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Citrus Genetic Resources Grown on the Ryukyu Islands, Japan

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Abstract

The Ryukyu Islands are located southwest part of Japan. Various local citrus are grown in this subtropical region. Since there are large geographical and climatic differences between the Ryukyu Islands and the main islands (Honshu, Kyushu, and Shikoku) of Japan, there are unique local citrus genetic resources on the Ryukyu Islands. Shiikuwasha (*Citrus depressa*) is an indigenous mandarin species in this region. This species is clearly distinguished from the mandarin grown in China and India based on the results of isozyme and DNA analyses. DNA analysis also revealed that wide diversity exists in Shiikuwasha. Although Kunenbo (*C. nobilis*) and Daidai (*C. aurantium*) have been grown in most the islands, they were introduced from China and/or Southeast Asia. Indigeneous Shiikuwasha and introduced species probably played a part in the origin of many local citrus on the Ryukyu Islands. Various unique species in this region such as Kabuchii (*C. keraji*), Oto (*C. oto*), and Rokugatsu-mikan (*C. rokugatsu*) have been widely cultivated. Fruit of some local citrus contains high levels of polymethoxyflavonoids, one of the most important health-promoting components of citrus.

Keywords: Amami, citrus, Okinawa, Ryukyu, Shiikuwasha

Introduction

The Ryukyu Islands are located southwest part of Japan. Various local citrus are grown in this subtropical region. Since there are marked geographical and climatic differences between the Ryukyu Islands and the main islands (Honshu, Kyushu, and Shikoku) of Japan, there are unique local genetic resources of citrus on the Ryukyu Islands. Thus, many accessions were recorded and classified in several studies (INAFUKU-TERAMOTO *et al.* 2010, ISHIHATA *et al.* 1997, KINJO 2007, KITA *et al.* 2013, NAKANO *et al.* 2001, YAMAMOTO *et al.* 2006, 2008a) since these local citrus accessions are very important for studies on genetic resources in citrus. The genetic relationships among them were partly clarified (YAMAMOTO *et al.* 1998, 2010a, 2010b, 2011). Recently, fruits produced in this region have attracted attention because they contain high levels of health-promoting components (YAMAMOTO *et al.* 2008b).

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Here, local genetic resources of citrus grown on the Ryukyu Islands, with regard to: 1) the distribution of local citrus accessions grown on the Ryukyu Islands, 2) phylogenetic relationships of local genetic resources of citrus grown on the Ryukyu Islands, and 3) phytonutrient components of local citrus grown on the Ryukyu Islands, are discussed.

Distribution of Local Citrus Accessions Grown on the Ryukyu Islands

Among the local citrus accessions grown on the Ryukyu Islands, Shiikuwasha (*Citrus depressa* Hayata) is the indigenous species (TANAKA 1926). Daidai (*C. aurantium* L.) and Kunenbo (*C. nobilis* Lour.) were introduced from China or Southeast Asia. Since then, several species, such as Kabuchii (*C. keraji* hort. ex Tanaka), Oto (*C. oto* hort. ex Yu. Tanaka), and Rokugatsu-mikan (*C. rokugatsu* hort. ex Yu. Tanaka), have grown as new seedlings that have arisen by chance from indigenous or introduced species (TANAKA 1948) (Fig. 1 and Table 1).



Fig. 1. Fruits of local citrus on the Ryukyu Islands. A: Shiikuwasha (*Citrus depressa*), B: Kunenbo (*C. nobilis*), C: Daidai (*C. aurantium*), D: Buntan (*C. maxima*), E: Akamikan (*C. tangerina*), F: Shimamikan (*C. sp.*), G: Kabuchii (*C. keraji*), H: Keraji (*C. keraji*), I: Oto (*C. oto*), J: Tarogayo (*C. tarogayo*), K: Rokugatsu-mikan (*C. rokugatsu*), L: Shiikuu, Kusa, or Tunugekunin (*C. sp.*).

Common name	Latin name	Origin
Shiikuwasha	Citrus depressa Hayata	Authigene
Kunenbo	C. nobilis Lour.	Introduction
Sour orange (Daidai)	C. aurantium L.	Introduction
Pummelo (Buntan)	C. maxima (Burm.) Merr.	Introduction
Dancy (Obeni-mikan)	C. tangerina hort. ex Tanaka	Introduction
Komikan (Shimamikan)	<i>C</i> . sp.	Probably introduction
Kabuchii	C. keraji hort. ex Tanaka	Chance seedling
Keraji	C. keraji hort. ex Tanaka	Chance seedling
Oto	C. oto hort. ex Yu. Tanaka	Chance seedling
Tarogayo	C. tarogayo hort. ex Tanaka	Chance seedling
Rokugatsu-mikan	C. rokugatsu hort. ex Yu. Tanaka	Chance seedling
Fusu, Kusa or Tunugekunin	<i>C</i> . sp.	Chance seedling

Table 1. Major local citrus grown on the Ryukyu Islands.

Phylogenetic Relationships of Local Genetic Resources of Citrus Grown on the Ryukyu Islands

The results of isozyme and cleaved amplified polymorphic sequence (CAPS) analyses of chloroplast DNA (cpDNA) are shown in Table 2 and Table 3, respectively (YAMAMOTO *et al.* 2011, 2013).

The indigeneous Shiikuwasha and introduced Kunenbo probably played a role in the origin of many local citrus on the Ryukyu Islands. Keraji, Kabuchii, and Oto are closely related to Kunenbo based on the results of isozyme and DNA analyses. The type of cpDNA of Keraji, Kabuchii, Tarogayo, and Oto is the same as that of Kunenbo. This suggests that they arose from Kunenbo as a female ancestor because cpDNA is inherited maternally. On the other hand, the genetic influence of Japanese mandarins such as Shiikuwasha on Kabuchii and Oto was observed. Their *GOT-2* genotype was *MA*, and *A* is a characteristic allele of the Japanese mandarins, Shiikuwasha and Tachibana (HIRAI *et al.* 1986). The involvement of Shiikuwasha in the development of Rokugatsu-mikan and Fusu was identified; all of them possessed *A* in *GOT-2*, a characteristic allele of Japanese mandarin.

In cpDNA analysis, Shiikuwasha belonged to both types 4 and 5 in Table 3. The cpDNA divergence of this species grown in Okinawa was also reported by URASAKI *et al.* (2005). These results suggest a polyphyletic origin of Shiikuwasha.

Common name	Latin name	Genotype of
		GOT-2
Shiikuwasha	Citrus depressa Hayata	MA
Kunenbo	C. nobilis Lour.	MM
Sour orange	C. aurantium L.	MM
Pummelo	C. maxima (Burm.) Merr.	MM
Dancy	C. tangerina hort. ex Tanaka	MM
Komikan (Shimamikan)	<i>C</i> . sp.	MM
Kabuchii	C. keraji hort. ex Tanaka	MA
Keraji	C. keraji hort. ex Tanaka	MM
Oto	C. oto hort. ex Yu. Tanaka	MA
Rokugatsu-mikan	C. rokugatsu hort. ex Yu. Tanaka	MA
Shiiku, Kusa,or Tunugekunin	<i>C</i> . sp.	MA
Control		
Tachibana	C. tachibana (Makino) Tanaka	AA
Kinokuni	C. kinokuni hort. ex Tanaka	MM
Satsuma mandarin	C. unshiu Marcow.	MM
Ponkan	C. reticulata Blanco	MM
Lemon	C. limon (L.) Burm. f.	SM

Table 2. Isozyme genotype of major local citrus grown on the Ryukyu Islands.

Modified from YAMAMOTO et al. (2011).

Table 3. Types of chloroplast DNA (cpDNA) based on CAPS analysis of major local citrus grown on the Ryukyu Islands.

Туре	Common name
1	Kunenobo, Sour orange, Pummelo, Kabuchii, Keraji, Oto, Tarogayo, Sweet orange, lemon
2	Khasi Papeda
3	Ichang Papeda, Yuzu
4	Shiikuwasha, Tachibana
5	Shiikuwahsa, Sunki, Cleopatra, Tankan
6	Dancy, Ponkan, Satsuma mandarin, Kinokuni, Clementine
Modif	ied from YAMAMOTO et al. (2013).

Phytonutrient Components of Local Citrus Grown on the Ryukyu Islands

Citrus fruits are essential sources of some phytonutrient components. Among them, polymethoxyflavonoids (PMFs), unique components of citrus, show efficacy against lifestyle-related diseases such as cancer and diabetes (KAWAI *et al.* 1999, LEE *et al.* 2010, MIYATA *et al.* 2008). The PMF content of Shiikuwasha and Kabuchii was much higher than that of Satsuma mandarin (*C. unshiu*), which is a leading citrus species in Japan (Table 4) (YAMAMOTO *et al.* 2008).

Accession	Date	Polymetoxyflavanoid	
	(y/m/d)	Juice	Peel
		(pg/mL)	(pg/g)
Shiikuwasha	2004/9/17	17.6	4,699.8
(C. depressa)	2004/10/27	9.9	3,523.4
	2004/12/8	0.9	2,848.3
Kuriha (Citrus keraji)	2004/9/17 2004/10/27 2004/12/8	20.6 12.1 1.9	3,584.7 2,833.4 2,628.0
Control Miyagawa-wase (C. unshiu)	2004/10/27	2.0	369.7

Table 4.	Polymethoxyflavonoid content of juice and pe	eel
	in Shiikuwasha and Kabuchii.	

Modified from YAMAMOTO et al. (2008).

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