Parentage Analysis of Pepper Cultivar ‘Manganji’ (*Capsicum annuum* L.) Characterized by SSR Markers

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Summary

Pepper local cultivar ‘Manganji’ has been cultivated at Maizuru in Kyoto Prefecture since the early 20th century. It is said that the ‘Manganji’ is an offspring of the cross between cultivars ‘Fushimi-amanaga (Fushimi)’ and ‘California Wonder (CW)’. However, there is no proof about the parentage relationship with the cultivars. Therefore, we investigated it using six cultivars with 113 simple sequence repeat (SSR) markers.

A phylogenetic tree was constructed by using 93 SSR loci which were polymorphic among the cultivars ‘Manganji’, ‘Fushimi’, ‘CW’, ‘LS2341’ (Malaysian origin) and two *Capsicum chinense*. Four *C. annuum* cultivars and two *C. chinense* ones were clearly divided into two clusters. It also revealed that ‘Fushimi’ was the closest to ‘Manganji’ and ‘LS2341’ was positioned next to it. ‘CW’ was most distantly located in the four *C. annuum* cultivars examined.

Seventy six SSR loci were polymorphic among ‘Manganji’, ‘Fushimi’ and ‘CW’. In these 76 loci, the alleles between ‘Manganji’ and ‘Fushimi’ were the same in the 33 loci (43.4%), while only the 10 loci (13.2%) had the same alleles between ‘Manganji’ and ‘CW’. Contrary to ‘CW’ alleles, the 32 alleles (42.1%) of ‘LS2341’ were the same as ones of ‘Manganji’. In other 33 loci (43.4%), the alleles of ‘Manganji’ were different from the ones of both ‘Fushimi’ and ‘CW’. These were serious discrepancy of the parentage assumption between ‘Manganji’ and ‘Fushimi × CW’.

In consequence, parent – offspring relationship between ‘Manganji’ and two candidate cultivars were denied. In addition, ‘Manganji’ may have close relationship with old Asian cultivars rather than modern western cultivars.

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Ⅰ Introduction

1. History of ‘Manganji’ pepper and its production

‘Manganji’ is a local sweet pepper cultivar (*Capsicum annuum* L.) at Maizuru in Kyoto prefecture (Fig. 1). It has triangular fruit shape such as New-Mex pepper. It is said that Manganji pepper was firstly cultivated at Nakasuji village or Maruyae village at Maizuru around 100 years ago (Takashima, 1982).

Production of ‘Manganji’ started to increase around 1960s, because of promotion by Japan Agricultural cooperatives ‘JA Maizuru-Nakasuji’. They introduced rootstock in 1981 with the cooperation of the Kyoto Prefectural Research Institute of Agriculture. JA Maizuru-Nakasuji firstly shipped Manganji to Kyoto Central Wholesale Market in 1983. Then, ‘Manganji’ was authenticated as a brand vegetable by Association for Price and Distribution Stabilization of Kyoto Furusato Products in 1989. The sales amount has been expanding since

1983 and reached 330 million yen in 2015 by JA Kyoto Ninokuni (Agriculture, Forestry Division Maizuru City Hall, 2016). At present, ‘Manganji’ pepper is widely cultivated in Chutan area (Maizuru, Ayabe and Fukuchiyama).

On the other hand, several breeding programs have been done by Kyoto Prefectural Institute of Agricultural Biotechnology (KAB). The fruit was appreciated by consumers for its distinctive flavor. However, it occasionally contains a substance that imparts an undesirable hot taste. A new pure bred cultivar, ‘Kyoto-Manganji No.1 (MDH)’, was bred from the local ‘Manganji’ cultivar by anther culture in 2007 (Minamiyama and Inaba, 2007). ‘MDH’ stands for ‘Manganji’ of double haploid. Thereafter marker-assisted selection was carried out in order to develop a novel non-pungent cultivar, ‘Kyoto Manganji No. 2’, by transferring the recessive gene for pungency to the original cultivar ‘Manganji’ (Minamiyama *et al.*, 2012).

Figure 1 Fruits of ‘Manganji’ pepper
This picture is presented by courtesy of Mr. Shun Ito

2. Unknown origin of ‘Manganji’

Although the recent breeding processes have been revealed by several literatures, there is no certain record about established process of ‘Manganji’ as a local cultivar. There are following two hypotheses for the origin of ‘Manganji’. One was the crossbred among local cultivars (Takashima, 1982), and the other was come from the cross between ‘Fushimi-amanaga (Fushimi)’ and ‘California Wonder (CW)’ (Kyoto Prefectural Research Institute of Agriculture, 1980).

Therefore, the objective of this study is to find certain information related to the parentage hypotheses of ‘Manganji’ pepper by using simple sequence repeat (SSR) markers.

Ⅱ Materials and Methods

1. Plant materials

Four *C. annuum* cultivars (‘MDH’, ‘Fushimi’, ‘CW’ and ‘LS2341’) and two *C. chinense* (‘PI152225’ and ‘PI159236’) were used in this study (Table 1). ‘MDH’ was suitable for DNA analysis because of the perfect homozygous scoring, and used for the representative of local cultivar ‘Manganji’.

2. Genotyping of SSR

Genomic DNA was extracted from young leaf tissues with the Nucleon PhytoPure Genomic DNA Extraction Kits (GE Healthcare, N.J., U.S.A.). SSR polymorphisms were scored according to the method described by Minamiyama *et al.* (2006). The SSR primer pairs used in this study were developed from genomic libraries and/or registered sequences at the databases (Minamiyama *et al.*, 2006, 2007, Yi *et al.*, 2006, Nagy *et al.*, 2007, Mimura *et al.*, 2010, 2012). Of these, 113 SSR markers were chosen in order to involve alleles derived from all the 12 chromosomes of pepper genome (Table 2). The number of SSR assigned in each chromosome was 12, 3, 10, 7, 16, 10, 6, 10, 13, 1, 7 and 7 from chromosome 1 to 12, respectively, whereas the assignment of 11 SSR markers was unknown.

3. Phylogenetic analysis

A neighbor-joining tree (Saitou and Nei 1987) was constructed based on Nei’s genetic distance (Nei *et al.* 1983) using Populations 1.2.32 (Langella, 2011).

Table 1. Charactaristic of Pepper cultivars 'Manganji', 'Fushimi', 'CW' and other 3 cultivars used for phylogenic tree

Cultivar	Origin	Obtained from	Fruits shape	Pungency	Species	References
Kyoto Manganji No. 1	Kyoto	KAFF*	Triangular	sweet	*Capsicum annuum*	Minamiyama and Inaba (2007), Mimura *et al* . (2010)
Fushimi-amanaga	Kyoto	KAFF	Elongated	sweet	*C. annuum*	Takashima (1982, 2003),
California Wonder	U.S.A.	NARO**	Blocky	sweet	*C. annuum*	Andrews(1995)
LS2341	Malaysia	NARO	Triangular	hot	*C. annuum*	Mimura et al. (2000, 2009ab, 2010, 2012)
PI152225	Peru	NARO	Triangular	hot	*Capsicum chinense*	http://www.gene.affrc.go.jp/index_en.php
PI159236	U.S.A.	NARO	Triangular	hot	*C. chinense*	http://www.gene.affrc.go.jp/index_en.php

* KAFF = Kyoto Prefectural Agricultural, Forestry and Fisheries Technology Center
** NARO = The Genetic Resources Center, National Agriculture and Food Research Organization
Fruit shape criteria was defined by "Descriptors for Capsicum" of IPGRI, AVRDC and CATIE (1995)

Table 2. SSR markers used in this study

Marker name	References
CAMS015-2, CAMS020, CAMS024, CAMS037, CAMS049, CAMS051, CAMS056, CAMS065, CAMS066, CAMS070, CAMS072-1, CAMS072-2, CAMS075, CAMS081, CAMS089, CAMS090, CAMS095, CAMS101, CAMS117, CAMS122, CAMS134, CAMS142, CAMS153, CAMS156-1, CAMS156-2, CAMS162, CAMS163, CAMS173-1, CAMS177, CAMS190, CAMS191, CAMS194, CAMS199, CAMS201, CAMS207, CAMS215, CAMS227, CAMS236, CAMS237, CAMS301, CAMS313, CAMS319, CAMS324, CAMS326-1, CAMS327, CAMS330, CAMS336, CAMS340, CAMS348, CAMS351, CAMS352, CAMS358, CAMS360, CAMS361, CAMS378, CAMS396, CAMS398-2, CAMS405, CAMS417, CAMS420, CAMS424, CAMS451-1, CAMS454, CAMS456, CAMS460, CAMS462, CAMS478, CAMS489, CAMS492, CAMS493, CAMS606, CAMS610, CAMS619, CAMS626, CAMS644, CAMS647, CAMS649, CAMS679, CAMS684-2, CAMS687, CAMS806-1, CAMS806-2, CAMS811, CAMS826-1, CAMS844, CAMS855, CAMS865, CAMS876, CAMS885, CAMS891, CAMS892-3	Minamiyama *et al.* (2006)
CAeMS035, CAeMS049	Minamiyama *et al.* (2007) Mimura *et al.* (2010, 2012)
HpmsE004, HpmsE005, HpmsE010, HpmsE020, HpmsE057, HpmsE062, HpmsE072, HpmsE075, HpmsE081, HpmsE082, HpmsE090, HpmsE110, HpmsE128, HpmsE132, HpmsE145, HpmsE149	Yi *et al.* (2006)
GPMS112, EPMS376, EPMS418, EPMS480	Nagy *et al.* (2007)

Ⅲ Results

1. Phylogenetic analysis by SSR polymorphisms

In the 113 SSR markers, three markers had no polymorphism among the six cultivars and genotypes of 17 SSRs involved data missing in two or more cultivars. Therefore, these 20 SSRs were omitted from the calculation. A phylogenetic tree was constructed by using 93 SSR loci which were

polymorphic among ‘MDH’, ‘Fushimi’, ‘CW’, ‘LS2341’ and two *C. chinense* cultivars. Two main clusters are easily distinguishable with four *C. annuum* cultivars and two *C. chinense* ones. It also revealed that ‘Fushimi’ was the closest to ‘MDH’ and ‘LS2341’ was positioned next to it (Fig. 2). The above mentioned clusters were supported with moderate to high bootstrap values (≥70%). ‘CW’ was most distantly located in the four *C. annuum* cultivars examined here.

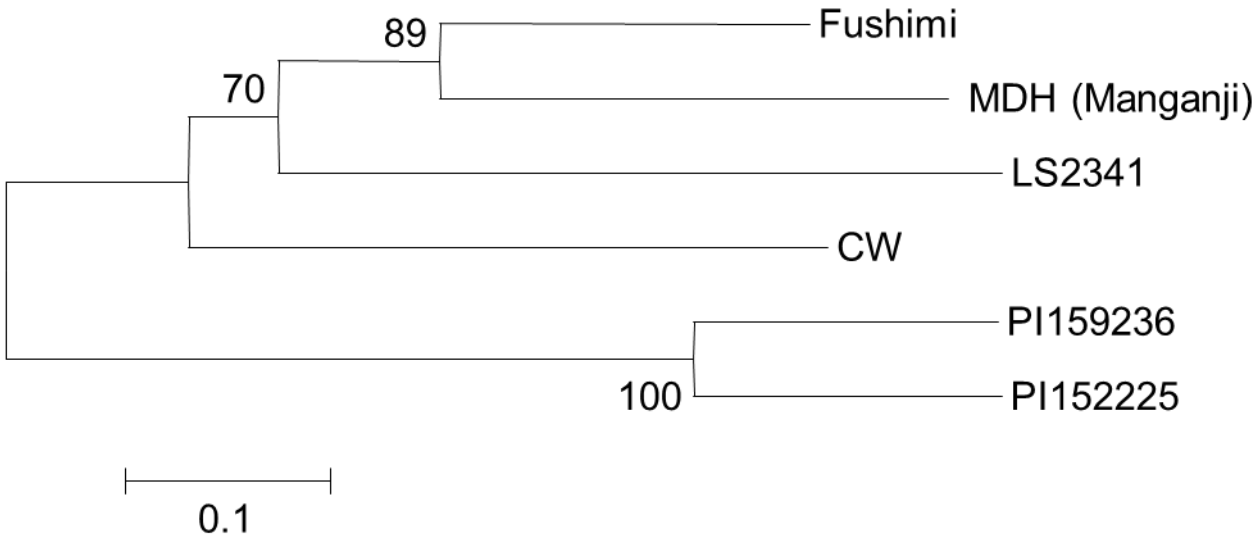

Fig. 2 Neighbor-joining (NJ) rooted phylogenetic tree of six pepper cultivars based on D_A genetic distance (Nei *et al.* 1983) of 93 SSR markers. Bootstrap values (percentages) were computed over 1000 replications

2. Parentage analysis of ‘Manganji’

In the 113 SSR markers, we obtained the complete genotyping data for 106 SSRs. Of these, 76 SSR loci were polymorphic among ‘MDH’, ‘Fushimi’ and ‘CW’. The alleles between ‘MDH’ and ‘Fushimi’ were the same in 33 loci (43.4%) out of the 76, while only 10 loci (13.2%) had the same alleles between ‘MDH’ and ‘CW’. Contrary to ‘CW’ alleles, the 32 alleles (42.1%) of ‘LS2341’ were the same as ones of ‘MDH’. In other 33 loci (43.4%), the alleles of ‘MDH’ were different from the ones of both ‘Fushimi’ and ‘CW’.

Ⅳ Discussion

In this study, the objective is to find certain information related the parentage hypotheses of ‘Manganji’ pepper.

‘Manganji’ used in this study was ‘MDH’ which is a pure bred line from original cultivar without using any outcrossing, and the ‘Fushimi’ is considered as an old local cultivar in Yamashiro area (Takashima 1982, 2003), because peppers have been cultivated in the area for more than 330 years (Kurokawa, 1684). ‘CW’ was obtained from U.S.A. These materials probably have minor genetic difference from the same cultivars in early 20th century. For example, ‘California Wonder’ was firstly released in 1928 (Andrews, 1995). Actually, Votaba and Bosland (2002) pointed out the genetic variability in heirloom bell pepper ‘California Wonder’ nowadays. However, Nicolai *et al*. (2013) revealed that three *C. annuum* clusters were significantly distinct for plant and fruit descriptors corresponding to cultivar types. It implies that genotyping data are relatively stable within the same cultivar types. Therefore, the genetic information in this study is considered to be relatively similar with the cultivars data in those days. The genotyping data in this study have serious discrepancy of the parentage assumption of ‘Fushimi × CW’. In consequence, parent – offspring relationship between ‘Manganji’ and two candidate cultivars is unduly suspicious. In contrast, ‘Manganji’ has close relationship with ‘Fushimi’

and ‘LS2341’.

‘LS2341’ was introduced as an accession JP187992 from Tropical Agriculture Research Center (Okinawa) collection. It originally came from Malaysia as a local cultivar before 1986 (The Genetic Resources Center, 2016). It is noteworthy that ‘Manganji’ may have close relationship with old Asian cultivars rather than modern western cultivars according to this study. To reveal ‘Manganji’ parentage in detail, further data for various cultivars are required.

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SSR マーカーを利用した‘万願寺とうがらし’(*Capsicum annuum* L.)の親子関係分析

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摘要

‘万願寺とうがらし（万願寺）’は、20 世紀初めから舞鶴市で栽培されてきた。この‘万願寺’は、‘伏見甘長とうがらし（伏見）’と‘カリフォルニアワンダー（ＣＷ）’との交配によるものと言われてきた。しかし、これらの品種の親子関係は証明されていない。そこで、本研究では、113 個のＳＳＲ（simple sequence repeat）マーカーを用いて、６品種について分析を行った。トウガラシ属アニューム種の‘万願寺’、‘伏見’、‘ＣＷ’、‘ＬＳ２３４１（マレーシア原産）’、およびトウガラシ属キネンセ種の２品種の間で多型のあった 93 個のＳＳＲマーカーから進化系統樹を作成した。その結果、アニューム種の４品種とキネンセ種の２品種は２つのクラスターに明確に分かれた。アニューム種内では、‘万願寺’と遺伝的に最も近い関係にあるのは‘伏見’、次いで‘ＬＳ２３４１’であり、’ＣＷ‘は、最も遠縁であった。

‘万願寺’、‘伏見’および‘ＣＷ’の間では、ＳＳＲマーカーの 76 遺伝子座で、多型があった。この 76 遺伝子座のなかで、‘万願寺’と‘伏見’の間で、33 遺伝子座（43.4%）において同じ対立遺伝子（アリル）を持っていたが、‘万願寺’と‘ＣＷ’の間では、10 遺伝子座（13.2%）のみアリルが一致した。‘ＣＷ’とは対照的に、‘ＬＳ２３４１’のアリルは、32 遺伝子座（42.1%）において‘万願寺’と一致した。その他の 33 遺伝子座（43.4%）では、‘万願寺’のアリルは、‘伏見’および‘ＣＷ’の両方と異なった。交配親の仮説と今回の分析結果は、大きく矛盾しており、‘万願寺’が‘伏見’と‘ＣＷ’の後代であるという仮説は否定された。‘万願寺’は、近代の西洋品種よりも、古いアジアの品種と近い関係なのかもしれない。

キーワード:‘万願寺’、トウガラシ、SSR、親子関係、系統樹