Chimerism of Kobayashi-mikan (*Citrus natsudaikai* × *unshiu*) Judged from Isozyme Patterns in Organs and Tissues¹

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Summary

The present experiment was undertaken to confirm the generally recognized assumption concerning the chimerism of Kobayashi-mikan. This cultivar is supposed to be a synthetic chimera which has arisen from the junction of satsuma mandarin (*Citrus unshiu* Marc.) scion and Natsudaikai (*C. natsudaikai* Hayata) stock. Peroxidase and esterase isozymes of foliage and fruit parts as well as immature leaves and rootlets of young seedlings were analyzed by starch gel electrophoresis. The results obtained from a zymographic comparison of Kobayashi-mikan, satsuma mandarin and Natsudaikai are summarized as follows.

1. In the case of samples taken in July, peroxidase isozyme patterns of new and old leaves as well as albedo of Kobayashi-mikan distinctly resembled those of Natsudaikai. The pattern of juice sac of Kobayashi-mikan considerably resembled that of satsuma mandarin. Esterase isozyme patterns of flavedo and albedo of Kobayashi-mikan were completely the same as those of Natsudaikai.

2. In the case of samples taken in October, peroxidase isozyme patterns of old leaves as well as flavedo of Kobayashi-mikan were nearly the same as those of Natsudaikai. The patterns of albedo and new leaves of Kobayashi-mikan resembled those of Natsudaikai. Esterase isozyme patterns of old leaves and seeds of Kobayashi-mikan were equal to those of Natsudaikai. The patterns of flavedo, albedo and new leaves of Kobayashi-mikan were rather similar to those of Natsudaikai.

3. Peroxidase and esterase isozyme patterns of immature leaves and rootlets of young seedlings grown from seeds of Kobayashi-mikan were similar to those of young seedlings grown from seeds of Natsudaikai.

From these results, the hypothesis that Kobayashi-mikan is a graft-induced periclinal chimera between satsuma mandarin and Natsudaikai has been further confirmed.

Introduction

In order to clarify the hypothesis that Kobayashi-mikan is a graft-induced periclinal chimera between satsuma mandarin and Natsudaikai, a number of experiments were performed including morphological observations with naked eyes(10), color reaction analysis of bark and leaf extracts(7, 8, 9), gas chromatographic analysis on the essential oils in leaves(2), and scanning electron microscopic observation on epidermal systems (15). In the present experiment, peroxidase and esterase isozymes in foliage and fruit of Kobayashi-mikan sampled in July and

October were examined by starch gel electrophoresis, and the results were compared with those of satsuma mandarin and Natsudaikai. The same procedures were also applied to immature leaves and rootlets of young seedlings grown from seeds of the three cultivars in question.

Materials and Methods

Mature trees of Kobayashi-mikan (*Citrus natsudaikai* × *unshiu*) in the orchard of Mr. Takemitsu Raisen (Kamikuranaga, Takaoka-cho, Higashimorokata-gun, Miyazaki Prefecture), Natsudaikai (*C. natsudaikai* Hayata, CV. 'Kawano') in the orchard of Mr. Torao Nakazuru (Takahama, Takaoka-cho, Higa-

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shimorokata-gun, Miyazaki Prefecture) and satsuma mandarin (*C. unshiu* Marc., CV. 'Nankan Yongo') in the experimental orchard of Miyazaki University (Funazuka-cho, Miyazaki City, Miyazaki Prefecture) were used as the experimental materials. In July and October, foliage and fruit were sampled, put into polyethylene bags and kept in a freezer at -18°C until used for analysis. In November, immature leaves and rootlets were sampled from young seedlings which had been grown from seeds for a month. Zymographic methods and staining procedures were the same as described in the previous paper(14). Based upon the isozyme patterns obtained, Similarity Index Values between cultivars were calculated as follows: Similarity Index Value (SIV)=Number of bands common to each other/Total number of bands.

Results

Tables 1~6 show the peroxidase and esterase isozyme patterns of new and old leaves, fruit parts as well as immature leaves and rootlets of young seedlings. Table 7 summarizes the results in Table 1~6 so that they can be understood clearly.

1. Zymograms from samples taken in July

Peroxidase isozyme patterns of old leaves and albedo of Kobayashi-mikan were completely the same as those of Natsudaiddai. The pattern of new leaves of Kobayashi-mikan was nearly the same as that of Natsudaiddai (SIV=0.933). The pattern of flavedo showed a little difference between the two cultivars (SIV=0.625). But the pattern of juice sac of Kobayashi-mikan resembled that of satsuma mandarin (SIV=0.778).

Table 1. Peroxidase isozyme patterns of foliage and fruit of 'Kobayashi-mikan', 'satsuma' mandarin, and 'Natsudaiddai' sampled in July

Organs or Tissues		Band Distribution															
New leaf	—	4.2~	3.4~	3.1~	2.5~	2.1~	1.9~	1.5~	1.1~	0.7~	0.3~	1.7~	1.9~	2.2~	2.6~	2.8~	+
	cm	4.3	3.5	3.3	2.7	2.4	2.0	1.6	1.3	0.9	0.4	1.8	2.0	2.4	2.7	2.9	cm
	A*	+	+	+	—	—	+	—	—	+	+	—	—	+	+	—	
	B	—	—	+	+	+	—	+	+	+	—	—	+	+	—	+	
Old leaf	C	—	—	+	+	+	—	+	+	+	—	+	+	+	—	+	
	—	1.9~	1.7~	1.5~	1.3~	1.1~	0.7~	0.5~	0.2~	0~	0~	1.1~	1.5~	1.8~	2.3~	2.6~	+
	cm	2.0	1.8	1.6	1.4	1.2	0.8	0.6	0.4	0.1	0.1	1.2	1.7	2.0	2.5	3.0	cm
	A	—	+	+	—	+	+	+	+	+	+	+	—	+	+	+	
Flavedo	B	+	+	—	+	—	+	—	+	+	+	—	+	+	+	+	
	C	+	+	—	+	—	+	—	+	+	+	—	+	+	+	+	
	—	2.5~	2.0~	1.7~	0.9~	0.3~	1.3~	2.0~	2.5~	+	1.3~	2.0~	2.5~	+	1.4	2.4	2.8
	cm	2.8	2.2	1.9	1.1	0.6	1.4	2.4	2.8	cm							
Albedo	A	—	—	+	+	—	—	+	+	—	—	+	—	—			
	B	+	+	—	+	—	+	—	+	+	+	+	+	+			
	C	+	+	—	+	—	+	—	+	+	+	+	+	+			
	—	2.5~	1.9~	1.1~	0.5~	1.0~	2.4~	3.3~	3.8~	+	1.0~	2.4~	3.3~	3.8~	+	1.2	2.7
Juice sac	A	—	—	+	+	—	—	+	+	—	—	+	—	—			
	B	+	+	—	+	—	+	—	+	+	+	+	+	+			
	C	+	+	—	+	—	+	—	+	+	+	+	+	+			
	—	2.6~	1.9~	0.6~	0~	0.6~	1.4~	2.5~	3.4~	5.2~	0~	0.6~	1.4~	2.5~	3.4~	5.2~	
	cm	2.8	2.2	0.8	0.2	0.9	1.6	2.8	3.5	5.5	0.2	0.9	1.6	2.8	3.5	5.5	

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaiddai

Table 2. Esterase isozyme patterns of foliage and fruit of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaiddai' sampled in July

Organs or Tissues					Band Distribution												
New leaf	— cm	2.0~ 2.1	1.7~ 1.9	1.2~ 1.4	0.4~ 0.6	0.8~ 1.0	1.2~ 1.4	1.7~ 1.8	2.1~ 2.3	2.4~ 2.6	2.7~ 2.8	3.0~ 3.2	3.3~ 3.4	3.7~ 3.8	+		
	A*	+	—	+	+	+	+	—	+	—	+	—	+	+	+		
	B	—	+	+	+	+	+	+	+	+	+	+	—	—	—		
	C	—	+	+	+	+	—	—	+	+	—	+	+	—	—		
Old leaf	— cm	0.3~ 0.6	0~ 0.1		0~ 0.1	0.2~ 0.4	0.5~ 0.6	0.7~ 0.9	1.0~ 1.1	1.4~ 1.6	2.0~ 2.2	2.4~ 2.6	2.7~ 2.9	3.0~ 3.2	3.3~ 3.4	3.5~ 3.7	+
	A		+	+	+	+	+	—	+	+	+	+	+	+	+	+	+
	B		+	+	+	+	—	+	—	—	—	—	+	+	+	—	—
	C		+	+	+	+	+	+	—	—	—	+	+	+	—	—	+
Flavedo		— cm	0.1~ 0.5		0.3~ 0.4	0.8~ 0.9	1.2~ 1.6	2.2~ 2.3	2.4~ 2.6	2.7~ 2.9	3.0~ 3.2	3.5~ 3.9	+	+			
	A			—	+	—	—	+	+	—	+	+					
	B			+	—	+	+	—	—	+	—	+					
	C			+	—	+	+	—	—	+	—	+					
Albedo		— cm	0~ 0.5		0.5~ 0.6	0.8~ 1.0	1.5~ 1.7	2.4~ 2.6	2.9~ 3.1	4.0~ 4.2	+	+					
	A			+	+	—	—	+	—	—							
	B			+	—	+	+	—	+	+							
	C			+	—	+	+	—	+	+							
Juice sac		— cm	0~ 0.5		0.5~ 0.6	1.2~ 1.5	1.8~ 2.0	2.8~ 3.0	4.2~ 4.7	9.0~ 9.5	+	+					
	A			+	—	—	—	—	—	—							
	B			+	—	+	—	+	+	+							
	C			+	+	—	+	+	+	+							

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaiddai

The similarity in esterase isozyme patterns is as follows. No difference was observed between Kobayashi-mikan and Natsudaiddai in the patterns of flavedo and albedo. The pattern of juice sac of Kobayashi-mikan resembled Natsudaiddai a little (SIV=0.571). But, no similarity in the patterns of new and old leaves was observed on the three cultivars in question.

2. Zymograms from samples taken in October

The peroxidase isozyme patterns of old leaves of Kobayashi-mikan was the same as that of Natsudaiddai. Little difference was observed between the patterns of flavedo of Kobayashi-mikan and Natsudaiddai (SIV=0.900). In addition, the two cultivars showed the same tendency in the patterns of albedo (SIV=0.727). The pattern of new leaves of Kobayashi-mikan was rather similar to that

of Natsudaiddai (SIV=0.778). As to the pattern of juice sac, there was no similarity on the three cultivars. The pattern of seeds of Kobayashi-mikan was similar to that of Natsudaiddai to some extent (SIV=0.615).

With respect to esterase isozyme patterns, Kobayashi-mikan and Natsudaiddai showed the same patterns for old leaves and seeds. They also had similar patterns for new leaves (SIV=0.733), flavedo (SIV=0.700) and albedo (SIV=0.818). Thus, Kobayashi-mikan differed from satsuma mandarin completely in the patterns of all organs tested.

3. Zymograms of leaves and rootlets of Young seedlings

Peroxidase and esterase isozyme patterns of immature leaves of young seedlings of Kobayashi-mikan grown from seeds were exactly the same as those of Natsudaiddai. The patterns of rootlets of young seedlings

Table 3. Peroxidase isozyme patterns of foliage and fruit of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaikai' sampled in October

Organs or Tissues		Band Distribution																		
New leaf	—	1.9	1.6	1.4	1.1	0.7	0.4	0.2	0	0	0.2	0.5	0.9	1.2	1.7	1.9	2.3	2.5	2.9	+
	cm	} 2.2	} 1.8	} 1.5	} 1.3	} 0.8	} 0.5	} 0.3	} 0.1	} 0.1	} 0.3	} 0.6	} 1.1	} 1.4	} 1.8	} 2.1	} 2.4	} 2.6	} 3.1	cm
	A*	+	+	+	+	+	+	—	+	+	+	+	—	+	—	+	+	+	+	+
	B	—	+	+	+	—	+	+	+	+	+	—	—	+	+	+	—	+	+	+
Old leaf	C	+	+	+	+	+	+	+	—	+	+	—	+	+	+	+	—	+	+	+
	—	2.5	2.3	1.9	1.4	1.2	0.8	0.6	0.3	1.7	1.9	2.1	2.4	2.6	2.8	3.3	3.7	+		
	cm	} 2.6	} 2.4	} 2.0	} 1.6	} 1.3	} 0.9	} 0.7	} 0.4	} 1.0	} 2.0	} 2.3	} 2.5	} 2.7	} 3.0	} 3.4	} 3.8			cm
	A	—	+	+	+	+	—	+	—	—	—	+	+	—	+	+	+			
Flavedo	B	+	—	+	+	—	+	—	+	+	+	+	—	+	+	+	—			
	C	+	—	+	+	—	+	—	+	+	+	+	—	+	+	+	—			
	—			—	1.8	1.5	1.3	0.3	0.1	0.7	0.9	1.6	2.1	2.5	+					
	cm			} 1.9	} 1.7	} 1.4	} 0.4	} 0.2		} 0.8	} 1.0	} 1.8	} 2.4	} 3.0	cm					
Albedo	A				+	+	+	—	—	+	—	—	—	+						
	B				+	—	+	+	+	+	+	+	+	+						
	C				+	—	+	+	+	—	+	+	+	+						
	—	2.5	2.2	1.8	1.2	0.8	0.5	0.2	0	1.9	2.6	3.0	+							
Juice sac	cm	} 2.7	} 2.4	} 2.0	} 1.4	} 0.9	} 0.6	} 0.3	} 0.1	} 2.1	} 2.9	} 3.1	cm							
	A	—	—	+	+	—	—	—	+	—	+	—								
	B	—	+	+	+	+	+	+	+	+	—	+								
	C	+	+	—	—	+	+	+	+	+	—	+								
Seed	—									3.5	4.5	5.2	+							
	cm									} 4.0	} 4.7	} 5.4	cm							
	A									—	+	—								
	B									—	—	+								
Seed	C									+	—	—								
	—	2.9	2.7	1.4	1.1	0.6	0.3	0	0	0	0.6	1.7	2.1	2.5	2.9	+				
	cm	} 3.1	} 2.8	} 1.6	} 1.2	} 0.7	} 0.5	} 0.1		} 0.1	} 0.7	} 1.8	} 2.2	} 2.6	} 3.0	cm				
	B	+	+	+	+	+	+	+	+	+	+	+	+	—	+					
Seed	C	+	+	+	—	+	+	+	+	+	—	—	—	+	+					

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaikai

of the two cultivars were almost the same (SIV of peroxidase and esterase isozyme patterns were 0.818 and 0.900 respectively) and were different from those of rootlets of young seedlings of satsuma mandarin.

Discussion

Using two kinds of polyploid chimeras whose histogenic layers in the growing point were composed of either 2n-4n-4n or 2n-4n-2n, Frost and Krug(1) clarified from what layer arises each organ or tissue of a

citrus plant. According to their results, epidermal systems arise from the first layer, while the tissue of reproductive organs including ovules and anthers, epidermis of stems, and hypodermal tissues of leaves arise from the second layer. Systematic examination to discover the details of morphological development of fruit tissue has not been carried out using such polyploid chimeras as mentioned above, but Iwamasa(3) stated that epidermis surrounding the whole fruit, as well as flesh itself, arises from the first

Table 4. Esterase isozyme patterns of foliage and fruit of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaikai' sampled in October

Organs or Tissues				Band Distribution														
New leaf	—	0.3~	0~	0~	0.2~	0.6~	1.1~	1.3~	2.3~	2.6~	2.9~	3.3~	3.5~	4.2~	4.7~	9.2~	+	
	cm	0.4	0.1	0.1	0.3	0.7	1.2	1.5	2.5	2.8	3.1	3.4	3.7	4.5	4.8	9.4	cm	
	A	+	+		+	+	+	—	+	—	+	+	—	—	—	—		
	B	+	+		+	—	+	+	+	+	+	+	+	—	+	+		
	C	+	+		+	—	+	+	+	+	+	+	—	+	—	—		
Old leaf		—			0.2~	1.0~	1.6~	1.8~	2.0~	2.5~	3.0~	3.6~	+					
		cm			0.5	1.2	1.7	1.9	2.1	2.9	3.5	3.7	cm					
	A				+	—	+	+	+	+	—	+						
	B				+	+	—	—	—	+	+	—						
	C				+	+	—	—	—	+	+	—						
Flavedo		—	0~		0.2~	0.5~	0.8~	1.3~	1.5~	1.8~	2.6~	4.9~	5.3~	+				
		cm	0.3		0.3	0.7	1.0	1.4	1.7	2.3	3.0	5.0	5.4	cm				
	A		—		—	+	+	—	+	—	+	+	+					
	B		+		—	+	+	—	—	+	+	—	—					
	C		+		+	—	+	+	—	+	+	—	—					
Albedo		—	0~		0~	0.5~	1.0~	1.4~	1.8~	2.2~	2.6~	2.9~	3.3~	4.0~	+			
		cm	0.5		0.2	0.7	1.2	1.5	1.9	2.4	2.8	3.2	3.6	4.4	cm			
	A		—		—	+	+	+	—	—	+	—	+	+				
	B		+		+	—	—	—	+	+	—	+	+	+				
	C		+		+	—	—	—	—	+	—	+	+	—				
Seed					0~	1.0~	1.4~	2.0~	2.7~	3.2~	3.8~	4.7~	5.3~	+				
					0.3	1.2	1.7	2.3	2.9	3.4	4.0	5.0	5.8	cm				
	B				+	+	+	+	+	+	+	+	+					
	C				+	+	+	+	+	+	+	+	+					

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaikai

Table 5. Peroxidase isozyme patterns of immature leaves and rootlets of young seedlings of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaikai'

Organs or Tissues		Band Distribution														
Immature leaf	—	2.3~	1.8~	1.4~	1.0~	0.7~	0~	0~	0.2~	0.7~	1.0~	1.7~	1.9~	2.3~	+	
	cm	2.6	2.0	1.5	1.1	0.9	0.1	0.1	0.3	0.8	1.1	1.8	2.2	2.4	cm	
	A	+	+	+	+	+	+	+	+	—	+	—	+	+	+	
	B	+	+	—	+	—	+	+	+	+	—	+	+	—	—	
C	+	+	—	+	—	+	+	+	+	+	—	+	+	—	—	
Rootlet	—	2.5~	1.9~	1.5~	1.3~	1.0~	0.8~	0~	0~	1.1~	1.8~	2.1~	+			
	cm	2.7	2.1	1.6	1.4	1.2	0.9	0.1	0.1	1.3	2.0	2.4	cm			
	A	—	+	—	+	—	+	+	+	—	—	+				
	B	+	+	+	+	—	+	+	+	+	+	+				
	C	+	+	+	—	+	+	+	+	+	+	+				

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaikai

layer; flavedo and a part of albedo arise from the second layer; segment, most of albedo, and a part of fruit core arise from the third layer.

Kobayashi-mikan has been supposed to be a periclinal chimera whose first and second

layers in the growing point are derived from satsuma mandarin and Natsudaikai, respectively(10,11). If this hypothesis is considered in connection with the explanation on the morphological development of citrus organs or tissues mentioned above, it can be easily

Table 6. Esterase isozyme patterns of immature leaves and rootlets of young seedlings of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaïdai'

Organs or Tissues	Band Distribution										
Immature leaf	0~0.3	0.6~0.7	1.9~2.0	2.1~2.3	2.8~3.0	3.1~3.4	3.7~3.9	4.0~4.2	4.3~4.6	+cm	
A*	+	—	+	+	—	+	—	+	+		
B	+	+	—	+	+	+	+	+	+		
C	+	+	—	+	+	+	+	+	+		
Rootlet	0~0.3	0.9~1.0	1.2~1.5	2.0~2.3	2.4~2.5	2.9~3.3	3.4~3.6	4.2~4.3	4.5~4.7	4.7~4.9	+cm
A	—	—	—	—	—	—	+	—	—	+	
B	+	—	+	+	+	+	—	+	+	—	
C	+	+	+	+	+	+	—	+	+	—	

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaïdai

Table 7. Similarity Index Values of 'Kobayashi-mikan', 'satsuma' mandarin and 'Natsudaïdai' judged from peroxidase and esterase isozyme patterns of organs or tissues

Sampled Time	Organs or Tissues	Similarity Index Value judged from peroxidase isozyme patterns	Similarity Index Value judged from esterase isozyme patterns
July	New leaf	B~A : 0.267 B~C : 0.933	B~A : 0.462 B~C : 0.692
	Old leaf	B~A : 0.533 B~C : 1.000	B~A : 0.786 B~C : 0.714
	Flavedo	B~A : 0.250 B~C : 0.625	B~A : 0.111 B~C : 1.000
	Albedo	B~A : 0.250 B~C : 1.000	B~A : 0.143 B~C : 1.000
	Juice sac	B~A : 0.778 B~C : 0.333	B~A : 0.429 B~C : 0.571
	Seed	B~C : 0.615	B~C : 1.000
October	New leaf	B~A : 0.667 B~C : 0.778	B~A : 0.600 B~C : 0.733
	Old leaf	B~A : 0.313 B~C : 1.000	B~A : 0.250 B~C : 1.000
	Flavedo	B~A : 0.400 B~C : 0.900	B~A : 0.500 B~C : 0.700
	Albedo	B~A : 0.364 B~C : 0.727	B~A : 0.182 B~C : 0.818
	Juice sac	B~A : 0.333 B~C : 0.333	B~A : — B~C : —
	Seed	B~C : 0.615	B~C : 1.000
November	Immature leaf of young seedling	B~A : 0.538 B~C : 1.000	B~A : 0.556 B~C : 1.000
	Rootlet of young seedling	B~A : 0.636 B~C : 0.818	B~A : 0.100 B~C : 0.900

* A : satsuma mandarin, B : Kobayashi-mikan, C : Natsudaïdai

assumed what kind of genetic constitution should be contained in each organ or tissue of Kobayashi-mikan. Based upon this assumption, several chemotaxonomic examinations have been carried out to date, such as zymographic observations by Ueno(12) or Yamashita and Imamura(13). The results of

the present experiment show the same tendency as described in the previous reports (12,13) and are likely to fit the assumption for the most part. But, in detail, some of them are a little inconsistent with the assumption. For instance, in October, Similarity Index Value in peroxidase isozyme

pattern of juice sac was lower than expected between Kobayashi-mikan and satsuma mandarin (SIV=0.333). In addition, the Similarity Index Values of new leaves of Kobayashi-mikan and Natsudaikai were not always close to 1.000, although they were more than 0.700. The reason why they were so is not clear at all, but it is presumably due to some physiological interaction between rind and flesh or between leaf epidermis and mesophyll, whose genetic constitutions are different from each other. Moreover, it might be due to some differences in the stage of the experimental materials used. In fact, some seasonal differences of peroxidase isozymes in peels of satsuma mandarin were pointed out by Kuraoka et al. (6). Thus, the zymographic method is considered to have a limit in deducing the chimerism of plant organs or tissues. Other chemotaxonomic methods tried up to date have not always been sufficient for the same reasons. Therefore, tissue culture should be adopted to obtain more direct and convincing evidence. Tissue blocks should be excised from various parts of the fruit and cultured on a suitable medium *in vitro*. If a satsuma mandarin plant arises from the callus of juice sac of Kobayashi-mikan and at the same time a Natsudaikai plant from its flavedo callus, it can be conclusive evidence. Based upon these considerations, the author tried tissue culture for two years, where the callus formation was observed only from the block of juice sac. It was extremely difficult to induce a bit of callus from flavedo blocks. But Kato(5) observed the formation of vessels and tracheids in the juice vesicle culture of Hassaku. Since the cells in the juice sac or flavedo of Kobayashi-mikan are sure to have totipotency, there remains a possibility of inducing bud and root formation in the tissue culture of Kobayashi-mikan, provided that some proper auxins, cytokinins and additives are used at favorable concentrations.

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コバヤシミカンのキメラ性に関する研究——特に器官，組織の アイソザイムパターンについて

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摘 要

本実験においては，ウンシュウミカンとナツダイダイの接木キメラと言われているコバヤシミカンとを材料として，新・旧葉，果実，実生のアイソザイムパターンを観察し，ウンシュウミカンおよびナツダイダイのそれと比較した。

1. 7月の新旧葉，果実各部位について得られたパーオキシダーゼアイソザイムパターンは，新・旧葉とアルベドについてはコバヤシミカンとナツダイダイとの間にはっきりとした類似性がみられた。しかし，砂じょうについては，コバヤシミカンとウンシュウミカンとの間に類似性がみられた。エステラーゼアイソザイムパターンについてみると，フラベドとアルベドに関してコバヤシミカンはナツダイダイと全く同一のパターンを示した。

2. 10月の新旧葉，果実各部位について得られたパーオキシダーゼアイソザイムパターンについてみると，コバヤシミカンは旧葉とフラベドに関してナツダイダイと

きわめてよく似たパターンを，またアルベドと新葉に関してもかなりよく似たパターンを示した。エステラーゼアイソザイムパターンについてみると，旧葉と種子に関してコバヤシミカンとナツダイダイは全く同じパターンを示した。フラベド，アルベド，新葉に関しても，コバヤシミカンはナツダイダイに類似したパターンを示した。

3. 実生の葉および根のパーオキシダーゼおよびエステラーゼアイソザイムパターンについてみると，コバヤシミカンはナツダイダイにきわめてよく似たパターンを示した。

以上の結果より，コバヤシミカンが生長点組織分化層の第Ⅰ層が温州ミカン因子，第Ⅱ層がナツダイダイ因子よりなる周縁キメラであることについて確証が深められた。