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An Analysis on DNA Fingerprints of Thirty Papaya Cultivars (*Carica papaya* L.), Grown in Thailand with the Use of Amplified Fragment Length Polymorphisms Technique

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Abstract: The experiment was carried out at the Department of Horticulture, Ubon Ratchathani University, Ubon Ratchathani province, Northeast Thailand during June 2002 to May 2003 aims to identify DNA fingerprints of thirty papaya cultivars with the use of Amplified Fragment Length Polymorphisms (AFLP) technique. Papaya cultivars were collected from six different research centers in Thailand. Papaya plants of each cultivar were grown under field conditions up to four months then leaf numbers 2 and 3 of each cultivar (counted from top) were chosen for DNA extraction and the samples were used for AFLP analysis. Out of 64 random primers being used, 55 pairs gave an increase in DNA bands but only 12 pairs of random primers were randomly chosen for the final analysis of the experiment. The results showed that AFLP markers gave Polymorphic Information Contents (PIC) of three ranges i.e., AFLP markers of 235 lied on a PIC range of 0.003-0.05, 47 for a PIC range of 0.15-0.20 and 12 for a PIC range of 0.35-0.40. The results on dendrogram cluster analysis revealed that the thirty papaya cultivars were classified into six groups i.e., (1) Kaeg Dum and Malador (2) Kaeg Nuan (3) Pakchong and Solo (4) Taiwan (5) Co Coa Hai Nan and (6) Sitong. Nevertheless, in spite of the six papaya groups all papaya cultivars were genetically related to each other where diversity among the cultivars was not significantly found.

Key words: AFLP random primers, dendrogram, diversity, genetic traits, papaya cultivars

INTRODUCTION

Papaya (*Carica papaya* L.), one of many important cash crops is believed to have its origin from Central America i.e., Mexico, Costa Rica and later spread to the nearby countries during the fifteenth century and soon this crop was introduced to West Indies and arrived in the Philippines in the sixteenth century and later to Indonesia and the nearby countries such as India, Thailand and others. This crop reached African countries in the eighteenth century (1874). Papaya is, more or less, a tropical crop and the plants can thrive on easily in most soils. Papaya cultivation can be found in many countries such as Australia, India, Burma, USA, Thailand, Taiwan and some other countries in Africa and also in most Latin American countries and many other countries in the tropics (Suksri, 1999). Papaya fruits provide nutritious value to man, particularly when ripe. It is rich in vitamins C and A, whilst green fruits contain an enzyme known as papain or proteolytic enzyme, which could be used to tenderise or soften animal meats when cooking, particularly meats of beef or pork and it is commercially used as a meat tenderiser and it is also used in many other chemical products (Williams, 1975; Purseglove, 1968; Smith *et al*., 1992). A large amount of papain has been imported to Thailand annually for industrial uses (Waraporn, 1990). In the 2002, Thailand used an area of approximately 29,048 hectares for papaya plantations with its annual production of fruits of approximately 467,983 metric tones. The popular varieties being cultivated in Thailand include Kaeg Dum, Kaeg Nuan and Co Coa, whilst other varieties such as Florida and Hawaiian received less attention from growers. Papaya plantations in Northeast Thailand occupied the largest land area of 57% followed by the Central Plane region, whilst growers in other regions grow papaya mostly for their household consumption (Anonymous, 2001 and 2002). The local population in each province of the country weekly consumed several tones of fresh papaya fruits apart from ripen fruits where they use papaya fresh fruits for papaya salad mostly in northeastern region. Papaya salad is normally prepared for a daily diet of the

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local population in Northeast Thailand and it is known as the fabulous Som Tam. It is a part of daily life of the people thus this crop signifies its tangible economic impact of the country.

For the past few decades there had been some changes in using different types of breeds due to the changing in environmental conditions and selection processes and the use of natural hybridization, thus growers were not able to distinguish vividly, which papaya plants could retain its true to type of its origins, hence growers had given their own appropriate names for their selected offsprings of cultivars. It has been advocated that AFLP technique could provide outstanding results since it could differentiate DNA of different living organisms with a high degree of accuracy (Cerrera *et al*., 1996) and the technique had been used by several workers to identify some certain orchard plant species such as in citrus (Ulubelde and Tan, 1986), mangoes (Degani *et al*., 1995), longan (Ratchadaporn, 2003) and others. It seems more likely that information on genetic code identification of papaya plants in Thailand is limited due to presumably the complication in carrying out the work or perhaps the lack of equipment. Therefore, it may be of tangible value to investigate and identify different types of popular papaya cultivars being cultivated within the country with the use of a technique known as AFLP. The technique includes the use of Polymerase Chain Reaction (PCR) to determine genomic DNA where it provides fingerprints of its actual DNAs of their genetic codes without the interference of environmental conditions (Sharma *et al*., 1996).

MATERIALS AND METHODS

This study was carried out at the Department of Horticulture, Faculty of Agriculture, Ubon Ratchatani University, Ubon Ratchatani province, Northeast Thailand during June 2002 to May 2003 to search for more information on popular papaya cultivars being cultivated in Thailand, particularly in northeastern region where a large amount of papaya fruits has been consumed annually. The search includes the collection of seeds of different types and local names of papaya cultivars from six different places namely: (1) Srisaket Horticultural Research Centre. There were twenty cultivars obtained from this research center, they include Tha Phra 1, Tha phra 2, Tha Phra 3, Khaeg Dum Dum Nern, Sitong, Khaeg Dum Dum Nern x Malador, Pak Chong, Khaeg Dum Dum Nern x Pak Chong, Khaeg Dum Srisaket-8-9, Solo, Co Coa Karn Dum, Khaeg Dum Srisaket-6-2, Khaeg Dum Srisaket, Khaeg Dum Nakorn Phanom, Mexico-Indonesia, Co Coa Klong Tor, Co Coa Som Dang, Malador, Taiwan x Khaeg Dum Dum Nern and Khaeg Dum Dum Nern x Mexico-Indonesia; (2) Loei Highland Agricultural Station, this location provided five papaya cultivars, they include Khaeg Dum Trat, Khaeg Dum Loei, Hai Nan, Taiwan and Taiwan Tissue; (3) Phichit Horticultural Research Centre, only one cultivar was attained, i.e., Khaeg Nuan; (4) Department of Horticulture, Kaset Sart University where Khaeg Nuan x Malador cultivar was obtained; (5) Khon Kaen Horticultural Research Centre with one cultivar i.e., Khaeg Dum Khon Kaen and (6) Department of Horticulture, Ubon Ratchathani University with one cultivar of Khaeg Dum Ubon Ratchathani. Some considerable amounts of matured seeds of 30 different papaya cultivars were obtained from these six locations and most of them had reached its generations of F7 after several years of selection. Seeds of each cultivar were sown separately into trays filled with moist compost and then placed the trays on germinating desks under glasshouse conditions. Watering was carried out daily to assure adequate amount of moisture content of the compost in each tray. Eight to fourteen days after sowing, papaya seeds were germinated. One week after germination healthy seedlings of each tray were transplanted into polythene pots, each pot contained approximately 500 g of moist compost. Thirty polythene pots were used for each cultivar and they were allowed to grow under glasshouse conditions for 2 months after germination. Watering of seedlings was carried out daily to assure no wilting signs of leaves found during this growing period. After 2 months of growing period under glasshouse conditions then 20 healthy seedlings of each cultivar were chosen at random and transplanted into the Experimental Field, Ubon Ratchatani University at a distance between rows and within rows of 4×4 m, respectively. Each seedling was allowed to grow in a drill where an amount of soil of 0.50 m^3 was taken out to make a hole with a dimension of 50×50×50 cm and then a similar quantity of topsoil was thoroughly mixed with cattle manure of 2-kg (approximately 30% moisture contents) together with 200 g of a complete chemical fertiliser 15-15-15 (N, P_2O_5, K_2O) and then the mixture was used to replace the amount of soil taken out from each hole of each papaya plant stand. Due to the fact that most soils in Northeast Thailand are the soils of poor fertility as reported by Suksri (1999), Kasikranan (2003) and Pholsen (2003), thus to attain normal growth and high yield of papaya plants in each hole of papaya plant stand, growing media for papaya seedlings must be thoroughly prepared in order to provide adequate amounts of soil nutrients and aeration for respiration of roots of papaya

plants. Leaf samples of papaya plants for the determinations of DNA were taken from a 4-moth old papaya plants. At 4 months after germination, all papaya plants started to bear a number of fruits hence uniformed papaya plants (observed by naked eye) were chosen at random for leaf samples. Papaya young leaves of numbers 2 and 3 (being counted from top to lower leaves) were cut at the edge of petiole of leaves and then collected for laboratory assessment. The bearing fruits of papaya plants facilitated uniform characteristics of papaya plants to be chosen, thus leaf samples of those bearing fruits were collected. Papaya leaf samples of the same cultivar were collected from 15 papaya plants and then they were used for DNA extraction. The extraction was carried out with the method of Aggarwal *et al.* (1999) where two types of enzymes were used i.e., EcoRI and MSel. The extracted DNA samples were mixed together according to their respective cultivars and then the samples were used for Amplified Fragment Length Polymorphisms (AFLP). The AFLP analysis was carried out with the use of the method of Vos *et al.* (1995) where 64 pairs of AFLP random primers were used and 55 pairs of random primers were able to produce DNA bands. However, with this study only 12 pairs of AFLP random primers were randomly chosen. They include E-AGG/M-CTC; E-AGG/M-CAT; E-AGC/M-CAA; E-ACT/M-CTG; E-ACT/M-CAG; E-ACG/M-CTC; E-ACC/M-CTG; E-ACC/M-CAT; E-ACA/M-CAA; E-AAG/M-CAC; E-AAC/M-CTA and E-AAC/M-CAC. The attained results were statistically calculated with the use of the computer programmes where the calculation started with the use of Numerical Taxonomy and Multivariate Analysis Systems (NTSYS, pc2.02i) of Rohlf (1998) to produce Nei and Li similarity correlation coefficients (Nei and Li, 1979) and then matrix genetic distances were used for cluster analysis of genotypic values for a dendrogram figure with the application of un-weighted pair group method on the basis of arithmetic averages (UPGMA) as carried out by Sneath and Sokal (1973).

RESULTS

AFLP primers, monomorphic bands, polymorphic bands and polymorphic %: With the use of 64 pairs of primers being applied to 30 papaya cultivars, the results showed that there were 9 pairs of primers where an increase in DNA bands was not found. However, there were 55 pairs of primers where an increase in DNA bands was possible but for this study only 12 pairs of primers were randomly chosen. They include (1) E-AGG/M-CTC, (2) E-AGG/M-CAT, (3) E-AGC/M-CAA, (4) E-ACT/M-CTG, (5) E-ACT/M-CAG, (6) E-ACG/M-CTC, (7) E-ACC/M-CTG, (8) E-ACC/M-CAT, (9) E-ACA/M-CAA, (10) E-AAG/M-CAC, (11) E-AAC/M-CTA and (12) E-AAC/M-CAC, these 12 pairs of primers were used for this investigation (Table 1). The results showed that DNA bands primer^{-1} ranged from 50 to 75 for E-AGC/M-CAA and E-ACC/M-CAT, respectively with a total DNA bands or AFLP markers of 734 bands with an average value of 63 bands. For monomorphic bands, it ranged from 11 to 33 bands for E-AGC/M-CAA and E-ACG/M-CTC, respectively and out of 734 bands it gave a genetic similarity of bands of 249. With the results on polymorphic bands, the results showed that polymorphic bands ranged from 32 to 43 bands for E-AAC/M-CAC and E-AGG/M-CTC, respectively with total polymorphic markers of 485 bands, i.e., 66.08% of all DNA bands (734 bands).

Table 1: Pairs of AFLP primers being used, numbers of bands primer^{-1}, monomorphic bands primer^{-1}, polymorphic markers primer^{-1} and % of polymorphic bands primer^{-1} of 36 popular papaya cultivars, grown in Thailand

No. of pairs of AFLP primers	No. of bands primer^{-1}	No. of monomorphic bands	No. of polymorphic markers	Polymorphic bands (%)
E-AGG/M-CTC	62	19	43	69.35
E-AGG/M-CAT	68	28	40	58.82
E-AGC/M-CAA	50	11	39	78.00
E-ACT/M-CAA	64	24	40	62.50
E-ACT/M-CAG	55	19	36	65.45
E-ACG/M-CTG	58	33	35	51.47
E-ACC/M-CTG	50	18	32	64.00
E-ACC/M-CAT	75	20	55	73.33
E-ACA/M-CAA	70	19	51	72.86
E-AAG/M-CAC	66	23	43	65.15
E-AAC/M-CTA	54	15	39	72.22
E-AAC/M-CAC	52	20	32	61.54
Total	734	249	485	66.08%

Polymorphic information content (PIC): With PIC, the results showed that polymorphic markers of 485 bands of the 30 papaya cultivars gave a highest value of AFLP markers of 235 where it lied on a PIC range of 0.003-0.05 i.e., equivalent to 48.45% out of all DNA bands. It was shown that values of AFLP markers gave a range of values from 12 to 235 where the second lower value was 47 with a PIC range of 0.15-0.20 and the lowest value of 12 gave a PIC range of 0.35-0.40 (Fig. 1).

Cluster analysis on genetic evaluation: The results on cluster analysis derived from a dendrogram (Fig. 2) revealed that papaya cultivars being used in this study could be classified into six groups of papaya cultivars i.e., Group I (Kaeg Dam and Malador group), this group includes 18 papaya cultivars they are: Tha Phra 1, Tha Phra 2, Tha Phra 3, Kaeg Dum Khon Kaen, Kaeg Dam Kalasin, Kaeg Dum Loei, Kaeg Dum Nakhon Phanom, Kaeg Dum Ubon, Kaeg Dum Trat, Kaeg Dum Dum Nern x Pak Chong, Kaeg Dum Dam Nern x Mlador, Kaeg Dum

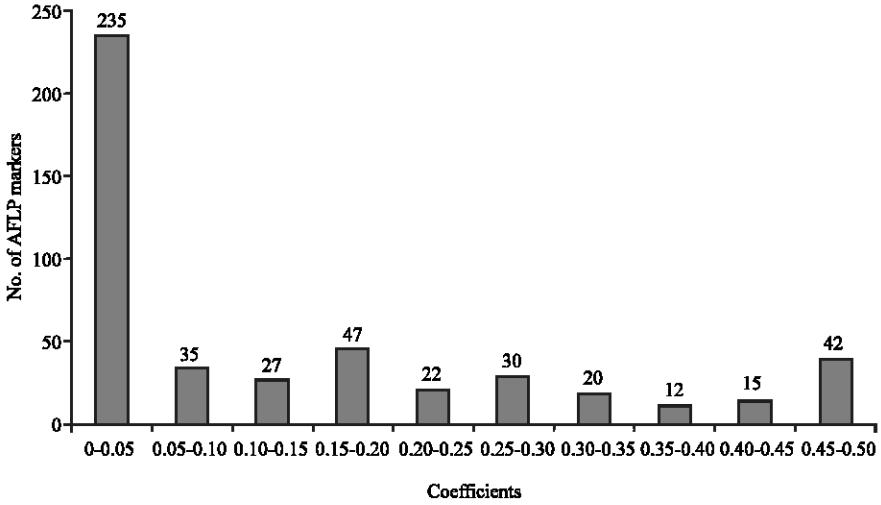

Fig. 1: The distribution of polymorphic information contents (PIC coefficients) of 30 papaya cultivars being popularly grown in Thailand as distributed by Amplified Fragment Length Polymorphisms (ALFP) markers

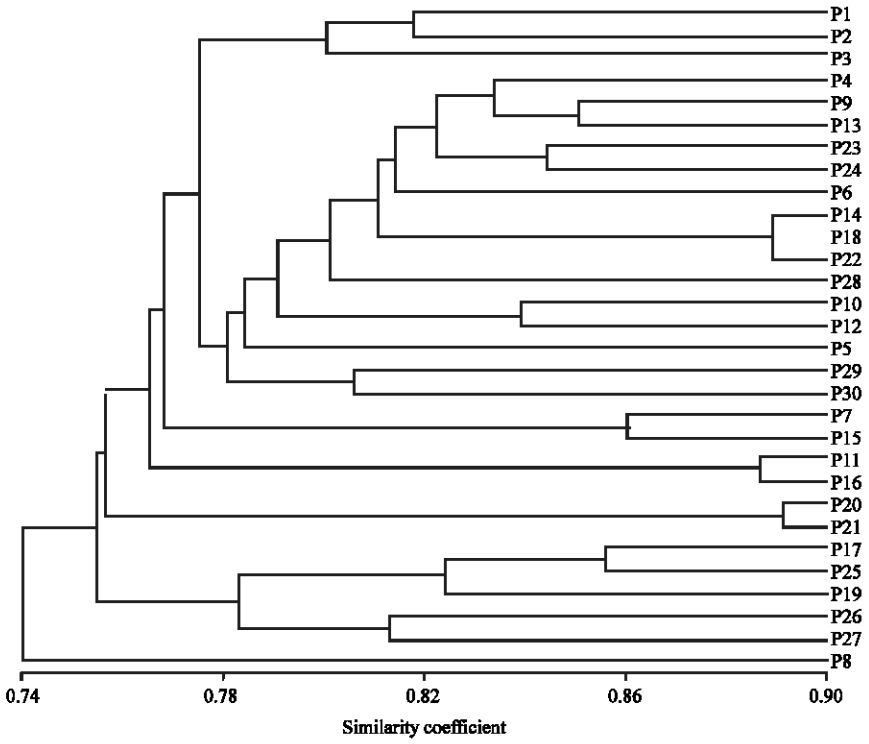

Fig. 2: A dendrogram structure illustrated similarity coefficients and relationships among 30 papaya cultivars based on cluster analysis of NTSYS-pc2.02i (Rohlf, 1998). P = Papaya cultivars. P1= Tha Phra1, P2 = Tha Phra2, P3 = Tha Phra3, P4 = Kaeg Dum Khon Kaen, P5 = Kaeg Dum Dum Nern, P6 = Kaeg Dum Trat, P7 = Kaeg Nuan, P8 = Sitong, P9 = Kaeg Dum Kalasin, P10 = Kaeg Dum Dum Nern x Pak Chong, P11 = Pak Chong, P12 = Kaeg Dum Dum Nern x Malador, P13 = Kaeg Dum Loei, P14 = Kaeg Dum Srisaket-8-9, P15 = Kaeg Nuan x Malador, P16 = Solo, P17 = Co Coa Karn Dum, P18 = Kaeg Dum Srisaket-6-2, P19 = Hai Nan, P20 = Taiwan, P21 = Taiwan Tissue, P22 = Kaeg Dum Srisaket, P23 = Kaeg Dum Nakhon Phanom, P24 = Kaeg Dum Ubon, P25 = Mexico-Indo, P26 = Co Coa Klong Tor, P27 = Co Coa Som Dang, P28 = Malador, P29 = Taiwan x Kaeg Dum Dum Nern and P30 = Kaeg Dum Dum Nern x Mexico-Indonesia

Srisaket 4-9, Kaeg Dum Srisaket 9-5, Kaeg Dum Srisaket, Kaeg Dum Dum Nern, Malador, Taiwan x Kaeg Dum Dum Nern and Kaeg Dum Dum Nern x Mexico-Indonesia; Group II (Kaeg Nuan group), this group consisted of 2 cultivars i.e., Kaeg Nuan and Kaeg Nuan x Malador; Group III (Pakchong and Solo group), this group has 2 papaya cultivars i.e., Pak Chong and Solo; Group IV (Taiwan group), this group has 2 cultivars i.e., Taiwan and Taiwan Tissue; Group V (Co Coa Hai Nan, Mexico-Indonesia group), this group consisted of 5 papaya cultivars i.e., Co Coa Karn Dam, Mexico-Indonesia, Hai Nan, Co Coa Klong Tor and Co Coa Som Dang; Group VI (Sitong group), this group has a single cultivar i.e., Sitong.

DISCUSSION

In Thailand, the plantation of papaya crop within a few decades was carried out mainly in the Central Plane region where growers planted a large number of papaya plants within a large piece of land such as those plantations carried out at Ratchaburi province and others. The plantations aimed to supply papaya fresh fruits mainly to northeastern region where tones of fresh fruits have been used mostly for papaya salad or by the local name of Som Tam. Within this decade many Thai government agencies had paid their attention to preserve and collect germ plasms of papaya cultivars in order to search for outstanding cultivars for growers in all regions of the country such as Srisaket Horticultural Research Center, Khon Kaen Horticultural Research Center and many other centers as stated earlier in this study. It is generally known to the Thai people that papaya is an important crop being grown for household consumption hence a large number of the families in each village grows a few plants of papaya for their own utilization. Thus growers normally preserve their own cultivars from generation to generation. It was found with the results carried out in Thailand by Sukhontip (2003) with the use of DNA amplification fingerprints (DAF) that from 11 papaya cultivars being used for the experiment, the papaya cultivars could be classified into three groups only i.e., (1) Kaeg Dum, (2) Co Coa Karn Dum and (3) Taiwan and Solo. She stated that Kaeg Dum cultivar has a close genetic relationship with Kaeg Nuan, Taiwan with Solo and Co Coa Karn Dum with Mexico-America. Therefore, there were only a few varieties of papaya cultivars have been used by growers within the past decades. Thus diversity in genetic traits among papaya cultivars was relatively small.

The results found with this study on the application of AFLP primers showed that an increase in DNA bands was attained with 55 pairs of primers and 9 pairs were not able to produce their DNA bands. However, out of 55 pairs of primers only 12 pairs were randomly chosen for this investigation and the results revealed that polymorphic markers of bands reached a value of 66.08% (485 bands). The results indicated a close relationship on genetic traits among the thirty papaya cultivars used where polymorphic information contents (PIC) were distributed into three ranges, i.e., 0.003-0.05 (48.45%), 0.15-0.20 and 0.35-0.40 (9.70%) and 0.15-0.20 (2.47%). When it comes to the effect due to genetic traits on similarity coefficients with the use of its dendrogram, it was found that the thirty papaya cultivars could be categorized into six groups but their ranges on similarity coefficients ranged from 0.728 to 0.920 only so this range of values indicates a close relationship among the thirty papaya cultivars used. The obtained results were similar to the study of Kim *et al.* (2000) where they collected 71 accessions of papaya cultivars of commercial, improved and unimproved breeding lines and able to identify the cultivars with the use of 9 pairs of ALFP primers. They attained 186 markers with a highest value of 0.880 from their cluster analysis. They stated that papaya accessions being used did not provide any information on the diversity of genetic traits. Furthermore, they stated that self-pollinated hermaphrodite cultivars gave some considerable levels of variations similar to those of open-pollinated cultivars. However, they were able to differentiate the 71 accessions from other 6 *Carica* species where identity of breeds of the 6 species was not similar to their 71 accessions. Similarly, Droogenbroeck *et al.* (2002) carried out papaya experiment to identify papaya breeds in Ecuador with the use of AFLP markers reported that from 95 papaya accessions, the papaya plants produced 491 markers where these markers derived from 5 pairs of random primers and the results on cluster analysis revealed that the papaya accessions used had its close relationship on genetic traits and the results did not agree with the results derived from taxonomic identification.

Although the results on cluster analysis attained with this work signified a close relationship among the thirty papaya cultivars but still the papaya cultivars could be categorized into six groups according to their similarity coefficients of the dendrogram figure. The results evidently showed that the thirty papaya cultivars could be differentiated into six different groups in spite of their close relationship on genetic traits. This must be attributable to perhaps (1) the limited varieties of papaya imported to Thailand in the past decades or perhaps even now and then, (2) Seeds of productive cultivars must have been always kept by growers for their own uses from generation to generation, (3) natural selection and natural

hybridization of papaya cultivars due to the mixing up or a closed planting distance among papaya plants of different breeds could have its effect on the mixing up of their genetic traits from generation to generation and (4) another reason could have been due to a failure in breeding programme and selection of the scientists within the country when some large amounts of investment budget must be used in order to produce new cultivars for use, (5) lack of well trained plant breeders of papaya crop or perhaps scientists ignore this prospect due to plentiful amount of papaya fruits available in the markets. Therefore, it is an urgent need for the Thai scientists both plant breeders and plant physiologists to pay more attention to this particular cash crop in order to provide both productive varieties and technologies of know how for prospective growers in the country since high quality papaya fruits could be exported annually and some considerable amounts both fresh and ripe are needed for domestic consumption, particularly in all large cities and even hotels and restaurants and etc.

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