agric. Exp. Stn., at Nienami, Nango. These findings indicate that wild trees of tachibana are growing naturally in the southern part of Miyazaki Prefecture.

In conclusion, it is obvious that tachibana occurs naturally from Izu Peninsula, Shizuoka Prefecture, westwards along the Pacific coastal area at least to south of Miyazaki Prefecture in Kyushu, but the natural habitations have been seriously damaged.

Isozyme analysis of tachibana

All tachibana trees surveyed in this study showed an identical genotype of *MD* at *Mdh-1*. Although all samples are not analyzed, genotypes for *Ppo, Sod-2* and *Sod-3* genes were *AA, AA* and *AA* as far as examined. Variations in the genotypes were observed for *Px, Got-2* and *Got-3* genes.

Table 1 shows the variation in the isozyme genes in the tachibana population. An allele *M* at *Got-2,* which was widely found in mandarins from China and India (7), was also observed in the tachibana population. But tachibana trees having this allele showed no morphological characters suggesting genetic factors from Chinese mandarins. Moreover, the possibility of hybrid origin with Chinese mandarin of these tachibana trees is undoubtedly ruled out by the occurrence of *CC* genotype for *Px* gene, because any Chinese mandarins introduced to Japan do not have *C* at *Px*. The allele *M* is, therefore, considered to occur originally in the tachibana population in Japan.

It is noteworthy that most of the tachibanas had *CC* at *Px,* while an allele *Px-D,* which is common to all Chinese mandarins (5), was only found on a single tree found in Ishinami, Kushima, Miyazaki Prefecture. Occurrence of *Px-C* in the mandarin population in China remains unclear. Peroxidase isozymes of Chinese wild mandarins were already analyzed (9), but the zymograms published were complex, and the isozyme pattern changes depending on developmental stage of the leaf (5). An allele *A* at *Got-2,* which was common to most of the tachibanas surveyed here, was not found in other citrus (7). A few trees in the western Kochi, southern Ehime and southern Miyazaki showed a rare allele *Got-3-7,* which is not found in the other citrus including Chinese mandarins, as far as examined (5).

A tachibana tree at Ishinami, Kushima, Miyazaki showed *AD* at *Px* locus. These two alleles were not found in the other tachibana trees. The allele *D* was also found in yuzu (*Citrus junos*), which is assumed to originate in China (14). It remains unclear whether these alleles originally occurred in the natural population of tachibana in Japan or have been introduced by crossing with the other mandarins from Chinese Continent.

These findings suggest that the tachibana population in Japan has been isolated from the wild mandarin populations in mainland China, and has developed the unique genes by mutation or has accumulated genes that had been lost or had become very rare in the Asian Continent.

Heterogeneity in isozyme genes and propagation of tachibana

The present isozyme analysis clearly demonstrates a genetic heterogeneity of the tachibana population in Japan. It is difficult to assume that this heterogeneous population has been introduced

as a whole from China. On the contrary, the cultivated citrus groups, such as satsuma mandarin or sweet orange were homogeneous for the isozyme genes (5), and for protein pattern (22). In spite of the heterogeneity for the isozyme genes, no variation of morphological characters was obvious in the tachibana population. All these tachibana trees showed smooth-surfaced, oblate and peelable fruit. The fruit were 7.0 to 16.4 g in average. The fruit from Heda, Shizuoka were smaller than those from western Kochi. It is, however, uncertain whether the variation in fruit weight is derived from genotypic difference or environmental effects.

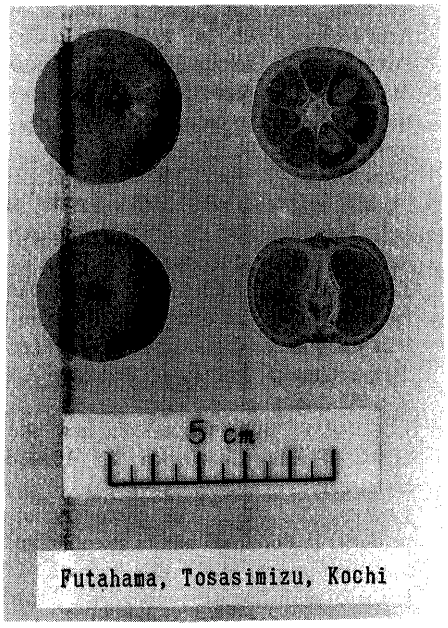

Fig. 2. A possible natural hybrid of tachibana found at Futahama, Tosashimizu, Kochi Prefecture.

Although tachibana is polyembryonic, it has a low embryo number per seed, and this suggests the possibility of zygotic in addition to asexual propagation as reported in trifoliate orange (6). It is interesting that only 4 genotypes were observed in the tachibana population in the present study. Most of the trees were *AA*, *E-* and *CC* for *Got-2*, *Got-3* and *Px*. While, 35 trees in Shizuoka Prefecture were or *MA*, *E-* and *CC*. Five were *MA*, *ET* and *CC*, and one tree was *MA*, *ET* and *AD* (Table 1). These findings indicate that tachibana populations are propagated mainly through nucellar seedlings, and rarely by zygotic ones.

Natural hybrids of tachibana

Several mandarin-type trees morphologically distinct from tachibana were observed in the present survey. These are listed in Table 2. A tree found at Muroto, Kochi showed *CD* at *Px* gene and larger fruit, and was monoembryonic. Trees in Tosashimizu produced coarse-surfaced and more reddish-colored fruit as compared with that of tachibana (Fig. 2). They are suggested to be hybrids between cultivated Chinese mandarins having *DD* at *Px* locus and tachibana. Monoembryony may be inherited from a Chinese mandarin, Kinokuni, which is common in Japan. A mandarin found in Atashika, Kumano, Mie showed coarse-surfaced fruit similar to that from Tosashimizu. This tree is also assumed to be a hybrid. A mandarin tree found at Ipponmatsu was morphological-

Table 2. Possible natural hybrids of tachibana.

Location	Genotype				Average wt. of fruit (g)	Morphological remarks
	Got-2	Got-3	Px	Mdh		
Atashika, Kumano, Mie[z]	MM	E-	n.e.[x]	n.e.	n.e.	coarse-surfaced fruit with fragrance of 'Yuzu'
Sakihama, Muroto, Kochi	MA	E-	CD	n.e.	43.6	monoembryonic
Futahama, Tosashimizu, Kochi[y]	MM	E-	CD	BB	19.0	coarse-surfaced fruit with fragrance of 'Hyuganatsu'
Masaki, Ipponmatsu, Ehime	MM	E-	CC	n.e.	20.2	reddish-colored and very smooth-surfaced fruit.

[z] Another tree having similar morphology was cultivated in the same yard.

[y] Two other trees having similar morphology were found in Futahama and Shimonokae, Tosashimizu.

[x] n.e.: not examined.

ly distinct from normal tachibana, but exhibited *CC* at *Px*. It is therefore, still uncertain whether this is an inbred seedling within tachibana or a hybrid with another mandarin. Some of the old and local Japanese cultivars of mandarin such as 'Koji' and 'Yatsushiro' have possibly been selected among these hybrid seedlings (5).

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摘　要

静岡県伊豆半島から宮崎県南部の石波までの100本以上のタチバナを調査し，そのアイソザイムを分析した．多くの木は庭や神社の境内に植栽されたものであったが，伊豆戸田の山林の群落，高知県室戸市立岩，宮崎県串間市石波海岸樹林で野生のタチバナが確認された．そのほか三重県紀伊長島町の豊浦神社では野生と思われるものが存在した．三重県熊野市，高知県西部でも野生のものの存在が示唆された．高知県室戸市佐喜浜や土佐清水市では多くの栽培樹が存在した．

アイソザイムの分析ではほとんど全てのタチバナが*Px*座に*CC*を持ち，同座が*DD*である中国産マンダリンと異なっていた．また*Got-2*座における対立遺伝子*A*は中国産マンダリンには見られず，タチバナに固有であるが，分析した全ての個体に見られた．また高知県西部，愛媛県南部及び宮崎県のタチバナの一部は*Got-3*座に*T*を持っていたが，これは今まで中国産のマンダリンを含めて他のカンキツ類に発見されていないものであった．分析したタチバナ集団のアイソザイム遺伝子には栽培されている他のカンキツ品種には見られない不均一性が見られた．以上の結果からタチバナがキシュウミカン等と共に中国から導入されたとは考え難く，日本に野生のものであり，中国大陸のカンキツ類から隔離されて進化したものと考えられた．またこの調査で果実形質がタチバナのそれとは明らかに異なるマンダリンがいくつか見いだされた．これらはアイソザイム分析の結果からキシュウミカンなどの中国系マンダリンとタチバナとの自然交雑樹と推定された．日本固有の古いマンダリン品種のあるものはこのような交雑樹から選抜されたものと考えられる．