南太平洋海域調査研究報告　No.54（2014年12月）
OCCASIONAL PAPERS　No.54（December 2014）

Citrus Genetic Resources Grown on the Ryukyu Islands, Japan

YAMAMOTO Masashi

Faculty of Agriculture, Kagoshima University

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).

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).

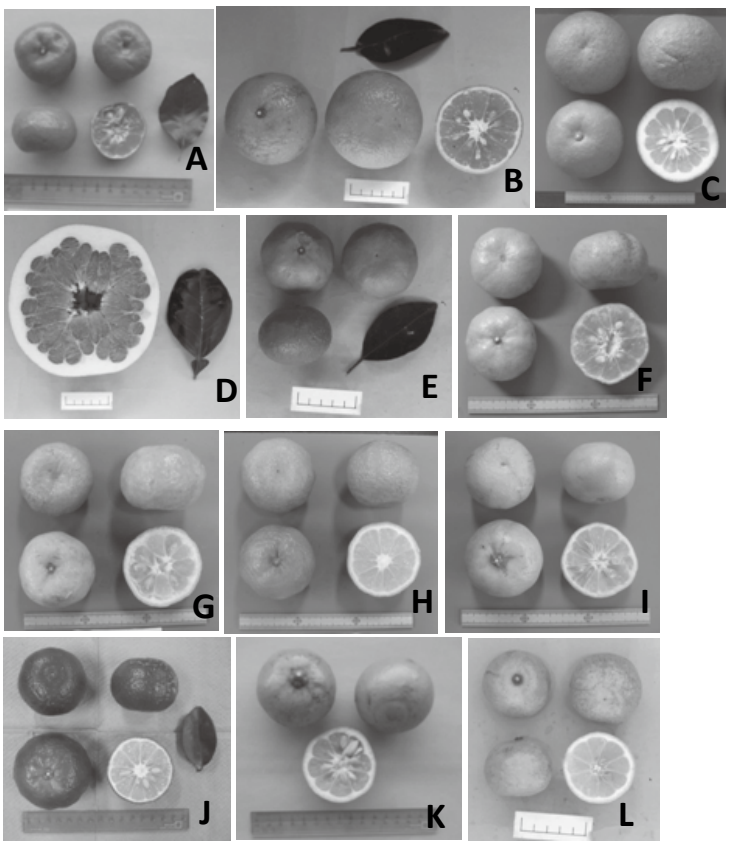

Fig. 1. Fruits of local citrus on the Ryukyu Islands. A: Shiikuwasha (*Citrus depressa*), B: Kunenbo (*C. nobilis*), C: Daidai (*C. aurantium*), D: Buntan (*C. maxima*), E: Akamikan (*C. tangerina*), F: Shimamikan (*C.* sp.), G: Kabuchii (*C. keraji*), H: Keraji (*C. keraji*), I: Oto (*C. oto*), J: Tarogayo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

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

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)	*C*. 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	*C*. sp.	Chance seedling

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.

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

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)	*C*. 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	*C*. 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

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.

Type	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

Modified 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).

Table 4. Polymethoxyflavonoid content of juice and peel in Shiikuwasha and Kabuchii.

Accession	Date (y/m/d)	Polymetoxyflavanoid	
		Juice (pg/mL)	Peel (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	2004/9/17	20.6	3,584.7
(*Citrus keraji*)	2004/10/27	12.1	2,833.4
	2004/12/8	1.9	2,628.0
Control			
Miyagawa-wase	2004/10/27	2.0	369.7
(*C. unshiu*)			

Modified from YAMAMOTO *et al*. (2008).

References

HIRAI, M., KOZAKI, I. and KAJIURA, I. 1986. Isozyme Analysis and Phylogenic Relationship of Citrus. Japanese Society of Breeding, 36: 377-389.

INAFUKU-TERAMOTO, S., YAMAMOTO, M., KINJO, H., KITAJIMA, A., WADA, K. and KAWAMITSU, Y. 2010. Local Citrus Genetic Resources and Their Polymethoxyflavones Content in Northern Part of Okinawa Island. Horticultural Research (Japan), 9: 263-271 (in Japanese with English Summary).

ISHIHATA, K., SHIOTA, H. and ONJO, M. 1997. Some Kinds of Tropical and Subtropical Fruit Trees and Characteristics of Some Native Citrus Fruits in Amami Islands. Bulletin of the Experimental Farm, Faculty of Agriculture, Kagoshima University, 22: 1-13 (in Japanese with English Summary).

KAWAI, S., TOMONO, Y., KATASE, E., OGAWA, K. and YANO, M. 1999. Antiprofiferative Activity of Flavonoids on Several Cancer Cell Lines. Bioscience, Biotechnology, and Biochemistry, 63: 896-899.

KINJO, H. 2007. Acid Citrus "Shiikuwasha" in Ryukyu Island. Studia Citrologica, 17: 137-148 (in Japanese).

KITA, M., YAMAMOTO, M., INAMORI, H., SAKAUE, H., SEKITA, T., TAHARA, A. and MINORU, H. 2013. Exploration of *Citrus* Genetic Resources in Amami Archipelago in Kagoshima Prefecture, Japan. Annual Report on Exploration and Introduction of Plant Genetic Resources, 29: 107-117 (in Japanese with English Summary).

LEE, Y. S., CHA, B. Y., SAITO, K., YAMAKAWA, H., CHOI, S. S., YAMAGUCHI, K., YONEZAWA, T., TERUYA, T., NAGAI, K. and WOO, J. T. 2010. Nobiletin Improves Hyperglycemia and Insulin Resistance in Obese Diabetic ob/ob Mice. Biochemical Pharmacology, 79: 1674-1683.

MIYATA, Y., SATO, T., IMADA, K., DOBASHI, A., YANO, M. and ITO, A. 2008. A Citrus Polymethoxyflavonoid, Nobiletin, Is a Novel MEK Inhibitor That Exhibits Antitumor Metastasis in Human Fibrosarcoma HT-1080 Cells. Biochemical and Biophysical Research Communications, 366: 168-173.

NAKANO, M., NESUMI, H. and YOSHIDA, T. 2001. Exploration for Citrus Genetic Resources on the Nansei Archipelago, Japan. Annual Report on Exploration and Introduction of Plant Genetic Resources, 17: 39-48 (in Japanese with English Summary).

TANAKA, T., 1926. Wild *Citri* of the Japanese Territories. Bulteno Scienca de la Fakultato Terkultura, Kjusu Imperia Universitato, 2: 51-58 (in Japanese with English Summary).

TANAKA, Y. 1948. An Iconograph of Japanese Citrus Fruits. Vol. 2, 525 pp., Yokendo, Tokyo (in Japanese).

URASAKI, N., YOSHIDA, K., UEHARA, T., INOUE, H., ONDA, S., KINJO, H. and KAWANO, S. 2005. Single Nucleotide Polymorphism in Shiikuwasha (*Citrus depressa* Hayata) Chloroplast DNA, trnL-trnF. Japanese Journal of Tropical Agriculture, 49: 246-251.

YAMAMOTO, M., FUKUDA, M., KOGA, T., KUBO, T. and TOMINAGA, S. 2010a. Examination of the Origin of Keraji (*Citrus keraji*), Local Citrus of Kikaijima Island, Kagoshima Prefecture. Horticultural Research (Japan), 9: 7-12 (in Japanese with English Summary).

YAMAMOTO, M., KOUNO, R., NAKAGAWA, T., USUI, T., KUBO, T. and TOMINAGA, S. 2011. Isozyme and DNA Analyses of Local *Citrus* Germplasm on Amami Islands, Japan. Journal of the Japanese Society for Horticultural Science, 80: 268-275.

YAMAMOTO, M., KUBO, T. and TOMINAGA, S. 2008a. Exploration of *Citrus depressa* Hayata Germplasm in Okinoerabu Island. Bulletin of the Experimental Farm, Faculty of Agriculture, Kagoshima University, 32: 7-12 (in Japanese).

YAMAMOTO, M., MATSUMOTO, R., UECHI, Y., IJICHI, T., KUBO, T. and TOMINAGA, S. 2008b. The Polymethoxy Flavones Content of Local Citrus Accessions on the Island of

Kikaijima in Kagoshima Prefecture, Japan. Bulletin of the Faculty of Agriculture, Kagoshima University, 58: 1-7 (in Japanese with English Summary).

YAMAMOTO, M., MATSUO, Y., KUNIGA, T., MATSUMOTO, R. and YAMADA, Y. 1998. Isozyme and RAPD Analyses of Shiikuwashas (*Citrus depressa* Hayata). Bulletin of the National Institute of Fruit Tree Science, 30/31: 39-51 (in Japanese with English Summary).

YAMAMOTO, M., MATSUSHIMA, K., IJICHI, T., UECHI, Y., KAWAGUCHI, S., NAKANO, H., NOMURA, T., TANIMURA, O., KUBO, T. and TOMINAGA, S. 2006. Exploration and Preservation of Local Citrus Germplasms in the Amami Archipelago. Bulletin of the Experimental Farm, Faculty of Agriculture, Kagoshima University, 29: 5-11 (in Japanese with English Summary).

YAMAMOTO, M., TSUCHIMOCHI, Y., NINOMIYA, T., KOGA, T., KITAJIMA, A., YAMASAKI, A., INAFUKU-TERAMOTO, S., YANG, X., YANG, X., ZHONG, G., NASIR, N., KUBO, T. and TOMINAGA, S. 2013. Diversity of Chloroplast DNA in Various Mandarins (*Citrus* spp.) and Other Citrus Demonstrated by CAPS Analysis. Journal of the Japanese Society for Horticultural Science, 82: 106-113.

YAMAMOTO, M., YAMASAKI, A., SHIMIZU, T., KITAJIMA, A., KUBO, T. and TOMINAGA, S. 2010b. SSR Analysis of Local Citrus Accessions Grown in Amami Islands. Horticultural Research (Japan), 9 (suppl. 2): 86 (in Japanese).