Variation of Peroxidase Isozymes in Sweet Potato Varieties

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Introduction

Sweet potato was introduced, early in the 17th century, to Okinawa in Japan from China, henceforward, propagated to Kagoshima and other districts of our country[4]. So, it may be inferred that Japanese old local varieties of sweet potato have genetically intimate relationships with Chinese varieties.

Many old breeding varieties in Japan were almost bred by being crossed among these several old local varieties and their offsprings: and here many varieties as breeding materials were introduced to our country from foreign countries, Formosa, U.S.A., Mexico and Brazil, while wild related species as breeding materials were introduced from Mexico. By the crossing between Japanese varieties and newly introduced foreign varieties or wild related species, new higher yielding varieties, Koganesengan and Minamiyutaka, superior to old breeding varieties, have been made up. These brilliant effects of breeding are considered to be depending on the spreading of narrow gentic diversity of Japanese breeding materials by the introduction of new gentic stock.

Enzyme polymorphism in plants has been reported by many investigators[1,2,7,8,9]. Sakai et al.[6] attempted to ascertain the genetic variation involved in natural populations of *Pinus*, with the aid of peroxidase isozyme variation. Nakagahra et al.[5] reported about the usefulness of zymogramic approach in advancing the working hypothesis on speciation and migration of the rice plant.

The present study was conducted with an objective to reveal genetic variation of peroxidase isozyme in sweet potato varieties.

Materials and Methods

Materials used are in total 126 varieties and strains of sweet potato, consisting of Japanese old local varieties, foreign varieties introduced from China, Formosa, U.S.A., Mexico and Brazil, leading varieties in Japan, interspecific hybrid between Japanese varieties and wild related species intoduced from Mexico, *Ipomoea trifida*, and *I. leucantha*, and other preserved breeding materials excepting the above mentioned strains, as shown in Table 1.

These varieties were planted in the laboratory field late in May and harvested early in November in 1976.

Semi-tuberous roots were sampled from sweet potato plant after harvesting, since

Table 1. Sweet potato varieties and interspecific hybrid strains used

Variety group	No. of varieties	Variety name
Japan (old local)	8	Motokajā, 4–33, Shichifuku, Hayato, Chōshū, Taihaku, Genji, Sakashita
China	8	Chūshi No.1, Chūshi No.5, Chūshi No.7 Chūshi No.8, Hakusanzairai, Kōkenkō Kōhi 70 nichi, Kōhikōshin
Formosa	7	Tainō No.7, Tainō No.16, Tainō No.56, Tainō No.57, Linnan No.1, C–456, Hiyake
U.S.A.	10	L–4–5, Southern queen, FV 62–41, Beikokuaka, Centenial, General grand, Gold rush, Heart gold, L 4–89, Pelican
Mexico	7	No.206(Me 6), No.226(Me 4), No.228(Me 10), Linea 24A, M 60–2, Tinian, Linea
Brazil	5	Santo Amaro, Visosa, NR515–20, F 682–38, F 683–4
Japan (leading varieties)	10	Nōrin No.1, Nōrin No.2, Nōrin No.3, Gokokuimo, Okinawa No.100, Benisengan, Ariakeimo, Nakamurasaki, Koganesengan, Minamiyutaka
Hybrid (interspecific)	11	I.11–126, I.12–95, I.42–2, I.83–109, I.95–193, I.120–110, I.3105–3, I.3108–15, BL.67–107–6, BL.67–107·–10
Japan (breeding materials)	60	Varieties or strains except leading varieties

the preliminary test gave stronger enzymatic activity and larger number of peroxidase isozyme bands for semi-tuberous root than those in other organs of sweet potato. Two grams of semi-tuberous roots were cut out of each sample into pieces in a bowl to which 6 *ml* of distilled water was added, then they were grounded and filtered with gauze, the filtrates were centrifuged at 10,000 *g* for 60 minutes. The supernatants were preserved in a refrigerator kept at $-20°C$ for a while until they were used for electrophoresis. Electrophoresis was performed by horizontal agar gel thin layer methods. The gel medium contained 0.7 % agar, 4 % polyvinylpyrrolidone and 5 % glycerin in a phosphate buffer solution adjusted at *pH* 6.85 and ion intensity 0.015. The hot gel medium was spread on to clean glass plate in a thin layer of 0.7 *mm*. After the gel was cooled down to room temperature, about 1 *cm* cotton threads were dipped in the crude extracts thawed just before, were inbedded in the original position of the gel plate. Both ends of the gel plate were connected with filtrating paper to a phosphate buffer solution adjusted at *pH* 6.85 and ion intensity 0.05.

Electrophoresis was carried out at 1 *mA/cm* for 90 minutes at 4°*C*. After electrophoresis, the plate was sprayed first with 1 % H_2O_2 solution and then with sodium acetate buffer solution (*pH* 4.5, 0.01 *M*) containing 0.1 % benzidine acetate.

Results

The peroxidase isozyme bands observed in our materials are diagramatically represented in Fig. 1. As shown in Fig. 1, we observed six bands on the cathodic side and twenty-two on the anodic side. Of these bands, 5C band was clearly discriminated from 6C band in some cases, but occasionally they did not make distinct separation, showing

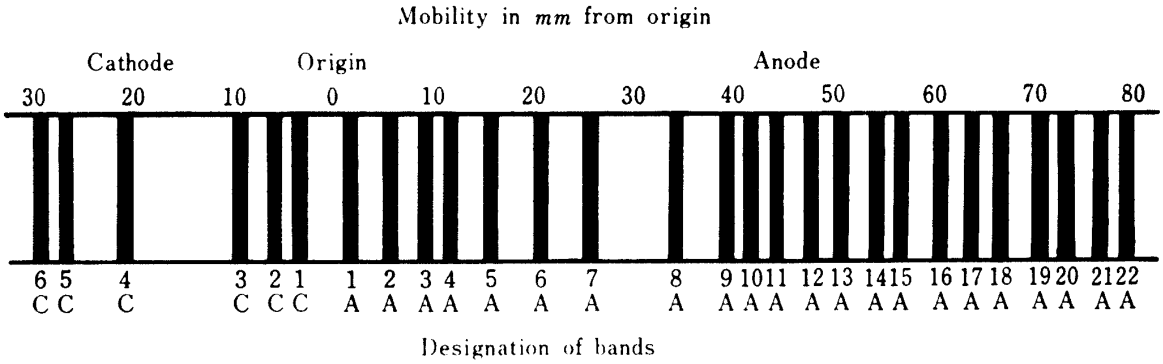

Fig. 1. Peroxidase isozyme bands observed in semi-tuberous root of sweet potato varieties used.

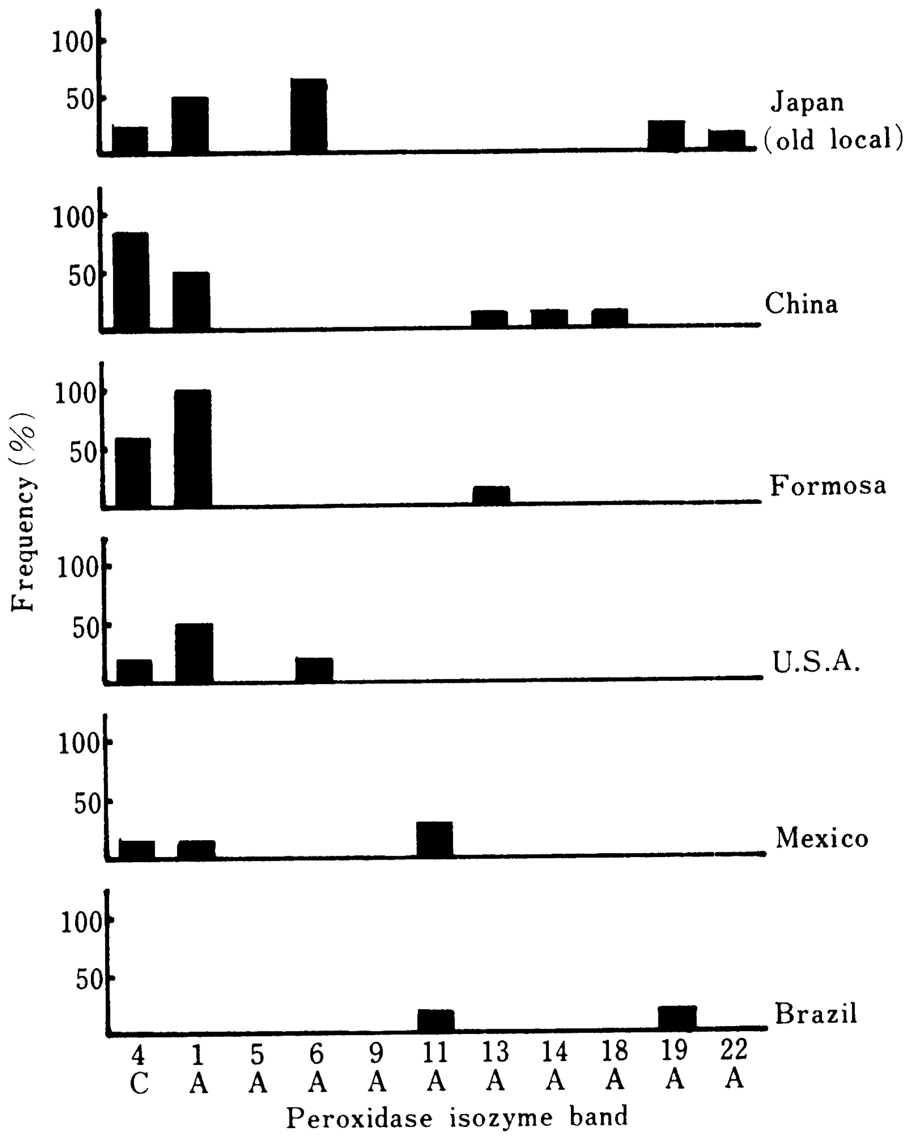

Fig. 2. Occurrence-frequencies of several peroxidase isozyme bands showing lower incidences.

Table 2. Occurrence-frequency in percent of peroxidase isozyme bands

Isozyme band	Variety group J* (O, L)	C	F	U	M	B	J (L)	H (I, S)	J (B, M)	Total
6C	100	100	100	90	100	100	100	91	92	94
5C	25	75	71	90	14	40	40	45	52	52
4C	25	87	57	20	14	0	20	27	32	32
3C	100	75	57	90	86	80	80	73	82	81
2C	25	50	71	40	29	40	30	36	42	40
1C	100	100	100	100	100	100	100	100	100	100
1A	50	50	100	50	14	0	40	0	27	32
2A	25	62	29	50	71	100	70	55	73	63
3A	75	100	71	70	86	100	90	100	100	93
4A	63	62	100	70	57	80	10	64	45	54
5A	0	0	0	0	0	0	0	27	5	5
6A	63	0	0	20	0	0	0	45	8	13
7A	100	100	100	90	71	80	100	91	95	94
8A	100	100	71	90	86	80	90	73	87	87
9A	0	0	0	0	0	0	0	9	0	1
10A	88	88	86	90	71	100	90	100	97	93
11A	0	0	0	0	29	20	0	0	0	2
12A	100	100	100	100	86	100	100	100	100	99
13A	0	13	14	0	0	0	10	45	15	13
14A	0	13	0	0	0	0	0	0	2	2
15A	88	75	86	80	71	60	80	45	52	63
16A	100	100	100	100	100	100	100	100	100	100
17A	63	75	43	60	57	80	50	64	67	63
18A	0	13	0	0	0	0	0	0	8	5
19A	25	0	0	0	0	20	10	0	8	6
20A	100	50	57	60	71	40	50	27	63	60
21A	88	50	100	80	71	100	100	82	88	86
22A	13	0	0	0	0	0	10	0	7	6

*Note; J (O, L): Japan (old local), C: China, F: Formosa, U: U.S.A., M: Mexico, B: Brazil, J (L): Japan (leading varieties), H (I, S): Hybrid (interspecific)
J (B, M): Japan (breeding materials)

a broad band. Then, when the band showed such a broad one, it was classified as two bands, 5C and 6C. Also, when the band showed a narrow band, it was classified as one band either 6C or 5C, according to the comparison with the mobility of the bands of other varieties showing clear separation on the same plate. As band 20A, 21A and 22A also showed similar tendencies, they were classified basing on the same method. Density of each band differed with band, and also the density of the band showing same mobility differed with varieties. But, in this report, the densities of respective bands were not compared, on account of the difficulty due to this experimental method.

Peroxidase isozyme bands observed in this experiment differed in occurrence-frequencies. They were classified into three groups, basing on their incidences. Table 2 shows occurrence-frequencies of each band. As shown in Table 2, band 4C, 1A, 5A, 6A, 9A, 11A, 13A, 14A, 18A, 19A and 22A showed lower incidences, band 6C, 3C, 1C, 3A, 7A, 8A, 10A, 12A, 16A and 21A showed higher incidences, and the other band 5C, 2C, 2A, 4A, 15A, 17A and 20A showed intermediate incidences. Several bands showing lower incidences entirely did not occur in some variety groups, such as band 9A occurred only in one strain of interspecific hybrid, and band 11A occurred in two varieties of Mexico and in one variety of Brazil, respectively.

Generally, lower incident bands were observed in near location of higher incident ones (Table 2, Fig. 1).

Fig. 2 shows occurrence-frequencies of peroxidase isozyme band showing lower incidence in six variety-groups from different sources. It is noticeable in Fig. 2 that though band 4C does not occur in Brazilian varieties, it shows 14% incidences in Mexican varieties and shows gradual increasing in incidence in American, Formosan and Chinese varieties, and again decreasing in Japanese old local varieties. Band 1A also shows almost the same tendency of incidences, but the highest incidence is shown in Formosan varieties. Band 11A occurred only in Mexican and Brazilian varieties and did not occur in other variety groups. But, band 5A, 6A, 9A, 13A, 14A, 18A, 19A and 22A did not occur in Mexican varieties, though they occurred in some of other variety-groups (Table 2, Fig. 2). Higher incidence bands, of course, showed higher occurrence-frequencies in any variety groups, irrespectively.

Intermediate incidence bands showed either lower or higher occurrence-frequency in other variety groups than in Mexican variety group (Table 2).

Frequency distributions of the number per variety of peroxidase isozyme bands in nine variety groups, the averages and the standard deviations are shown in Table 3. It is shown in Table 3 that Mexican varieties show particularly lower number per variety of peroxidase isozyme bands. This is more distinctly presented in Table 4 in which differences of average number per variety of peroxidase isozyme bands among variety groups are shown. As shown in Table 4, the differences of average number per variety of peroxidase isoyzme bands between Mexican variety group and Japanese, Chinese or Formosan variety groups were significant, respectively. And also, the differences of those among Japanese, Chinese and Formosan variety groups were very small, respectively.

Differences between each zymogram of two varieties were compared with, by using the similarity index value.

$$S = \frac{\text{Similarities}}{\text{Similarities} + \text{Dis-similarities}} \times 100$$

S: similarity index value (S value) between all the combinations of the entries.
Similarity: the number of isozymes in similar mobility in the two entries compared.
Dis-similarity: the number of isozymes occurring in one of the entries but not in the other.

These similarity index values were calculated in the whole two combinations of total 66 varieties in 8 variety groups, except Japanese breeding materials, then these values were averaged within and between variety groups.

Average similarity index values within and between variety groups are shown in Table 5. As shown in Table 5, average similarity index value within Formosan varieties showed the highest value (70%), and the values within other variety groups, excepting Mexican varieties and hybrid strains, ranged from 64.4 percent to 66.4 percent, while the values within Mexican varieties and hybrid strains showed somewhat lower ones, 59.4 and 58.8 percent, respectively. Namely, these values clearly show the fact that the difference of peroxidase zymograms within Formosan varieties are smaller, while the

Table 3. Number per variety of peroxidase isozyme bands in 9 variety groups

Variety group	Number per variety of peroxidase isozyme bands 9	10	11	12	13	14	15	16	17	18	19	20	No. of variety	Average	Standard deviation
Japan (old local)				1	1		3	2				1	8	15.25	2.37
China				1		2	1	2		2			8	15.38	2.07
Formosa						1	4	2					7	15.14	0.69
U.S.A.			1	1	3		1	2	1	1			10	14.40	2.32
Mexico			2	1	1	2	1						7	12.86	1.57
Brazil			1		1	1	1			1			5	14.20	2.59
Japan (leading varieties)		1	1	1	2	1	1	2	1				10	13.70	2.13
Hybrid (interspecific)			2		2	2	4			1			11	14.00	2.00
Japan (breeding materials)	1	2	3	3	11	12	10	10	2	2	2	2	60	14.45	2.32

Table 4. Differences of average number pervariety of peroxidase isozyme bands among variety groups

Variety group		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Japan (old local)	(1)								
China	(2)	0.13							
Formosa	(3)	0.11	0.24						
U.S.A.	(4)	0.85	0.98	0.74					
Mexico	(5)	2.39*	2.52*	2.28*	1.54				
Brazil	(6)	1.05	1.18	0.94	0.20	1.34			
Japan (leading variety)	(7)	1.55	1.68	1.44	0.70	0.84	0.50		
Hybrid (interspecific)	(8)	1.25	1.38	1.14	0.40	1.14	0.20	0.30	
Japan (breeding materials)	(9)	0.80	0.93	0.69	0.05	1.59	0.25	0.75	0.45

*: Significant at 5% level

Table 5. Average similarity index values within and between variety groups

Variety group		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Japan (old local)	(1)	64.9							
China	(2)	61.9	66.0						
Formosa	(3)	62.2	70.0	70.0					
U.S.A.	(4)	64.3	64.3	65.3	64.5				
Mexico	(5)	62.0	60.4	58.8	60.6	59.4			
Brazil	(6)	63.4	59.7	59.7	63.7	63.9	64.4		
Japan (leading variety)	(7)	63.7	62.9	61.9	62.8	62.8	62.8	66.4	
Hybrid (interspecific)	(8)	59.9	58.0	58.0	61.6	57.7	61.6	59.0	58.8

differences of those within Mexican varieties and interspecific hybrid are larger.

In comparison of average similarity index values within and between variety groups, it is shown in Table 5 that average similarity values within variety groups generally show larger values than those between variety groups, though there are few exceptions, while the average similarity index values within Mexican varieties and interspecific hybrid strains show more or less lower ones than those between variety groups. And also, when average similarity index values between variety groups with geographically nearer distance of sources were compared, the similarity index values between Japanese old local variety group and Chinese or Formosan variety groups did not show especially higher ones, while those between Chinese variety group and Formosan variety group showed somewhat higher ones.

Discussion

Peroxidase isozyme bands of sweet potato varieties observed in this experiment could be classified into three groups, lower, higher and intermediate incident band ones, according to their occurrence-frequency. Some of lower and intermediate incident bands showed the lowest occurrence-frequency in Mexican or Brazilian variety groups, and showed gradual increasing in American, Formosan or Chinese variety groups, decreasing in the Japanese old local variety group, again. Moreover, several bands showing higher mobility in anodic side, not occurring in Mexican variety group, were observed in Formosan, Chinese or Japanese variety groups, though it was with lower occurrence-frequencies. Namely, concerning the occurrence-frequencies of these bands, Japanese, Chinese and Formosan variety groups showed somewhat similar tendencies and Mexican variety group showed different ones.

The average number per variety of peroxidase isozyme bands of the variety group of Mexico showed especially smaller value, and the differences of the average number between the variety group of Mexico and Japanese, Chinese or Formosan variety groups were significantly large, respectively, though the differences of the average number among variety group of Japan, China and Formosa were very small. Basing on the above mentioned result concerning several band incidences and average number per variety of peroxidase isozyme band, it may be estimated that Japanese old local varieties have genetically intimate relationships with the varieties of China or Formosa, and have not so intimate ones with the varieties of Mexico or U.S.A. . These relationships between Japanese old local varieties and Chinese or Formosan ones probably seem to have the relation with the migration course of sweet potato varieties to Japan.

Finally, it is a result of great interest that Mexican varieties showed lower similarity index values within the variety group as well as between other variety groups, as interspecific hybrid strains do. The following fact seems to have been shown that Mexican variety group contains various genetical variations. And such results seem to support the postulation that the center of genetic diversity of sweet potato exists in the Central America.

Summary

The variations of peroxidase isozyme of semi-tuberous root of sweet potato varieties were investigated, by electrophoresis using horizontal agar gel thin layer. The

results obtained are as follows.

1) Six bands on the cathodic side and twenty-two on the anodic side were observed.

2) They were classified into three groups according to their incidences, lower, higher and intermediate incident one, respectively.

3) Some of lower and intermediate incident bands showed the lowest occurrence-frequency in Mexican or Brazilian variety groups and showed gradual increasing in American, Formosan or Chinese variety groups, showing decreasing in Japanese old local variety group, again.

4) The average number per variety of the bands in Mexican variety group showed especially smaller value, and its differences between the Mexican variety group and Japanese, Chinese, or Formosan variety groups were significantly large, respectively, while the differences of the average number among variety group of Japan, China and Formosa were quite small.

5) Mexican variety group showed lower similarity index value within the variety group as well as between other variety groups.

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