Variation of Esterase Isozymes in Sweet Potato Varieties

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Introduction

In the previous paper[2], variation of peroxidase isozymes of semi-tuberous root of sweet potato among the different variety-groups consisting of the Japanese varieties containing several old local varieties, introduced foreign varieties and interspecific hybrid strains was reported. Moreover, using the tuberous root of almost the same varieties as those in the previous experiment, variation of esterase isozymes in sweet potato varieties was investigated.

The present paper describes about the relationships between the variation of zymogramic patterns of esterase isozymes and the sources of the varieties.

Materials and Methods

Materials used are in total 127 varieties and strains, they are the same varieties as those in the previous paper, except one strain added into Japanese breeding materials in this experiment. Tuberous roots were sampled from sweet potato plants after harvesting, since the preliminary test gave stronger enzymatic activity and larger number of esterase isozyme bands for tuberous root than for other organs of sweet potato. Tuberous root was cut out in transection of the portion showing the largest diameter and grated in a cutting phase by a radish grater. By the same method as mentioned in the previous paper, about five grams of the grated materials were filtered with gauze, the filtrates were centrifuged, and the supernatants were preserved in a refrigerator for some time until they were used for electrophoresis. An electrophoretic method used was just same one as in the case of peroxidase in the previous paper. After electrophoresis, the plate was sprayed first with distilled water and then with an aceton solution containing 1% naphthyl acetate, following the incubation for 45 minutes at 37°C, the plate was sprayed with 2% solution of naphthanil diazo blue B.

Results

The esterase isozyme bands observed in this experiment are diagramatically represented in Fig. 1. One band in the cathodic side and twelve bands in the anodic side were observed. As shown in Fig. 1 they were designated as 1C and 1A~12A, according to the mobility of each band from the origin. Band 1C and 11A occurred in all the varieties used. Band 11A was sometimes vague one, but it separated clearly into two bands only

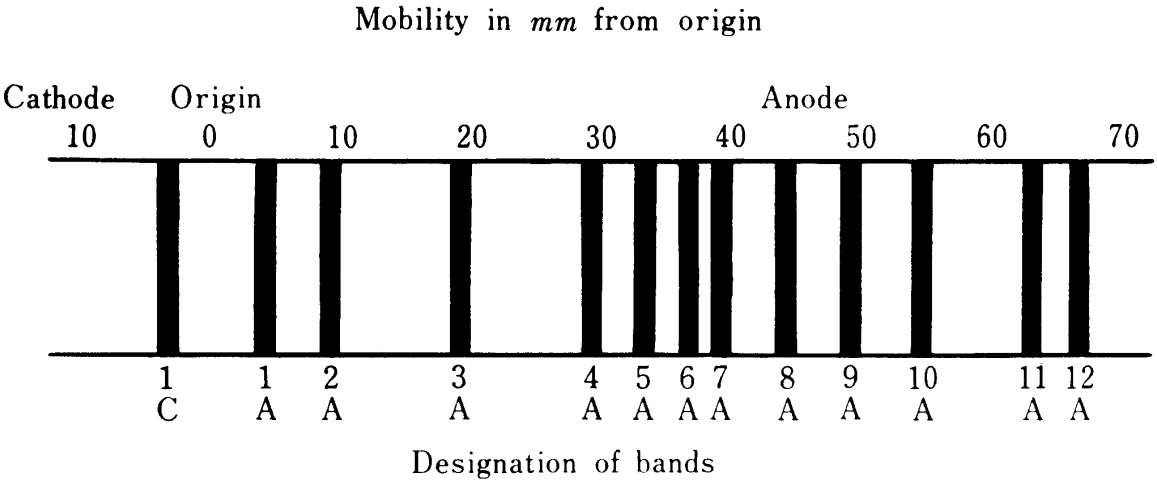

Fig. 1. Esterase isozyme bands observed in tuberous root of sweet potato varieties used.

in two varieties, Koganesengan and Kyushu No. 18. So, this band, situated fartherest from the origin in the anodic side, was designated as 12A. Band 1A and 5A respectively occurred in only one variety, 1A, in Chugoku No. 31, 5A, in Beikokuaka. Band 2A, 3A, 4A and 6A were sometimes unstable in activity and the discriminations of them were often difficult for their vague separation. Band 7A, 8A, 9A and 10A showed clear separation and stable activity. So, they were mainly noticed for ascertaining the variation of esterase isozymes in sweet potato varieties.

The occurrence-frequencies, in percentage, of each esterase isozyme band in 9 variety groups are shown in Table 1. And the incidences of nine esterase isozyme bands, except the band occurring in all varieties, 1C and 11A, and the band occurring in only a few varieties, 1A, 5A and 12A, are shown in Fig. 2. It is noticeable in Table 1 that band 3A shows the highest occurrence-frequencies among vague bands, 2A, 3A, 4A and 6A, in any variety groups, and also band 8A shows the highest occurrence-frequencies among clear bands, 7A, 8A, 9A and 10A, in any variety groups, excepting that it shows lower occurrence-frequency in Brazilian variety group than band 9A. In the comparisons of occurrence-frequencies of the respective band among the variety groups, barring the

Table 1. Occurrence-frequency in percent of esterase isozyme bands in 9 variety groups

Variety group	No. of varieties	Isozyme bands												
		1C	1A	2A	3A	4A	5A	6A	7A	8A	9A	10A	11A	12A
Japan (old local)	8	100	0	0	50	50	0	25	88	100	88	50	100	0
China	8	100	0	50	63	13	0	50	75	88	88	75	100	0
Formosa	7	100	0	43	100	43	0	0	57	100	100	29	100	0
U.S.A.	10	100	0	40	80	40	10	10	40	70	70	80	100	0
Mexico	7	100	0	0	43	29	0	14	29	86	86	43	100	0
Brazil	5	100	0	0	80	40	0	20	60	80	100	40	100	0
Japan (leading varieties)	10	100	0	20	70	30	0	20	50	70	60	70	100	10
Hybrid (interspecific)	11	100	0	0	91	36	0	27	73	100	64	27	100	0
Japan (breeding materials)	61	100	2	18	70	25	0	8	42	98	65	43	100	2
Total	127	100	1	19	71	30	1	15	51	92	72	48	100	2

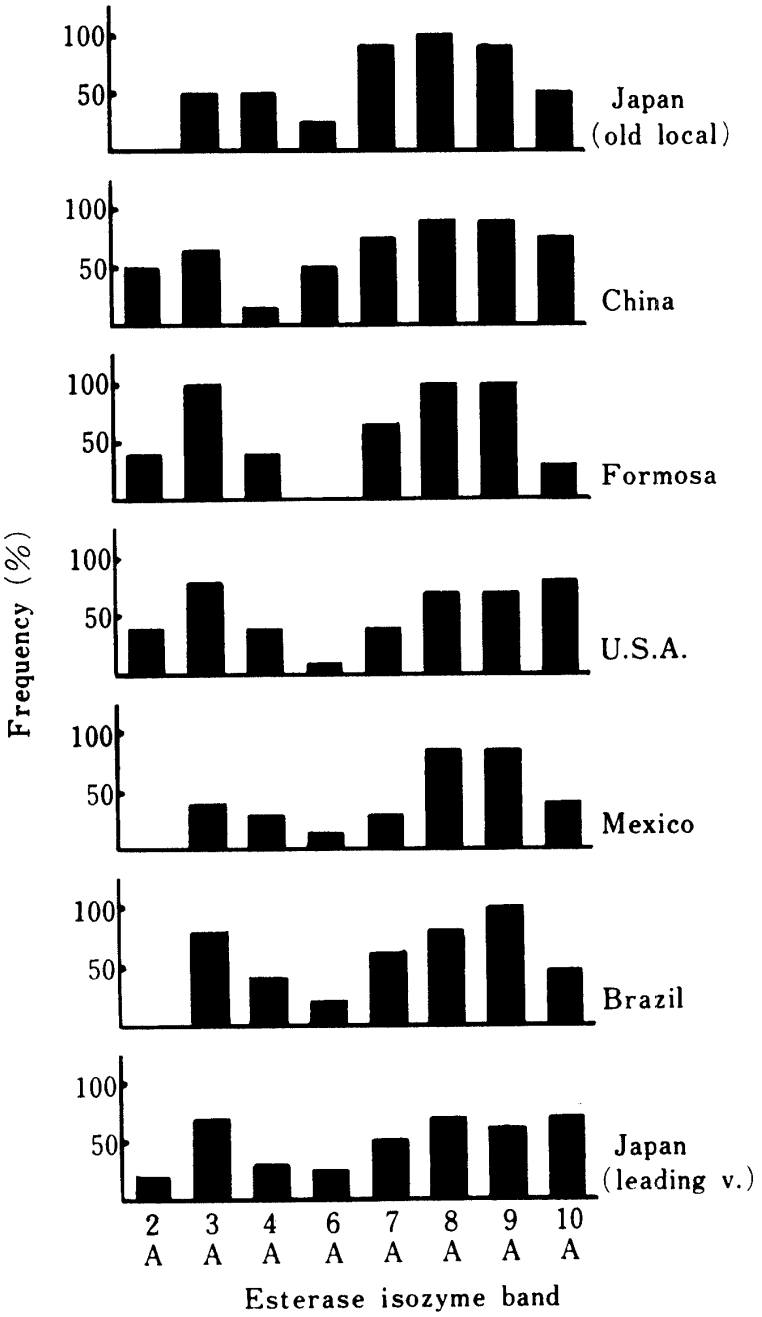

Fig. 2. Occurrence-frequenceis of several esterase isozyme bands in 7 variety groups of sweet potato.

fact that no occurrence was to be noted in band 2A in Mexican and Brazilian variety groups, gradual increasing was noted in incidence ranging from 40 percent to 50 percent in American, Formosan and Chinese variety groups, again disapeared in the Japanese old local variety group. Band 3A showed 43 percent incidences in Mexican varieties, and in American, Brazilian and Formosan variety groups it showed gradual increasing tendency, and in Chinese and Japanese old local varieties, decreasing tendency, again. Moreover, band 7A showed the lowest occurrence-frequency in Mexican

varieties and showed gradual increasing tendency of the occurrence-frequencies in Brazilian, American, Formosan, Chinese and Japanese variety groups (Table 1, Fig. 2). Namely, it is clear that several bands show lower incidence in Mexican variety group than in other district variety groups. Occurrence-frequency distributions per variety of esterase isozyme bands, the averages and the standard deviations in 9 variety groups are shown in Table 2. Although any clear tendency is not showed in Table 2, somewhat lower values are noted at the average number of occurrence per variety in Mexican varieties and Japanese breeding materials. Some differences among these average numbers per variety of esterase isozyme bands between the respective groups are shown in Table 3. The differences between Mexican variety group and Japanese, Chinese or Formosan variety groups showed somewhat larger values, showing almost no statistical significances; while the differences between the Japanese breeding material group and the Japanese old local, Chinese or Formosan variety groups were statistically significant.

Table 2. Frequency distributions of number per variety of esterase isozyme bands in 9 variety groups

Variety group	Number per variety of esterase isozyme bands									No. of varieties	Averages	Standard deviation
	3	4	5	6	7	8	9	10	11			
Japan (old local)		1	2	1	2		2			8	6.50	1.85
China			3		2		3			8	7.00	1.85
Formosa				4	1	2				7	6.71	0.95
U.S.A.	1	1		1	5	2				10	6.40	1.65
Mexico		3		3	1					7	5.29	1.25
Brazil		1	1			2	1			5	6.20	1.64
Japan (leading varieties)		1	3	2	3	1				10	6.00	1.25
Hybrid (interspecific)		1	3	3	2	1	1			11	6.19	1.47
Japan (breeding materials)	1	6	23	20	8	2			1	61	5.73	1.25

Table 3. Differences of average numbers per variety of esterase isozyme bands among variety groups

Variety group		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Japan (old local)	(1)								
China	(2)	0.50							
Formosa	(3)	0.21	0.29						
U.S.A.	(4)	0.10	0.60	0.31					
Mexico	(5)	1.21	1.71	1.42	1.11				
Brazil	(6)	0.30	0.80	0.41	0.20	0.91			
Japan (leading varieties)	(7)	0.50	1.00	0.71	0.40	0.71	0.20		
Hybrid (interspecific)	(8)	0.31	0.81	0.52	0.21	0.90	0.01	0.19	
Japan (breeding materials)	(9)	0.77*	1.27*	0.98*	0.67	0.44	0.47	0.27	0.46

*Significant at 5% level

Distributions of occurrence-frequency of zymogram patterns comprising band 7A, 8A, 9A and 10A showing clear separation and strong activity, are shown in Table 4. Concerning the occurrence of these four bands, it is expected that it exists in sixteen patterns; namely, in addition to the patterns in this Table, two patterns are expected; namely the one pattern in which only one band 10A occurs and the other in which all the four bands do not occur. But these two patterns were not observed in this experiment. In Table 4, through all variety groups, 8A-9A patterns showed the highest occurrence-frequency (22.8%), and next to this, 7A-8A-9A and 7A-8A-9A-10A patterns showed rather high frequencies: 17.3 percent and 15.7 percent, respectively.

Table 4. Frequency distributions of occurrence of the zymogramic patterns comprising band 7A, 8A, 9A and 10A in 9 variety groups

Zymogramic pattern	Variety group* J (C, L)	C	F	U	M	B	J (L, V)	H (I, S)	J (B, M)	Total No.	%
7A–8A–9A–10A	3	3		2	1	1	2	1	7	20	15.7
7A–8A–9A	3	2	4		1	1		3	8	22	17.3
7A–8A–10A		1						1	8	10	7.9
7A–9A–10A						1	1			2	1.5
8A–9A–10A	1	1	2	3	1			1	4	13	10.2
7A–8A	1			1			1	3	1	7	5.6
7A–9A									1	1	1.0
7A–10A				1			1			2	1.6
8A–9A			1		2	2	2	2	20	29	22.8
8A–10A				1			2		7	10	7.9
9A–10A		1		1	1		1			4	3.1
7A									1	1	1.0
8A					1				4	5	3.9
9A				1						1	1.0
Total	8	8	7	10	7	5	10	11	61	127	

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

In the comparisons of occurrence-frequencies of band patterns among variety groups, Japanese old local variety, Chinese variety and Formosan variety group showed higher occurrence-frequency of the band patterns comprising four or three bands than those comprising two bands or one band, while Mexican variety group showed similar occurrence-frequency in any band patterns.

These band patterns comprising 7A, 8A, 9A and 10A, were compared with each other between the whole two varieties excepting Japanese breeding materials, and the similarity index value was calculated as in the previous paper[2]. Table 5 shows average similarity index values within and between the variety groups. As shown in Table 5, average similarity index value within Japanese old local variety group shows the highest values (72.4%), and contrasting to this, the values within American, Mexican and Japanese

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)	72.4							
China	(2)	70.6	65.8						
Formosa	(3)	72.4	66.6	71.5					
U.S.A.	(4)	54.6	57.6	52.4	47.4				
Mexico	(5)	57.9	56.1	62.3	50.0	50.8			
Brazil	(6)	66.1	62.8	64.6	50.8	59.1	58.4		
Japan (leading variety)	(7)	52.6	55.9	54.5	50.6	50.7	52.7	47.7	
Hybrid (interspecific)	(8)	67.0	60.1	65.6	54.5	53.6	59.4	54.3	60.5

leading variety groups showed rather lower ones. Namely, by these results it was clearly showed that Japanese old local variety group is made of the varieties showing rather similar esterase zymogramic patterns in relation to 7A, 8A, 9A and 10A; on the other hand, American, Mexican and Japanese leading variety groups are made of those showing somewhat different esterase zymogramic patterns.

In comparison of average similarity index values within and between variety groups, Japanese old local variety, Chinese variety, and Formosan variety groups show generally higher value in case of within the groups than in that of between the groups, but American, Mexican, Brazilian and Japanese leading variety groups show rather smaller values in the case of within the groups than in that of between the groups.

Moreover, Japanese old local variety, Chinese variety and Formosan variety groups showed higher average similarity index values in case of between the groups, but they showed somewhat lower values between the American, or Mexican variety groups. Namely, Japanese old local variety, Chinese variety and Formosan variety groups have similar esterase zymogramic pattern between them, but rather different one in case of between American or Mexican variety groups.

Discussion

Useful informations have been obtained from the investigation of isozymes in several enzymes, not only for the analysis of genomic relationships and the detection of recombination in hybrid between species[1,4,5,8] but also for the research in speciation and migration and the determination of the purity of F_1 hybrid within species[3,6,7].

Esterase isozyme bands observed in this experiment were one band in cathodic side and twelve bands in anodic side. Among these thirteen bands, band 7A, 8A, 9A and 10A showed stronger enzymatic activity and clearer separation.

In the comparison of the occurrence-frequency of esterase isozyme patterns comprising these four bands, 7A, 8A, 9A and 10A, among the respective variety groups, Japanese old local variety, Chinese variety and Formosan variety groups showed higher occurrence-frequency of the band patterns comprising four or three bands than those comprising two or one band, while Mexican variety group showed similar occurrence-frequency in any band patterns.

These relationships were clearly represented by the similarity index values. Namely, the average similarity index value within Japanese old lcal variety, Chinese variety and

Formosan variety groups showed generally higher ones and those between them also showed higher values. These results seem to show the fact that these variety groups have genetically intimate relationships with each other.

On the other hand, the average similarity index values within American or Mexican variety group showed somewhat lower values, and those values between American or Mexican variety groups and Japanese variety, Chinese variety or Formosan variety groups also showed rather low ones.

Basing on these results, it seems to be shown that American and Mexian variety groups contain more genetical diversities in them than in Japanese old local variety, Chinese variety and Formosan variety groups.

It is an interesting result that Japanese leading varieties showed lower similarity values within variety group, and also showed rather lower values between Japanese old local variety groups. These values seem to show the result that Japanese leading varieties used in this experiment consist of two groups, namely, the old varieties produced by being crossed only between old local varieties or their offsprings, and the new varieties produced by being crossed between Japanese breeding varieties or strains and newly introduced foreign varieties or wild related species, so they contain large variations in them, and also are different from old local varieties.

Summary

The variations of esterase isozymes of tuberous root in sweet potato varieties were investigated, by electrophoresis using horizontal agar gel thin layer.

The resluts obtained are as follows.

1) One band on the cathodic side and twelve bands on the anodic side were observed.

2) Among these thirteen bands, four bands, 7A, 8A, 9A and 10A, showed strong enzymatic activity and clear separation.

3) Concerning zymogram patterns comprising these four bands, Japanese old local variety, Chinese variety and Formosan variety groups showed higher similarities within and between them, while Mexican variety and American variety groups showed lower similarities within and between them.

4) And also, concerning the above mentioned zymogram patterns, it was showed that there were lower similarities between Japanese old local variety, Chinese variety or Formosan variety groups and Mexican variety or American variety groups.

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References

1) Katayama, T. and Chern, J. L.: Zymographic studies on diploid *Oryza punctata* and its related species. *Japan. J. Breed.*, **23,** 329–333 (1973)
2) Kokubu, T. and Maeda, K.: Variation of peroxidase isozymes in sweet potato varieties. *Mem. Fac. Agr. Kagoshima Univ.*, **23,** 77–84 (1978)
3) Nakagahra, M., Akihama, T. and Hayashi, K.: Genetic variation and geographic cline of esterase isozymes in native rice varieties. *Japan. J. Genetics*, **50,** 373–382 (1975)
4) Nakata, Y. and Tsunewaki, K.: Isozyme variations in *Aegilops and Triticum*, I. Esterase isozymes in *Aegilops* studied using the gel isoelectrofocusing method. *Japan. J. Genetics*, **46,** 321–336 (1971)
5) Nakata, Y.: Isozyme variation in *Aegilops* and *Triticum*, II. Esterase and acid phosphatase isozymes studied by the gel isoelectrofocusing method. *Seiken Zihô*, **24,** 25–75 (1975)
6) Sakai, K. I., Hayashi, S. and Iyama, S.: Genetic studies in natural population of *Pinus*, I. Genetic variability in local populations from several prefectures. *Mem. Fac. Agr. Kagoshima Univ.*, **19.** 37–49 (1974)
7) Woods, S. and Thurman, D. A.: The use of seed acid phosphatases in the determination of the purity of F_1 hybrid Brussels sprout seed. *Euphytica*, **25,** 707–724 (1976)
8) Yamamoto, K.: Estimation of genetic homogenity by isozymes from interspecific hybrid of *Vicia*, I. Amylase isozyme pattern in the hybrid progenies between *Vicia pilosa* and *V. macrocarpa*. *Japan. J. Breed.*, **25,** 60–64 (1975)