Variation of Mitochondrial DNA of Potatoes Cultivated in Japan

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Abstract

One of the plant genomes, mitochondrial (mt) DNA, of potato has not been as extensively investigated as the other 2 genomes, nuclear and chlroplast DNAs. Restriction fragment length polymorphisms of mt DNA of potato were studied to analyze the mt DNA variation of potato. The mt DNA was isolated from leaves of 21 Japanese potato cultivars (*Solanum tuberosum* ssp. *tuberosum*) and a stain of *S. tuberosum* ssp. *andigena*. The mt DNAs were analyzed by restriction endonuclease analysis using 8 endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I) and Southern hybridization analysis using the endonucleases and 3 rice mt DNAs (*coxI, atpA* and *atp 6* genes) as probes. The *Hind* III, *Sma*I and *Xho*I digests gave 2 different patterns, and *Pvu*II and *Sal*I gave 3. Based on the fragment patterns, the mt genome of the potatoes was classified into 4 types. The combination of *Hind* III/*atpA* revealed 3 hybrid band patterns, and 5 combinations of *Bam*HI/*atpA*, *Eco*RI /*atpA*, *Eco*RI/*coxI*, *Sal*I/*atpA* and *Xho*I/*coxI* revealed 2. The mt genome was classified into 3 types based on the hybrid band patterns. Based on the data of both analyses, the mt genome of the potatoes was divided into 5 groups.

Introduction

Three organelles, nucleus, chloroplast (ct) and mitochondrion (mt), in a plant cell contain genetic information. Genetic information in the nucleus is inherited from both parents, whereas that of ct and mt is usually inherited maternally. Although genomes in all three organelles show an autonomous and independent inheritance, they interact with each other for gene expression.

Analysis of DNA variation has been used to study the genetic diversity and the phylogenetic relationships among wild and cultivated species of potato. Bonierbale *et al*. (1988) and Gebhardt *et al*. (1989 b) analyzed the nuclear DNA variation and constructed restriction fragment length polymorphism (RFLP) linkage maps corresponding to 12 linkage groups of potato. Gebhardt *et al*. (1989 a) identified potato varieties and lines with RFLP-fingerprints. Bonierbale *et al*. (1988) revealed the modes of chromosomal evolution between potato and tomato. Phylogenetic relationships among *Solanum* species were inferred using DNA RFLPs as discriminating characters (Debener *et al*., 1990). The ct DNA varition has been studied extensively. Hosaka *et al*. (1984) studied the phylogenetic relationship between the tuberous *Solanum* species by restriction endonuclease analysis of ct DNA. Hosaka and Hanneman (1988 a) detected a geographical cline from the Andean region to coastal Chile for tetraploid potato by using restriction enzyme analysis of ct DNA. The relationships of ct DNA types among cultivated potato species and their wild relatives were described (Hosaka, 1986; Hosaka *et al*., 1988). The ct DNA of cultivated potato differs from that of the wild type by one physical deletion (Hosaka *et al*., 1988). Hosaka (1993) also determined the ct DNA types for most of the Japanese potato varieties. He then suggested that old varieties are relic potatoes of early European potatoes and similar ct DNA intoroduction and incorporation occurred in both Europe and Japan.

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The mt DNA variation of potato, however, has not been investigated extensively, compared with the other 2 genomes. Perl *et al*. (1990) only used potato mt DNA variation among 4 species and identified the organelle donor of protoplast-fusion-derived cybrids by Southern blot analysis using heterogeneous mt genes as probes. As the information on mt DNA variation of potato is scarce, in this paper, I report the mt DNA variation of Japanese cultivated potatoes revealed by RFLP analysis.

Materials and methods

1 Materials

Twenty-one Japanese cultivars of potato (*Solanum tubersoum* ssp. *tuverosum*) and a strain of Andigena (*S. tuberosum* ssp. *andigena*) were used for mt DNA isolation (Table 1). These materials were provided from Hokkaido National Agricultural Experiment Station (Shimamatsu, Hokkaido). The potato plants were grown at the experimental farm. Their female parents are also listed because the mt genome is usually inherited maternally.

2 Restriction endonuclease analysis

For mt DNA isolation from leaves, restriction endonuclease reaction and agarose gel electrophoresis were basically adopted the procedures described by Kadowaki *et al*. (1986). The homogenization buffer was modified and contained 1% polyvinylpyrrolidone (PVP). Eight endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I) were used to digest mt DNA.

3 Southern hybridization analysis

After electrophoresis, DNAs were transferred to a nylon membrane (Hybon−N+; Amersham) by using a vacuum blotting apparatus (LKB 2016 VacuGene) according to the instructions of the manual. The

Table 1 Materials and their female parents

Cultivar	Female parent
Irish Cobbler	Early Rose
Norin No. 1	Irish Cobbler
Kita-akari	Irish Cobbler
Hatsuhubuki	Irish Cobbler
Tachibana	Irish Cobbler
Shimabara	Irish Cobbler
Unzen	Norin No. 1
Dejima	Hokkai No. 31
Nishiyutaka	Dejima
Waseshiro	Konkei No. 7
Toyoshiro	Hokkai No. 19
Hokkaikogane	Toyoshiro
Konahubuki	Toyoshiro
Setoyutaka	Saikai No. 10
Meihou	Chijiwa
Ezo-akari	Tunika
Toyo-akari	Tunika
May Queen	(Garnet Chili)
Yukijiro	Kennebec
Eniwa	Shimakei No. 267
Benimaru	Lembke Fruhe Rosen
Andigena	−

−, Unknown

DNA was refixed by drying the nylon membrane at 80°C for 2 hours. The membrane was hybridized with DNA probes. Three rice mt genes, cytochrome oxidase subunit I (*coxI*), F_1-ATPase a sub-unit (*atpA*) and ATPase subunit 6 (*atp 6*), were as probes. These probes were probided by Dr. K. Kadowaki, National Institute of Agrobiological Resources (Tsukuba, Ibaraki). Hybridization was carried out based on the ECL gene detection system (Amersham) according to the supplier's instructions.

4 Cluster analysis

The mt genome types were clustered by average linkage analysis (SAS) which is similar to the UPGMA method (Sneath and Sokal, 1973).

Results

1 Restriction endonuclease analysis

RFLPs of mt DNA among the potatoes were studied by restriction endonuclease analysis using 8 endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I). The *Bam*HI, *Eco*RI and *Pst*I digests did not give different patterns among the cultivars. The *Hin*dIII, *Sma*I and *Xho*I digests gave 2 patterns, and the *Pvu*II and *Sal*I gave 3 (Fig. 1). Table 2 shows the fragment constitution and the molecular sizes of individual fragments revealed by RFLP analysis. *Hin*dIII, *Pvu*II, *Sal*I, *Sma*I and *Xho*I digests gave 37, 31, 21, 36 and 34 common fragments other than the fragments listed in Table 2, respectively. Based on these fragment patterns, the mt DNAs of the potatoes were classified into 4 types. Out of 22 cultivars, the mt DNA types of 15 cultivars were grouped into Types 1 to 4 (Table 3). The mt DNAs of 9 cultivars belonged to Type 1, those of 4 cultivars to Type 2, that of 1 cultivar (May Queen) to Type 3 and that of 1 cultivar (Yukijiro) to Type 4.

Based on the results presented in Table 2 and the total restriction fragment positions, the numbers of all the restriction fragment positions and unique restriction fragment positions were calculated between

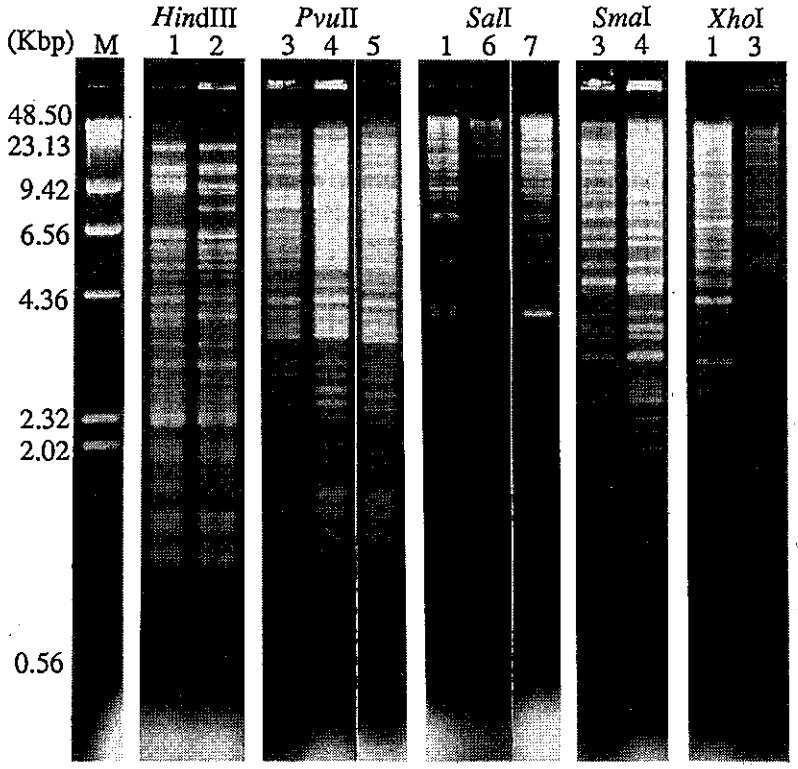

Fig. 1 Restriction fragment patterns of mt DNA of potatoes
1, Unzen. 2, Setoyutaka. 3, Meiho. 4, Toyoshiro
5, Yukijiro. 6, May Queen. 7, Andigena. M, Marker.

Table 2 Fragemnt constitution and molecular sizes of the individual fragments revealed by RFLP analysis based on the electrophoretic patterns of 5 endonuclease digests of mt DNA of potatoes

Fragment size (Kbp)	Type 1	2	3	4	Fragment size (Kbp)	Type 1	2	3	4
*Hin*dIII					*Sal*I				
11.5	+	−	+	+	21.7	+	+	−	+
8.0	−	+	−	−	9.4	−	+	−	−
6.4	+	−	+	+	8.8	+	−	+	+
5.2	−	+	−	−	7.0	+	+	−	+
5.0	+	−	+	+	6.0	−	+	−	−
4.9	−	+	−	−	5.3	+	−	−	+
*Pvu*II					5.0	−	+	−	−
8.6	+	−	+	+	4.8	+	−	+	+
7.8	−	+	−	−	3.2	+	−	+	+
7.7	+	−	+	+	3.1	+	+	−	+
5.3	−	−	−	+	2.2	+	−	+	+
3.2	−	−	−	+	*Xho*I				
3.0	−	+	−	−	23.7	−	+	−	−
2.9	+	−	+	+	22.6	+	−	+	+
2.8	−	+	−	−	20.5	−	+	−	−
*Sma*I					19.6	+	−	+	+
30.3	+	−	+	+					
2.3	−	+	−	−					

+, copy. −, no copy.

Table 3 Classification of mt genome of potatoes by endonuclease and Southern hybridization analyses

Cultivar	Mt genome type		
	Endonuclease	Hybridization	Total
Irish Cobbler	1	a	I
Norin No. 1	1	a	I
Kita-akari	1	a	I
Unzen	1	a	I
Dejima	1	a	I
Nishiyutaka	1	a	I
Waseshiro	1	a	I
Toyoshiro	1	a	I
Hokkaikogane	1	a	I
Setoyutaka	2	b	II
Meihou	2	b	II
Ezo-akari	2	b	II
May Queen	3	a	III
Yukijiro	4	a	IV
Benimaru	−	c	V
Andigena	2	b	II

−, Not determined.

every pair of the 4 mt genome types (Table 4) and clustering was performed by average linkage analysis (Fig. 2). The distance obtained by cluster analysis is not the same as, but corresponds to Nei's genetic distance (Nei, 1987). Type 2 was very differnt from the other types.

2 Southern hybridization analysis

RFLPs of the mt DNA of the potato cultivars were studied by Southern hybridization analysis using 3 rice mt genes (*coxI*, *atpA* and *atp 6*) as probes. The probe/enzyme combinations which enabled to detect polymorphic hybrid band patterns are listed in Table 5. Fig. 3 shows the polymorphic and monomorphic patterns. Out of 16 combinations, 6 combinations enabled to detect polymorphic patterns. The combination of *atpA*/*Hin*dIII revealed 3 patterns, and 5 combinations of *cox*I/*Eco*RI, *coxI*/*Xho*I, *atpA*/*Bam*HI, *atpA*/*Eco*RI, *atpA*/*Sal*I revealed 2. Table 6 shows the hybrid band constitutions and molecular sizes of individual hybrid bands detected as polymorphic. The mt DNAs were classified into 3 types, Types a to c, based on the hybrid band patterns. Out of 22 cultivars, the mt DNA types of 16 cultivars were determined (Table 3). The mt DNAs of 11 cultivars belonged to Type a, those of 4 cultivars to Type b, that of 1 cultivar (Benimaru) to Type c.

Based on the results of both restriction endonuclease and Southern hybridization analyses, the mt genomes of the potatoes were divided into 5 groupes, Types I to V. Nine cultivars belonged to Type I, 4 cultivars to Type II, and Types III to V included 1 cultivar each. May Queen, Yukijiro and Benimaru contained Type III, IV and V mt genome, respectively.

Based on the results listed in Tables 5 and 6, the numbers of all the hybrid band positions and unique hybrid band positions were calculated between every pair of the 3 mt genome types (Table 7). Type b

Table 4 Number and percentage of unique mt DNA restriction fragment positions among 4 mt genome types

Types compared	No. of fragment positions		% of unique fragment positions
	Total	Unique	
1, 2	187	26	13.9
1, 3	176	4	2.3
1, 4	178	2	1.1
2, 3	186	28	15.1
2, 4	189	28	14.8
3, 4	178	6	3.4

Five restriction enzymes (*Hin*dIII, *Pvu*II, *Sal*I, *Sma*I, *Xho*I) were used.

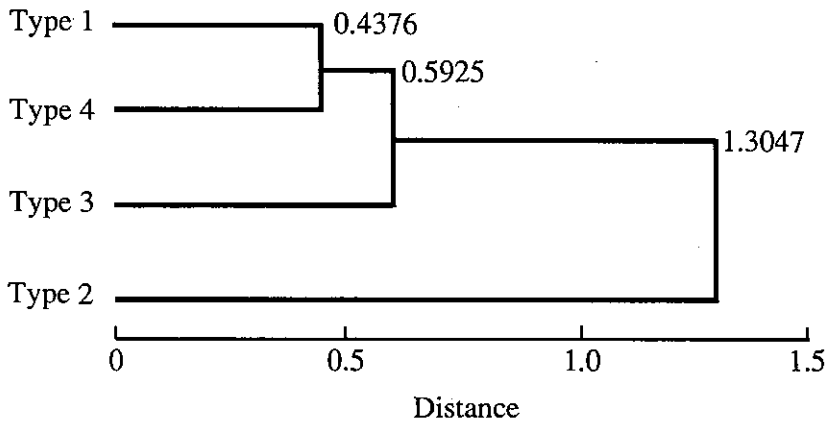

Fig. 2 Dendrogram showing the relationships among mt genome types clustered by average linkage analysis (SAS)

Table 5 Detection of RFLPs of potato mt DNA by Southern hybridization analysis using 3 rice mt genes as probes

Probe/enzyme combination	No. of hybrid band positions	No. of hybrid patterns
coxI/*Bam*HI	2	1
*Eco*RI	2	2
*Hin*dIII	1	1
*Pvu*II	1	1
*Sal*I	1	1
*Xho*I	4	2
atpA/*Bam*HI	4	2
*Eco*RI	3	2
*Hin*dIII	3	3
*Pvu*II	1	1
*Sal*I	3	2
*Xho*I	1	1
atp 6/*Bam*HI	1	1
*Eco*RI	1	1
*Sal*I	1	1
*Xho*I	2	1

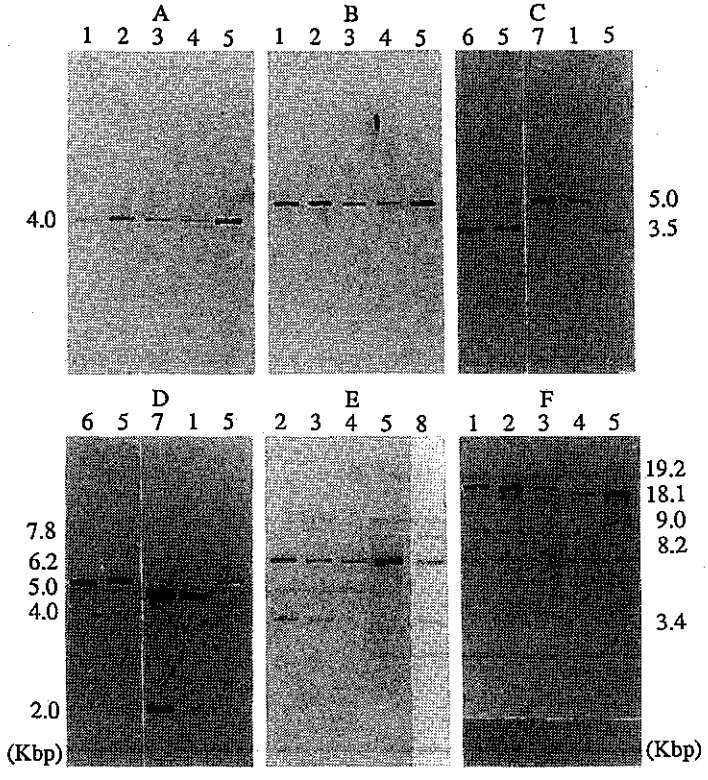

Fig. 3 Southern hybridization patterns of potato mt DNA probed with rice mt genes
A, *coxI*/*Hin*dIII. B, *coxI*/*sal*I, C, *coxI*/*Eco*RI.
D, *aptA*/*Eco*RI. E, *atpA*/*Hin*dIII, F, *coxI*/*Xho*I.
1, Norin No. 1. 2, Unzen. 3, May Queen. 4, Ezo-akari.
5, Andigena. 6, Setoyutaka. 7, Dejima. 8, Benimaru.

Table 6 Hybrid band constitution and molecular size of individual hybrid band in Southern hybridization patterns revealed by RFLP analysis of mt DNA of potatoes

Probe/enzyme combination	Hybrid band molecular size (Kbp)	Type		
		a	b	c
coxI/*Eco*RI	5.0	+	−	+
	3.5	−	+	−
/*Xho*I	19.2	+	−	+
	18.1	−	+	−
	9.0	−	+	−
	8.2	+	−	+
atpA/*Bam*HI	22.5	−	+	−
	18.1	+	−	+
	14.2	+	+	+
	6.9	+	+	+
/*Eco*RI	5.0	−	+	−
	4.0	+	−	+
	2.0	+	−	+
/*Hin*dIII	7.8	−	−	+
	6.2	+	+	+
	3.4	+	−	+
/*Sal*I	70.0	+	−	+
	12.5	+	+	+
	8.4	+	+	+

+, Present. −, Absent.

Table 7 Number and percentage of unique hybrid band positions among 3 mt genome types

Types compared	No. of band positions		% of unique band positions
	Total	Unique	
a, b	30	13	43.3
a, c	26	1	3.8
b, c	31	14	45.2

showed a long distance to the other 2 genome types based on cluster analysis as Type 2 in Fig. 2 (Data not shown). Type b was very different from the other types.

Discussion

The ct DNA types were determined for most of the Japanese potato varieties by Hosaka (1993). The ct DNAs were classified into 3 types. Out of 68 varieties, 55 (80.9%) showed the T type ct DNA similar to that of the current European potatoes. The T type ct DNA was found in both modern varieties and landraces. The W type ct DNA was found only in the modern varieties derived from *S. demissum*. The A type ct DNA was found only in the landraces, which is typical of Andean potatoes and has been found in derivatives of the oldest European variety, Myatt's Ashleaf (Hosaka and Hanneman, 1988).

The varieties with the T type ct DNAs contained Type I mt DNAs. Type II mt DNA was found in the varieties with the W type ct DNA. The varieties (May Queen, Yukijiro and Benimaru) which showed

different mt genome types (Types III, IV and V, respectively) had the common T type ct DNA. These findings suggest that the mt genome is more variable and more useful to analyze intraspecific variability than the ct genome. The Type II mt genome was found in the varieties with the A type ct DNA. The A type ct DNA is specific to ssp. *andigena* and is found in the early European potatoes. A strain of ssp. *andigena* had the Type II mt genome. As for Japanese potatoes, A type ct DNA was only found in old varieties. These results suggest that Japanese potatoes with the A type ct genome and Type II mt genome originated from early European potatoes.

Hosaka (1993) determined ct DNA types based only on *Bam*HI digestion patterns. The T type ct genome was derived from the W type ct genome with one physical deletion (about 400 bp) (Hosaka *et al*., 1988). Hosaka and Hanneman (1988 a ; b) reported the presence of a wide ct DNA diversity in the Andean cultivated potatoes. The Type II (Type 2 or Type b) mt genome was very different from the other types (Tables 4, 7 and Fig. 2). Based on the ct genome variation, the ct DNA type of cultivar with Type II mt genome differed from that with Type I mt genome only by one physical deletion, whereas Type II was genetically very different from Type I based on the mt genome variation. The mt DNA type of Benimaru could not be determined without the application of the Southern hybridization analysis. Therefore, the success of RFLP analysis depends on which analysis, enzyme and combination of probe/enzyme are employed. The analysis of ct DNA variation with a few enzyme digestion patterns was effective for elucidating the genetic variabililty among related wild and cultivated species. For the determination of intraspecific variability, the analysis of mt DNA variation seems more effective.

The information about genetic variability in *Solanum* species at the DNA level is still limited to the nuclear and chloroplast genomes. It is necessary to study mt DNA variation in all wild and cultivated species to identify comprehensive phylogenetic relationships among wild and cultivated *Solanum* species.

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Discussion

Valkoun, J. (ICARDA): You used RFLP to gain information on the DNA structure and composition of your crop, did you use other DNA techniques such as RAPD or PCR for molecular characterization and what was the correlation with the RFLP technique?

Answer: I did not use these techniques, although I know that many researchers are using them for potato.

Yonezawa, K. (Japan): I understand that the analysis of organelle DNA may provide information about the evolution or phylogenetic relationships of species and cultivars. It is difficult for me to understand how the variation of mitochondorial DNA can be utilized in genetic resources for plant improvement.

Answer: The variation of mitochondrial DNA can be used as optional criterion in addition to other coventional methods. The usefulness depends on how much further you should or could characterize materials. As for corn, mitochondorial variation was used to select hybrid parents after the epidemic of southern leaf late blight. When they make hybrids using male sterility, they often consider cytoplasmic traits (mitochondrial and/or chloroplast variation).