

**MOLECULAR PHYLOGENY OF SELECTED MANGO CULTIVARS
BASED ON INTERNAL TRANSCRIBED SPACER (ITS) REGION**

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A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

**Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia**

JANUARY 2013

I dedicate my research in the name of Allah to Islam and mankind

ACKNOWLEDGEMENTS

Bismillah Ar-Rahman Ar-Raheem

All gratitude and praise to Allah for the ocean of blessings He has showered on me. Alhamdulillah.

First and foremost, I wish to extend my deepest gratitude and profound appreciation to my supervisor, Dr. Azman Abd Samad and Dr. Topik Hidayat, for their kindness, invaluable supervision and helpful suggestion throughout the study until this research was completed.

A note of thanks also goes to Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI), Serdang, Selangor, for their generosity in providing samples that has been used in this study and for the all laboratory staffs for providing facilities, valuable guidance and generous advice in performing my laboratory works.

To all my lecturers and friends, for their support, kindness and warm co-operation all this time. My sincere appreciation also extended to my UTM colleagues who had always supported me.

Finally, I am wholeheartedly grateful for the support received from my family, especially my mother, for all the loves, understanding and encouragement throughout my long days of study and life.

ABSTRACT

Mango (*Mangifera indica* L.) is an important species of the family Anacardiaceae and is one of the economical value crops in many parts of the world. Hence, the phylogeny study of this crop is crucial as a step for improvement of its breeding programs. Phylogenetic relationships among 13 *Mangifera indica* species from Malaysia, Indonesia and Taiwan were inferred by comparing DNA sequence data sets derived from the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and analyzed using maximum parsimony (MP) method. The results showed that the cultivars were clustered into two major groups. The first group had low bootstrap value which consisted of Malaysian cultivars. The second group divided into two subclades, the first subclade consisted mainly of Taiwan cultivars. The second subclade further divided into two small subclades, which comprised of Indonesian cultivars in the first small subclade and Malaysian cultivar (1) and Indonesian cultivars (2) in another one. The findings suggested that some cultivars had a close relationship with each other even it is originates from different countries which gives better insight with regards to the relationship among these cultivars, thus providing useful information for generating new cultivar.

ABSTRAK

Mangga (*Mangifera indica* L.) merupakan spesies penting di dalam keluarga Anacardiaceae dan merupakan salah satu dari tanaman yang mempunyai nilai ekonomi di banyak tempat di dunia. Oleh itu, kajian filogeni tanaman ini adalah penting sebagai langkah untuk penambahbaikan bagi program pembiakannya. Hubungan filogenetik di antara 13 spesies *Mangifera indica* dari Malaysia, Indonesia dan Taiwan telah ditafsir dengan membandingkan data jujukan DNA yang berasal dari daerah *Internal Transcribed Spacer* (ITS) rantau DNA nuklear ribosom (nrDNA) dan dianalisis menggunakan kaedah parsimoni maksimum (MP). Hasil kajian menunjukkan bahawa kultivar-kultivar ini dapat dikelompokkan ke dalam dua kumpulan utama. Kumpulan pertama mempunyai nilai *bootstrap* yang rendah terdiri daripada kultivar Malaysia. Kumpulan kedua terbahagi kepada dua klad kecil, klad kecil pertama hampir keseluruhannya adalah kultivar Taiwan. Klad kecil kedua dibahagikan lagi kepada dua klad kecil, ia terdiri daripada kultivar Indonesia di klad kecil pertama dan kultivar Malaysia (1) dan kultivar Indonesia (2) di klad kecil yang satu lagi. Penemuan ini mengusulkan bahawa terdapat beberapa kultivar yang mempunyai hubungan rapat antara satu sama lain walaupun ia berasal dari negara-negara yang berbeza, maka memberikan gambaran yang lebih baik dari segi hubungan antara kultivar ini, sekali gus memberikan maklumat yang berguna untuk menjana kultivar baru.

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LIST OF ABBREVIATIONS

μ l	-	Microliter
μ M	-	Micromolar
AFLP	-	Amplified fragment length polymorphism
BS	-	Bootstrap
bp	-	Base pair
CI	-	Consistency index
cpDNA	-	Chloroplast DNA
et al	-	and other persons
ITS	-	Internal transcribed spacer
kb	-	Kilo base
kcal	-	Kilo calories
<i>matK</i>	-	Maturase K
mg	-	Miligram
min	-	Minute
ml	-	Mililiter
ML	-	Maximum likelihood
MP	-	Maximum parsimony
ME	-	Minimum evolution
MEGA	-	Molecular Evolutionary Genetics Analysis
mtDNA	-	Mitochondria DNA
NJ	-	Neighbour-joining
nm	-	Nanometer
nrDNA	-	Nuclear ribosomal DNA
PCR	-	Polymerase chain reaction
RAPD	-	Random amplified polymorphic DNA
<i>rbcL</i>	-	Ribulose biphosphate carboxylase
RFLP	-	Restriction fragment length polymorphism

RI	-	Retention index
rpm	-	Rotation per minute
Sec	-	Second
SSRs	-	Simple sequence repeats
UPGMA	-	Unweighted Pair Group Method with Arithmetic Mean

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree Celcius
∞	-	Infinity
>	-	More than

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Mango (*Mangifera indica* L.) is one of the most prominent members of the family Anacardiaceae that is grown commercially in many parts of the world, particularly tropical and subtropical countries (Rajwana *et al.*, 2011). This is due to several characteristics possessed by this climacteric fruit such as stupendous quality (attractive appearance, great taste and adorable flavour) and excellent nutritional composition (diverse amount of fibre, minerals, vitamins and various antioxidant compounds) (Kim, 2005).

Its genotypes are classified into two categories, namely monoembryonic which is mostly from subtropical regions (Indian types) and polyembryonic which is from tropical regions (Southeast Asian types) (Luo *et al.*, 2011). Over 4000 years ago, mango is originated from India and Burma and has been spread to Eastern Asia, Eastern Africa and Malaysia (Mitra and Baldwin, 1997). Malaysia is among countries that cultivates mango for commercial production in Southeast Asia. There are several cultivars of mango grown in Malaysia, including Chokanan (MA 224), Harumanis (MA 128), Maha 65 (MA 165) and Nam Doc Mai (223).

In terms of production, it is ranked as the fifth major fruit grown throughout the world after apples, bananas, grapes and oranges and the second most vital tropical fruit

(Bally *et al.*, 2009). Generally, there are numbers of mango cultivars that arise from naturally occurred open-pollinated seedlings (Iyer and Degani, 1997). As for commercially grown cultivars, seedling selections with respect to characteristics like colour, flavour, size and taste has been done and subsequently, these cultivars are propagated and cultivated in a huge area (Ravishankar *et al.*, 2004).

According to Ravishankar *et al.*, (2000), information with regards to genetic diversity is one of the most crucial parts in designing breeding programmes in order to maintain mango production. Furthermore, another factor that contributes to the effective production of hybrids is the well-defined phylogenetic relationship between the parents (Nishiyama *et al.*, 2006). However, the phylogenetic relationship among numbers of mango cultivars is poorly studied and remains unclear as there are numbers of mango cultivars and sometimes different or synonym names are used referring to a single cultivar. Additionally, the morphological based approach has led to confusion on cultivars identification. Thus, creating a conflict in classification of mango cultivars.

Rapid advances of molecular techniques such as polymerase chain reaction (PCR) bring an impact for the use of DNA sequences in molecular phylogenetic studies (Topik and Adi, 2010). According to Germano and Klein (1999), DNA sequencing-based method is very reliable due to the reproducibility of the results and the scope of application. Duneman, (1994) has mentioned that DNA-based markers are very advantageous in characterizing and studying genetics similarities among cultivars, land races and varieties.

Nowadays, various DNA markers namely restriction fragment length polymorphism (RFLP) (Ravishankar *et al.*, 2004), random amplified polymorphic DNA (RAPD) (Karihaloo *et al.*, 2003, Ravishankar *et al.*, 2004), amplified fragment length polymorphism (AFLP) (Yamanaka *et al.*, 2006) and simple sequence repeats (SSRs) (Viruel *et al.*, 2005, Schnell *et al.*, 2006) have been utilized to determine taxonomic

identity (Schnell *et al.*, 2006), estimate genetic diversity (Viruel *et al.*, 2005) and infer evolutionary histories of mango (Yamanaka *et al.*, 2006).

In this study, phylogenetic relationship of selected mango cultivars was inferred by using DNA sequences of Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA), a widely used DNA marker in order to resolve phylogenetic relationship at various taxa (Karehed *et al.*, 2008). This DNA marker is very useful due to several features such as high copy number (Hillis and Dixon 1991), small in size, possess highly conserved flanks (Baldwin, 1992), rapid concerted evolution and universality of primers (Baldwin *et al.*, 1995).

1.2 Problem statement

Mango is well known as one of the most prominent commercial tropical fruit in the world that has a great taste and beneficial for health. However, the phylogenetic relationship among mango cultivars is poorly studied and remains unclear as there are thousands of mango cultivars and sometimes different or synonym names are used for a single cultivar. Moreover, it is often identified due to its morphological traits which add difficulties and confusion in identifying the cultivars. Thus, lead to conflicting in classification of mango cultivars which result difficulty in designing effective breeding programmes. Therefore, in this study DNA marker, namely Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA) has been used to infer the phylogenetic relationship among selected mango cultivars.

1.3 Objectives

The objectives of this study are:

- I. To amplify and sequence the ITS region of Malaysian mango cultivars
- II. To construct pylogenetic tree of selected mango cultivars from Malaysia, Indonesia and Taiwan
- III. To determine the utility of ITS as promising phylogenetic marker

1.4 Scope of study

This study focused on phylogenetic relationship among 13 selected mango cultivars from Malaysia, Indonesia and Taiwan that has been constructed based on DNA sequences of Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA) by using maximum parsimony (MP) method.

1.5 Significance of study

It is crucial to study the phylogenetic relationship among mango cultivars in order to provide useful information for cultivar identification, cultivar development and data resource for future study, improvement in breeding strategies for future gains in productivity and genome mapping.

REFERENCES

- Alvarez, I., and Wendle, J. F. (2003). Ribosomal ITS Sequences and Plant Phylogenetic Inference. *Molecular Phylogenetics and Evolution*, 219, 417-434.
- Bailey, C. D., Koch, M. A., Mayer, M., Mummenhoff, K., O'Kane, S. L. Jr., Warwick, S. I., Windham, M. D., Al-Shehbaz, I. A. (2006). Towards a Global Phylogeny of the Brassicaceae. *Molecular Biology and Evolution*, 23, 2142-2160.
- Baldwin, B. G. (1992). Phylogenetic Utility of the Internal Transcribed Spacers of Nuclear Ribosomal DNA in Plants: An Example from the Compositae. *Molecular Phylogenetics and Evolution*, 1(1), 3-16.
- Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., and Donoghue, M. J. (1995). The ITS Region of Nuclear Ribosomal DNA: A Valuable Resource of Evidence on Angiosperm Phylogeny. *Annals of the Missouri Botanical Garden*, 82(2), 247-277.
- Bally, I. S. E. (1998). *Mango Variety R2E2 fact sheet*. Queensland Horticultural Institute, QDPI. Brisbane.
- Bally, I. S. E. (2006). *Mangifera indica* (mango), ver. 3.1. *Species Profiles for Pacific Island Agroforestry*. Permanent Agriculture Resources (PAR), Holualoa. <http://www.traditionaltree.org>
- Bally, I. S. E., Lu, P., and Johnson, P. R. (2009). *S.M. Jain, P.M. Priyadarshan (eds.), Breeding Plantation Tree Crops: Tropical Species*: Springer.

- Brinkman, F. S. L., and Leipe, D. D. (2001). Phylogenetic Analysis. In D. Andreas, Baxevanis, B. F., and Ouellette, F. (Ed.), *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Second Edition* (pp. 358): John Wiley & Sons, Inc.
- Buettner, G. R. (1993). The Pecking Order of Free Radicals and Antioxidants: Lipid Peroxidation, Alpha-tocopherol, and Ascorbate. *Archives of Biochemistry and Biophysics*, 300, 534–543.
- Calonje, M., Martin-Bravo, S., Dobes, C., Gong, W., Jordon-Thaden, I., Kiefer, C., Kiefer, M., Paule, J., Schmickl, R., Koch, M. A. . (2009). Noncoding Nuclear Markers in Phylogenetic Reconstruction. *Plant Syst Evol.* , 282, 257–280.
- Carvalho, A. C., Guedes, M. M., Souza, A. L., Trevisan, M. T., Lima, A. F., Santos, F. A., and Rao, V. S. (2007). Gastroprotective Effect of Mangiferin a Xanthonoid from *Mangifera indica*, Against Gastric Injury Induced by Ethanol and Indomethacin in Rodents. *Planta Medica*, 73, 1372–1376.
- Clarke, A. B., and Clarke, B. A. (1987). A Description of Preharvest Factors Affecting Yield in Mango (*Mangifera indica* L.) in Mangoes'- A Review. *Commonwealth Science Council*.
- Clegg, M. T., and Durbin, M. L. (1990). Molecular Approaches to the Study of Plant Systematics. In: Ladiges PY, Martinelli LW (eds). *Plant Systematics in the Age of Molecular Biology*. CSIRO, Australia, 1-8.
- Crane, J. H., and Campbell, C. W. (1994). *The Mango*: University of Florida IFAS Extension.

- Duneman, F. (1994). *Molecular classification of Malus with RAPD markers*. In: Schmidt, H., Kellerhals, M. (Eds.), *Progress in Temperate Fruit Breeding*. Dordrecht: Kluwer Academic Publishers.
- Eiadthong, W., Yonemori, K., Sugiura, A., Utsunomiya, N., Subhadrabandhu, S. (1999). Analysis of Phylogenetic Relationships in *Mangifera* by Restriction Site Analysis of an Amplified Region of cpDNA. *Scientia Horticulturae* 80, 145-155.
- Eiadthong, W., Yonemori, K., Kanzaki, S., and Sugiura, A. (2000). Amplified Fragment Length Polymorphism Analysis for Studying Genetic Relationships among *Mangifera* Species in Thailand. *J. Amer. Soc. Hort. Sci.*, 125(2), 160–164.
- Endress, P. K., Baas, P., and M Gregory, M. (2000). Systematic Plant Morphology and Anatomy-50 Years of Progress. *Taxon*, 49, 401-434.
- Farihul, I., and Sukarmin. (2008). Teknik Persilangan Mangga (*Mangifera indica*) Untuk Perakitan Varietas Unggul Baru. *Buletin Teknik Pertanian* 13(1), 33-36.
- Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution*, 39, 783–791.
- Fitch, W. M., and Margoliash, E. (1967). The Construction of Phylogenetic Trees. *Science*, 155(279-284).
- Fitch, W. M. (1971). Toward Defining the Course of Evolution: Minimal Change for a Specific Tree Topology. *Systematic Zoology*, 20, 406-416.
- Fitmawati. (2008). *Biosistematis of Indonesian Mangoes*. Thesis for Doctor of Philosophy (Biology). Bogor Agricultural University, Bogor, Indonesia.

- Garrido, G., González, D., Delporte, C., Backhouse, N., Quintero, G., Núñez-Sellés, A. J., Morales, M. A. (2001). Analgesic and Anti-inflammatory Effects of *Mangifera indica* L. extract (Vimang) *Phytother Res* 15(1), 18–21.
- Germano, J., and S. Klein, A. (1999). Species-specific Nuclear and Chloroplast Single Nucleotide Polymorphisms to Distinguish *Picea glauca*, *P. mariana* and *P. rubens*. *Theoretical and Applied Genetics*, 99(1-2), 37-49.
- Griesbach, J. (2003). *Mango Growing in Kenya*. Nairobi, Kenya: World Agroforestry Centre (ICRAF).
- Hall, B. G. (2008). *Phylogenetic Tree Made Easy: A How-To Manual* (3 ed.). Sunderland, Massachusetts, USA: Sinauer Associates, Inc.
- Hamby, R. K., and Zimmer, E. A. (1988). Ribosomal RNA Sequences for Inferring Phylogeny Within the Grass Family (Poaceae). *Plant Syst Evol*, 160, 29-37.
- Harrison, C. J., and Langdale, J. A. (2006). A Step by Step Guide to Phylogeny Reconstruction. *The Plant Journal*, 45, 561–572.
- Hillis, D. M., and Dixon, M. T. (1991). Ribosomal DNA: Molecular Evolution and Phylogenetic Inference. *Quarterly Rev Biol* 66, 411-453.
- Hillis, D. M., and Bull, J. J. (1993). An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis *Syst. Biol.*, 42, 182–192.
- Hillis DM, M. C., Porter CA, Baker RJ. . (1991). Evidence for Biased Gene Conversion in Concerted Evolution of Ribosomal DNA. *Science*, 251, 308-310.
- Huelsenbeck, J. P. (1995). Performance of Phylogenetic Methods in Simulation. *Syst. Biol.*, 44(17-48).

- Hyam, R., and Pankhurst, R. (1995). *Plants and their names; A concise dictionary* Walton street, Oxford, UK: Oxford University Press.
- Iyer, C. P. A., and Degani C. (1997). *Classical breeding and genetics*. In: Litz R. E. (ed.) *The mango: Botany, Production and Uses*. Wallingford, UK: CAB Intl.
- Jagetia, G. C., and Baliga, M. S. (2005). Radioprotection by Mangiferin in DBAxC57BL Mice: A Preliminary Study. *Phytomed*, 12(3), 209–215.
- John, S. K. (2001). *Biochemical Investigations on Mango Sap and Sap-injury*. Thesis for Doctor of Philosophy (Biotechnology) University of Mysore, Mysore, India.
- Jones, M. D. M., Forn, I., Gadelha, C., Egan, M. J., Bass, D., Massana, R., Richards, T. A. (2011). Discovery of Novel Intermediate Forms Redefines the Fungal Tree of Life. *Nature*, 474, 200–203.
- Karehed, J., Groeninckx, I., Dessein, S., Motley, T. J., Bremer, B. (2008). The Phylogenetic Utility of Chloroplast and Nuclear DNA Markers and the Phylogeny of the Rubiaceae Tribe Spermaceae. *Molecular Phylogenetics and Evolution*, 49(843–866).
- Karihaloo, J. L., Dwivedi, Y. K., Archak, S., Gaikwad, A. B. (2003). Analysis of Genetic Diversity of Indian Mango Cultivars Using RAPD Markers. *Journal of Horticultural Science & Biotechnology*, 78, 285-289.
- Kaur, A., Ha, C. O., Jong, K., Sands, V. E., Chan, H. T., Soepadmo, E., and Ashton, P. S. (1980). Apoximis May be Widespread Among Trees of the Climax Rainforest. *Nature*, 270, 440-442.
- Kebun Penelitian Cukurgondang (2010). Ciri Morfologi Mangga. Balai Penelitian Buah Tropika. Retrieved December 8, 2012. [http.:// www.balitbang.deptan.go.ind](http://www.balitbang.deptan.go.id).

- Kelly, A., Mohamed M. P., and Donna, M. (2012). Assessment of DNA Yield and Purity: an Overlooked Detail of PCR Troubleshooting. *Clinical Microbiology Newsletter*, 34(1), 1-6.
- Kim, Y. M. (2005). *Changes in Polyphenolics and Resultant Antioxidant Capacity in 'Tommy Atkins' Mangos (Mangifera indica L.) by Selected Post Harvest Treatments*. Thesis for Master of Science (Biology) University of Florida, Florida.
- Kostermans, A. J. G. H., and Bompard, J. M. (1993). *The mangoes: Their botany, nomenclature, horticulture and utilization*. London, England: IBPGR Academic Press.
- Lemus-Molina, Y., Sánchez-Gómez, M. V., Delgado-Hernández, R., Matute, C. (2009). *Mangifera indica* L. Extract Attenuates Glutamate-induced Neurotoxicity on Rat Cortical Neurons. *Neurotoxicol* 30(6), 1053–1058.
- Li, W. H., and Graur, D. (1991). *Fundamentals of Molecular Evolution*. Sunderland, Massachusetts, USA: Sinauer Associates.
- Li, W. H. (1997). *Molecular Evolution*. Sunderland, Massachusetts, USA: Sinauer Associates.
- Li, W. H. (1998). *Molecular Evolution*. Sunderland, Massachusetts, USA: Sinauer Associates.
- Lim, T. K. (2012). *Edible Medicinal and Non-Medicinal Plants: Volume 1, Fruits*: Springer.
- Lipscomb, D. (1998). *Basics of Cladistic Analysis*. Washington D. C., USA: George Washington University.

- Loria, C. M., Klag, M. J., Caulfield, L. E., and Whelton, P. K. (2000). Low vitamin C Status May Increase the Risk of Mortality from Cancer and Cardiovascular Disease. *American Journal of Clinical Nutrition*, 72, 139–145.
- Luo, C., He, X. H., Chen, H., Ou, S.J., Gao, M. P. (2010). Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. *Biochemical Systematics and Ecology*(38).
- Luo, C., He, X. H., Chen, H., Ou, S. J., Gao, M. P., Steven Brown, J. S., Tondo, C. T., Schnell, R. J. (2011). Genetic Diversity of Mango Cultivars Estimated Using SCoT and ISSR Markers. *Biochemical Systematics and Ecology*, 39, 676–684.
- Jabatan Pertanian Malaysia (2009). *Pakej Teknologi Mangga (Edisi kedua)*. Malaysia: Kementerian Pertanian dan Industri Asas Tani.
- Jabatan Pertanian Semenanjung Malaysia (2000). *Pakej Teknologi Mangga Malaysia*: Malaysia: Kementerian Pertanian dan Industri Asas Tani.
- Jabatan Pertanian Perak (2010). Panduan Menanam Mangga. Retrieved July 1, 2012. <http://www.pertanianperak.gov.my/>
- Marzia, M. Z., and Ngizailah, Z. (2010). *Maklumat Tanaman Harumanis (MA 128) di Negeri Perlis*. Malaysia: Kementerian Pertanian dan Industri Asas Tani.
- Mathew, P. A., and Dhandar, D. G. (1997). Cardozo Mankurad – A Breakthrough in Mango (*Mangifera indica* L.) Selection. *Acta Horticulturae* 455, 236–240.
- Mitra, S. K., and Baldwin, E. A. (1997). *Mango*. In: *Postharvest physiology storage of tropical and subtropical fruit*. New York, USA: CAB International.

- Mohamad, O. (1994, September 30 -October 2). *Proceedings: Tropical Fruit Crops of Malaysia*. Paper presented at the 4th Annual Hawaii Tropical Fruit Growers Conference, Keauhou, Hawaii.
- Moritz, C., and Hillis, D. M. (1996). Molecular systematics: context and controversies. In D. M. Hillis, Moritz, C., and Mable, B. K. (Ed.), *Molecular Systematics, 2nd edition*. Sunderland, Massachusetts, USA: Sinauer Associates.
- Mukherjee, S. K. (1950). Mango: Its Allopolyploid Nature. *Nature*, 166, 196-197.
- Mukherjee, S. K. (1953). Origin, Distribution and Phylogenetic Affinity of the Species of *Mangifera* L. *J Linn Soc Bot*, 55, 65-83.
- Mukherjee, S. K., and Litz, R. E. (2009). Introduction: Botany and Importance. In R. E. Litz (Ed.), *The mango, 2nd Edition: Botany, Production and Uses*. UK: CAB International.
- Nakasone, H. Y., and Paul, R. E. (1998). *Tropical fruits*. Willingford, UK: CAB Intl.
- Nei, M., and Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press, Oxford.
- Ng'uni, D., Geleta, M., Fatih, M., Bryngelsson, T. (2010). Phylogenetic Analysis of the Genus *Sorghum* Based on Combined Sequence Data from cpDNA Regions and ITS Generate Well-supported Trees with Two Major Lineages. *Annals of Botany* 105, 471–480.
- Nishiyama, K., Choi, Y. A., Honsho, C., Eiadthong, W., Yonemori, K. (2006). Application of Genomic *in situ* Hybridization for Phylogenetic Study Between *Mangifera indica* L. and Eight Wild Species of *Mangifera*. *Scientia Horticulturae* 110, 114-117.

- Noratto, G. D., Bertoldi, M. C., Krenek, K., Talcott, S. T., Stringheta, P. C., Mertens-Talcott, S. U. (2010). Anticarcinogenic Effects of Polyphenolics from Mango (*Mangifera indica*) Varieties *J. Agric Food Chem.*, 58(7), 4104–4112.
- Pandey, S. N. (1986). *Mango cultivars: Nomenclature and Registration*. Paper presented at the ISHS Acta Hort, 182: 1 International Symposium on Taxonomy of Cultivated Plants.
- Prabhu, S., Jainu, M., Sabitha, K. E., Shyamala Devi, C. S. (2006). Effect of Mangiferin on Mitochondrial Energy Production in Experimentally Induced Myocardial Infarcted Rats. *Vasc Pharmacol.*, 44(6), 519–525.
- Prasad, S., Kalra, N., Shukla, Y. (2007). Hepatoprotective Effects of Lupeol and Mango Pulp Extract of Carcinogen Induced Alteration in Swiss Albino Mice. *Mol Nutr Food Res*, 51(3), 352–359.
- Priya, T. T., Sabu, M. C., Jolly, C. I. (2009). Role of *Mangifera indica* Bark Polyphenols on Rat Gastric Mucosa Against Ethanol and Cold-restraint Stress. *Nat Prod Res*, 14, 1-12.
- Project, A. F. H. (1988). Nutrient Composition of Malaysian Foods. In: Food Habits Research and Development.
- Purnomo, S. (2000). *Buku Komoditas Mangga*. Solok, Indonesia: Balai Penelitian Tanaman Buah, Solok.
- Rahman, M. L., Rabbani, M. G., Siddique, M. N. A., Rahman, M. A., Garvey, E. J., Rahaman, E. H. M. S. (2007). Molecular Characterization of 28 Mango Germplasm Using RAPD. *Plant Tissue Cult. Biotech.*, 17(1), 71–77.

- Rajwana, I. A., Khan, I. A., Malik, A. U., Saleem, B. A., Khan, A. S., Ziaf, K., Anwar, R., Amin, M. (2011). Morphological and Biochemical Markers for Varietal Characterization and Quality Assessment of Potential Indigenous Mango (*Mangifera indica*) Germplasm. *Int. J. Agric. Biol.*, 13, 151–158.
- Ramirez, I. M., Rodriguez, N. N., Valdes-Infante, J., Capote, M., Becker, D., Rohde, W. (2004). Isolation of Genomic DNAs from the Tropical Fruit Trees Avocado, Coconut, Guava and Mango for PCR-based DNA Marker Application. *Cult Trop*, 25(1), 33-38.
- Ravishankar, K. V., Anand, L., Dinesh, M. R. (2000). Assessment of Genetic Relatedness Among Mango Cultivars of India Using RAPD Markers. *J. Hortic. Sci. Biotechnol.*, 75, 198–201.
- Ravishankar, K. V., Chandrashekara, P., Sreedhara, S. A., Dinesh, M. R., Anand, L., Saiprasad, G.V.S. (2004). Diverse Genetic Bases of Indian Polyembryonic and Monoembryonic Mango (*Mangifera indica* L.) Cultivars. *Curr. Sci.*, 87, 870–871.
- Ribeiro, S. M. R., and Schieber, A. (2010). *Bioactive Foods in Promoting Health: Fruits and Vegetables*: Elsevier Inc.
- Satish Rao, B. S., Sreedevi, M. V., Nageshwar Rao, B. (2009). Cytoprotective and Antigenotoxic Potential of Mangiferin, a Glucosylxanthone Against Cadmium Chloride Induced Toxicity in HepG2 Cells. *Food Chem Toxicol* 47(3), 592–600.
- Sauco, G. V. (2004). Mango Production and World Market: Current Situation and Future Prospects. *Acta References Horticulturae*, 645, 107–116.

- Schnell, R. J., Brown, J. S., Olano, C. T., Meerow, A. W., Campbell, R. J., Kuhan, D. N., . (2006). Mango Genetic Diversity Analysis and Pedigree Inferences for Florida Cultivars Using Microsatellite Markers. *J. Am. Soc. Hortic. Sci.*, *131*, 214–224.
- Sharma, S. K., Dkhar, J., Kumaria, S., Tandon, P., Rao, S. R. (2012). Assessment of Phylogenetic Inter-relationships in the Genus *Cymbidium* (Orchidaceae) Based on Internal Transcribed Spacer Region of rDNA. *Gene* *495*, 10–15.
- Simon, U. K., Trajanoski, S., Kroneis, T., Sedlmayr, P., Guelly, C., Guttenberger, H. (2012). Accession-Specific Haplotypes of the Internal Transcribed Spacer Region in *Arabidopsis thaliana*—A Means for Barcoding Populations. *Molecular Biology and Evolution*, 1-9.
- Singh, L. B. (1976). *Mango*. In: *Simmonds N. W. (ed.) Evolution of crop plants*. London, UK: Longman Group Limited.
- Slotta, T. A. B. (2000). *Phylogenetic Analysis of Iliamna (Malvaceae) Using the Internal Transcribed Spacer Region*. Thesis of Master of Science. Virginia Polytechnic Institute and State University, Blacksburg.
- Spallholz, E. J., Boylan, L. M., and Driskell, A. J. (1999). *Nutrition Chemistry and Biology (second edition)*: CRC press.
- Strimmer, K., and Robertson, D. L. (2001). Inference and Applications of Molecular Phylogenies: An Introductory Guide. In C. Sansom, and Horton, R. M. (eds.) (Ed.), *The Internet for Molecular Biologists (Practical Approach Series)*. Oxford, UK: University of Oxford.

- Sukarmin, F., I., and Endriyanto. (2009). Teknik Perbanyakkan FI Mangga Dengan Menggunakan Batang Bawah Dewasa Melalui Sambung Pucuk. *Buletin Teknik Pertanian*, 14(2), 58-61.
- Sun, Y., Skinner, D. Z., Liang, G. H., Hulbert, S. H. (1994). Phylogenetic Analysis of *Sorghum* and Related Taxa Using Internal Transcribed Spacers of Nuclear Ribosomal DNA. *Theoretical Applied Genetics*, 89, 26–32.
- Tipperry, N. P., and Les, D. H. (2008). Phylogenetic Analysis of the Internal Transcribed Spacer (ITS) Region in Menyanthaceae using Predicted Secondary Structure. *Molecular Phylogenetics and Evolution* 49, 526–537.
- Topik, H., Yukawa, T., and Ito, M. (2005). Molecular Phylogenetics of Subtribe Aeridinae (Orchidaceae): Insights from Plastid *matK* and Nuclear Ribosomal ITS Sequences. *Journal of Plant Research*(118), 271–284.
- Topik, H., and Adi, P. (2006). *Short Communication: DNA Technology and Studies in Phylogenetic Relationship of Tropical Plant: Prospect in Indonesia*. Paper presented at the International Conference on Mathematics and Natural Sciences.
- Topik, H. (2010). Molecular Phylogenetic of Selected Mango Cultivars Using Internal Transcribed Spacer (ITS). Unpublished Research Report. Universiti Pendidikan Indonesia.
- Topik, H., and Adi, P. (2010). Single Strand Conformation Polymorphism Method for Initial Detection DNA Sequences Homogeneity. *HAYATI Journal of Biosciences*, 17(1), 50-52.

- Topik, H., Adi, P., Diah, K., Eiadthong, W. (2011, 14 - 15 January). *Molecular Diversification and Phylogeny of Mangifera (Anacardiaceae) in Indonesia and Thailand*. Paper presented at the International Conference on Advanced Science, Engineering and Information Technology 2011, Bangi-Putrajaya, Malaysia.
- Varma, A., Padh, H., Shrivastava, N. (2007). Plant genomic DNA isolation: an art or a science. *Biotechnology Journal*, 2, 386-392.
- Villegas, V. N. (2002). Breeding New Fruit Varieties in the Philippines. *Acta Horticulturae*, 575.
- Viruel, M. A., Escribano, P., Barbieri, M., Ferri, M., Hormaza, J. I. (2005). Fingerprinting, Embryo Type and Geographic Differentiation in Mango (*Mangifera indica* L.) (Anacardiaceae) with Microsatellites. *Mol. Breed.*, 15, 338–339.
- Wang, X., Xiao, H., Zhao, X., Li, C., Ren, J., Wang, F., Pang, L. (2012). Isolation of High-Quality DNA from a Desert Plant *Reaumuria soongorica*. In M. Caliskan (Ed.), *Genetic Diversity in Plants* (pp. 498): InTech.
- Weider, L. J., Elser, J. H., Crease, T. J., Mateos, M., Cotner, J. B., Markow, T. A. (2005). The Functional Significance of Ribosomal (r)DNA Variation: Impacts on the Evolutionary Ecology of Organisms. *Annu Rev Ecol Evol Syst.* , 36, 219–242.
- Wen, J., and Zimmer, E. A. (1996). Phylogeny and Biogeography of *Panax* L. (the Ginseng Genus, *Araliaceae*): Inferences from ITS Sequences of Nuclear Ribosomal DNA. *Molecular Phylogenetics and Evolution*, 167-177.

- Wilkinson, A. S., Monteith, G. R., Shaw, P. N., Lin, C. N., Gidley, M. J., Roberts-Thomson, S. J. (2008). Effects of the Mango Components Mangiferin and Quercetin and the Putative Mangiferin Metabolite Norathyriol on the Transactivation of Peroxisome Proliferator-activated Receptor Isoforms. *J Agric Food Chem* 56(9), 3037–3042.
- Yamanaka, N., Hasran, M., Xu, D. H., Tsunematsu, H., Idris, S., Ban, T. (2006). Genetic Relationship and Diversity of Four *Mangifera* Species Revealed Through AFLP Analysis. *Genet Resour Crop Evol* 53(5), 949–954.
- Yonemori, K., Honsho, C., Kanzaki, S., Eidthong, W., Sugiura, A. (2002). Phylogenetic Relationships of *Mangifera* Species Revealed by ITS Sequences of Nuclear Ribosomal DNA and a Possibility of Their Hybrid Origin. *Plant Syst Evol*, 231, 59-75.
- Zheng, M. S., Lu, Z. Y. (1990). Antiviral Effect of Mangiferin and Isomangiferin on Herpes Simplex Virus. *Chin Med J* 103 (2), 160–165.