Kasetsart J. (Nat. Sci.) 29 : 266-278 (1995)

Classification of Some Tamarind Varieties by Using Peroxidase Isozymes

Sawanee Suriyapananont[1], Suranant Subhadrabandhu[2], Chirayupin Chandraprasong[2] and Ngampong Kongkathip[3],

ABSTRACT

The peroxidase isozymes (PER) were studies in eighty-one varieties of *Tamarindus indica* Linn. by using vertical polyacrylamide gel electrophoresis. The typical zymograms of PER of the sour and the sweet tamarinds showed three different coloration of bands : the light brown bands, the light grey bands and the dark brown bands. The dark brown bands was a key band that can be used to classify the tamarind varieties.

The PER isozyme patterns of the sour and the sweet tamarinds were shown to be genotypically different. The genotypes of tamarind plants were closely related if the plants were collected from the same habitat. The environment had an effect on the position of light brown bands and light grey bands but not on the dark brown bands.

Key words : *Tamarindus indica* Linn., Peroxidase isozymes

INTRODUCTION

Tamarind (*Tamarindus indica* Linn, LEGUMINOSAE) is one of the famous tropical fruit crops of the world (Bailey, 1949.) There are two strains of tamarind trees in Thailand, the sweet and the sour types. These tamarinds have been commonly cultivated for hundreds of years. Tamarind is one of the fruit crops of high economic value with high export potential for Thailand (Anon, 1989.).

There are more than one hundred varieties in each strain, these varieties were resulted from previous propagation by seeds. The morphological and genetical characters may be different even the plants were grown from seeds of the same tree, some characters showed the variation which were noticeable but some characters did not. The plantsof good fruit characters of the sweet tamarind were selected and named such as Meoun Chong, Sri Thong, Sri Chomphu, Indha Plum, Petch Kaset, etc., but in the sour plants, these varietal names were not known. The varietal names may not be true to the taxonomic name as they were derived unsystematically. The diversity of these plants is known and so far no one has grouped the varietal

1 Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Rama IV, Phayathai,Bangkok 10400, Thailand
2 Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
3 Department of Chemistry, Faculty of Sciences, Kasetsart University, Bangkok 10900, Thailand.

names to their proper nomenclature name. The chemotaxonomic method is one of the accurate way in grouping the plants by using the enzyme controlling biosynthesis of the biochemical compounds. The patterns of PER isozyme in leaves of the tamarind varieties were studied and analyzed as chemical marker for their identification. Hence, the objective of this study was to find the variation of PER isozyme patterns in the tamarind plants.

Isozymes have been successfully used for the identification of cultivars in several crops including fruit species such as citrus (Torress *et al*, 1978, 1982), bean (Bassiri and Rouhani 1977, Bassiri and Adams, 1978), apple (Bournival and Korban, 1987), avocado (Torress and Bergh, 1980), almond (Cerezo, *et al*, 1989), but these isozymes methods have not been used in tamarind.

MATERIALS AND METHODS

The peroxidase isozyme (PER) was assayed for the variation using vertical electrophoresis methods.

Fifty-four of tamarind varieties were collected from :

1) Mr. Kayan Ooncharoen's orchard (-A) in Phetchabun province,

2) Mr. Denchai Kartaipeuk's orchard (-B) in Phetchabun province,

3) Si Sa Ket Horticultural Research Center (-C) in Si Sa Ket province.

4) Nuan Noi's orchard (-D) in Loei province.

A total of 81 leaf samples, in four orchards (Table 1) were selected for study and the evaluation of possible variation within a cultivar was assayed by analysing PER isozyme patterns.

Fresh leaf samples (5 gm) from the trees listed in Table 1 were ground thoroughly in 8 ml of 0.5 M Tris HCl (pH 8.5) in a cold mortar. Polyvinyl pyrrolidone (PVP 5% W/V) was added in the extraction buffer to prevent the diffusion of phenolic compounds. The mixture was filtered through 2 layers of net clothes and the homogenate solution was centrifuge at 17,000 rpm for 30 minutes at 0°C. The clear supernatant (80 µl) was loaded onto electrophoresis gel. DISC polyacrylamide gel (1.5 mm thicked) were prepared by the modified method of Laemmli (1970) having 4.5 % stacking gel solution and 10 % resolving gel solution. Hoefer Scientific Instruments Electrophoresis model SF 600 and Fujox FS-150 was used to seperate PER isozyme, the electrode buffer (0.25 M Tris, 0.133 M glycine, pH 8.3) was used (Pasteur et al. 1988). After loading, the gels were run at 25 mA in 5°C incubator for 30 mim and the higher electric current was applied at 40 mA. The horse radish PER isozyme was a standard marker and the bromophenol blue (BB) was used as the indicator, the migration of the BB front was stopped when it moved to 11 cm from the top.

The gels were stained using the staining procedure for PER and was fixed in the fixing mixture (Sriprasertsark, 1988; Pasteur *et al.* 1988). The PER bands were examined and photographed. The zymogram was drown and the percentage of similarity among the varieties was studied by calculation from the similar index (Whitney *et al.*, 1968).

$$\text{Percentage of similarity} = \frac{\text{no. of pairs of similar band} \times 100}{\text{no. of different bands} + \text{no. of pairs of similar band}}$$

RESULTS

PER isozyme detection on acrylamide gels

The PER isozyme from tamarind leaf extracts found in different zymograms consisted of 3 different color bands : 1) dark brown PER color band, 2) light brown PER color band and 3) light grey PER color band. The light brown bands and light grey bands were found in the upper part and

Table 1 Plant names and locations where samples were collected.

Plant Name	Location
001 - 007	Ubon Ratchathani province
008 - 011	Mukdahan province
012 - 014	Nakhon Phanon province
015	-
016	-
017 - 018	Sakon Nakhon province
019	Udon Thani province
020 - 025	Loei province
026 - 027	Chanthaburi province
029 - 036	Songkhla province
Nam Peung	Phetchabun, SiSaKet and Loei provinces
Pai Yai	Phetchabun and Loei provinces
Poo Yai Ma	Phetchabun province
Lang Tag	Phetchabun province
Nang Nguan	Phetchabun province
Nang Beung	Phetchabun province
Panna Nikom	Loei province
Kanom Pang Fag Loei	Loei province
Baan Pra-roj	Phetchabun and Loei province
Nim Nuan	Loei province
Aree	Loei province
Baan-na Sinuan	Loei province
Petch Kaset	Phetchabun, Si Sa Ket and Loei province
Pak Dook	Phetchabun province
Sang Arthit or Koat Petch	Phetchabun province
Sri Chomphu	Phetchabun, Si Sa Ket and Loei province
Indha Plum	Phetchabun, Si Sa Ket and Loei province
Sri Thong	Phetchabun, Si Sa Ket and Loei province
Kru Inn	Phetchabun, Si Sa Ket and Loei province
Meoun Chong	Phetchabun, Si Sa Ket and Loei province
Kan Ti	Phetchabun, Si Sa Ket and Loei province
Na Sai	Phetchabun provinces
Chao Nua Settakit	Phetchabun and Loei provinces
Pramuan Wit	Loei province
Kru Bua-pan	Loei province

lower part of the gel while the dark brown PER color bands was in the middle part. The number and the position of dark brown bands were consistent among the same variety, thus, it was perceived as the key bandset for identifying tamarind varieties (Figure 1;a-d).

Therefore, the groups of tamarind plant can be classified by using the dark brown PER bands. Five groups of tamarind were found : group I contained two thick brown PER isozyme bands (=), group II had three thick dark brown PER isozyme bands ( ), group III had two thick dark brown PER isozyme bands and one thin dark brown isozyme band ( ), group IV consisted of three thick dark brown PER isozyme bands and one thin dark brown PER isozyme bands ( ) and group V had four thin dark brown PER isozyme bands ( ). Most tamarind varieties were found in group I (Figure 2).

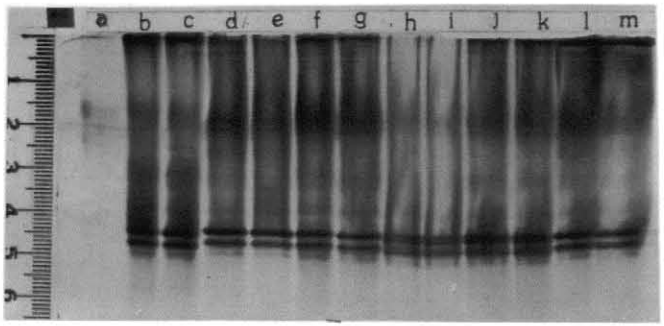
The similarity of PER isozyme bands among the tamarind varieties

Similarity index was used as an indicator to calculate and compare the difference between the sour and sweet tamarind types. The sour tamarind varieties contained more PER isozyme bands than the sweet tamarind (12-21 bands and 6-19 bands respectively). The migration of PER isozyme bands showed a high degree of reproducibility for all the genotypes investigated. The position of faster migration bands was light grey moving ahead of the dark brown PER bands, the faster migrating band moved to 9.1 cm. The slower migrating band was on the upper part of dark brown PER, it moved to 2.0 cm. (Figure 1,a-d).

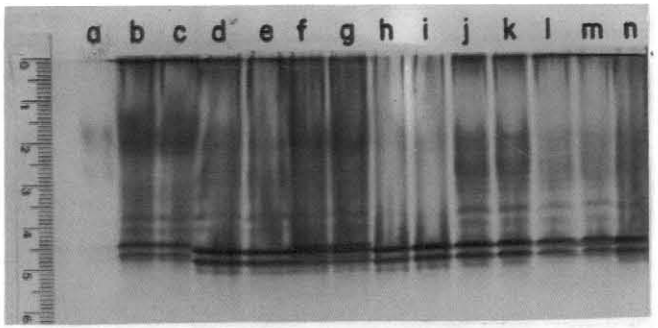
The sour and the sweet tamarind varieties in group I showed different PER zymogram patterns (Figure 2). The 001C, 002C, 003C and 006C varieties were closely related. The percentage of similarity were 81-95. The percentage of similarity between Pai Yai C and Nam Peung C or Indha plum C were 79-63. Lang Tag A variety was closely related to Meoun Chong A, Petch Kaset D and Sri Chomphu B. In group II, the percentage of similarity for this group was low (43-60 percent). Group III, the 007C, 011C, 012C and 013 C varieties had a high value of 90-100 percent of similarity. The plants in group IV and group V had high value of similarity 73-91 percent and 70 percent, respectively

DICUSSION

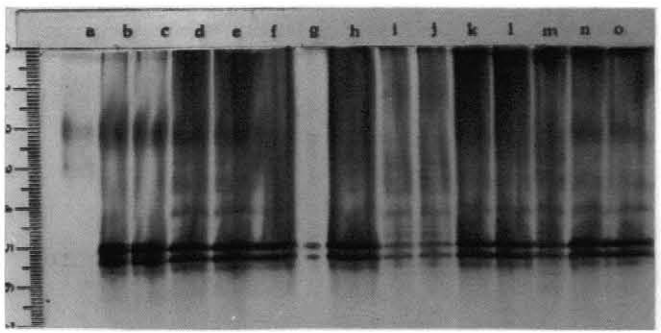
The migration, the arrangement and the characteristics of PER isozyme bands can be used as an important guide for classifying the tamarind varieties. The dark brown PER isozyme bands was a key band in identifying the group of tamarind varieties. The position and the number of dark brown PER bands were consistent among the same variety. Five patterns of dark brown PER bands were found in 83 plants, where 61 plants were in group I, 3 plants in group II, 4 plants in group III, 8 plants in group IV and 5 plants in group V (Figure 2).There are two different tastes in tamarind pulp: the sweet and the sour.Generally people can only classify tamarind plant by sweetness of the pulp but in this study the differences in PER isozyme pattern has opened a new way to classily the sour and the sweet tamarind. The dark brown PER bands made us realize that they were divided into 5 group while the sour and the sweet varieties always mixed in these 5 groups. The classification of this plants by using the taste of pulp differedfrom that of using zymogram. This suggested that, the gene which control the taste of pulp and the PER isozyme may not have the same control effect. In the taxonomic way, we can not used the taste of pulp for identifying the tamarind plant, whereas chemotaxonomic approach can be used to classify the tamarind varieties. Moreover the environment had some effects on the distribution of light brown color bands and light grey color bands but not on



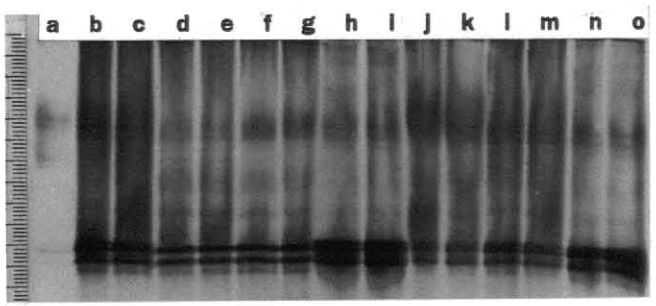
1a Std(a), 001(bc), 002(de), 003(fg), 004(hi), 005(jk), 006(lm) from Si Sa Ket Hort. Research Centre.



1b Std(a), 007(bc), 008(de), 009(fg), 010(hi), 011(jk), 012(lm), 013(n) from Si Sa Ket Hort. Research Centre.



1c Std(a), 014(bc), 015(de), 016(fh), 017(ij), 018(kl), 019(m), 020(no) from Si Sa Ket Hort. Research Centre.



1d Std(a), 029(bc), 030(de), Petch Kaset(fg), Srithong(hi), Kru Inn(jk), Meoun Chong(lm), Sri Chomphu(no) from Si Sa Ket Hort. Research Centre.

Figure 1 The PER isozymes patterns of the sweet and the sour tamarind in disc acrylmide gel; Std = Horse radish PER isozyme.

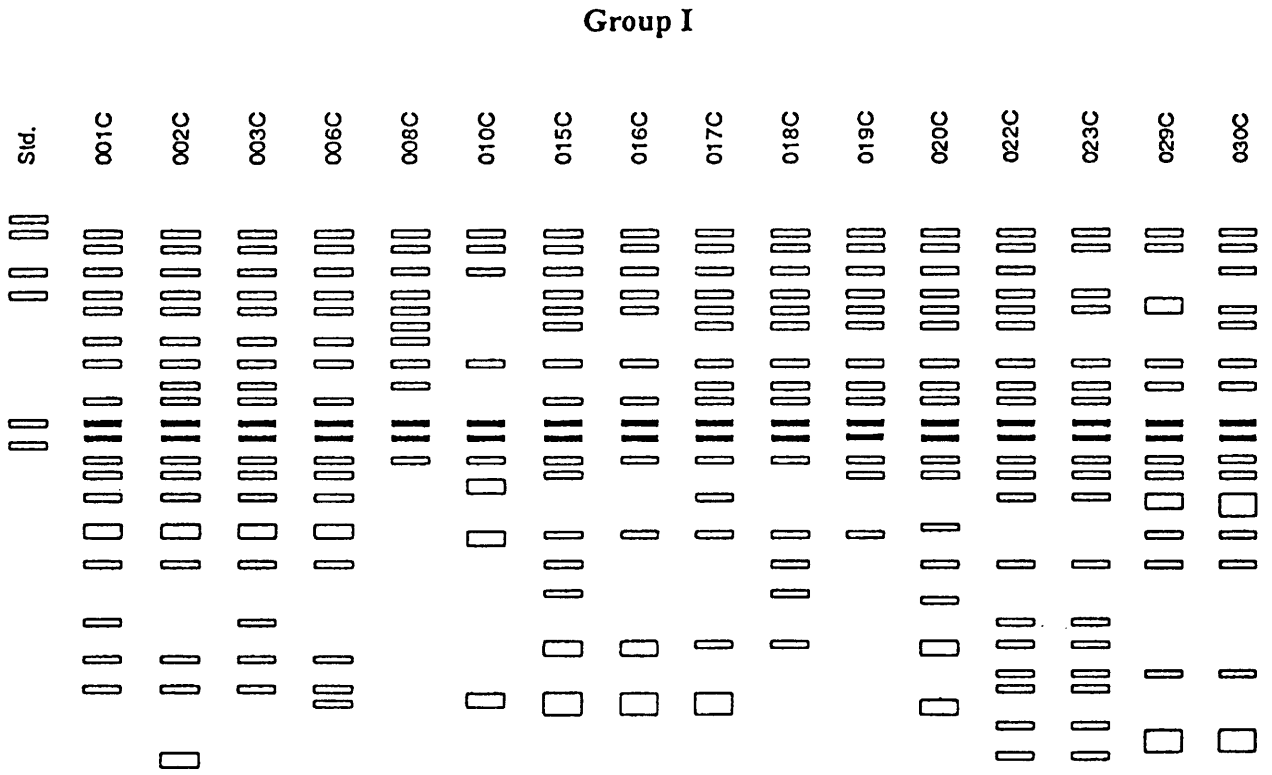


Figure 2 Diagrammatic representation of electrophoresis banding patterns for peroxidase isozyme from tamarind leaf.

A = Kayan's
B = Denchai's Orchard
C = Si Sa Ket Hort. Research Centre
D = Nuan Noi's Orchard

Figure 2 (Continued).

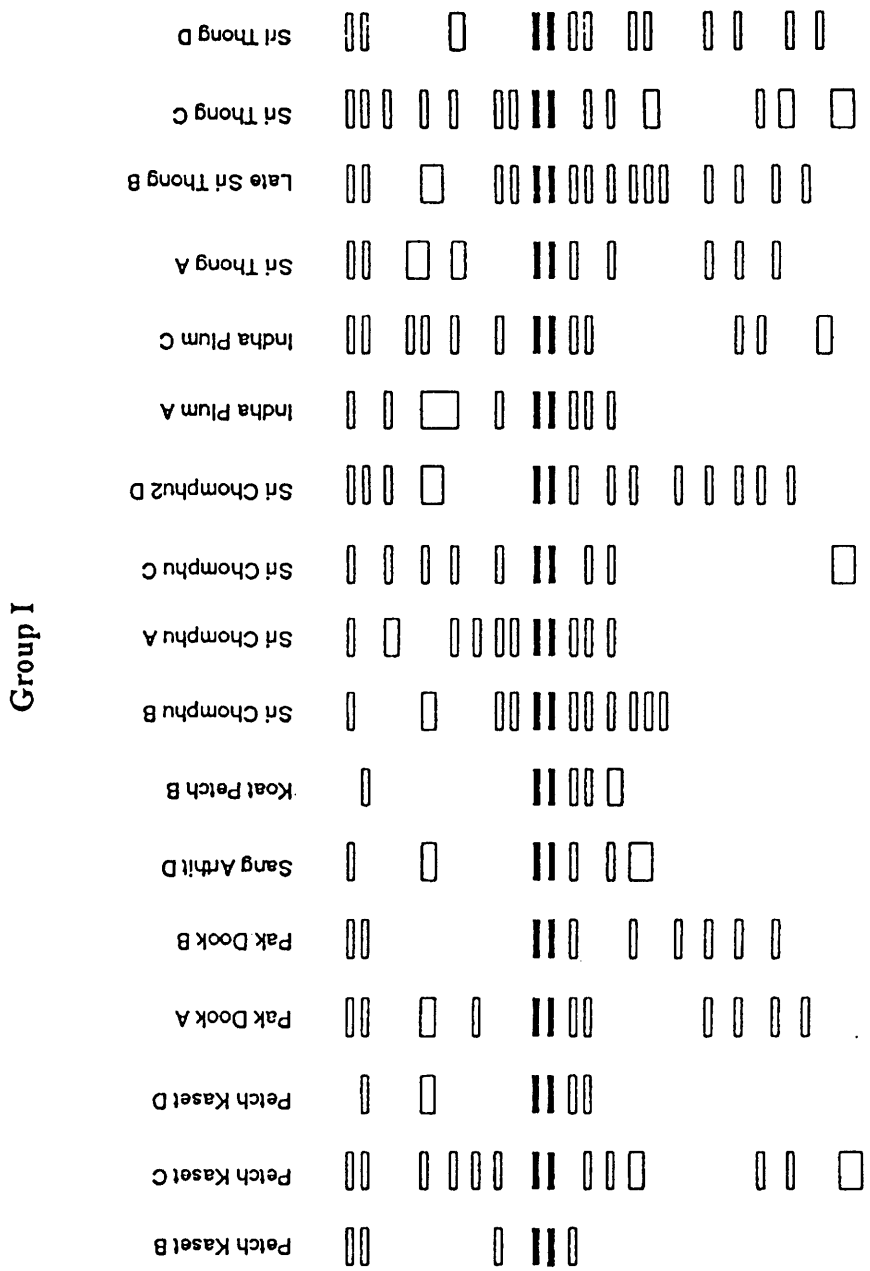


Figure 2 (Continued).

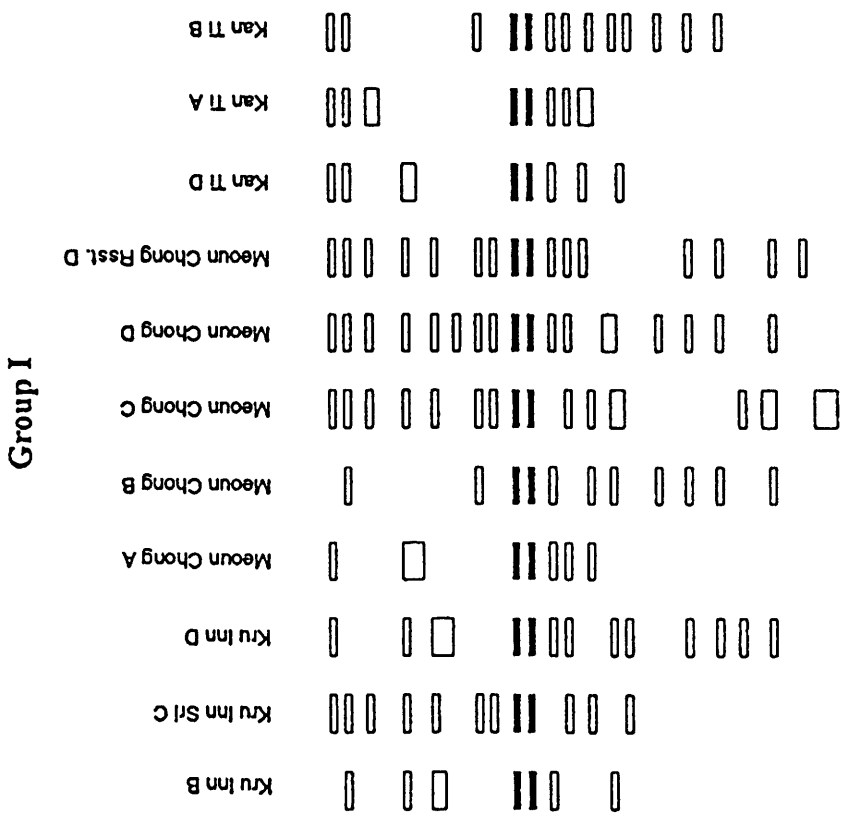


Figure 2 (Continued).

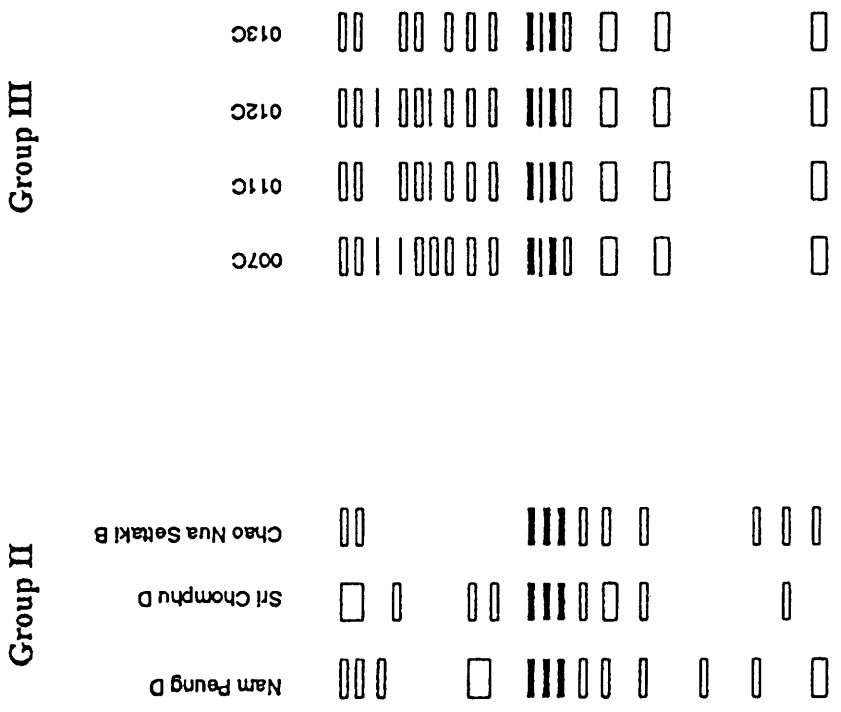


Figure 2 (Continued).

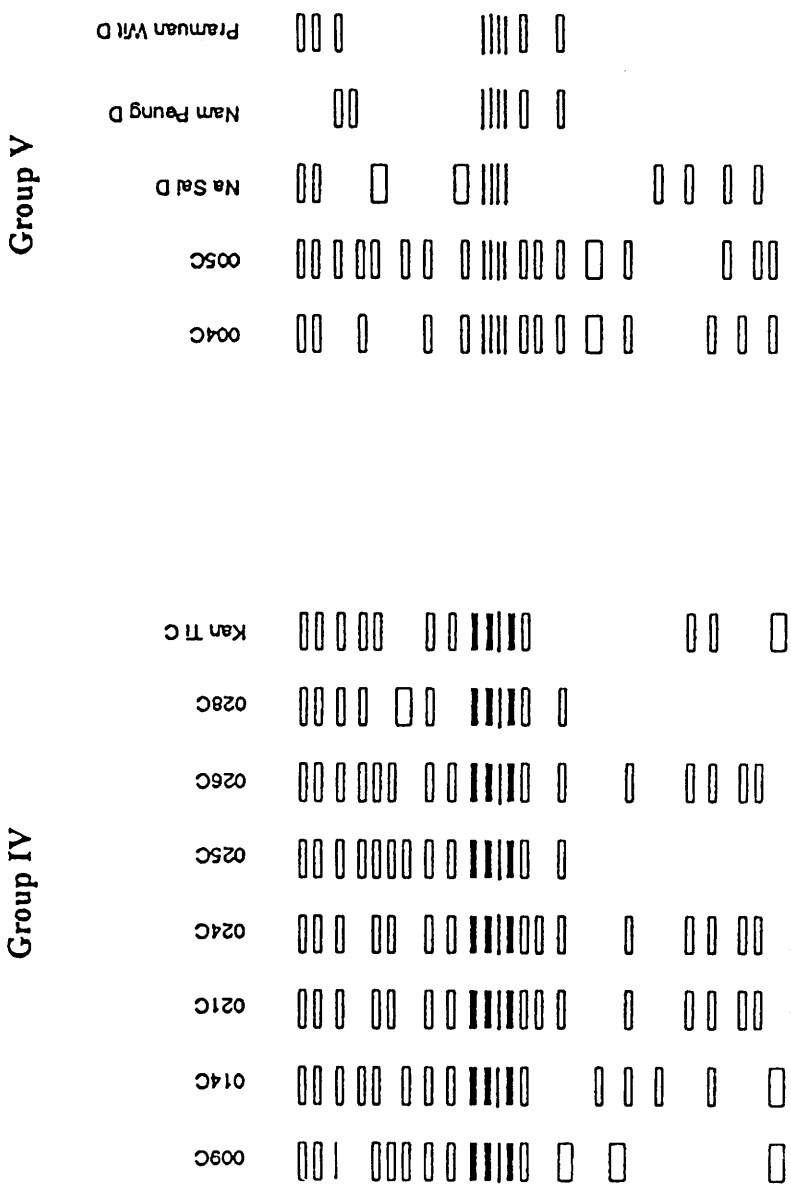


Figure 2 (Continued).

the dark brown color bands. The dark brown PER bands looked like the key bands for classifying the varietal names of tamarind plants they were not changeable in different environment. The relationship of the tamarind varieties could be studied by using the percentage of similarity. The results of this research showed that, the percentage of similarity could be used to divied the sweet tamarind varieties into two lines; line one consisted of the varieties from the northern part of Thailand, and line two included the varieties from the northeastern part. The result of percentage of similarity of the sour varieties showed that the sour tamarind varieties from the same habitat had high value.

The character of tamarind varieties which were originated in the same location or in the same habitat may be closely related. The sour and the sweet tamarind were not closely relationship except some varieties of sweet tamarind which were originated in the same habitat of the sour showed the similar band varieties. The chemotaxonomy method seems to be a better way in grouping the many varieties of tamarind plants.

CONCLUSION

The PER isozyme pattern studied can be concluded as follows :

1. There are five groups of tamarind plant. The results of PER isozyme patterns showed that the sweet and the sour tamarind varieties had different genotype.

2. The habitat of tamarind plants had an effect on the patterns of PER isozymes. The varieties in the same habitat had high value of percentage of similarity

3. The environment had an effect on the minor bands but these factors had no effect on the dark brown bands.

ACKNOWLEGEMENTS

The authors wished to thank Mr. Kayan Ooncharoen, Mr. Denchai Krataipenk, Mr. Chusak Sajjapongse and Mr. Savien Hom-nan for their kind assistance in providing the plant materials.

LITERATURE CITED

Anonymous 1989. Statistics of Fruit Tree Production in 1988/1989. Dep. of Agriculture Extension, Ministry of Agriculture Thailand, 41p.

Bailey L.H. 1949. *Tamarindus indica* L. The Standard Encyclopedia of Horticulture. vol III. The Macmillan Company. London. 3307p.

Bassiri, A. and M.W. Adams 1978. An electrophoretic survey of seedling isozyme in several *Phaseolus* species. Euphytica. 27 : 447-459.

Bassiri, A. and I. Rouhani. 1977. Identification of broad bean cultivars base on isozyme patterns. Euphytica. 26 : 276-286.

Bournival, B.L. and S.S. Korban 1987. Electrophoretical analysis of genetic variability in the apple. Scientia Horticulturae. 31 : 233- 243.

Cerezo M., R.S. Company, and A. Prus. 1989. Identification of almond cultivars by pollen isoenzymes. J. Amer. Soc. Hort. Sci. 114 : 164-169.

Laemmli, U.K. 1970. Cleavage of Structure proteins during the assembly of the head of bacteriophage T4. Nature. 227 : 680-685.

Pasteur, N., F. Bonhomme, J. Catalan, and J. Britton-Davidian. 1988. Practical Isozyme Genetic. Ellis Horwood Limited, London. 215 p.

Sriprasertsak, P. 1988 Electrophoresis method. Electrophoresis Technique for Plant Identification. The Central Laboratory, Kasetsart University, Kamphangsaen Campus. 117 p.

Suriyapananont, S. 1993. Identification of tamarind base on isozyme patterns in leaf. Thesis of Doctor of Philosophy (Horticulture), Kasetsart University, Thailand.

Torress, A.M. and B.O. Bergh. 1980. Fruit and leaf isozymes as genetic marker in avocado. J. Am. Soc. Hort. Sci. 105 : 614-619.

Torress, A.M., R.K. Soost, and U. Diendenhoten, 1978. Leaf isozymes as genetic markers in Citrus. Am. J. Bot. 65 : 869-886.

Torress, A.M., R.K. Soost, and T.M. Lastovicka. 1982. Citrus isozymes. J. Hered. 78 : 385-389.

Whitney, P.J., J.G. Vanghum, and J.B. Heale. 1968. A disc electrophoretic study of the proteins of *Verticilium alboratum, V. dahliae* and *Fusarium oxysporium* with reference to their taxonomy. J.Exp. Bot. 19 : 415-426.