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Genetic Characterization of Pili (*Canarium ovatum* Engl.) from Albay, Camarines Norte, and Camarines Sur Through Isozyme Analysis

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Based on esterase (EST), acid phosphatase (ACP), and alkaline phosphatase (ALP), genetic variability was noted in 19 accessions of pili (*Canarium ovatum* Engl.) from Albay, Camarines Norte, and Camarines Sur and in the 11 accessions of unknown origin. Seven presumptive loci were determined. Two presumptive loci were observed in EST (EST1 and EST2), ACP (ACP1, and ACP2) while three for ALP (ALP1, ALP2, and ALP3). Percent polymorphism was 100%. Using a similarity coefficient of 0.60, the 30 accessions were divided into 5 clusters. Accessions of different origin grouped together which would indicate that variability exists in the accessions considered. To further prove that genetic diversity exists in pili, 25 accessions collected from Oas, Albay were also subjected to isozyme analysis. Eighteen presumptive loci were determined: 3 for glucose-6-phosphatase dehydrogenase (G6PD), 2 each for EST, phosphogluconate dehydrogenase (PGD), malate dehydrogenase (MDH), ACP, ALP, and phosphoglucomutase (PGM), and 1 each for glutamate oxaloacetate (GOT), phosphoglucoisomerase (PGI), and alcohol dehydrogenase (ADH). Only ALP2, G6PD2, and G6PD3 were monomorphic. The observed heterozygosity for ACP1, ACP2, ALP1, EST1, EST2, and PGD2 was higher compared to the expected heterozygosity. Fifteen of the presumptive loci were polymorphic (83.33%). Considering a similarity coefficient of 0.70; 4 clusters were obtained although the 25 accessions were collected only from Oas, Albay. This would indicate that accessions were genetically different. Pili being dioecious is an obligate cross-pollinating crop. Genetic variability observed can be explained through recombination occurring during sexual reproduction.

Key Words: *Canarium ovatum*, isozymes, heterozygosity, presumtive loci

INTRODUCTION

The Philippines, a tropical country, is very rich in edible fruit and nut bearing trees, of which about 167 are indigenous (Villegas & Coronel 1979). Several fruit trees that bear edible nuts are claimed to have their center of diversity in the Philippines. The most important of these is pili, *Canarium ovatum* (Figure 1), of which geographic distribution in the country remains limited to areas located relatively closer to its center of origin (Coronel 1996). Pili is considered to be the most important nut-producing species indigenous to the country. It has a nationwide acceptance and has great potential to develop into a major industry (Philippine Fruit Network 2001). As a nut, the development of this crop is promising as demand for processed kernel is rising. The pili nut kernel (Figure 2) is the most important part of the tree and has many uses (Coronel 1996). Pili nuts are mainly used to manufacture

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Figure 1. Pili (*Canarium ovatum* Engl.) tree planted at the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

Figure 2. Pili nut from *Canarium ovatum* and other processed products

candies and confectioneries, while pili nut oil is highly in demand local and in foreign countries like Guam, Australia, Canada, and United States (PCARRD 1997).

The National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, University of Philippines Los Baños maintains a germplasm collection of pili. This collection is a potential reservoir of outstanding genes. There is a need to genetically characterize them to assess the degree of diversity available. Genetic diversity refers to the variation of genes within species and the heritable variation within and between populations of organisms (de Vicente & Fulton 2003). This is important not only in conservation but also in species improvement as well (Yu et al. 2001). Gene conservation management aimed to save adaptive genetic diversity is necessary. Assessment of genetic diversity is very important for efficient utilization of genetic resources by plant breeders and for rationalization of gene bank management to minimize the conservation of redundant samples which are sometimes useless or costly (Balma et al. 1996).

Since isozymes are proteins, these can directly reflect alterations in the DNA sequence through changes in the amino acid composition (Weeden & Wendel 1989). Changes in electrophoretic mobility of the enzymes are extremely useful to evaluate genetic differences among individuals. Isozyme marker is still one of the best genetic markers specially in studying natural populations like pili nuts. Isozyme data are easily generated and the resulting data are not only simple co-dominant Mendelian characters, but more importantly, are for the genes that code for various enzymes of critical cellular pathways such as gylcolysis and citric acid cycle (May 1992).

Genetic diversity analysis based on isozymes was done on *Cucumis sativus* germplasm collection in the US (Knerr et al. 1988), wild emmer wheat (*Triticum dicoccoides*) in Israel and Turkey (Nevo and Beiles 1989), 90 accessions of spontaneous taxa of sorghum (*Sorghum bicolor*) section sorghum (Morden et al 1990), Algerian cultivars of date palm, *Phoenix dactylifera* (Bennaceur et al. 1991), Chinese, Seguin, and American species of chestnut, *Castanea* spp.(Huang et al. 1994), four populations of South American wild rice (*Oryza glumaepatula*) populations (Buso et al. 1998), 31 accessions of turnip (*Brassica rapa* var. *rapa*) from the Nordic area (Persson et al. 2001), and on 18 landraces of rye, *Secale cereale* from Northern Europe (Persson & von Bothmer 2002). Isozyme analysis was also used to determine the amount and distribution of variation in 48 sorghum landraces from Ethiopia and Eritrea (Ayana et al. 2001) and the geographic variation of 206 accessions of cherimoya from Madeira, Bolivia, Spain, Chile, California, Peru, and Ecuador (Perfectti & Pascual 2005).

The study was conducted to genetically characterize the different accessions of pili in Camarines Norte, Camarines Sur, Albay and accessions of unknown origin through isozyme analysis, estimate the degree of genetic diversity available by computing the gene frequencies and genetic polymorphism, and analyze the genetic relationship of the different accessions by comparing their similarity coefficient.

MATERIALS AND METHODS

Thirty accessions of pili maintained by the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding (IPB), Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños were initially studied using three enzymes. Nineteen of these accessions were collected from Albay, Camarines Norte, Camarines Sur, and 11 of unknown origin (Table 1). Isozyme analysis for nine enzymes was also conducted on 25 accessions, all of which originated from Oas, Albay.

Leaf samples of the different pili accessions were separately powdered using liquid nitrogen, transferred to microcentrifuge tube, and 700 µL of cold extraction buffer (0.5M Tris-Histidine, pH 8.0, 0.02% 2-mercaptoethanol, 1.0% polyethylene glycol) was added (Glaszman et al. 1988). The samples were individually mixed using vortex mixer and centrifuged for 15 min (4° C, 13,000 rpm). The clear supernatant was transferred to a pre-labeled microcentrifuge tube. Five hundred µL Ethyl acetate was added, and each tube was slowly inverted 5 to 10 times and centrifuged for one minute. Approximately 4 µL of the aqueous layer was transferred to another pre-labeled microcentrifuge tube. Samples were loaded in 12%, 60 mm x 108 mm (L x W), 10 mm thick starch gel. Bromphenol blue was used as tracking dye. Electrophoresis was performed inside a refrigerator (approximately 10° C) at 50 volts for the first 2 h and 100 volts for the remaining time of the total 3-4 h until the tracking dye has traveled 50 mm from the origin. The gel and running buffer used was the 1x dilution of the stock buffer (0.009 M Tris, 0.005 M Histidine, pH 8). After the run, the gel was horizontally sliced resulting to four 2.0-2.5 mm thick gels. The different slices were used to detect different enzyme systems. The following enzymes were detected: malate dehydrogenase (MDH), esterase (EST), acid phosphatase (ACP), alkaline phosphatase (ALP), glucose 6 phosphate dehydrogenase (G6PD), glutamate-oxaloacetate transaminase (GOT), 6-phosphogluconate

Table 1. Accessions of pili (*Canarium ovatum* Engl.) maintained at the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, Crop Science Cluster, College of Agriculture University of the Philippines Los Baños

Accession Number	Origin
7363	Unknown
7075	Unknown
7090	Macasdi, Guinobatan Albay
7079	CSSAC, Pili, Camarines Sur
7093	Matbog, Basud, Camarines Norte
7088	Macasdi, Guinobatan Albay
7361	Unknown
7362	Unknown
7383	San Jose, Camarines Sur
7089	Macasdi, Guinobatan Albay
7372	Unknown
7383	San Jose, Camarines Sur
7089	Macasdi, Guinobatan Albay
7372	Unknown
7074	CSSAC, Pili, Camarines Sur
7085	San Jose, Camarines Sur
7076	Pili, Camarines Sur
7371	Unknown
7087	Macasdi, Guinobatan Albay
7077	CSSAC, Pili, Camarines Sur
7078	Unknown
7081	Public Market Guinobatan, Albay
7376	Unknown
7448	Albay
7083	San Jose, Camarines Sur
7139	Miti, Camalig Albay
7084	San Jose, Camarines Sur
7385	Unknown
7359	CSSAC, Pili, Camarines Sur
7092	Guinobatan Albay
7360	Unknown
7082	Mauro, Camarines Sur
7374	Unknown

dehydrogenase (6-PGD), phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM). The enzyme banding patterns were observed by placing the gel slabs over a light box. The number of bands per enzyme was noted and the relative mobility (Rf) values were obtained using the formula:

$$Rf = \frac{\text{distance traveled by the band}}{\text{distance traveled by the tracking dye}}$$

Photographs of the gels were taken. Numerical Taxonomy System (NTSYS) version 2.1 program was used to determine similarity coefficient between accessions and dendrogram was generated based on similarity coefficient using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Bootstrap analysis with 1000 iterations was done using WinnBoot (Yap & Nelson 1996). POPGENE version 1.31 (Yeh & Boyle 1997) a freeware for population genetic analysis was used for the computation of gene frequencies and percent polymorphism. Lavene's calculation for observed and expected heterozygosity was done using POPGENE.

RESULTS AND DISCUSSION

Isozyme Profile in Pili

Thirty accessions of pili were initially studied for esterase, acid phosphatase, and alkaline phosphatase.

Esterase (EST). Two zones of activity were observed in EST (Figure 3), EST1, and EST2 with an average Rf value range of 0.04-0.18 and 0.19-0.45, respectively. Alleles were designated as S and F for slow and fast allele, respectively. For EST1, two genotypes were noted (Figure 3), SS and FS while for EST2, the genotypes were SS and FF. Four banding patterns (BP) were observed. No particular banding pattern was noted to be specific to a place of origin.

Acid phosphatase (ACP). Two zones of activity (Figure 4) were observed, ACP1 (0.02-0.23) and ACP2 (0.24-0.34). Only one genotype (SS) was noted for both loci. Three banding patterns were noted and no specific pattern was noted to be exclusive for the area of collection.

Alkaline phosphatase (ALP). Three zones of activity were observed (Figure 5), ALP1 (0.04-0.19), ALP2 (0.20-0.41), and ALP3 (0.43-0.65). Only 1 genotype (SS) was noted for the 3 loci. Six banding patterns were noted. No specific banding pattern was noted that is directly associated with the site of collection.

Genetic Frequencies and Heterozygosity in Accessions of Pili Collected from Different Locations

The frequencies of the total 9 presumptive loci for esterase, acid, and alkaline phosphatases are presented in Table 2. For the S allele, EST3 showed the highest frequency of 0.711 followed by ACP2 (0.526). For the F allele, ALP1 showed the highest frequency of 0.804 followed by ALP3 (0.604). The 9 loci identified were all polymorphic, meaning 100% polymorphism.

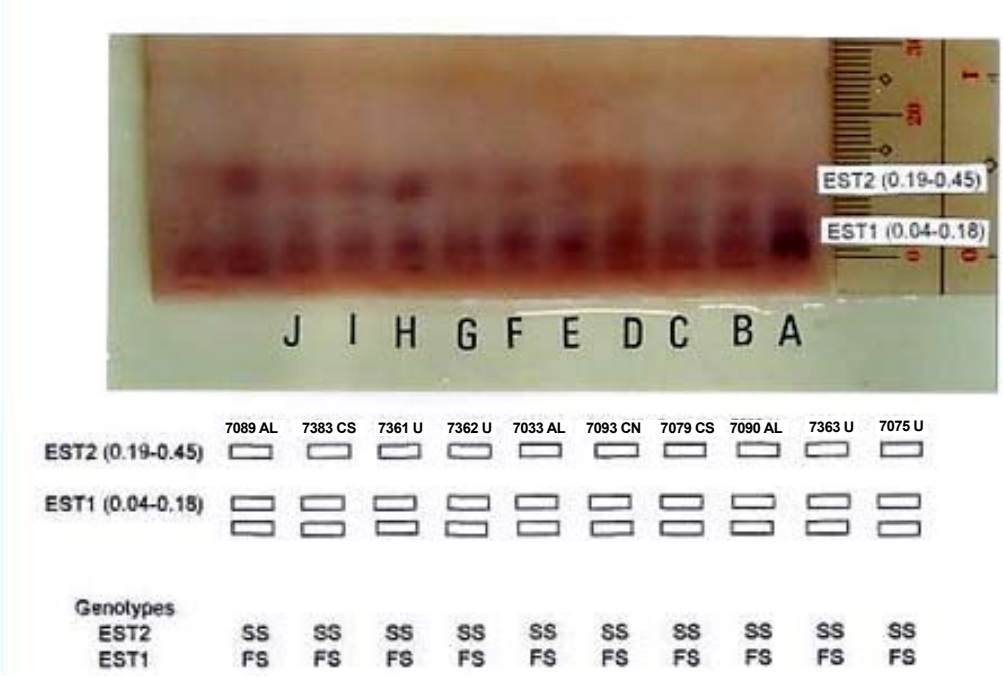

Figure 3. Representative photograph of the gel, zymogram, and genotype for esterase (EST) in pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources, Crop Science Cluster, University of the Philippines Los Baños

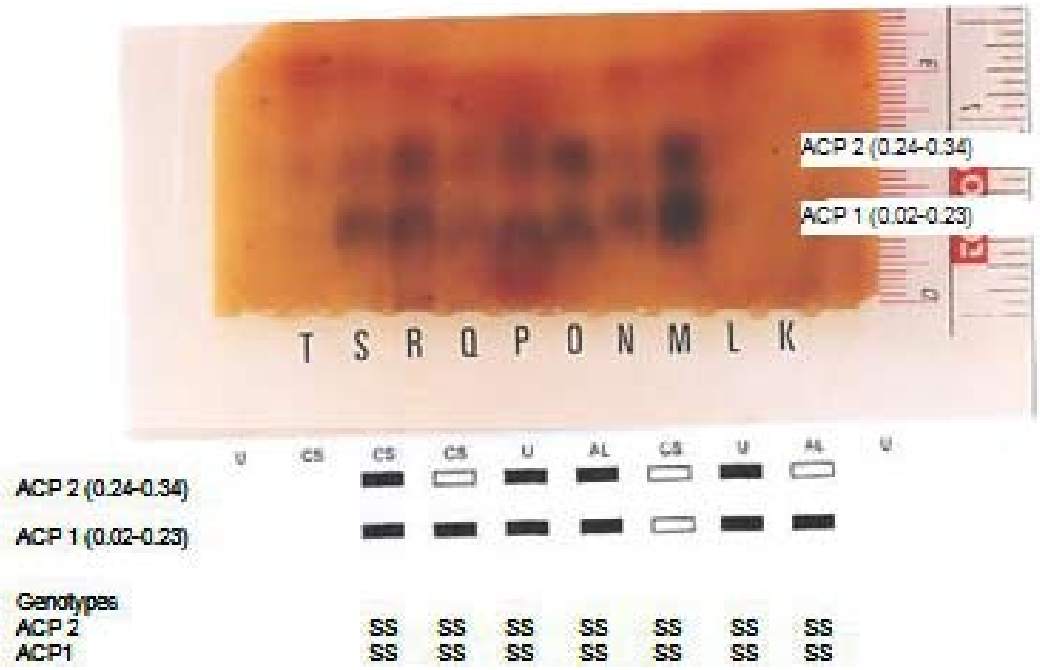

Figure 4. Representative photograph of the gel, zymogram, and genotypes for acid phosphatase (ACP) in pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources Laboratory, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

The observed heterozygosity in ACP1 (0.621) and ACP2 (0.526) was higher compared to the expected heterozygosity of 0.508 and 0.512, respectively (Table 3). Pili is a dioecious crop with separate male and female plants. As such it is considered to be an obligate cross-pollinating crop. Recombination that happens during sexual reproduction could explain the observed high heterozygosity.

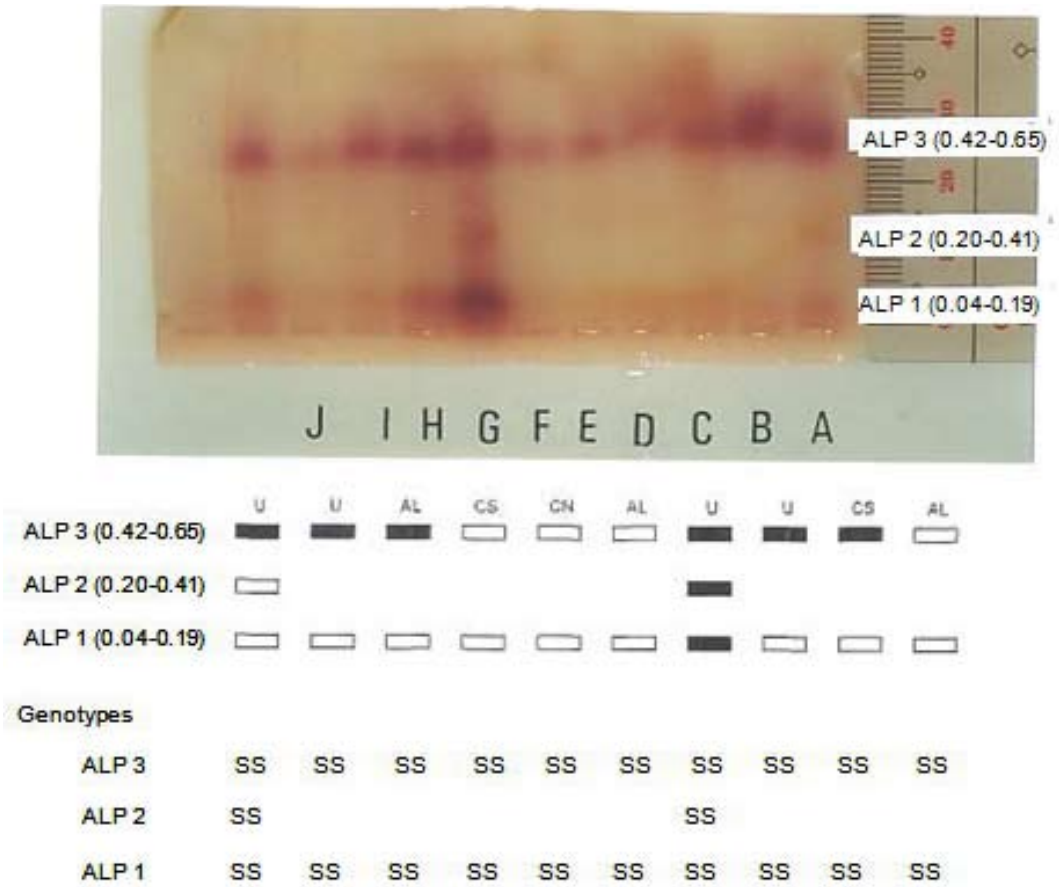

Figure 5. Representative photograph of the gel, zymogram, and genotype for alkaline phosphatase (ALP) in pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños.

Table 2. Gene frequencies of the nine presumptive loci in pili (*Canarium ovatum*) from the collection of the National Plant Genetic Resources Laboratory, Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños[1].

Locus	Gene Frequencies	
	Allele S	Allele F
ACP1	0.517	0.483
ACP2	0.526	0.474
ACP3	0.500	0.500
ALP1	0.196	0.804
ALP2	0.463	0.537
ALP3	0.396	0.604
EST1	0.444	0.556
EST2	0.500	0.500
EST3	0.711	0.289

[1]Values were obtained using POPGENE (Yeh and Boyle 1997).

Table 3. Levene's observed and expected heterozygosity for the nine presumptive loci in pili (*Canarium ovatum*) from the collection of the National Plant Genetic Resources Laboratory, Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños[1]

Locus	Observed Heterozygosity	Expected Heterozygosity
ACP1	0.621	0.508
ACP2	0.526	0.512
ACP3	0.263	0.513
ALP1	0.178	0.321
ALP2	0.333	0.506
ALP3	0.241	0.487
EST1	0.444	0.503
EST2	0.217	0.511
EST3	0.157	0.422

[1]Values were obtained using POPGENE (Yeh and Boyle 1997).

Genetic Relationships of Accessions of Pili Collected from Different Locations

Figure 6 shows the genetic relationships based on the isozymes of 3 enzymes systems of the 30 accessions of pili. Using a similarity coefficient of 0.60, the 30 accessions formed 5 clusters. Accessions from different collection sites grouped together. Using bootstrap analysis, 5 accessions namely, Acc 7077 (Camarines Sur), 7087 (Albay), 7371 (U), 7076 (Camarines Sur), and 7085 (Camarines Sur) were found to cluster at 94.4%. Thirteen of the accessions were included in cluster1 which is composed of 4 accessions from Albay, 3 from Camarines Sur, and 1 from Camarines Norte. Five accessions of unknown origin were also included in this cluster. Cluster2 is composed of 5 accessions from Camarines Sur, and 3 from Albay, and two of unknown origin. The third cluster was composed of only 2 accessions, 1 from Camarines Sur, and 1 that is of unknown origin. The fourth cluster is composed of only 3 accessions, 2 of which are of unknown origin, and one from Camarines Sur. The fifth cluster is composed of an accession from Albay, and an accession from an unknown origin. Pili found in nurseries are commonly propagated through cleft grafting and budding. Small seedlings that are easy to handle may be transported to any area in Bicol region. This could possibly explain why no association was observed between genotype and origin of collection. Some of the accessions may be products of introduction.

To further determine the extent of genetic diversity existing in the gene bank's pili collections, 25 accessions planted in the vicinity of IPB were also subjected to isozyme analysis. All these accessions were collected from Oas, Albay. Ten enzymes were studied namely: glucose 6 phosphate dehydrogenase, esterase, phosphogluconate dehydrogenase, malate dehydrogenase, alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, phosphoglucoisomerase, alcohol dehydrogenase, and phosphoglucomutase.

Glucose 6 phosphate dehydrogenase (G6PD). Three zones of activity were noted (Table 4) designated as G6PD1 (Rf value range of 0.04-0.12), G6PD2 (0.18-0.22) and G6PD3 (0.32-0.36). Genotypes SS and FS were noted for G6PD1, while only FF was observed for G6PD2 and G6PD3. G6PD2 and G6PD3 were not observed in all accessions.

Esterase (EST). EST1 and EST2 loci were noted (Table 4). SS, FS, and FF were observed for EST1 and EST2. Figure 7 shows the 2 zones noted for EST. EST1

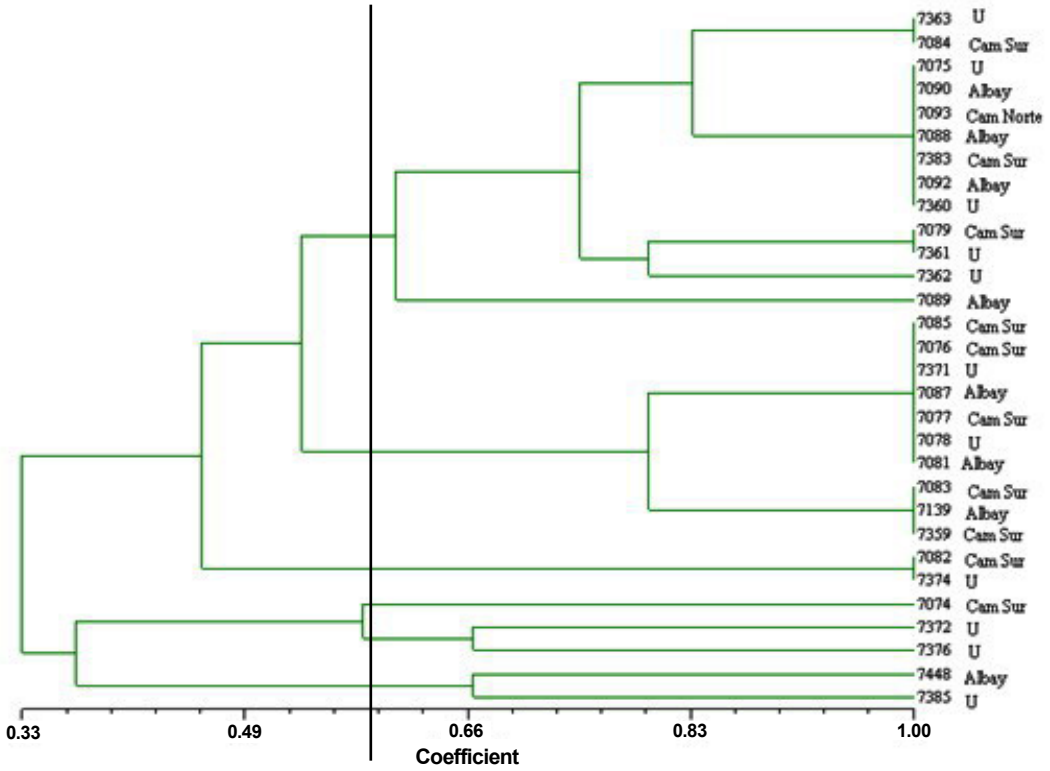

Figure 6. Dendrogram to show genetic relationship of the thirty accessions of pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

Table 4. Gene frequencies of the 18 presumptive loci in pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños[1]

Locus	Gene Frequencies	
	Allele S	Allele F
ACP1	0.475	0.525
ACP2	0.682	0.318
ADH1	0.542	0.458
ALP1	0.409	0.591
ALP2		1.0
EST1	0.5	0.5
EST2	0.587	0.413
GOT1	0.44	0.560
G6PD1	0.54	0.460
G6PD2		1.0
G6PD3		1.0
MDH1	0.125	0.875
MDH2	0.833	0.166
PGD1	0.1	0.900
PGD2	0.412	0.588
PGI1	0.636	0.364
PGM1	0.292	0.708
PGM2	0.143	0.857

[1]Values were obtained using POPGENE.

and EST2 have an Rf value range of 0.12-0.20 and 0.24-0.34, respectively. Two accessions namely 7421 and 7422 failed to show the two loci.

Phosphogluconate dehydrogenase (PGD). Two zones of activity were observed, PGD1 (0.15-0.20) and PGD2 (0.22-0.34).Only SS and FF genotype were observed for PGD1 (Table 4) while SS, FS, and FF were noted for PGD2. Accessions 7402, 7411, 7415, 7416, and 7429 failed to show PGD1 and PGD2.

Malate dehydrogenase (MDH). Two zones (Table 4) were noted, MDH1 (0.02-0.16) and MDH2 (0.16-0.26). Two genotypes were noted for MDH1, FF, and FS. Although SS and FF were noted for MDH2, most of the accessions did not exhibit MDH2.

Alkaline phosphatase (ALP). ALP1 (0.04-0.12) and 2 (0.12-0.20) were observed (Table 4). SS and FF were noted for ALP1 and only FF was observed for ALP2.

Acid phosphatase (ACP). Two zones were also observed for ACP (Table 4). For ACP1 (0.02-0.14) and ACP2 (0.15-0.26), SS, FF, and FS genotypes were observed. Fourteen of the accessions did not show ACP2.

Glutamate oxaloacetate (GOT). Only 1 zone of activity was noted for GOT (Table 4). Three genotypes were noted, SS, FS, and FF (Figure 8). All 25 accessions were positive for GOT1.

Phosphoglucoisomerase (PGI). Although Tanksley (1980) observed 2 loci in tomato, only 1 zone was noted for PGI in pili (Table 4). Average Rf range is 0.29-0.54. Only SS and FF genotypes were noted. Not all accessions were positive for PGI1 namely, 7402, 7410, 7411, 7416, 7419, 7427, 7429, 7432, 7435, 7436, 7440, 7442, and 7443.

Alcohol dehydrogenase (ADH). Only 1 zone was noted for ADH (Table 4). Average Rf range is 0.11-0.32. Three genotypes were observed for ADH1, SS, FS, and FF. Twelve accessions were negative for ADH.

Phosphoglucomutase (PGM). Two zones of activity were observed for PGM (Table 4), PGM1 (0.06-0.38) and PGM2 (0.40-0.58). Only FS and FF genotypes were noted for PGM1 while SS and FF were noted for PGM2. This is different from what Lebot et al. (1993) observed in banana where they reported 3 zones of activity.

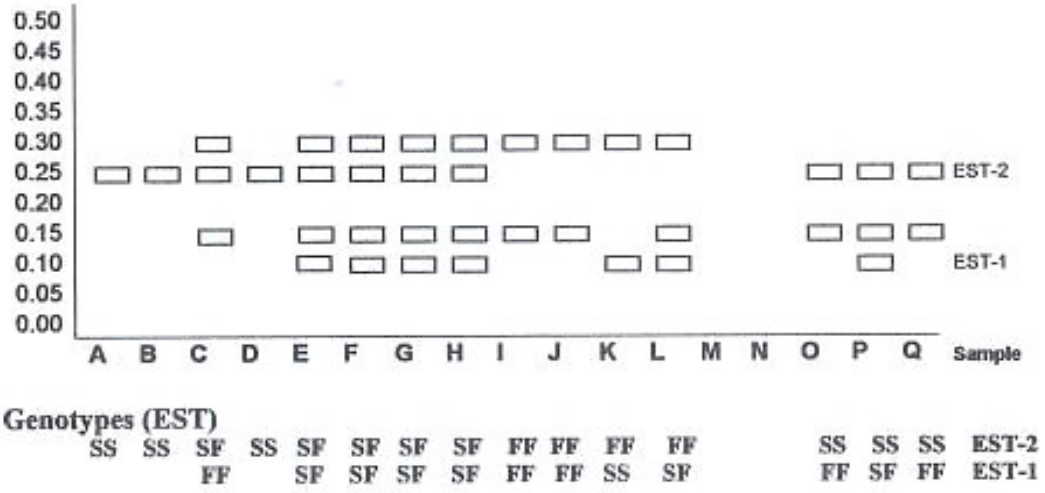

Figure 7. Zymogram of esterase (EST) in pili (*Canarium ovatum*) from the collection of National Genetics Resources Laboratory, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

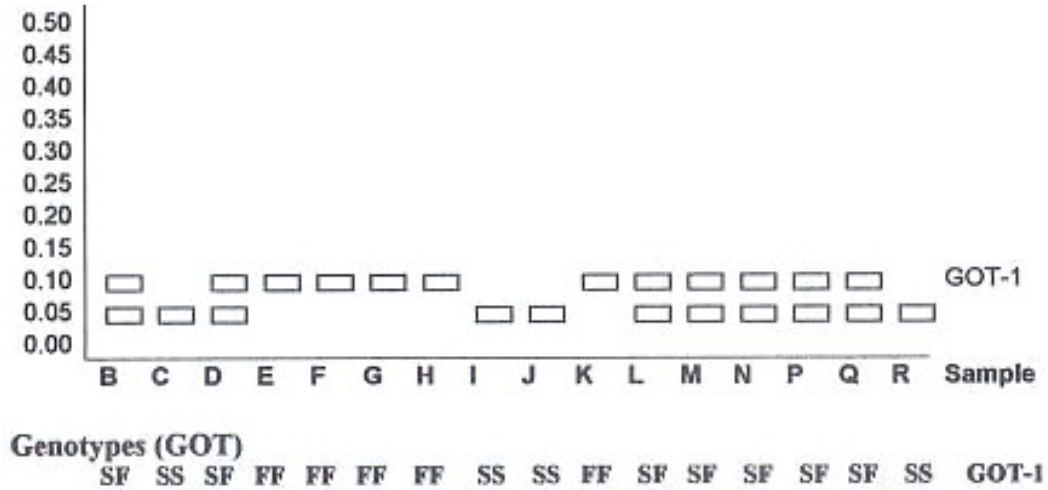

Figure 8. Zymogram of glutamate-oxaloacetate transaminase (GOT) in pili (*Canarium ovatum*) from the collection of the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

Gene Frequencies and Heterozygosity in Twentyfive Accessions of Pili

Considering the 18 presumptive loci, 15 were polymorphic and percent polymorphism is 83.33. ALP2, G6PD2, and G6PD3 were all monomorphic. The frequencies of the different alleles are shown in Table 4. The S allele for MDH2 gave the highest frequency of 0.833 followed by allele S of ACP2 (0.682). PGI1 S allele has a frequency of 0.636. For the F allele, the highest frequency of 1.0 was shown by ALP2, G6PD2, and G6PD3, followed by PGD1 (0.900), MDH1 (0.875), PGM2 (0.857), and PGM1 (0.708).

The expected and observed heterozygosity are presented in Table 5. The observed heterozygosity for ADH1 (0.75) and G6PD1 (0.92) is high compared to the expected 0.51 and 0.50, respectively.

Genetic Relationships of the Twenty-five Accessions of Pili

Using NTSYS, a dendrogram was created to analyze the genetic relationship of the 25 accessions (Figure 9). Considering a similarity coefficient of 0.70, 4 clusters were obtained. The first cluster includes 7402, 7443, 7411, 7429, 7432, 7435, 7427, 7416, and 7419. The second cluster is composed of 5 accessions (7408, 7442, 7410, 7436, and 7440. Five accessions were included in cluster 3 (7409, 7413, 7414, 7412, and 7431). Cluster 4 is composed of 6 accessions (7415, 7420, 7425, 7421, 7423, and 7422). Although the accessions were obtained from the same place (Oas, Albay), variations do exist thus various clusters were formed. Bootstrap analysis indicates that the different accessions were indeed different from each other. The highest value obtained was only 65.6%, which involve only Acc 7409, 7413, and 7414. The different collections could have been derived through sexual reproduction. The fact that pili is dioecious, there are male and female plants, cross pollination would result to genetic recombination resulting to higher variability.

Table 5. Levene's observed and expected heterozygosity for the 18 presumptive loci from the collection of the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños[1]

Locus	Observed	Expected
	Heterozygosity	Heterozygosity
ACP1	0.15	0.51
ACP2	0.27	0.45
ADH1	0.75	0.51
ALP1		0.49
EST1	0.3	0.51
EST2	0.39	0.49
GOT1	0.48	0.5
G6PD1	0.92	0.5
MDH1	0.25	0.22
MDH2		0.3
PGD1		0.18
PGD2	0.23	0.49
PGI1		0.48
PGM1	0.58	0.43
PGM2		0.26

[1]Values were obtained using POPGENE (Yeh and Boyle 1997).

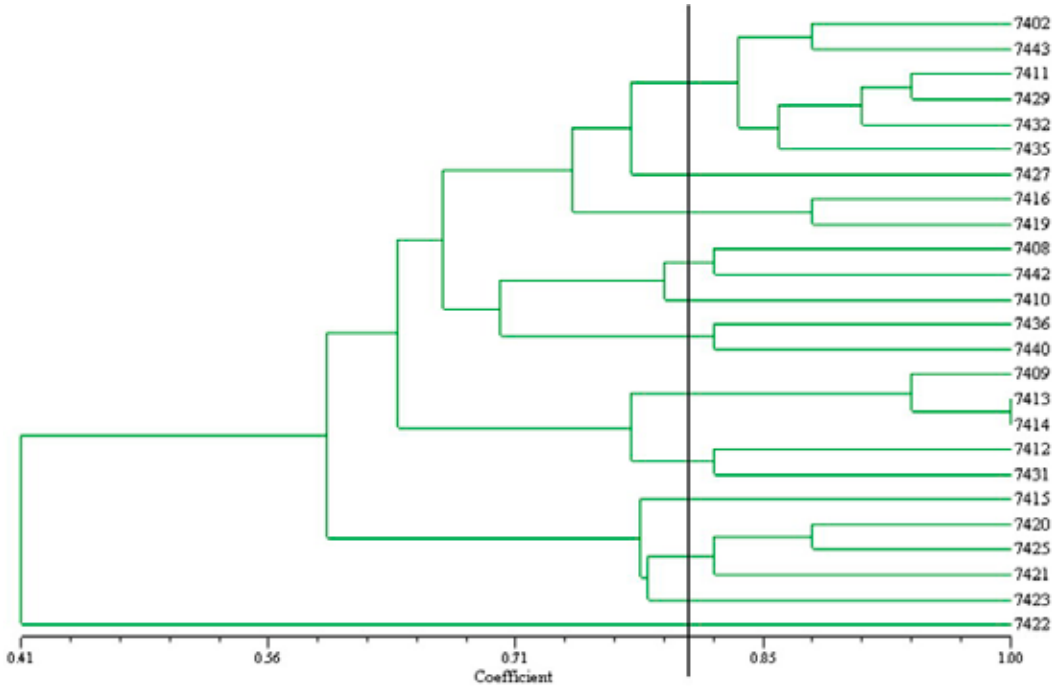

Figure 9. Dendrogram to show genetic relationship of the twenty five accessions of pili (*Canarium ovatum*) from the National Plant Genetics Resources, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños

SUMMARY AND CONCLUSION

Isozyme profile of 19 accessions of pili from Albay, Camarines Norte, Camarines Sur, and 11 accessions of unknown origin showed polymorphism. EST and ACP showed 2 zones of activity while ALP showed three. A total of 7 presumptive loci were detected namely: EST1, EST2, ACP1, ACP2, ALP1, ALP2, and ALP3. For EST1, SS and FS genotypes were noted while for EST2, SS, and FF were detected. Only SS was detected for ACP1, and ACP2. The same is true for ALP1, ALP2, and ALP3. No specific banding pattern (BP) was noted to be exclusive to the site of collection. Percent polymorphism was 100%. Considering 9 enzyme systems, 25 accessions of pili all collected from Oas, Albay showed genetic variations. A total of 18 presumptive loci were detected; 3 for G6PD, 2 for EST, PGD, MDH, ALP, ACP, and PGM. Only 1 zone was detected in GOT, PGI, and ADH. Of the 18 presumptive loci, 15 were polymorphic (83.33% polymorphism). Using NTSYS, the genetic relationships of the 25 accessions showed 4 clusters at 0.70 similarity coefficient. Although the accessions were obtained from the same place, the observed groupings indicate that the different accessions were genetically different and this could be attributed to the fact that the accessions in the collections were derived through sexual reproduction. Genetic variability indeed exists in the germplasm collection of pili at the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, University of the Philippines Los Baños. Bootstrap analysis supports the genetic variation noted in the different accessions. Even accessions collected from the same place are different from each other. Considering that pili is a dioecious crop, (an obligate cross pollinating species) could explain the high level of heterozygosity and the polymorphism noted in pili. The genetic recombination that happens during meiosis and random association of genes from the gametes of male and female parents could be the reason why high variability was observed in pili.

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