GENETIC DIVERSITY AMONG DURIAN (*DURIO ZIBETHINUS* MURR.) CULTIVARS ORIGINATED FROM VIETNAM, THAILAND AND MALAYSIA AS REVEALED BY INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

Van Quoc Giang[1], Mai Van Tri*[2], Huynh Ky[1],
Pham Thi Muoi[2] and Nguyen Loc Hien[1]

Abstract

Genetic diversity of 16 durian cultivars originated from Vietnam, Malaysia and Thailand was analyzed by using ISSR markers. Among 181 fragments that were generated by 13 ISSR primers, 165 fragments (91.1%) were shown polymorphism, on average, 12.7 polymorphic fragments per primer. Genetic relationships of 16 durian cultivars were clustered by UPGMA to demonstrate the differentiation of all cultivars and revealed the correlation between molecular grouping and geographical origin. The first cluster grouped the durian cultivars derived from Thailand. The second cluster included three cultivars from Vietnam, Chuong Bo, Sau Huu and Kho Qua, while the third cluster grouped two Malaysian cultivars, D10 and D24. The fourth and fifth clusters only contained D197 cultivar originated from Malaysia and HB11 cultivar supposed from Vietnam, respectively. Furthermore, ISSR primers, such as ISSR B2 and ISSR BB7, can be used to identify HB11 durian cultivar. Thus, DNA profile of 16 durian cultivars based on ISSR markers revealed the potential of digital fingerprinting of all cultivars examined.

Keywords: Durian, genetic diversity, ISSR markers, polymorphism

INTRODUCTION

'The king of fruits', durian (*Durio zibethinus* Murr.) is one of the most popular and economically important fruit crops in the Southeast Asia. Durian is indigenous to the hot equatorial rain forests of Borneo, Malaysia and Indonesia. However, currently durian is mainly grown in Thailand, Malaysia, Indonesia, Philippines and Vietnam (Ahmad and Nanthachai, 1994). Over 200 durian cultivars were named in Thailand (Tinggal *et al*., 1994) but Chanee, Monthong, Kanyao, and Kradumthong are four Thai durian cultivars that are favorite among growers and consumers. In Malaysia, more than 200 durian cultivars were also recorded but only 10 cultivars are recommended for production. In which, D24, D99, D123 (Chanee), D145 (Beserah) and D197 (Raja Kunyit/Musang King) are for general field planting while some cultivars are recommended for specific locations, such as D148 for Perak, D158, D159 and D169 for Kedah, Penang, Kelantan and Terengganu as well as D168 for Johore. In Vietnam, the durian industry is small but rapidly expanded by the effort of Vietnamese government. There are more than 35 durian cultivars were documented in the country but the common cultivars are Chin Hoa (Com Vang Sua Hat Lep), Ri 6, Monthong and Kho Qua Xanh. To date, there is little information on genetic relationship among durian cultivars including common durian cultivars originated from Vietnam, Thailand and Malaysia.

Earlier classification and evaluation of durian relationship were primarily based on the phenotypic expression, such as fruit shape, the size of thorns on durian skins and other morphological characters (Somsri, 2007). However, morphological variation is limited to distinguish genetic relationship among similar individuals. In contrast, molecular markers have become a standard method to study genetic variability among closely related taxa (Weising *et al*., 1995). The relatively similar Inter-simple sequence repeats (ISSR) were introduced as the efficient molecular markers that are based on quick PCR amplification of polymorphic DNA fragments starting from small amounts of templates (Zietkiewicz *et al*., 1994). ISSR technique has been successfully exploited to identify genetic diversity of some crops including mango (Rocha *et al.,* 2011) and durian (Vanijajiva, 2012).

In this study, ISSR markers were to apply for evaluation of genetic diversity and relatedness of 16 durian cultivars originated from Vietnam (10 cultivars), Thailand (3 cultivars), and Malaysia (3 cultivars).

MATERIALS AND METHODS

Plant materials

List of 16 cultivars along with origin and collected locations in this study were presented in Table 1. Fresh leaf samples were used to extract DNA for ISSR analysis.

[1] College of Agriculture and Applied Biology, Can Tho University
[2] Southeast Horticultural Research Center (SOFRI)
* Corresponding author: mv_tri@yahoo.com

Table 1. The list of 16 durian cultivars sampled and used in this study

Sample code	Name of cultivars	Origin	Collected locations
1	Chanee	Thailand	Vietnam
2	Tam Son	Vietnam	Vietnam
3	HB11	Vietnam	Vietnam
4	Kanyao	Thailand	Vietnam
5	B31	Vietnam	Vietnam
6	Bi (D6)	Vietnam	Vietnam
7	Chin Hoa (Com Vang SHL)	Vietnam	Vietnam
8	La Queo	Vietnam	Vietnam
9	Ri 6	Vietnam	Vietnam
10	Monthong	Thailand	Vietnam
11	Chuong Bo	Vietnam	Vietnam
12	Kho Qua	Vietnam	Vietnam
13	Sau Huu	Vietnam	Vietnam
14	D10	Malaysia	Malaysia
15	D24	Malaysia	Malaysia
16	D197	Malaysia	Malaysia

Genomic DNA isolation

Genomic DNA was extracted from the leaves of 16 durian cultivars by CTAB method (Doyle and Doyle, 1990), and was then stored at -20°C for further use. The DNA quantity and quality were measured by using Eppendorf BioPhotometer D.30 spectrometer (Eppendorf, German) before ISSR analysis.

ISSR-PCR analysis

To conduct ISSR-PCR analysis, the extracted genomic DNA was used as the template for PCR reactions with 20µl mixture containing 2X master mix (Taq DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers from NEXpro, Korean), Ultrapure water and ISSR primers (Table 2). PCR reactions were performed by GeneAmp PCR System 2700 (Amplied Biosystems – USA). There were two melting steps at 95°C, the first step was started for 5 min and the second for 20 sec, followed by 40 cycles. Annealing step was setup based on Tm of each primer such as 42.7°C and 42°C for ISSR B1 and ISSR B2, respectively. The primer extension step was performed at 72°C for 30 sec and 5 min, as the final step. All ISSR-PCR products were separated by 8% polyacrylamide gel electrophoresis. Then, the gels were stained with GelRed (Ethidium bromide substitution) and photographed using UV transluminator. To determine the size of each DNA band, 1 kb plus DNA ladder was used as the molecular weight marker (M).

Table 2. Listed of 13 ISSR primers and annealing temperature (Tm) used in this study

Name	Nucleotide sequence	Tm (°C)
ISSR B1	5' – TCCTCCTCCTCCTCC – 3'	42.7
ISSR B2	5' – CACACACACACAAG – 3'	42.0
ISSR B3	5' – AGGTCCAGCAGCAGCAG – 3'	51.4
ISSR B4	5' – CACGTACACTGTGTGTGTGT-GTGT – 3'	55.4
ISSR B5	5' – GACGATATGAGAGAGAGAGA-GA – 3'	46.5
ISSR B6	5' – CGTAATGAGAGAGAGAGA-GAGA – 3'	46.9
ISSR P3	5' – GACAGACAGACAGACA – 3'	48.2
ISSR P6	5' – GAGAGAGAGAGAGAGAC – 3'	52.4
ISSR P8	5' – GAGAGAGAGAGAGAGAT – 3'	49.9
ISSR P11	5' – CACCACCACGC – 3'	38.0
ISSR P14	5' – AGCAGCAGCAGCGT – 3'	46.6
ISSR P15	5' – TCCTCCTCCTCCTCC – 3'	51.7
ISSR BB7	5' – GGGCGAGAGAGAGAGAGA – 3'	55.0

(*Nirmaladevi et al., 2016)*

Data analysis

The molecular weights of polymorphic bands were measured by GelAnalyzer (Istvan, 2010), which was 1D gel electrophoresis image analysis software. The number and sizes of DNA bands were scored in binary data and determined by the distance matrix method to connect the relationships among individual samples. Eventually, a dendrogram of 16 durian cultivars was constructed using the hierarchical cluster analysis based on the unweighed pair-group method algorithm (UPGMA) by STATISTICA ver. 5.5 (StatSoft, 2000).

RESULTS AND DISCUSSION

DNA profiles

CTAB method gave A_{260}/A_{280} range of 1.84 (D24) to 1.86 (Tam Son, Kanyao, Chuong Bo), which indicated that the DNA was none degraded and free from protein or polysaccharides contaminant (Table 3).

Table 3. The quality and quantity of total DNA isolated from 16 cultivars

Sample number	Durian cultivars	Concentration (ng/µl)	A_{260}/A_{280}
1	Chanee	76.1	1.67
2	Tam Son	51.2	1.86
3	HB11	17.4	2.09
4	Kanyao	54.6	1.86
5	B31	10.5	1.73
6	Bi (D6)	62.1	1.77
7	Chin Hoa (Com Vang SHL)	47.2	1.77
8	La Queo	51.2	2.20
9	Ri 6	64.0	1.69
10	Monthong	93.0	1.78
11	Chuong Bo	44.3	1.86
12	Kho Qua	28.3	1.68
13	Sau Huu	46.8	1.72
14	D10	140.0	1.68
15	D24	119.0	1.84
16	D197	42.7	1.72

ISSR profiles

The ISSR polymorphisms were presented in detail in Table 4 that showed total of 181 distinct bands generated by 13 primers. Thus, there was an average of 13.92 scorable bands per primer and 165 bands to be considered as polymorphic among 181 bands. The highest number of polymorphic band was 17 (100%) and the lowest was 5 (62.5%), generated by ISSR P14 and ISSR P15 primers, respectively. The ISSR B1, ISSR B4, ISSR B5, ISSR P6, ISSR P8, ISSR P11 primers had totally 100% of band that were polymorphic loci.

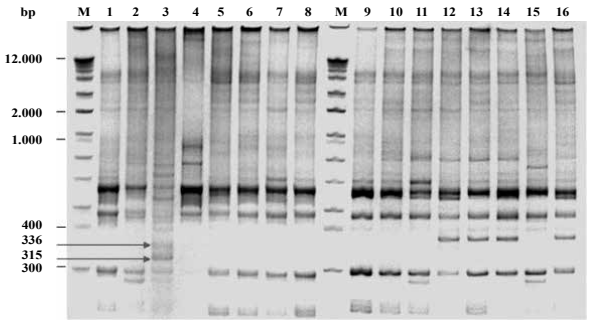

Figure 1. ISSR profile from 16 durian cultivars using ISSR BB7 primer (M: 1 kb plus molecular DNA ladder - Invitrogen, USA; lane 1-16 stand for individual durian cultivars as listed in Table 1). The pattern of HB11 in lane 3 could be separated from other cultivars by the specific bands at 315 bp and 336 bp.

Table 4. Sizes and number of amplified bands, and the percentage of polymorphic bands attained from the ISSR analysis

Primer number	Size range (bp)	Number of ISSR bands	Number of polymorphic bands	Proportion of polymorphicloci (%)
ISSR B1	300 - 1,850	11	11	100.0
ISSR B2	200 - 2,500	10	8	80.0
ISSR B3	200 - 5,000	16	15	93.8
ISSR B4	850 - 12,000	8	8	100.0
ISSR B5	300 - 6,000	17	17	100.0
ISSR B6	350 - 2,000	13	11	84.6
ISSR P3	300 - 2,500	11	10	90.9
ISSR P6	350 - 2,000	16	16	100.0
ISSR P8	200 - 2,000	17	17	100.0
ISSR P11	300 - 2,000	15	15	100.0
ISSR P14	500 - 2,000	18	17	94.4
ISSR P15	200 - 1,000	8	5	62.5
ISSR BB7	300 - 5,000	21	15	71.4
	Total	*181*	*165*	
	Average	*13.92 ± 4.07*	*12.69 ± 4.07*	*90.58 ± 12.45*

According to report of Cahyarini *et al*. (2004), the difference in cultivars could be observed from the difference in number of bands, the band thickness and mobility. Thus, the result of ISSR marker analysis for durian diversity in this study was in agreement with Cahyarini *et al*. (2004). Presently, ISSR BB7 is able to be used as a marker in order to identify HB11 cultivar, a newly-registered one, by the specific polymorphic fragments at 315 bp and 336 bp as shown in Figure 1.

Relationship among cultivars

A dendrogram as shown in Figure 2 indicated that the relationship analysis of 16 durian cultivars originated from Vietnam, Thailand and Malaysia by grouping based on the band pattern from ISSRs analysis. According to the results as shown Figure 2, the coefficients of relationships in this study ranged from 1.00 to 4.69, consisted of five clusters with the average genetic distance of approximately 2.86. The first cluster included nine cultivars, with three cultivars from Thailand (Chanee, Kanyao, and Monthong) and six cultivars from Vietnam (B31, Chin Hoa, La Queo, Bi, Ri 6 and Tam Son). In the first cluster, Chanee and Kanyao derived from Thailand were classified in the same sub-group. In addition, Tam Son cultivar found to be closely related to Monthong cultivar because they were clustered in the sub-group of the first cluster. The second cluster consisted of three cultivars, Chuong Bo, Sau Huu and Kho Qua while the third cluster were two Malaysian cultivars (D10 and D24). Two clusters included only one cultivar. D197 in the fourth cluster was a cultivar from Malaysia cultivar and HB11 in the fifth cluster was a newly-selected cultivar of Vietnam.

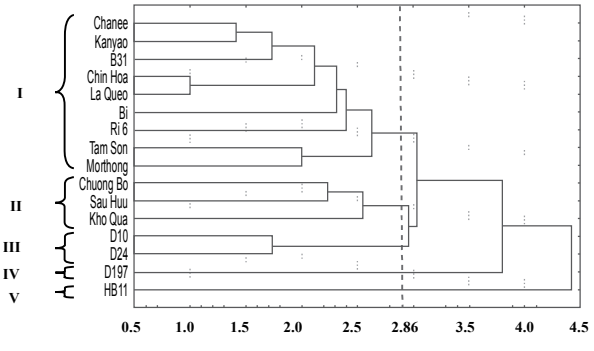

Figure. 2. The dendrogram illustrated genetic similarity and relationship of 16 durian cultivars obtained from ISSR markers based on UPGMA cluster analysis

Figure 2 showed that the dendrogram that almost durian cultivars (Cluster I) originated in Vietnam, including B31, Chin Hoa, La Queo, Bi, Ri 6, Tam Son, were grouped along with three cultivars from Thailand (Chanee, Kanyao and Monthong) which revealed an interesting information that most durian varieties of Vietnam could be the same source with Thailand's durian cultivars. In addition, ISSR might be used as DNA marker for identification of durian cultivars, exploiting ISSR BB7 to determine HB11 cultivar in this study.

CONCLUSION

In conclusion, the study has provided a specific picture of the relationships and classification of 16 durian cultivars in this study. Furthermore, this result also aimed to detect the conservation genetic resources of durian, which will be a potential value for durian breeding programs in the future.

ACKNOWLEDGEMENTS

The authors would like to give very special thanks to Dr. Yeap Swee Keong for his generosity in providing some plant materials and useful information.

REFERENCES

Ahmad, T.S., Nanthachai, S., 1994. Chapter 4: Fruit growth and development. In S. Nanthachai (ed.). *Durian: Fruit Development, Post-harvest Physiology, Handling and Marketing in ASEAN*. ASEAN Food Handling Bureau, KL, Malaysia, pp. 44-47.

Cahyarini, R.D., Yunus, A., Purwanto, E., 2004. Identification of the genetic diversity of some local soybean cultivars in Java based on isozyme analysis. *Agrosains*, 6(2): 79-83.

Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Istvan, L., 2010. *GelAnalyzer version 2010*, available at: http://www.gelanalyzer.com.

Nirmaladevi, D., Venkataramana, M., Srivastava, R.K., Uppalapati, S.R., Gupta, V.K., Yli-Mattila, T., Tsui, K.M.C., Srinivas, C., Niranjana, S.R., Chandra, N.S., 2016. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. *Scientific Reports*, pp. 1-14.

Rocha, A., Salomão, L.C.C., Salomão, T.M.F., Cruz, C.D., de Siqueira, D.L., 2011. Genetic diversity of 'Ubá' mango tree using ISSR markers. *Molecular Biotechnology,* 50(2): 108-113.

Somsri, S., 2007. *Thai Durian*. Horticulture Research Institute, Department of Agriculture, Chatuchak, Bangkok.

StatSoft, Inc., 2000. *STATISTICA for Windows*. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK 74104, phone: (918) 749-1119, fax: (918) 749-2217, available at: http://www.statsoft.com.

Tinggal, S., Tirtosoekotjo, R.A.B.S., Zainal Abidin, M., Espino, R.R.C., Huat, K.S., Sadokorn, J., 1994. Durian cultivars in ASEAN. In S. Nanthachai (ed.) Durian, *Fruit Development, Postharvest Physiology, Handling, Marketing in ASEAN*. ASEAN Food Handling Bureau, Kuala Lumpur, Malaysia, pp. 7-26.

Vanijajiva, O., 2012. The application of ISSR markers in genetic variance detection among Durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand. *Journal of Agricultural Science and Technology,* 32: 155-159.

Weising, K., Nybom, H., Wolff, K., Meyer, W., 1995. *DNA Fingerprinting in Plants and Fungi*. CRC Press, Boca Rato, pp. 1-336.

Zietkiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics,* 20: 176-183.

Date received: 15/10/2016
Date reviewed: 9/11/2016
Reviewer: Dr. Khuat Huu Trung
Date approved for publication: 20/12/2016

INSIGHT INTO THE *NUCLEAR FACTOR-YA* TRANSCRIPTION FACTOR FAMILY IN SWEET ORANGE (*Citrus sinensis*)

Chu Duc Ha*[1], Le Quoc Oai[2], Nguyen Van Giang[3], Pham Thi Ly Thu[1], Le Hung Linh[1]

Abstract

Nuclear factor Y (NF-Y), including NF-YA, NF-YB and NF-YC is well-established to play an important role in regulation of the developmental processes and stress responses in plants. In this study, 6 members of the *NF-YA* gene family were identified in the sweet orange (*Citrus sinensis*) genome. Among them, 2 members, CsNF-YA1 and CsNF-YA6 might be localized in the chloroplast, while CaNF-YA3 was thought to be associated with the secretary pathway. The common motif of the exon/intron organization of the *CaNF-YA* contains 5 exons and 4 introns. The conserved region of NF-YA is characterized by 2 typical domains, including the interaction NF-YB/C domain and the DNA binding domain. Next, various *cis*- regulatory elements related to the light responsiveness, tissues-specific expression and hormonal/stress responsiveness were predicted to be distributed on the promoter regions of *NF-YA* family, suggesting that this gene family might be related to the light conditions, tissue-specific expression and response to hormonal/stresses. Based on the available RNA-Seq, all of *CsNF-YA* genes were up-regulated in at least 1 organ/tissue. Interestingly, two genes, *CsNF-YA1* and *CsNF-YA6* were specific in callus, while *CsNF-YA4* was preferentially expressed in fruit. Three remaining genes were highly expressed in all 4 tissues/organs, including callus, flower, fruit and leaf.

Keywords: *Citrus sinensis,* NF-YA, *in silico,* sweet orange, transcription factor, stress

INTRODUCTION

NF-YA (Nuclear factor Y), one of the most important transcription factor (TF) family, was likely found in all eukaryotes. Based on the binding to the CCAAT elements, TF NF-Y proteins have roles in the regulation of diverse genes, therefore be related to various biological processes, such as the signaling pathway, and in plant stress responses (Laloum *et al*., 2013). Generally, NF-YA, including 3 subunits - NF-YA, NF-YB and NF-YC, of which NF-YA, with DNA binding sites and interaction NF-YB/C, is known as the most important subunit. Therefore, study on the genes encoding *NF-YA* is one of the interests that could be provide an insight into the regulation of NF-Y family in cellular biological processes, especially in the defense mechanisms of plant cells against the adverse environmental conditions.

Up to now, taking the advantages of omics era, NF-YA gene families have been identified and characterized in many plant species, such as in the model plant *Arabidopsis thaliana* (Siefers *et al*., 2009), *Brachypodium distachyon* (Cao *et al*., 2011), and several important crops - rice (*Oryza sativa*) (Thirumurugan *et al*., 2008), canola (*Canola napus*) (Liang *et al*., 2014), soybean (*Glycine max*) (Quach *et al*., 2015), foxtail millet (*Setaria italica*) (Feng *et al*., 2015). More recently, *NF-YA* gene family was also discovered in several newly annotated plant's

[1]Agricultural Genetics Institute; [2] Phuong Dong University
[3] Vietnam National University of Agriculture
* Corresponding author: hachu_amser@yahoo.com