

Genetic Relationships in Longan var. Daw as Revealed by Isozyme Analysis

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Abstract : Twenty clones of longan var. Daw were fingerprinted, classified and evaluated for phylogenetic relationships using isozyme pattern analysis. Ten enzyme systems were screened. It is found that two enzyme systems failed to be stained in polyacrylamide gel including alcohol dehydrogenase (ADH) and isocitrate dehydrogenase (IDH). The eight enzyme systems that produced clear and reproducible isozyme patterns were selected, including acid phosphatase (ACP), aspartate amino transferase (AAT), esterase (EST), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GDH), glyceraldehyde-3-phosphate dehydrogenase (G3PD), malate dehydrogenase (MDH) and peroxidase (POX). The number of schematic zymograms were 15, 13, 11, 19, 13, 11, 14 and 9, respectively with the total of 105. At 95 % similarity, cluster analysis based on molecular data of band distribution in all samples showed 19 clusters and 1 individual not belonging to any of the clusters. Daw Bai Hod (DBH) and Daw Kan On (DKO) could not be separated with 95 % similarity while the rest of the clones could be separated with similarity less than 95 %. Daw Kan Kaeng (DKK) and Daw Nam Pueng (DNP) as well as Daw Luang (DLG) and Daw Sukhum (DSK) had 80 % similarity. Daw Lum Nam Ping (DLP) and Daw Soy (DSY) had 68 % similarity while Daw Nan Sao (DNS) and Daw Ta Noi (DTN) had 64 % similarity. Daw Yod Kao (DYK) was the only individual that had 0 % similarity and was completely different from the others.

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บทคัดย่อ: จากการถ่ายภาพพิมพ์ลายนิ้วมือ จัดจำแนก และประเมินความสัมพันธ์ทางพันธุกรรมของลำไยพันธุ์คอ 20 สายพันธุ์ โดยการวิเคราะห์รูปแบบไอโซไซม์จากระบบเอนไซม์ 10 ชนิด พบว่า ระบบเอนไซม์ 2 ชนิดที่ไม่ติดสี ได้แก่ ADH และ IDH ส่วนระบบเอนไซม์ 8 ชนิดที่ให้รูปแบบไอโซไซม์ที่ชัดเจนและเหมาะสม ได้แก่ ACP, AAT, EST, G6PD, GDH, G3PD, MDH และ POX โดยให้รูปแบบไอโซไซม์เท่ากับ 15, 13, 11, 19, 13, 11, 14 และ 9 รูปแบบ ตามลำดับ รวม 105 รูปแบบ และที่ค่าความคล้ายคลึงกัน 95 % พบว่า การวิเคราะห์กลุ่มพืชตามการกระจายตัวของแถบสีของตัวอย่างทั้งหมดสามารถจำแนกได้พืช 19 กลุ่ม และมีสายพันธุ์เดียวที่ไม่มีความสัมพันธ์กับสายพันธุ์อื่น คอใบหัดและคอก้านอ่อนไม่สามารถแยกออกจากกันได้ เนื่องจากมีค่าความคล้ายคลึงกันที่ 95 % ส่วนสายพันธุ์อื่นสามารถแยกออกจากกันได้ เนื่องจากมีค่าความคล้ายคลึงกันน้อยกว่า 95 % คอก้านแข็งกับคองน้ำผึ้ง และ คอหลวงกับคอสุ่ม มีความคล้ายคลึงกันที่ 80 % คอคุ่มน้ำปิงกับคอสร้อยมีค่าความคล้ายคลึงกันที่ 68 % เช่นเดียวกับคอหนานขาวกับคอกานน้อยมีค่าความคล้ายคลึงกันที่ 64 % ส่วนคอยอดขาวมีความแตกต่างจากสายพันธุ์อื่นโดยสิ้นเชิงเนื่องจากมีค่าความคล้ายคลึงกันกับสายพันธุ์อื่นที่ 0 %

Index words: ลำไย ความสัมพันธ์ทางพันธุกรรม รูปแบบไอโซไซม์ การวิเคราะห์กลุ่ม
longan (*Euphoria longana* Lam.), phylogenetic relationships

Introduction

Longan (*Euphoria longana* Lam.; *Euphoria longan* Steud.; *Dimocarpus longan* Lour.; *Nephelium longana* Cambess.) is a subtropical fruit crop which is originated in southern China as well as lowlands in India, Sri Lanka and Myanmar (Ramingwong, 2003). It is one of the most important fruit crops in Thailand especially in Chiang Mai and Lamphun provinces. Approximately 90 per cent of longan grown on commercial scale is variety Daw (Juengyusuk, 1998) and genetic diversity always widely exists among different clones of longan var. Daw (Ramingwong and Chiewsilp, 1994).

Longan can be identified by different methods e.g. morphology, biochemistry and cytogenetics. Biochemical method is considered to

be one of the reliable methods. The result by biochemical method is consistent, precise and irreversible. It minimizes many problems occur in other methods. Traditionally, identification of plant cultivars is based on morphological traits, but assessment of morphological traits is very difficult among closely related cultivars. Moreover, morphological characters usually show genetic variation among varieties and among individual of the same variety due to genetic, developmental and environmental factors. Chromosome number is not sufficient as it is usually constant within a plant species and variety. Chromosome types, chromosome sizes and karyotype formula are always essential in identification (Ramingwong *et al.*, 1998).

Biochemical method using molecular markers are increasingly important in genetic

studies of horticultural crops. The different applications can be done either by Isozyme Patterns Analysis, Restriction Fragment Length Polymorphisms (RFLPs) or Randomly Amplified Polymorphic DNAs (RAPD) (O'Brin, 1990).

Isozyme pattern is the different molecular forms of an enzyme in a plant species that shares a common catalytic activity. Isozyme electrophoresis shows high potential discriminating between varieties in *Citrus* (Torres *et al.*, 1982), pineapple (DeWald *et al.*, 1988), *Musa* (Bhat *et al.*, 1992a; 1992b), ber (Kumar *et al.*, 1995), longan (Kanjana, 1998), lychee (Aradhya *et al.*, 1995; Yapwattanaphun, 1998), sweet tamarind (Subhadrabandhu and Suriyapananont, 1998), strawberry (Kumnuan, 2001; Thongthieng, 2002), silverberry (Kuntapanom and Sirikam, 2001).

The objectives of the present study are (a) to identify longan var. Daw using isozyme pattern analysis (b) to classify longan var. Daw using cluster analysis and (c) to rank the genetic relationships among longan var. Daw.

Materials and Methods

1. Plant Materials

Twenty clones of longan var. Daw included : DYK = Daw Yod Kao คอยอดขาว, DYD = Daw Yod Daeng คอยอดแดง, DKK = Daw Kan Kaeng คอก้านแข็ง, DKO = Daw Kan On คอก้านอ่อน, DBY = Daw Bai Yoke คอใบหยก, DKL = Daw Kam Lang คอคำล่าง, DHM = Daw Hom คอหอม, DBH = Daw Bai Hod คอใบหัด, DPT = Daw Puang Tong คอพวงทอง, DNP = Daw Nam

Pueng คอน้ำผึ้ง, DLG = Daw Luang คอหลวง, DSK = Daw Sukhum คอสุขุม, DSY = Daw Soy คอสร้อย, DLP = Daw Lum NamPing คอลุ่มน้ำผึ้ง, DDC = Daw Don Chai คอคอนไชย, DJE = Daw Jae คอแจ้, DTN = Daw Ta Noi คอทาน้อย, DNS = Daw Nan Sao คอหนานขาว, DBA = Daw Ban Hong 60 คอบ้านโฮ่ง 60, DNK = Daw Nong Chang Kuen คอหนองช้างคื่น

2. Field Experiment

All clones of longan var. Daw were collected from orchard plantation in Chiang Mai and Lamphun provinces as well as the Royal Agriculture Research Centre, Chiang Mai. Mature leaves at the third to the sixth nodes from apices were picked and cleaned before placing in an ice box. All samples were stored at - 20 degree Celcius prior to use.

3. Laboratory Experiment

3.1 Protein sample preparation: Two grams of fresh leaves were ground to fine powder in liquid nitrogen and homogenized in 3 millilitres extraction buffer and 0.4 grams polyvinylpyrrolidone (PVPP), then centrifuged for 30 minutes at 15,000 rounds per minutes at 4 degree Celcius. The supernatant was then transferred to a new Eppendorf tube. Marker dye solution was added to the supernatant at the ratio of 1:9 before use.

3.2 Enzyme systems and staining: Ten enzyme systems analysed were ACP = acid phosphatase, ADH = alcohol dehydrogenase, AAT = aspartate amino transferase, EST = esterase,

G6PD = glucose-6-phosphate dehydrogenase, GDH = glutamate dehydrogenase, G3PD = glyceraldehyde-3-phosphate dehydrogenase, IDH = isocitrate dehydrogenase, MDH = malate dehydrogenase and POX = peroxidase

Polyacrylamide vertical slab gel as well as staining recipes were prepared and run following the methods described by Hiratsuka *et al.* (1986). Discontinuous buffer, 4 per cent stacking gel and 8.5 or 10 per cent separating gel were applied on all enzyme systems.

3.3 Data analysis: Phenotypes or the different enzyme banding patterns were observed as difference in relative mobility (Rm) (Schmid, 1980). Profiles of isozyme patterns and zymograms of each enzyme system were recorded. Bands across all enzyme systems and entries were coded as present (1) or absent (0). Cluster analysis using computer programme SPSS for window release 9.0 by Jaccard's coefficient similarity was presented as dendrogram (Sokal and Sneath, 1973).

Results and Discussion

Ten enzyme systems were used in investigation of twenty Daw clones. It showed polymorphic and consistent isozyme banding patterns for eight enzymes. ADH and IDH could not be stained in polyacrylamide gel (Table 1). Identification of all clones could be determined by isozyme phenotypes (Table 1) and schematic zymograms (Figure 1) while their relationships could be determined by dendrograms (Figure 2).

1. Schematic zymograms of ACP revealed 10 different banding patterns with Rm 0.114 – 0.471 and each clone consisted of 1 – 3 bands (Figure 1a). All clones were separated into 15 groups.

2. Schematic zymograms of AAT revealed 8 different banding patterns with Rm 0.054 – 0.204 and each clone consisted of 0 - 4 bands (Figure 1 b). All clones were separated into 13 groups.

3. Schematic zymograms of EST revealed 9 different banding patterns with Rm 0.009 – 0.553 and each clone consisted of 1 – 7 bands (Figure 1 c). All clones were separated into 11 groups.

4. Schematic zymograms of G6PD revealed 12 different banding patterns with Rm 0.033 – 0.417 and each clone consisted of 2 - 5 bands (Figure 1 d). All clones were separated into 19 groups.

5. Schematic zymograms of GDH revealed 10 different banding patterns with Rm 0.036 – 0.446 and each clone consisted of 2 - 4 bands (Figure 1 e). All clones were separated into 13 groups.

6. Schematic zymograms of G3PD revealed 8 different banding patterns with Rm 0.063 – 0.944 and each clone consisted of 2 - 5 bands (Figure 1 f). All clones were separated into 11 groups.

7. Schematic zymograms of MDH revealed 10 different banding patterns with Rm 0.051 – 0.449 and each clone consisted of 1 – 5 bands (Figure 1 g). All clones were separated into 14 groups.

Table 1 Isozyme phenotypes of twenty Daw clones.

No.	Clone	Isozyme / Pattern									
		ACP	*ADH	AAT	EST	G6PD	GDH	G3PD	*IDH	MDH	POX
1.	DYK	A	-	A	A	A	A	A	-	A	A
2.	DYD	B	-	M	B	B	B	B	-	B	B
3.	DKK	C	-	B	C	B	C	B	-	C	A
4.	DKO	D	-	M	D	C	D	C	-	D	A
5.	DBY	B	-	C	E	D	E	D	-	E	A
6.	DKL	E	-	D	C	E	F	D	-	F	A
7.	DHM	B	-	E	F	F	G	B	-	G	C
8.	DBH	F	-	M	C	G	H	C	-	D	A
9.	DPT	G	-	M	C	H	I	B	-	H	B
10.	DNP	H	-	F	C	I	D	B	-	I	D
11.	DLG	I	-	G	C	J	J	E	-	D	E
12.	DSK	J	-	M	C	K	E	E	-	J	F
13.	DSY	J	-	G	C	L	K	F	-	K	E
14.	DLP	K	-	M	C	M	L	F	-	L	G
15.	DDC	L	-	H	G	N	M	G	-	D	A
16.	DJE	M	-	I	H	O	M	E	-	M	H
17.	DTN	N	-	H	I	P	F	H	-	C	G
18.	DNS	N	-	J	J	Q	E	I	-	C	G
19.	DBA	O	-	K	C	R	E	J	-	N	I
20.	DNK	D	-	L	K	S	E	K	-	D	G
No. Patterns		15	-	13	11	19	13	11	-	14	9
Total = 105											

* no staining

8. Schematic zymograms of POX revealed 8 different banding patterns with R_m 0.276 – 0.553 and each clone consisted of 4 – 6 bands (Figure 1 h). All clones were separated into 9 groups.

The result showed that neither ADH nor IDH was suitable as a tool in investigation due to failure in staining in polyacrylamide gel. Therefore, these two enzyme systems are not recommended. There was no specific enzyme system that could separate the twenty Daw clones. G6PD was the best as it could separate all clones into 19 groups. Only DYD and DKK were equivalent to G6PD. ACP, MDH, AAT, GDH, EST, G3PD and POX could separate all clones into 15, 14, 13, 13, 11, 11 and 9 groups, respectively. Some enzymes showed one or more bands in all clones as band 7 in EST (Figure 1c), band 1 in G3PD (Figure 1f) and bands 1, 2, 3 in POX (Figure 1h).

The cluster analysis based on enzyme systems using dendrogram is an appropriate method for comparison of the similarity among populations. The higher number of enzyme systems used the better efficiency can be achieved (Huang *et al.*, 1994; Obara-Okeyo *et al.*, 1998). Therefore, eight enzyme systems were used to rank the relationships among the twenty Daw clones (Figure 2).

The two most closely related were DBH and DKO. They had 95 % similarity and could not be separated. The rest of the samples could be separated as the similarity ranged between 0-80 %.

DKK and DNP as well as DLG and DSK had 80 % similarity. Less relationships appeared in DLP and DSY which had 68 % similarity while DNS and DTN had 64 % similarity. There were 5 clusters which had 50 % similarity i.e. (a) DBH, DKO, DKK, DNP, DYD, (b) DBY, DKL, (c) DNS, DTN, DBA, DNK, (d) DLP, DSY, and (e) DLG, DSK, DPT, DDC. DBH, DKO, DKK, DNP, DYD, DBD, DKL, DHM as well as DNS, DTN, DBA, DNK, DLP, DSY, DLG, DSK, DPT, DDC had 40 % similarity. DJE had only 20 % similarity to the others with 0 % similarity to DYK. DYK had 0 % similarity to the rest of the clones. It was the only individual clone that was completely different from the others.

Conclusion

In conclusions, a considerable amount of genetic diversity existed in all clones of longan var. Daw. Eight enzyme systems, which produced clear and reproducible isozyme patterns, were ACP, AAT, EST, G6PD, GDH, G3PD, MDH and POX. The number of schematic isozyme banding patterns were 15, 13, 11, 19, 13, 11, 14 and 9, respectively, with the total of 105. DBH and DKO were closely related at 95 % similarity while DKK and DNP as well as DLG and DSK were at 80 % similarity. DLP and DSY were at 68 % similarity as well as DNS and DTN were at 64 % similarity. DYK was the only individual that was completely different from the others at 0 % similarity.

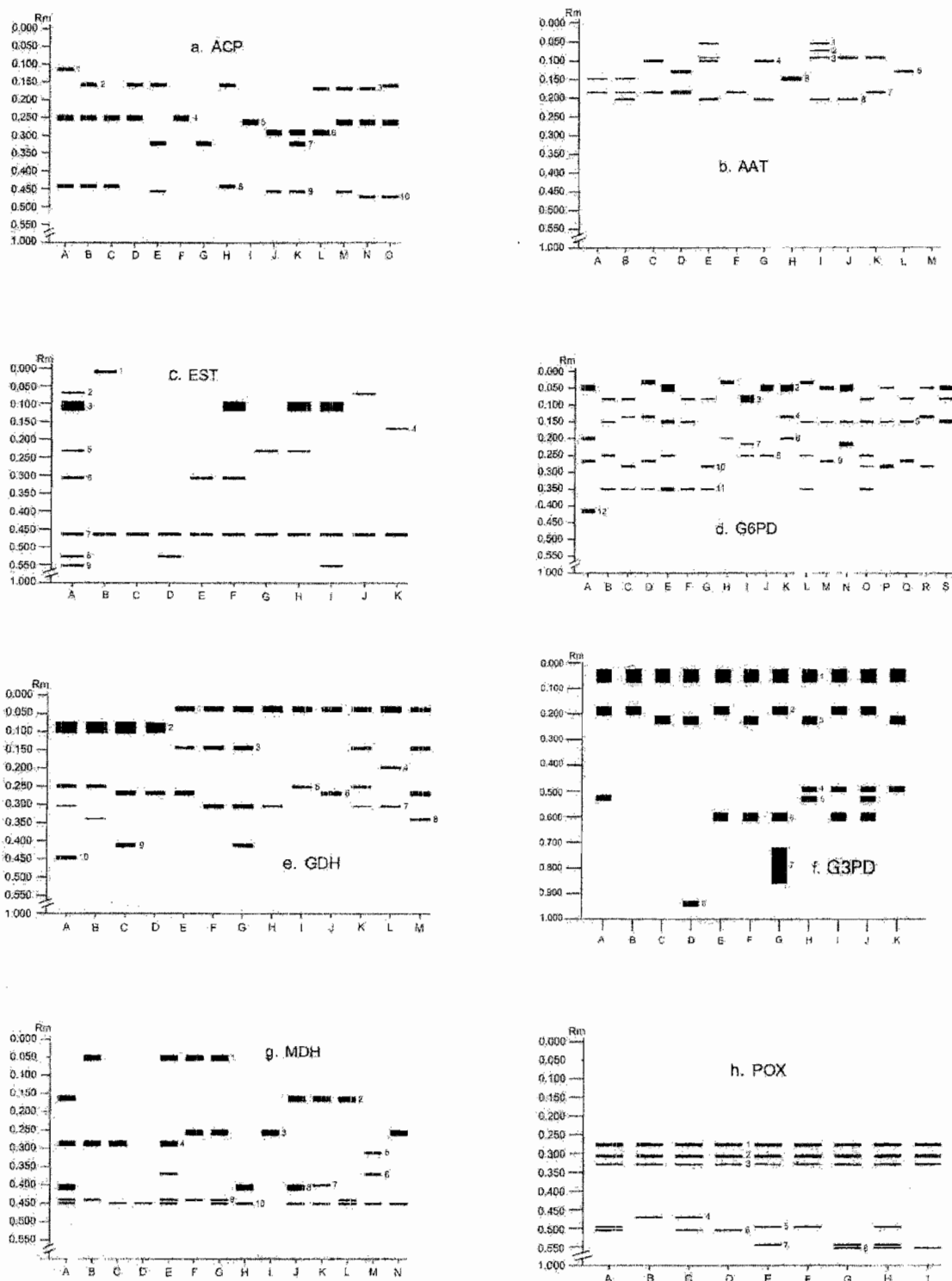


Figure 1 Schematic zymograms of twenty Daw clones.

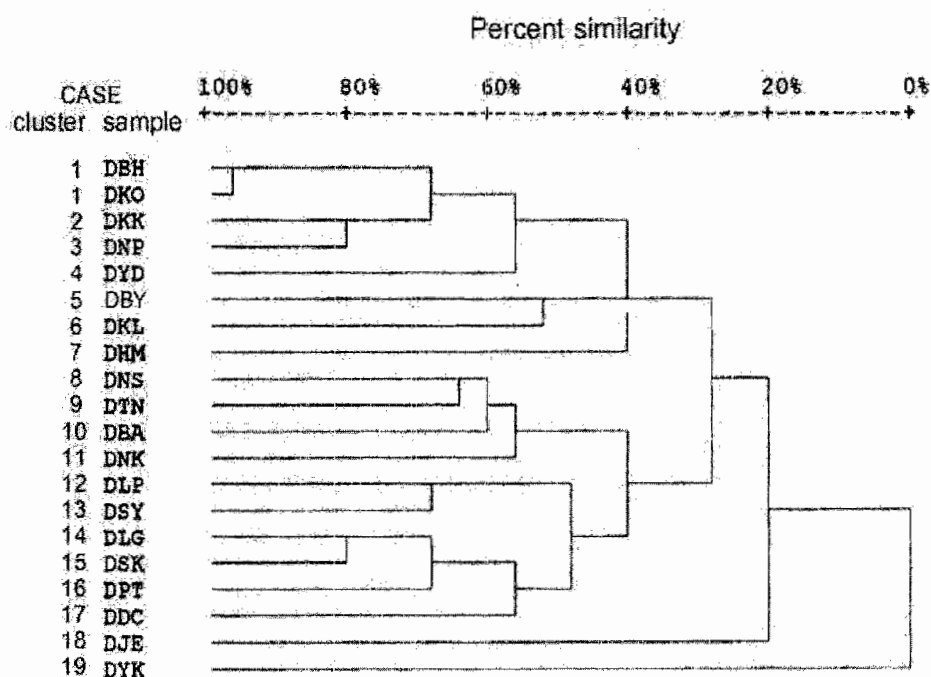


Figure 2 Dendrogram representing the relationships of twenty Daw clones based on eight isozyme patterns by Jaccard's coefficient similarity.

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