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Random Amplified Polymorphism DNA Method to Authenticate Indonesian medicinal Plant Ciplukan (*Physalis angulata*; Solanaceae)

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ABSTRACT

Ciplukan (*Physalis angulata*) is a medicinal plant in Indonesia, belonging to the family of Solanaceae. Based on molecular phylogenetic analysis, this plant is relative to Ashwaganda (*Withania somnifera*), the famed South Asia medicinal plant touted to kill cancer cells. A study was conducted on genetic variation of Ciplukan using Random Amplified Polymorphic DNA (RAPD) marker. A total of 23 plants from Northern, Southern, Central, Eastern, and Western part of Bandung were examined The RAPD analyses were performed using three selected random primers (OPA1, OPB17, and OPB10). Clustering analysis was conducted based on Unweighted Pair-Group Method with Arithmetic Average (UPGMA) using MEGA 4. Dendrogram showed that the sample plants were not grouped by their geographic localities, suggesting that a genetic interaction occurs among plants from five different locations. This result was also supported by the high level of estimated gene flow (Nm= 1.0919). It is likely due to the nature of self-incompatibility in Ciplukan which requires cross-pollination, creating a higher exchange of genes and leads to homogenization of genetic composition. Overall, these results indicated no genetic differentiation, meaning that all individuals remain taxonomically under the same species.

Keywords: Ashwaganda, Ciplukan, Polymorphism, RAPD fingerprinting

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INTRODUCTION

Ciplukan (*Physalis angulata*) is a herb belonging to the family Solanaceae. It is indigenous to Indonesia and grows well in tropical and subtropical climates. In Indonesia, Ciplukan is the most popular local name for this plant, although different regions have different names for it, such as Cecenet/

Cecendet (West Java), Nyurnyuran (Madura), and Kopok-kopokan (Bali). For many years, Indonesians have used Ciplukan as food and as traditional medicine. Previous molecular phylogenetic analysis based on the DNA sequences of internal transcribed spacer (ITS) region suggest that Ciplukan is a relative of Ashwaganda (*Withania somnifera*) (Hidayat, Priyandoko, Wardiny, & Islami, 2016). This molecular data was supported by morphological characters Ciplukan and Ashwaganda resemble in term of their fruits which are covered by expanded calyx, like a balloon. As Ashwaganda is a well-known medicinal plant which has anticancer activities and indigenous to South Asia, Ciplukan could serve as an alternative for Indonesians.

Ciplukan contains saponin (in shoot), flavonoids (in leaf and shoot), phenols, physalin, tannin, cryptoxantine, ascorbic acid, sugar, Withangulatin A (in fruit), palmitate and stearat acid (in seed), alkaloid (in root), and chlorogenic acid (in stem and leaf) (Licodiedoff, Koslowski, & Ribani, 2013). These chemical components are important to treat various diseases such as schitosomiasis, trypanosomiasis, inflammation, malaria, leismania, asthma, and tuberculosis (Rengifo-Salgado & Vargas-Arana, 2013; Mahalakshmi & Nidavani, 2014).

The chemical compounds and pharmacological activities in the plant are influenced by genetic factors. Different plants of the same species may have different pharmacological activities due to their genetic variations (Hao, Gu, & Xiao, 2015). Therefore, a study on genetic variation is important.

Authentication of species is important to eliminate doubts so that the ordinary Indonesians can use the plant without being wary (Mei et al., 2014). The genetic variation analysis is also crucial before undertaking research. There are several molecular markers available to measure genetic variation among populations, of which Random Amplified Polymorphic DNA (RAPD) is the most common marker (Freeland, 2005). The advantages of RAPD among others are it (1) does not require prior knowledge of DNA sequence information; (2) requires only a small amount of DNA; and (3) is not time consuming as well as being a simple and efficient technique (Kordrostami & Rahimi, 2015).

This study was aimed at analysing genetic variation of Ciplukan in Bandung city and its surrounding area using RAPD as a marker locus, in order that the local peoples obtain valuable information before using the plant. Genetic variation refers to genetic composition of a particular area, so that if the genetic composition homogen, then the locals are able to easily use the plant, and vice versa.

MATERIALS AND METHODS

Plant Materials

The 23 plants studied were from Northern, Southern, Centre, Eastern, and Western parts of Bandung (Figure 1; Table 1).

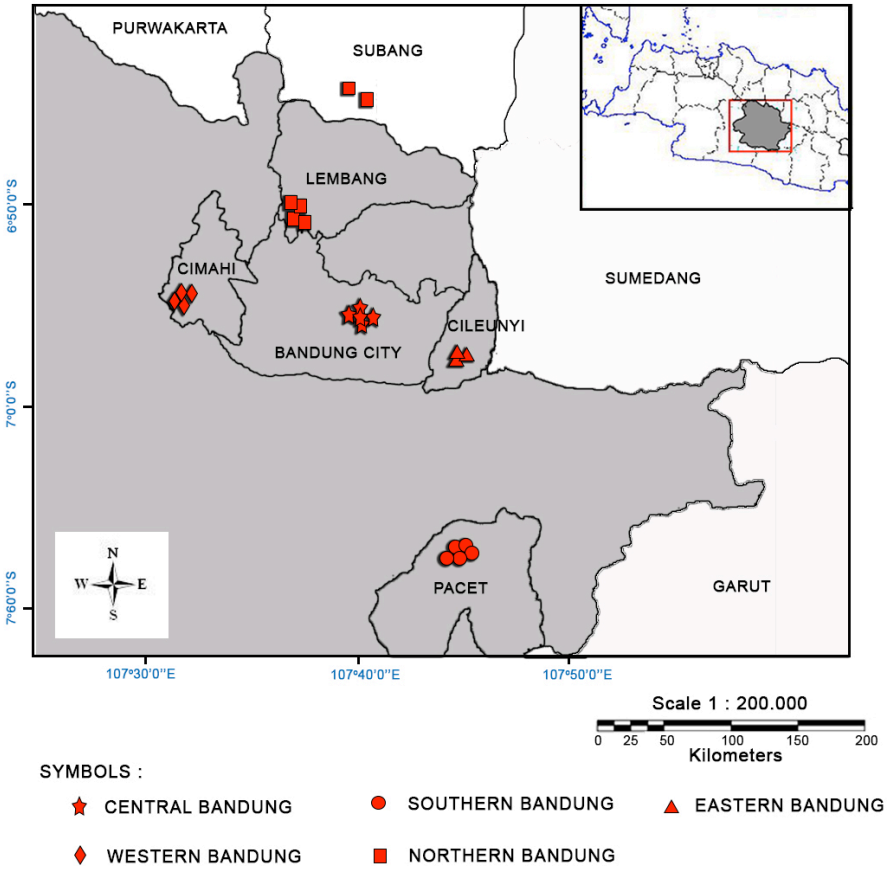

Figure 1. Map of sampling location of this study

Table 1
Plant materials examined in this study

Area	Location	Code
Northern Bandung	Komplek Pondok Hijau-1	14
	Komplek Pondok Hijau-2	15
	Cihideung-1	11
	Cihideung-2	12
	Ciater-1	20
	Ciater-2	21
Eastern Bandung	Komplek Bumi Harapan Cibiru-1	10
	Komplek Bumi Harapan Cibiru-2	19
	Komplek Bumi Harapan Cibiru-3	13
Central Bandung	Tegalega-1	3
	Tegalega-2	4
	Tegalega-3	16
	Tegalega-4	8
	Tegalega-5	5

Table 1 *(continue)*

Southern Bandung	Pacet-1	7
	Pacet-2	17
	Pacet-3	6
	Pacet-4	22
	Pacet-5	23
Western Bandung	Cibaligo-1	18
	Cibaligo-2	9
	Cibaligo-3	1
	Cibaligo-4	2

DNA Extraction and Amplification

The genomic DNA was extracted from fresh materials using a GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific, USA). Quantity and quality of the extracted DNA was determined using UV spectrophotometer (JASCO, Tokyo, Japan). The amplification of RAPD loci was carried out using three selected random primers (Table 2). The polymerase chain reaction (PCR) profile consisted of an initial 4-minute pre-denaturation at 94°C and 60 cycles of 1 minute at 94°C (denaturation), 1 minute at 32°C (annealing), and 2 minutes at 72°C (extension), followed by a final 10 minute extension at 72°C. The PCR products were separated on agarose gel (Sigma Co., USA) in 1x TAE buffer and the size of amplified fragments were estimated on the basis of 1 Kb DNA ladder (DNA marker) (Thermo Scientific, USA). 0.8 ul of PeqGreen (Peqlab Biotech., USA) were used for staining instead of ethidium bromide in 30 ml of 1.4% agarose gel, and was run for 80 minutes at 45 V, and subsequently documented using a camera phone (Oppo Smartphone F1, USA).

Table 2
The random primers used in this study

Primers	Sequences
OPA1	5' CAGGCCCTTC 3'
OPB10	5' CTGCTGGGAC 3'
OPB17	5' AGGGAACGAG 3'

Data Analysis

Amplified products were scored qualitatively for the presence (1) or absence (0) of bands. Only informative bands (clear and unambiguous) were subjected to analysis. The effectiveness of the primers was gauged based on polymorphic information content (PIC) value (Mir, Zaman-Allah, Sreenivasulu, Trethowan, & Varshney, 2012; Noormohammadi et al., 2015). Clustering analysis was conducted based on Unweighted Pair-Group Method with Arithmetic Average (UPGMA) using MEGA 4 (Tamura, Dudley, Nei, & Kumar, 2007). Gene flow was estimated based on number of migrants per generation (Nm) using POPGEN32 (Yeh, Yang, Boyle, Ye,

& Mao, 2000). Nm value was obtained from the calculation of coefficient differentiation (GST) based on frequency of allele (Nei, 1973). All analyses were based on data from the three random primers used.

RESULTS AND DISCUSSIONS

In total, 24 primers were screened, and only three primers produced bands (Figure 2). They provided information in terms of their percentage of polymorphism as well as PIC (Table 3). These three primers amplified 23 loci (7.66 loci per primer) and among them, 16 loci (5.33 loci per primer) were polymorphic. The average polymorphism was 71.58% with the highest from primer OPA1 (83.33 %).

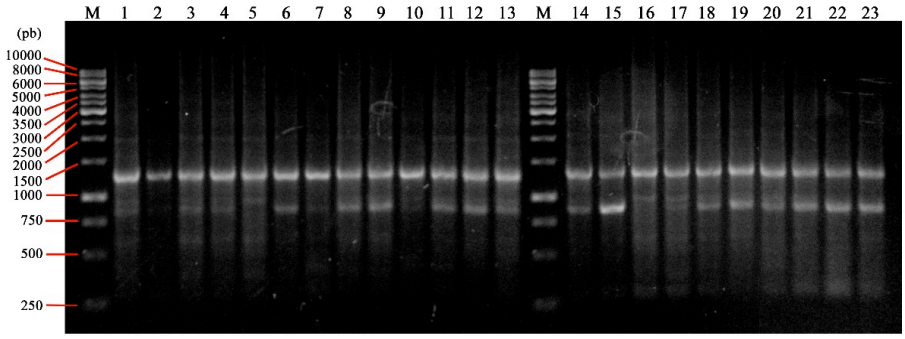

Figure 2. "The band pattern of RAPD product amplified by primer OPA1. Lane 1-23 correspond to code of individual as described in Table 1. M = DNA marker"

One important factor that influences the polymorphism in RAPD analysis is the quality of primers. As shown in Table 3, the primers used in this study were effective and informative indicated by good PIC value which is 0.425 in average. As for dominant markers such as RAPD, a good primer must have PIC value between 0 and 0.5 (De Riek, Calsyn, Everaert, Van Bockstaele, & De Loose, 2001).

Table 3
Effectivity of primers used

Primers	Number of loci	Polymorphic loci	% Polymorphism	PIC
OPA1	6	5	83.33	0.462
OPB10	7	5	71.42	0.395
OPB17	10	6	60	0.420
Average	7.66	5.33	71.58	0.425

As mentioned earlier, dataset from individual primers was combined for further analysis. Every single primer produced a unique band pattern which is different from other primers. By combining all three primers, a comprehensive and plausible results will be achieved (Zybartaite

et al., 2011). Combining dataset can also be done among different markers, such as RAPD and microsatellite (Tripathi, Saini, Mehto, Kumar & Tiwari, 2012). Clustering analysis showed three major groups among the 23 samples used in this study (Figure 3). However, the groups did not correspond with their geographical origin. This result was supported by value of gene flow (Nm= 1.0919; see Table 4), which means 1.09 individual per generation migrate among different plants randomly.

Genetic composition of particular populations is examined as well as its relationships with other populations in the particular area, so that genetic differentiation that leads to speciation event in the populations can be identified. This situation may cause confusion of the local peoples to access the plant, and therefore, authentication is needed (Mei et al., 2014).

The dendogram reconstructed in this study (Figure 3) showed that the individuals examined were not grouped by their geographic localities, suggesting that a genetic interaction occurs

Table 4
Gene flow estimation for Ciplukan in Bandung

Loci	Sample Size	Diversity of gene in total (Ht)	Diversity of gene in population (Hs)	Coefficient Differentiation (Gst)	Estimation of gene flow(Nm)
2004 bp	23	0,1629	0,0989	0,3929	0,7729
1862 bp	23	0,4883	0,3872	0,207	1,9149
1634 bp	23	0,4602	0,2222	0,5171	0,467
1478 bp	23	0,4593	0,2747	0,4019	0,7441
1215 bp	23	0,15	0,966	0,556	0,9059
1132 bp	23	0,1454	0,1355	0,0683	6,8241
1119 bp	23	0	0	****	****
1083 bp	23	0	0	****	****
1028 bp	23	0,2667	0,1831	0,3136	1,0943
992 bp	23	0	0	****	****
947 bp	23	0	0	****	****
849 bp	23	0,2428	0,0828	0,6589	0,2589
840 bp	23	0,4604	0,4147	0,0993	4,5373
818 bp	23	0,3868	0,3492	0,097	4,6532
770 bp	23	0,3504	0,193	0,4492	0,613
737 bp	23	0,4728	0,2734	0,4217	0,6857
671 bp	23	0	0	****	****
622 bp	23	0,4355	0,2941	0,3246	1,0401
608 bp	23	0	0	****	****
583 bp	23	0,3509	0,2794	0,2036	1,9552
547 bp	23	0	0	****	****
509 bp	23	0,4355	0,2941	0,3246	1,0401
392 bp	23	0,288	0,1907	0,1642	2,544
Mean	23	0,239	0,1639	0,3141	1,0919
St. Dev		0,0368	0,0195		

among the plants from five different regions. It is likely due to the nature of self-incompatibility in Ciplukan which requires cross-pollination (Sullivan, 1984). Human activities are also another factor that causes the interaction (Martinez, Freire, Arias-Perez, Mendez, & Insua, 2015). As people in Bandung use Ciplukan as a medicine, they may cultivate the plant in other locations.

This genetic interaction as depicted in Figure 3 was also supported by the high level of estimated gene flow (Table 4). The higher the gene flow, the lower the genetic differentiation among the samples (Mahjoub, Mguis, Rouaissi, Abdellaoui, & Brahim, 2012). The high level of gene flow can prevent differentiation or speciation (in the context of taxonomy) due to exchange of gene among populations through migration of individuals and pollination in order to obtain a homogeneous genetic composition (Morjan & Rieseberg, 2004).

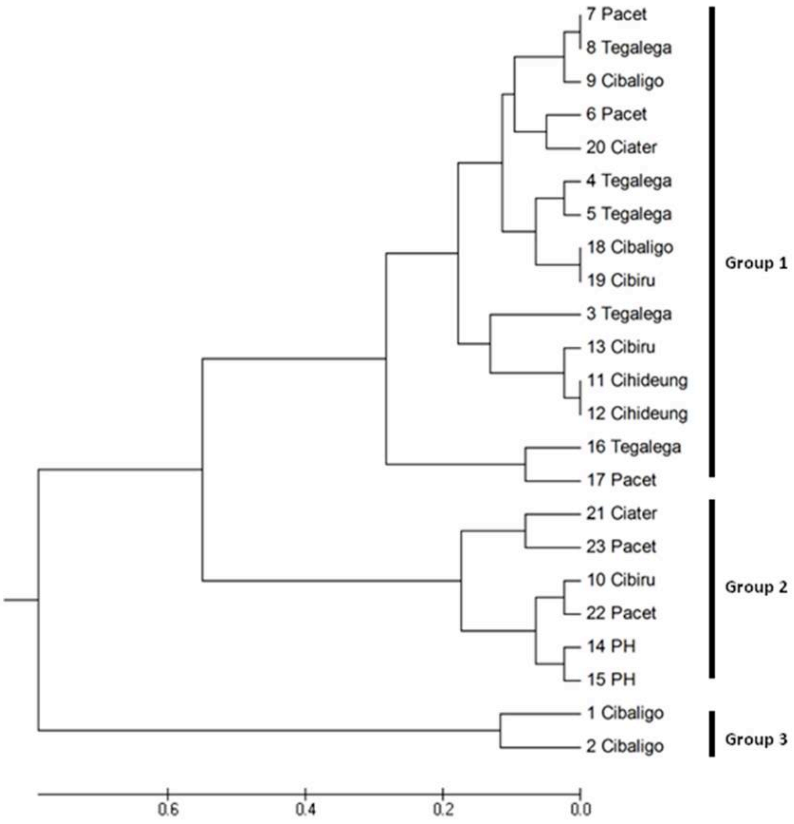

Figure 3. " Dendogram (UPGMA) of combined data (three primers) shows remakable genetic interaction among individuals from five different populations. Three major groups were recognised."

CONCLUSION

This study found that the genetic composition of Ciplukan in Bandung was homogenous. All the Ciplukan plants in Bandung were authentic, and taxonomically under the same species. This means the locals in Bandung are able to easily access the plant without hesitation. Moreover, this study has proved that the RAPD analysis can be applied to authenticate Ciplukan in other regions.

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REFERENCES

De Riek, J., Calsyn, E., Everaert, I., Van Bockstaele, E., & De Loose, M. (2001). AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. *TAG Theoretical and Applied Genetics, 103*(8), 1254-1265.

Freeland, R. J. (2005). *Molecular ecology*. England: John Wiley & Sons.

Hao, D.C., Gu, X.J., & Xiao, P.G. (2015). *Medicinal plant: Chemistry, biology and omics*. England: Woodhead Publishing Series in Biomedicine.

Hidayat, T., Priyandoko, D., Wardiny, P. Y., & Islami, D. K. (2016). Molecular Phylogenetic Screening of Withania somnifera Relative From Indonesia Based on Internal Transcribed Spacer Region. *HAYATI Journal of Biosciences, 23*(2), 92-95.

Kordrostami, M., & Rahimi, M. (2015). Molecular markers in plants: concepts and applications. *G3M, 13*, 4024-4031.

Licodiedoff, S., Deitos Koslowski, L. A., & Hoffmann Ribani, R. (2013). Flavonols and antioxidant activity of Physalis peruviana L. fruit at two maturity stages. Acta Scientiarum. *Technology, 35*(2).

Mahalakshmi, A. M., & Nidavani, R. B. (2014). Physalis angulata L.: An ethanopharmacological review. *Indo American Journal of Pharmaceutical Research*.

Mahjoub, A., Mguis, K., Rouaissi, M., Abdellaoui, R., & Brahim, N. B. (2012). RAPD analysis of genetic diversity in natural populations of Aegilops geniculata Roth and Triticum durum Desf from Tunisia. *Agriculture and Biology Journal of North America, 3*, 466-475.

Martínez, L., Freire, R., Arias-Pérez, A., Méndez, J., & Insua, A. (2015). Patterns of genetic variation across the distribution range of the cockle Cerastoderma edule inferred from microsatellites and mitochondrial DNA. *Marine Biology, 162*(7), 1393-1406.

Mei, Z. Q., Fu, S. Y., Yu, H. Q., Yang, L., Duan, C., Liu, X., ... & Fu, J. (2014). Genetic characterization and authentication of Dimocarpus longan Lour using an improved RAPD technique.*Genet Mol Res, 13*(1), 1447-55.

Mir, R. R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R., & Varshney, R. K. (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theoretical and Applied Genetics, 125*(4), 625-645.

Morjan, C. L., & Rieseberg, L. H. (2004). How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology, 13*(6), 1341-1356.

Nei, M. (1973). Analysis *of gene diversity in subdivided populations.* Proceedings of the National Academy of Sciences, 70(12), 3321-3323.

Noormohammadi, Z., Shafaf, T., Farahani, F., Sheidai, M., Talebi, S. H., & Faharani, Y. H. A. (2015). Within and among-genetic variation in Asian falx *Linum austriacum* (Linaceae) in response to latitude changes: cytogenetic and molecular analyses. *Biodiversitas, 16*, 145-150.

Rengifo-Salgado, E., & Vargas-Arana, G. (2013). Physalis angulata L.(Bolsa Mullaca): a review of its traditional uses, chemistry and pharmacology. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 12*(5).

Sullivan, J. R. (1984). Pollination biology of Physalis viscosa var. cinerascens (Solanaceae). *American Journal of Botany,* 815-820.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*(8), 1596-1599.

Tripathi, N., Saini, N., Mehto, V., Kumar, S., & Tiwari, S. (2012). Assessment of genetic diversity among Withania somnifera collected from central India using RAPD and ISSR analysis. *Med Aromatic Plant Sci Biotechnol, 6*(1), 33-39.

Yeh, F. C., Yang, R., Boyle, T. J., Ye, Z., & Xiyan, J. M. (2000). PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. *Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada*.

Zybartaite, L., Zukauskiene, J., Jodinskiene, M., Janssens, S. B., Paulauskas, A., & Kupcinskiene, E. (2011). RAPD analysis of genetic diversity among Lithuanian populations of Impatiens glandulifera. *Žemdirbystė=Agriculture, 98*, 391-398.