Genetic Control of Four Enzymes in Diploid Taro, *Colocasia esculenta* (L.) Schott

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Summary

In order to understand the genetic variation and relationship of taro, *Colocasia esculenta* (L.) Schott, isozyme analyses were initiated using fertile diploid taro accessions. Polyacrylamide gel electrophoreses were performed on leaf tissue of wild or cultivated taro accessions from Nepal and Thailand, and their progeny to determine genes controlling four enzyme systems: alcohol dehydrogenase, esterase, leucine aminopeptidase and phosphoglucumutase. A total of nine loci with two or three alleles were discovered encoding the four enzymes in leaf tissue among the materials used in this study. The isozymes at the two loci of alcohol dehydrogenase and esterase were in a dimeric form. Observed segregation ratios were generally not significantly different ($P > .05$) from expected Mendelian segregation ratios; however, distortions were observed at some loci and possible causes were discussed. These findings suggest the presence of allelic variants in other enzyme systems and precise classification of a wide range of diploid taro based on allozyme variations. Most of taro cultivars are triploids, which are considered to have evolved from fertilization of unreduced gametes of a diploid. The genetic analyses of isozymes on the diploids will provide useful information to investigate genetic variation, genetic relationship and evolution of not only diploid but also triploid taro.

Key Words : *Colocasia esculenta*, taro, isozymes, inheritance.

Introduction

Taro, *Colocasia esculenta* (L.) Schott, is one of the oldest crops grown throughout much of tropical and subtropical areas. In Japan, this root crop was ranked sixth in production area among major vegetables harvested in 1993. Despite such agricultural importance, no extensive studies of phylogeny on taro have ever been conducted. Even in a conventional taxonomy of the genus *Colocasia* and *Alocasia*, there seem to be some confusions in classifications proposed by different botanists, Hotta (1970), Kumazawa (1956) and Kitamura (1949).

The isozyme analysis has provided useful data for plant systematics and evolution studies. With the use of allelic data, it is possible to quantify genetic similarity among intraspecific taxa or species of the plant based

solely on the gene variability of isozymes under investigation (Crawford 1989). This feature of isozymes may be useful for defining species of the plants that are difficult to classify based on the morphology.

There have been a few isozyme studies on taro (Lebot and Aradhya 1991, Tanimoto and Matsumoto 1986, Isshiki *et al.* 1995) and related taxa (Murata *et al.*, 1995). Lebot and Aradhya (1991) studied isozyme variations among 1,417 taro accessions from Asia and Oceania and described seven polymorphic enzyme systems. Based on the presence or absence of electromorphs of these seven enzymes, they classified the accessions and estimated phylogenetic closeness among the accessions. Tanimoto and Matsumoto (1986) analyzed peroxidase and esterase isozymes of Japanese taro cultivars and observed variation in banding patterns. It was concluded that cluster analysis based on the isozyme banding patterns was a suitable method for classifying cultivars into groups. Isshiki *et al.* (1995) studied inheritance of isozyme variations for aspartate aminotransferase, shikimate dehydrogenase and glucose-6-phosphate isomerase of wild taro introduced from Bangladesh. Using diploid accessions and their progeny, three loci with two or three alleles were identified. The triploid accessions had the same alleles at these isozyme loci in common. They postulated the triploid taro was originated as an autopolyploid of the diploid. To our knowledge, the work by Isshiki *et al.* (1995), is the first and only study to investigate inheritance of taro isozyme systems. When loci and alleles controlling isozymes are determined, they become more useful and reliable genetic markers in a phylogeny study and breeding program than isozymes *per se*, some of which are products of the genes at different loci. To identify the loci and alleles responsible for isozymes, it is necessary to make a cross between individuals with different isozyme phenotypes and examine its progeny for segregation. Taro, where diploid and triploid forms exist together, normally propagates asexually. While the triploids are sterile, the diploids usually set viable seeds by either selfing or outcrossing and can be used for genetic analyses of isozymes.

The present study was conducted in the beginning of our taro phylogeny investigation using isozymes. Here, eight diploid taro accessions from Nepal and Thailand and their progeny were used to investigate the genetic control of isozyme variants of four enzymes : alcohol dehydrogenase, esterase, leucine aminopeptidase and phosphoglucumutase.

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Materials and Methods

Plant materials

The original plant materials used in this study were seven accessions of wild diploid taro, *C. esculenta* var. *aquaticus* (L.) Schott, C81081, C81079, C81135, C81080, C81019, TC83001 and C81027, and one accession of cultivated diploid taro, *C. esculenta* var. *esculenta* (L.) Schott, C81125. All accessions, except TC83001 from Thailand, were collected in Nepal in 1981, classified according to Hotta (1970), and have been vegetatively propagated at Okayama University.

The original accessions and their progeny derived from self- or cross-pollinations of the accessions were used for electrophoretic analyses of isozymes. Two F_1 plants (CR94310 and CR94334) of the C81027 and C81125 cross and their selfed progeny populations were also included in the analyses. Self- or cross-pollinations of the accessions were made during 1993 to 1995 growing seasons. Harvested seeds were sown in the following season and grown along with the clonal plants of the original accessions in an experimental field for an enzyme study.

Enzyme extraction and electrophoresis

The isozyme analysis was performed on extracts from a newly-extended leaf. Approximately 100mg of the sample was ground in a 1.5ml polypropylene tube with 0.3ml of 0.1M Tris-hydrochloride buffer (pH7.5), containing 20% (v/v) glycerol, 1% (v/v) 2-mercaptoethanol and 0.005M ethylenediaminetetraacetic acid (EDTA). The extracts were centrifuged for 10 minutes at 4,000rpm and 25 μ l of the supernatant was used for the electrophoresis. Four enzyme systems were assayed: alcohol dehydrogenase (ADH, E.C. 1.1.1.1), esterase (EST, E.C. 3.1.1.), leucine aminopeptidase (LAP, E.C. 3.4.11.1) and phosphoglucosmutase (PGM, E.C. 5.4.2.2). The enzymes were resolved by the vertical slab gel electrophoresis in which a separation gel contained 7.5% (w/v) acrylamide for EST and LAP analyses or 6.0% (w/v) acrylamide for PGM and ADH analyses, and a stacking gel contained 3.75% (w/v) acrylamide.

Electrophoreses were carried out at 4–7 °C with constant current of 15mA per gel in 0.025M Tris-glycine electrode buffer (pH 8.3) until bromophenol blue tracking dye migrated to the bottom of the gel.

Staining for isozyme activity

The substrates and buffers used for active staining of the isozymes were as follows: for ADH, those used by Wendel and Weeden (1989) with minor modification that 3ml ethanol instead of 0.75ml was used as substrate; for EST, 50mg of α -naphthyl acetate, 50mg of β -naphthyl acetate and 150mg of Fast Blue RR Salt in 100ml of 0.1M phosphate buffer (pH 6.3); for LAP, 40mg of L-leucyl- β -naphthylamide hydrochloride, 100mg of Fast Garnet GBC Salt and 1 ml of 1M

magnesium chloride in 100ml of 0.1M Tris-maleate buffer (pH 5.8); for PGM, those used by Vallejos (1983).

The gels were incubated in the staining solution at 34 °C in the dark until the isozyme bands developed to sufficient intensity. For each plant material, the electrophoreses were repeated at least three times using independent samples and only electromorphs with a consistent result over the replications were subjected for genetic analyses.

The isozyme phenotypes were determined for individual plants of the original accessions, two F_1 plants and their progeny based on the zymograms. The genetic interpretation of loci and alleles for each enzyme system was first inferred from the banding patterns of the accessions and later verified by Mendelian inheritance in the self- or cross-pollinated progeny. The loci for a given multilocus enzyme system were designated sequentially by number starting with "1" for the most anodal locus. The alleles at each locus were designated sequentially by letter starting with "a" for the most anodal allozyme. The letter "n" was used when referring to an inactive (null) allele.

Data analysis

A chi-square (χ^2) analysis was used to test single locus goodness-of-fit for expected Mendelian segregation ratios of codominant alleles. Inactive "n" alleles were found to behave as a recessive allele to their active counterpart and when "n" alleles were involved, dominant-recessive relation was assumed in χ^2 tests.

When an observed segregation ratio of two alleles, e.g. "a" and "b", was significantly different from an expected value, the χ^2 value was partitioned into orthogonal comparisons: $\chi_1^2 = (z_1 - z_2 + z_3)^2/n$ and $\chi_2^2 = 2(z_1 - z_3)^2/n$ where z_1 , z_2 , z_3 and n represented the numbers of the genotypes *aa*, *ab*, *bb* and total number of individuals, respectively. The χ_1^2 value tests for deviation in the ratio of heterozygotes and homozygotes, whereas the χ_2^2 value tests for deviations in the ratio of two homozygotes. The latter is a strong indicator of gamete selection, since selection in pollen grains and ovules distorts the ratio of homozygotes without affecting the frequency of heterozygote individuals (Westphal and Wricke, 1991).

Results and Discussion

All four enzyme systems gave clear zymograms. Figure 1 illustrates the banding patterns of the eight accessions and two F_1 plants used as a parental material for a segregation test.

Alcohol dehydrogenase: Four zones of isozyme activities were distinguishable on ADH zymograms. However, the isozyme bands in the most anodal zone, designated as ADH-1, and the most cathodal zone, ADH-4, were excluded from the analysis because of inconsistent band appearances. Faint bands appeared in

Fig. 1. Schematic representation of banding patterns and isozyme phenotypes of four enzymes in taro (*Colocasia esculenta* (L.) Shott) accessions used as parental plants.

(1):C81125, (2):C81081, (3):C81079, (4):C81135, (5):C81080, (6):C81019, (7):TC83001, (8):C81027, (9):CR94310, (10):CR94334.

*: Clear bands appeared only in progeny plants.

the ADH-3 zone and single-banded and double-banded patterns were recognized among the eight accessions (Fig. 1). However, the bands were not distinguishable in some of the progeny populations, and this zone was also excluded from the analysis.

Isozymes in the ADH-2 zone exhibited the most intensive staining. Two phenotypes, single-banded A and triple-banded AB, were observed among the eight accessions (Fig. 1). Self-pollination of the AB phenotype resulted in three progeny classes A, AB and B, a slow-migrating single band type (Fig. 2), with a 1:2:1 segregation ratio (Table 1). The crosses between the AB and A phenotypes resulted in the parental phenotypes in the progeny. The segregation ratio did not significantly differ from 1:1 when a male parent was the A phenotype, but it differed when a male parent was the AB

Fig. 2. Alcohol dehydrogenase zymograms of taro (*Colocasia esculenta* (L.) Shott) showing segregation in the ADH-2 zone and interlocus heterodimer bands.

phenotype (Table 1). Crosses between these A phenotypes showed no segregation (data not shown). These results suggest that the enzyme in the ADH-2 zone is in a dimeric form and that two codominant alleles, *Adh-2^a* and *Adh-2^b*, are present in the locus *Adh-2* for the materials used in this study. An active zone with two single-banded phenotypes and a triple-banded phenotype was also reported in the taro plants collected from Asia and Oceania (Lebot and Aradhyia 1991).

There are at least two different bands in the area between the ADH-1 and ADH-2 zones. Each of the two bands appeared to migrate to an intermediate position between two bands of the ADH-1 and ADH-2 zones. Because the triple band in the ADH-1 zone was not consistently detected, only the phenotypes of a clear fast-migrating single band in ADH-1 zone were selected and the banding patterns in the middle area were studied in relation to those in the ADH-2 zone. It was found that the fast-migrating single band type in the area was observed only when the phenotype of the ADH-2 was A. Similarly, the slow-migrating single band type was found only when the phenotype of the ADH-2 was B. The double-banded type in the middle area was found when both fast- and slow-migrating bands existed in the ADH-2 zone. These results strongly suggest that the isozyme bands in the middle area of the ADH-1 and ADH-2 zones were formed as an interlocus heteromer of subunits from the putative *Adh-1* and *Adh-2* loci. An interlocus heteromer of dimeric

Table 1. Segregation of alcohol dehydrogenase variants in self- or cross-pollinated populations of diploid taro (*Colocasia esculenta* (L.) Shott) accessions

Isozyme zone	Cross	Parental phenotype	Progeny phenotypes			Total	Expected ratio	χ^2 value
			A	AB	B			
ADH-2	C81079 ¹⁾	AB	16	21	12	49	1:2:1	1.653
	C81079×C81135	AB×A	45	52		97	1:1	0.505
	C81027×C81080	A×AB	19	45		64	1:1	10.563**

¹⁾ self-pollination.

** significant at the 0.01 level of probability.

ADH was reported in other plants (Roose and Gottlieb 1979, Crawford 1990, Gottlieb 1983). The formation of interlocus hybrid isozymes indicates that the ADH-1 and ADH-2 isozymes are active in the same subcellular compartment in taro leaf tissue.

Esterase: Several zones of EST activities were observed in taro, but four zones, EST-1, EST-3, EST-4 and EST-8, were found polymorphic in the eight accessions used in this study.

A double-banded phenotype A and a quadruple-banded phenotype AB were observed in the EST-1 zone among the eight accessions (Fig. 1). Self- or cross-pollinations of the identical double-banded phenotypes showed no segregation in the progeny (data not shown). Self- or cross-pollinations of the quadruple-banded phenotypes produced three progeny phenotypes,

A, AB and B, a slow-migrating double-banded type (Fig. 3a). Significant deviation from the expected 1:1 or 1:2:1 ratio did not occur in any of the six progeny populations (Table 2). These results demonstrate that the esterase isozymes at the EST-1 are under single locus control with two codominant alleles, *Est-1^a* and *Est-1^b*.

In the EST-3, three different phenotypes, A, AB and AC, and a total of five bands with different levels of staining intensities were observed in the original accessions (Fig. 1). Among five bands, the furthestmost migrated band was common to all three phenotypes. The band with intermediate mobility in the triple-banded phenotypes AB or AC, was stained intensely compared to the two outer bands. Table 2 shows the results of the segregation involving the isozymes in the EST-3. Self- or cross-pollinations of the AB phenotypes resulted in

Table 2. Segregation of esterase variants in self- or cross-pollinated populations of diploid taro (*Colocasia esculenta* (L.) Shott) accessions

Isozyme zone	Cross	Parental phenotype	Progeny phenotypes							Total	Expected ratio	χ^2 and its partitions		
			A	AB	AC	BC	B	C	N			χ^2	χ_1^2	χ_2^2
EST-1	C81081 ¹⁾	AB	13	32			17			62	1:2:1	0.581		
	C81135 ¹⁾	AB	15	30			19			64	1:2:1	0.750		
	C81079 × C81135	AB × AB	20	56			21			97	1:2:1	2.340		
	TC83001 × C81019	A × AB	35	35						70	1:1	0.000		
	C81027 × C81080	A × AB	26	38						64	1:1	2.250		
	C81081 × C81027	AB × A	36	33						69	1:1	0.130		
EST-3	C81135 ¹⁾	AB	11	24			29			64	1:2:1	14.125**	4.000*	10.125**
	C81081 ¹⁾	AB	14	36			12			62	1:2:1	1.742		
	C81079 × C81135	AB × AB	21	45			31			97	1:2:1	2.567		
	TC83001 × C81019	A × AB	17	53						70	1:1	18.514**		
	C81027 × C81125	AC × A	15		16					31	1:1	0.032		
	C81027 × C81080	AC × AB	19	13	17	15				64	1:1:1:1	1.250		
	C81081 × C81027	AB × AC	29	25	7	8				69	1:1:1:1	22.536**		
	CR94310 ²⁾	AC	9		23			2		34	1:2:1	7.118*	4.235*	2.882
EST-4	C81027 ¹⁾	AC	22		34			0		56	1:2:1	19.857**	2.571	17.286**
	CR94310 ²⁾	A	25						9	34	3:1	0.721		
	C81125 ¹⁾	A	25						8	33	3:1	1.227		
	C81079 × C81135	A × N	56						41	97	1:1	2.319		
	C81027 × C81125	N × A	20						11	31	1:1	2.613		
EST-8	C81027 × C81080	N × A	35						29	64	1:1	0.563		
	C81027 ¹⁾	AB	15	29			12			56	1:2:1	0.393		
	C81081 ¹⁾	AB	16	31			15			62	1:2:1	0.032		
	C81135 ¹⁾	AB	15	30			19			64	1:2:1	0.750		
	C81079 × C81135	AB × AB	22	45			30			97	1:2:1	1.825		
	C81081 × C81027	AB × AB	19	34			16			69	1:2:1	0.275		
	C81027 × C81080	AB × AB	17	30			17			64	1:2:1	0.250		
	TC83001 × C81019	B × AB		42			28			70	1:1	2.800		
	C81027 × C81125	AB × B		17			14			31	1:1	0.290		

¹⁾ self-pollination.

²⁾ self-pollination of the F₁ plant (CR94310) derived from the cross between C81027 and C81125.

*, ** significant at the 0.05 and 0.01 levels of probability, respectively.

Fig. 3. Esterase zymograms of taro (*Colocasia esculenta* (L.) Shott) showing segregation in the EST 1, EST 3 and EST 8 zones (a), EST 3, EST 4 and EST 8 zones (b) and EST 3 and EST 4 zones (c).

three progeny phenotypes, A, AB and B, a slow migrating single-banded type; the segregation ratio was 1:2:1 in the progeny after the cross between C81079 and C81135 or self-pollination of C81081, but the significant deviation was detected for the selfed progeny of C81135. Crosses of a triple-banded phenotype, either AB or AC, with the A phenotype produced the parental phenotypes in the progeny, although the segregation ratio was significantly different from 1:1 in the TC83001 and C81019 cross. Crosses between two triple-banded phenotypes, AB and AC, resulted in four phenotype classes: A, AB, AC and a different triple-banded type in the progeny. Two outside bands of the third triple-banded type corresponded to the slowest moving band of AB and that of AC, respectively. This phenotype was, thus, designated as BC, where its middle band was stained more intensely than two outer bands. The segregation ratio of the AB and AC cross was determined as 1:1:1:1 when the AC phenotype (C81027) was a female parent, but not so when the AC was a male parent (Table 2).

Self- or cross-pollinations of the triple-banded phenotype AB yielded the parental phenotype and two single-banded phenotypes, A and B, in the progeny. Similarly, self-pollinations of the triple-banded phenotype AC yielded the AC, A and C phenotypes (Fig. 3b). These results, in addition to the band characteristics of the triple-banded phenotype BC described above, indicate that the triple-banded phenotypes in the EST-3 are a heterozygote and the middle band is produced by a heterodimer molecule. The isozymes in this zone of *C. esculenta* are, therefore, formed as a dimeric molecule and governed by a single locus (*Est-3*) with three different alleles, designated as *Est-3^a*, *Est-3^b* and *Est-3^c*.

The selfed progeny of the triple-banded AC phenotypes (CR94310 and C81027) showed the segregation into A, AC, and C; however, the segregation ratio was significantly apart from the 1:2:1 ratio expected from a

pair of codominant alleles in a single locus (Table 2). When the χ^2 value was partitioned, either χ_1^2 or χ_2^2 was found significant indicating that 1) significant reduction in the total number of the homozygotes unproportional to that of the heterozygote, or 2) significant reduction in the number of the C phenotype compared to that of the A phenotype. These results suggest strong selection against the gamete of a *Est-3^c* allele. Significant reduction in the number of the phenotypes carrying the *Est-3^c* allele also occurred in the progeny after the crosses where the phenotype AC was used as a male parent. This was not the case in the crosses where the phenotype AC was only a female parent. The *Est-3^c* may be associated with the characteristics which impair development or viability for fertilization of the pollen carrying this allele.

In the EST-4 zone, the presence or absence of a single band was observed in the original accessions, of which the phenotypes were designated as A or N, respectively (Fig. 1). Self-pollinations of the A phenotypes resulted in two phenotypes, A and N, in a 3:1 ratio (Figs. 3b and 3c and Table 2). The crosses between A and N phenotypes yielded the same phenotypes in the progeny with a 1:1 ratio suggesting that the phenotype A accessions in the crosses, C81079, C81125 and C81080, were heterozygous at this locus. These results strongly indicate that isozyme variants in the EST-4 are governed by a single locus (*Est-4*) with two alleles, an active allele, *Est-4^a*, and a recessive null allele, *Est-4ⁿ*.

Three isozyme phenotypes, single-banded A and B, and double-banded AB, were found in the EST-8 zone among the original accessions (Fig. 1). Self- or cross-pollinations of AB phenotypes produced the A, AB and B phenotypes in a 1:2:1 segregation ratio (Fig. 3a and Table 2). Crosses between B and AB phenotypes produced parental phenotypes in the progeny with the segregation ratio of 1:1. The isozymes in the EST-8 zone are, thus, functionally monomeric and under single locus control (*Est-8*) with two codominant alleles, *Est-8^a* and *Est-8^b*.

Leucine aminopeptidase : Two zones of LAP activities were observed in original accessions (Fig. 1). The cathodal zone was monomorphic for all materials analyzed in this study.

Three phenotypes were present in the anodal zone, single-banded A and B and double-banded, AB (Fig. 1). Self- or cross-pollinations of the AB phenotypes produced three phenotype classes, A, AB and B, (Fig. 4a) in a 1:2:1 segregation ratio (Table 3). Self-pollination of single-banded B individuals (C81081 and C81135) resulted in two progeny phenotypes, active B and inactive N phenotypes (Fig. 4b). These results indicate these two B phenotype accessions are heterozygous of active and null alleles. The crosses of these B phenotype accessions with the AB phenotype resulted in the segregation of A, AB and B phenotypes with a 1:1:2 ratio confirming the heterozygosity of these two accessions. A cross between TC83001 (A phenotype) and

Table 3. Segregation of leucine aminopeptidase (LAP-1) variants in self- or cross-pollinated populations of diploid taro (*Colocasia esculenta* (L.) Shott) accessions

Cross	Parental phenotype	Progeny phenotypes				Total	Expected ratio	χ^2 value
		A	AB	B	N			
C81027 ¹⁾	AB	8	28	16		52	1:2:1	2.633
C81125 ¹⁾	AB	9	15	9		33	1:2:1	0.273
CR94334 ²⁾	AB	10	17	10		37	1:2:1	0.243
C81081 ¹⁾	B			41	3	44	3:1	7.758*
C81135 ¹⁾	B			49	15	64	3:1	0.083
C81027 × C81080	AB × AB	17	32	15		64	1:2:1	0.125
C81079 × C81135	AB × B	23	29	45		97	1:1:2	1.247
C81081 × C81027	B × AB	24	18	27		69	1:1:2	4.304
TC83001 × C81019	A × B	33	37			70	1:1	0.228

¹⁾ self-pollination.²⁾ self-pollination of the F₁ plant (CR94334) derived from the cross between C81027 and C81125.

* significant at the 0.05 level of probability.

Fig. 4. Leucine aminopeptidase zymograms of taro (*Colocasia esculenta* (L.) Shott) showing codominant expression (a) and active or null expression (b).

C81019 (B phenotype) produced A and AB phenotypes, in a 1:1 ratio (Table 3). This implies that TC83001 is a homozygote, but C81019 is a heterozygote of active and null alleles. Based on these results, the isozymes in the LAP-1 zone are coded by a single locus (*Lap-1*) with three alleles, *Lap-1^a*, *Lap-1^b* and *Lap-1ⁿ* (null), where *Lap-1ⁿ* is recessive to *Lap-1^a* and *Lap-1^b*.

Phosphoglucumutase: A total of ten bands were observed in the original accessions. They were possibly grouped into five zones of activities based on segregation analyses and relative band intensities (Fig. 1). In the PGM-1 zone, two single-banded phenotypes, A and B, and a double-banded AB were present in the original accessions. Additionally, a null phenotype, N, appeared in the progeny after the self-pollination of the A phenotype (C81027) or B phenotype (CR94310). The segregation ratio for the single-banded type (A or B) and N after the self-pollination of C81027 or CR94310 was 3:1, which suggested a null allele was recessive (Table 4). The presence of the null allele was confirmed when the cross between the A and AB phenotypes (C81027 × C81125) produced the B phenotype in the progeny or when the cross between the AB and B phenotypes (TC83001 × C81019) produced the A phenotype. Self-pollination of the AB phenotype resulted in the A, AB and B progeny phenotypes in a 1:2:1 ratio (Table 4 and Fig. 5). Based on these observation,

Fig. 5. Phosphoglucumutase zymograms of taro (*Colocasia esculenta* (L.) Shott) showing segregation in the PGM-1, PGM-2 and PGM-3 zones.

the isozymes in the PGM-1 zone are governed by a single locus, *Pgm-1*, with two codominant alleles, *Pgm-1^a* and *Pgm-1^b*, and a recessive null allele, *Pgm-1ⁿ*.

A single-banded phenotype, A, and a null phenotype, N, were observed in the PGM-2 zone. Self-pollination of C81027 or CR94310 produced the A and N phenotypes in a 3:1 ratio in the progeny. When C81027 was crossed with the N phenotype, C81125, the segregation ratio of the A and N phenotypes was 1:1 (Table 4). The PGM-2 zone is, thus, governed by a single locus with a dominant active allele, *Pgm-2^a*, and a recessive null allele, *Pgm-2ⁿ*. As far as reviewing references, the occurrence of null alleles in the PGM system is rare and the possibility of the single locus control over the PGM-1 and PGM-2 zones was examined. However, several plants in the progeny populations were found to have the phenotype of AB in the PGM-1 and A in the PGM-2 zones or that of N in both PGM-1 and PGM-2 zones, indicating these two zones were independently controlled. Segregation of an inactive phenotype in the PGM system was reported in *Brassica* plants (Chen and Heneen, 1990 and Nozaki *et al.*, 1995).

Single-banded A and B, and double-banded AB, were observed in the PGM-3 zone. Self- or cross-

Table 4. Segregation of phosphoglucosmutase variants in self- or cross-pollinated populations of diploid taro (*Colocasia esculenta* (L.) Shott) accessions

Isozyme zone	Cross	Parental phenotype	Progeny phenotypes					Expected ratio	χ^2 value and its partitions		
			A	AB	B	N	Total		χ^2	χ_1^2	χ_2^2
PGM-1	C81027 ¹⁾	A	40			12	52	3:1	0.103		
	CR94310 ²⁾	B			26	11	37	3:1	0.441		
	C81027 × C81125	A × AB	14	9	8		31	2:1:1	0.351		
	TC83001 × C81019	AB × B	14	27	29		70	1:1:2	6.891*		
	C81125 ¹⁾	AB	11	15	7		33	1:2:1	1.242		
	CR94334 ²⁾	AB	12	15	9		36	1:2:1	1.500		
PGM-2	C81027 ¹⁾	A	35			17	52	3:1	0.010		
	CR94310 ²⁾	A	25			12	37	3:1	0.014		
	C81027 × C81125	A × N	14			17	31	1:1	0.290		
PGM-3	C81125 ¹⁾	AB	7	18	8		33	1:2:1	0.333		
	C81027 ¹⁾	AB	13	33	6		52	1:2:1	5.654		
	C81027 × C81125	AB × AB	6	18	7		31	1:2:1	0.871		
	CR94310 ²⁾	AB	7	30	0		37	1:2:1	16.946**	14.297**	2.649
	CR94334 ²⁾	AB	4	30	2		36	1:2:1	16.222**	16.000**	0.222
	TC83001 × C81019	A × AB	36	34			70	1:1	0.057		

¹⁾ self-pollination.²⁾ self-pollination of the F₁ plant (CR94310 or CR94334) derived from the cross between C81027 and C81125.

*, ** significant at the 0.05 and 0.01 levels of probability, respectively.

pollinations of the AB phenotypes yielded three phenotypes in the progeny, A, B and AB. The observed segregation ratios fitted to a 1:2:1 in three cross combinations, however, segregation distortion was found in the two lineages (Table 4). When the significant χ^2 value of the two populations was partitioned, only the χ_1^2 value was significant indicating that the total number of the two homozygotes was significantly less than that of the heterozygote in these crosses. Natural selection against the homozygotes possibly occurred at this locus. A cross between the AB and A phenotypes resulted in parental phenotypes with a 1:1 segregation ratio (Table 4). A single locus controls the PGM isozymes in this zone with two codominant alleles, *Pgm-3^a* and *Pgm-3^b*.

Triple and double bands were found in the zones 4 and 5, respectively. However, no segregation was detected among the materials used in this study.

Conclusions

A total of nine polymorphic loci were discovered encoding for ADH, EST, LAP and PGM systems in leaf tissue of the diploid taro accessions from Nepal and Thailand. Isozymes of the *Adh-2* and *Est-3* loci were functionally dimeric molecules, whereas those at the loci of the *Est-8*, *Lap-1*, *Pgm-1* and *Pgm-3* were functionally monomeric. The subunit structure of the *Est-4* and *Pgm-2* was not specified because only a single active allele along with a null allele was detected in the taro materials used in this study. Null alleles were found at four loci (*Est-4*, *Lap-1*, *Pgm-1*, and *Pgm-2*).

This is the first report to determine the loci and alleles of the ADH, EST, LAP and PGM systems in taro. Isshiki *et al.* (1995) determined three loci of isozyme variations for aspartate aminotransferase, shikimate dehydrogenase and glucose-6-phosphate isomerase in wild taro introduced from Bangladesh. These findings may suggest the presence of allelic variations in other enzyme systems of taro. Further allozyme analyses will enable precise estimation of genetic similarity and classification of a wide range of diploid taro.

Most of taro cultivars are triploids. The genetic study on the triploid taro has been fairly difficult because of their complete gametic sterility. The triploid taro is believed to have evolved from the diploid by union of an unreduced gamete with a normal gamete because the tetraploid form of taro has never been discovered. As far as we investigate, the triploid taro has the similar allozymes to those found in the diploid species. The allozyme analyses on the diploid will provide quite useful information to understand genetic variation, genetic relationship and evolution of the triploid taro, which otherwise is difficult to obtain. We are currently investigating allozymes in other enzyme systems and linkage relationships among isozyme loci using diploid taro accessions. The discovery of the isozyme genetics can serve as the base to understand the evolution and phylogeny of taro and can be used to classify and evaluate germplasm for genetic improvement.

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