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Variation of Peroxidase Isozymes in the Wild Related Species of Sweet Potato

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Introduction

With the purpose of utilizing as breeding materials, many wild related species of sweet potato have been introduced into Japan from the U.S.A., Mexico and other countries in the Central America and the northern part of South America[1]. Concerning the phylogeny of these introduced *Ipomoea* species and the possibility of their utilization into sweet potato breeding, intensive investigations have been carried out[1,4-9]. From these investigations it has been clarified that these wild species consist of different polyploidies, showing the chromosome number $2n=30$, $2n=45$, $2n=60$, $2n=90$, and they may be classified into two groups, the Group 1 crossable with sweet potato and the Group II uncrossable with that. As the common characteristics of the plants belonging to the Group 1, the bell-shaped corolla, dark colour interior of the tube, prominent glands and self-incompatibility have been pointed out.

The present paper describes the variation of peroxidase isozymes in these introduced *Ipomoea* species.

Materials and Methods

The species used, which were spared from Kyushu National Agricultural Experiment Station, Ibusuki, Kagoshima, are shown in Table 1. The semi-tuberous roots or fiberous ones of the respective strains of these species were collected and washed with water, then drained off water. The juice pressed from the roots, was 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 electrophoreses. The electrophoresis was carried out by the same method as in the previous paper[2,3].

Results

The peroxidase isozyme bands discriminated in these related species were almost the same ones as those in sweet potato varieties reported in the previous paper[2], excepting that new 4 bands which were not observed in sweet potato varieties, were discriminated in a few species and that the 1 band which was discriminated in sweet potato varieties was not observed at all in the related species.

As reported in the previous papers[2], the difference between the respective zymograms of the two strains was shown by means of the similarity index value.

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

Table 1. Sweet potato and the wild related species used

Species number	Species	Group* and Ploidy	Strain	No. of strain used
(1)	*I. batatas*	I*-6x	Norin No. 1, Norin No. 2, Norin No. 7, Koganesengan, L-4-5	5
(2)	*I. trifida*	I-6x	K123-1, -2, -4, -5, -6, -7, -8, -9, -10, -11, -13, -14, -15, -16, -17, -18, -19, -20, K177-2, -3	20
(3)	*I. littolalis*	I-4x	K233-1, -2,	2
(4)	*I. trifida*	I-4x	K300-1, -2, -3, -5, -7, K500, K501	7
(5)	*I. gracilis*	II-4x	K134-1, -2	2
(6)	*I. tiliacea*	II-4x	K270-1, -2	2
(7)	*I. trifida*	I-3x	K222-1, -5	2
(8)	*I. leucantha*	I-2x	K221-A, -B, -S	3
(9)	*I. lacunosa*	II-2x	K61	1
(10)	*I. triloba*	II-2x	K121, K176-5	2
(11)	*I. ramoni*	II-2x		1
(12)	*I. trichocarpa*	II-2x		1

note: *Group I, species crossable with sweet potato
Group II, species uncrossable with sweet potato

Table 2. Average similarity index values* of peroxidase isozymes within and between the species

	Species number											
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1)	75.1											
(2)	54.6	81.8										
(3)	57.5	77.3	78.0									
(4)	60.0	74.1	70.7	77.6								
(5)	57.4	81.0	81.0	74.9	83.0							
(6)	46.2	65.8	69.3	64.4	72.8	90.0						
(7)	62.3	72.3	78.3	73.9	74.3	64.3	94.0					
(8)	42.9	54.7	64.2	55.5	58.2	65.0	60.2	63.7				
(9)	52.8	72.4	71.0	70.6	75.0	66.5	65.5	55.7	—			
(10)	48.3	57.1	62.8	53.6	60.0	72.8	50.0	64.0	61.5	65.0		
(11)	39.2	75.3	73.0	61.7	71.5	75.5	66.5	59.0	67.0	57.0	—	
(12)	51.6	70.9	68.0	64.1	63.5	67.5	58.5	59.0	75.0	74.0	68.0	—

Note: Difference between the respective zymograms of two strains was shown by means of the similarity index value.

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

S: Similarity index value (S value) between all the combination 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 all strains of the species used, then the values were averaged within and between species.

S: Similarity index value (S value) between all the combination 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

Table 3. Significance of average similarity index value of interspecies against intraspecies

	Species number											
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1)		**2)	**	**	**	**	**	*	—	**	—	—
(2)	**1)					**	**		—	**	—	—
(3)	**	**				**	**		—		—	—
(4)	**	**	**		**	**	**		—	**	—	—
(5)	**					*	**		—		—	—
(6)	**	**	*	**			**		—		—	—
(7)	**	**			*	**			—	*	—	—
(8)	**	**	**	**	**	**	**		—		—	—
(9)	**	**				*	*				—	—
(10)	**	**	*	**	*	*	**		—		—	—
(11)	**	**		**			*		—	—		—
(12)	**	**		*			*		—		—	

Note: At each vertical column the difference between intraspecies value and interspecies value was calculated, and when the difference was significant at 5% or 1% level, it was shown by * or **. For example 1) shows that the difference between intraspecies value of species (1) and interspecies value of species (1) and (2) was significant, and 2) shows that the difference between intraspecies values of species (2) and interspecies value of species (2) and (1) was significnat.

These similarity index values were calculated in the whole two combinations of all strains of the species used, then the values were averaged within and between the species. Average similarity index values of peroxidase isozyme within and between the species are shown in Table 2. As shown in Table 2, the largest value of them was that within (7), counting 94.0, and the smallest was that between (1) and (11), counting 39.2. Generally, the average similarity index value within the respective species was almost larger than that between the species and the other one.

Significance of average similarity index value of interspecies against intraspecies, is shown in Table 3. In table 3, at each vertical column the difference between intraspecies value and interspecies value was calculated, and in the case when the difference happened to be significant at 5% or 1% level, it was shown by * or **. For example 1) shows that the difference between intraspecies value of species (1) and interspecies value of species (1) and (2) was significant, and 2) shows that the difference between intraspecies values of species (2) and interspecies value of species (2) and (1) was significant.

All the differences among the intraspecies value and the interspecies values of species (1) and those among the intraspecies value and the interspecies value of species (7) were significant, respectively. In the species (8), only the difference between its intraspecies value and the interspecies value of species (8) and (1) was significant. In the other species, the differences among the intraspecies value of the respective species and their interspecies values were largely significant.

In order to compare the similarity index value within and between the Group and ploidy, the values were averaged in the respective categories. The average similarity index values gained, are shown in Table 4. Among these average values, the average value within Group and polidy 1–3x was the largest of them, counting 94.0, and that between Group and ploidy 1–6x and 1–2x the smallest, counting 48.8. Generally, the average similarity index values within the respective Group and ploidy were larger than those between the former and the other one. And also, the average similarity index value between the respective Group and ploidy and the other one, generally became

Table 4. Average similarity index values of peroxidase isozymes within and between the group and ploidy

	Group and ploidy					
	I–6x	I–4x	II–4x	I–3x	I–2x	II–2x
I–6x	70.5					
I–4x	67.2	75.4				
II–4x	62.6	72.4	81.9			
I–3x	67.3	76.1	69.3	94.0		
I–2x	48.8	59.8	61.6	60.2	63.7	
II–2x	58.5	65.6	69.0	60.1	59.4	66.0

Table 5. Significance of the average similarity index value between Group and ploidy against the average similarity index value within Group and polidy

	Group and Ploidy					
	I–6x	I–4x	II–4x	I–3x	I–2x	II–2x
I–6x		**	**2)	**	*	**
I–4x			*	**		
II–4x	*1)			**		
I–3x			*			*
I–2x	**	**	**	**		
II–2x	**	*	*	**		

Note: At each vertical column the difference between the value within Group and ploidy and the value between Group and ploidy was calculated, and when the difference was significant at 5% or 1% level, it was shown by * or **.
For example 1) shows that the difference between the value within Group and ploidy I–6x and the value between Group and ploidy I–6x and II–4x was significant and 2) shows that the difference between the value within Group and ploidy II–4x and the value between Group and ploidy II–4x and I–6x was significant.

smaller according to the difference of ploidy between the two, while it did not always show a certain tendency due to the Group.

Significance of the average similarity index value between Group and ploidy against the average similarity index value within Group and ploidy is shown in Table 5. In Table 5, at each vertical column the difference between the value within Group and ploidy and the value between Group and ploidy was calculated, and in the case when the difference happened to be significant at 5% or 1% level, it was shown by * or **. For example 1) shows that the difference between the value within Group and ploidy I–6x and the value between Group and ploidy I–6x and II–4x was significant, and 2) shows that the difference between the value within Group and ploidy II–4x and the value between Group and ploidy II–4x and I–6x was significant. All the differences among the average similarity index value within Group and ploidy II–4x and the average similarity index values between Group and ploidy II–4x and the other one and those among the value within Group and ploidy I–3x and the values between Group and ploidy I–3x and the other one were all significant, respectively. Generally, the differences among the value within the respective Group and ploidy and the values between the former and the other one were significant in the case when the difference of ploidy was large.

Whereas, the difference between the value within Group and ploidy I–4x and the value between

Group and ploidy I–4x and II–4x was not significant, and the difference between the value within Group and ploidy II–4x and the value between Group and ploidy II–4x and I–4x was significant, and also the difference between the value within Group and ploidy I–2x and the value between Group and ploidy I–2x and II–2x, and that between the value within Group and ploidy II–2x and the value between Group and ploidy II–2x and I–2x were not significant. Namely, the difference between the value within the respective Group and ploidy and the value between the former and the other ones was not always significant in the case when the ploidy was the same.

Discussion

Nishiyama et al[5–8] reported that based on morphological, cytological and genetical evidence, *I. leucantha* (2n=30), *I. littoralis* (2n=60) and *I. trifida* (2n=90), were found to be probable progenitors of sweet potato, *I. batatas* (2n=90), and these wild species and sweet potato were grouped in a series of autoploidy with doubling of the chromosome set of the B genome of *I. leucantha* (Group I), weheras the other species in the section *Batatas* as *I. triloba* (2n=30), *I. trichocarpa* (2n=30), *I. ramoni* (2n=30), *I. tiliacea* (2n=60) and *I. gracils* (2n=60) were not likely to be related to the sweet potato (Group II).

Generally, as shown in Table 4, the average similarity index value between the respective group and ploidy and the other ones, became smaller according to the difference of ploidy between the two. In spite of this tendency as shown in Table 2, the average similarity index value between (1), *I. batatas*, and (2), *I. trifida* −6x, the value between (1) and (4), *I. trifida*−4x, and the value between (1) and (7), *I. trifida* −3x, were relatively higher ones (54.6, 60.0, 62.3). These data seem to support that *I. trifida* is closely related to sweet potato.

Concerning the similarity index value between sweet potato and the related species of Group I or Group II, in sweet potato and tetraploid species, the average similarity index value between (1), *I. batatas*, and (3), *I. littoralis*, (57.5), the value between (1) and (4), *I. trifida*, (60.0) were somewhat larger than the value between (1) and (5), *I. gracilis*, (57.4), and the value between (1) and (6), *I. tiliacea* (46.2), while, in sweet potato and the diploid species, the average similarity index value between (1), *I. batatas*, and (8), *I. leucantha* (42.9) was not always larger than the value between (1) and (9), *I. lacunosa*, (52.8), the value between (1) and (10), *I. triloba*, (48.3), the value between (1) and (11), *I. ramoni*, (39.2), and the value between (1) and (12), *I. trichocarpa*, (51.6).

Namely, the average similarity index value between sweet potato and tetraploid species of Group I which is crossable with sweet potato, was somewhat larger than the value between sweet potato and tetraploid species of Group II which is uncrossable with sweet potato, but the value between sweet potato and diploid species of Group I was not always larger than the value between sweet potato and the diploid species of Group II.

Summary

The variation of peroxidase isozymes in semi-tuberous roots or fiberous ones of sweet potato varieties and its wild relative species was investigated by electrophoresis method. The results obtained are as follows:

1) The difference between the respective zymograms of the two strains was shown by means of similarity index value. These similarity index values were calculated in the whole two combinations of all strains of the species used. The values were averaged within and between species, the species

Group crossable with sweet potato or not and ploidy, then the average similarity index value of the respective categrories was gained.

2) Average similarity index value within the respective species was almost larger than those between the former and the other one.

3) The differences among the intraspecies value of the respective species and their interspecies value were largely significant.

4) The value within the respective Group and ploidy was generally larger than those between the former and the other ones.

5) The value between the respective Group and ploidy and the other one, generally became smaller according to the difference of ploidy between the two, while it did not always show a certain tendency due to the Group.

6) The differences among the value within the respective ploidy and the value between the former and the other one were significant in the case when the difference of ploidy between the two was large.

7) The values between sweet potato and the species of Group I which is crossable with sweet potato, were not always larger than the value between sweet potato and the species of Group II which is uncrossable with sweet potato.

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