RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
50 (2) 156-167, 2018

VARIABILITY AND INTRASPECIFIC CLASSIFICATION OF INDONESIAN EDIBLE CANNA (*Canna indica* L.) BASED ON RAPD MARKER ANALYSIS

N. SARI[1], SURYADIANTINA[2], B.S. DARYONO[3] and PURNOMO[4*]

[1]Faculty of Biology, Universitas Gadjah Mada, Bulaksumur Yogyakarta 55281, Indonesia
[2]Indonesian Center for Agricultural Biotechnology Research and Development, Indonesia
[3]Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Bulaksumur Yogyakarta 55281, Indonesia
[4]Laboratory of Plant Systematic, Faculty of Biology, Universitas Gadjah Mada, Bulaksumur Yogyakarta 55281, Indonesia
*Corresponding author's email: nomo@ugm.ac.id
Email addresses of coauthors: novitabiougm90@gmail.com, budi.daryono@ugm.ac.id, dianpbt11@yahoo.com

SUMMARY

Edible canna (*Canna indica* L.) is herbaceous plant originated from South America which its rhizome commonly used and its utillization not optimize in Indonesia. Molecular characterization are needed to determine characters of *C. indica* for genetic variability to improve edible canna varieties. For that reason, the purpose of this research was to determine diversity and phenetic relationship of *C. indica* based on molecular characters using RAPD analysis. Samples were collected from Indonesian Center for Agricultural Biotechnology Research and Development (BB-Biogen). Data was analysed by RAPD analysis using OPA-01, OPA-02, OPA-10, OPD-02, and OPN-05 primers. Similarity index was counted by Gower General Similarity Coefficient formula based on molecular scoring. Cluster analysis was conducted by Unweighted Pair Group Methods using Arithmetic averages (UPGMA) method to create a dendogram. Principal component analysis (PCA) was also performed to defined role of each molecular character in grouping of accessions with Multivariate Statistical Program (MVSP) v.3.1 software. The results showed that *C. indica* has divided into two main clusters. There were the green and red cultivar groups with 56% similarity. The green cultivar group also divided into green and green purple based on colour of sheats, tip of bud, rachis inflorescence, petals, brachtea, and colour pattern of staminodia with 73 % similarity. Red cultivar divided into red and red purplish based on colour of sheats, rachis inflorescence, and petals with similarity index 74%.

Key words: *Canna indica* L., cultivar group, molecular, phenetic relationship

Key findings: This research work will provide basic information about Indonesian edible canna which has potential to be applied in a commercial breeding and industrial program.

Manuscript received: February 5, 2018; Decision on manuscript: March 14, 2018; Accepted: May 15, 2018.

Communicating Editor: Dr. Sambasivam Periyannan

INTRODUCTION

Edible canna (*Canna indica* L.) is herbaceous plant originated from South America which its rhizome commonly used (Tanaka, 2001). Its origin and center of genetic diversity was from South American (Ugent *et al*., 1984; Gade, 1966). This plants dispersed through all tropical state (Piyachomkwan *et al*., 2002; Matoba *et al*., 2011). It's usually grow wild in dry field as auxiliary plants (Gade, 1966). In Indonesia the utilization of its rhizome has not been optimized as people only boiled its rhizome to consume without further processing (Sastrapradja *et al.,* 1977; Ugent *et al*., 1984; Gade, 1966; Matoba *et al*., 2011).

Supriyanta *et al*. (2000) classified 89 accession of *Canna indica* based on morphological characterization such as leaves colour, flowers colour, bract colours, plants high, rhizomes diameter, and size of leaves, but still the morphological traits were not sufficient to distinguish them based on colour of flowers, spathe and rhizomes.

Suhartini and Hadiatmi (2010) reported that potential tubers like edible canna are not in the list of priority for research and development in Indonesia. But keeping in view the importance of edible canna and its extinction from Indonesia, characterization of edible canna provides the unique opportunity for their identification, conservation, and management along with subsequent utilization in crop improvement programmes. Molecular approach represent a potential tool for effective characterization of germplasm which circumvents the limitations associated with morphological

While characterization based on RAPD markers have been extensively studied in species like Dioscorea spp. (Purnomo *et al*., 2016), *Jatropha curcas* L. (Susantidiana *et al*., 2009), *Phalaenopsis* orchid (Dwiatmini *et al*., 2003), *Allium cepa* L. (Laila *et al*., 2013), and *Cucumis sativus* L. (Julisaniah *et al*., 2008) due to simplicity, cost effectiveness, does not require any specific knowledge of the DNA sequence of the target organism (Wiliam *et al*., 1990) .This study describes an investigation of the genetic variability based on RAPD markers to identify and classify Indonesian edible canna cultivars groups.

MATERIALS AND METHODS

Plant material

Edible canna samples were collected from Indonesian Center for Agricultural Biotechnology Research and Development (BB-Biogen) which is collected from Java Island and around it. Determination of accession was used in this study based on the

result of genetic variability; to compare the clusters formed morphologically (Sari *et al*., 2016). The accession number, local name, origin, and morphological characters of samples are listed in Table 1. The specimen was deposited in Indonesian Center for Agricultural Biotechnology Research and Development (BB-Biogen) field as a life plants.

Table 1. Accession number, cultivar (local names), origin of accession, morphological characters of *C. indica*. The specimen was deposited in Indonesian Center for Agricultural Biotechnology Research and Development (BB-Biogen) field as a life plants.

No.	Accession number	Local name of cultivar	Origin of accession	Morphological characters (bud, bract, midrib of leaves, outside staminode, rachis, and fruit's colour)
1.	CI-01	Ganyong bodas	Loji, Karawang, West Java	Green, Green, transparent, yellow with red spot, orange, green
2.	CI-02	Ganyong Putih	Selomerto, Wonosobo, Central of Java	Green purple, green purple, transparent, red, orange, green with purple spot
3.	CI-16	Ganyong	Dorowati, Tulung, Klaten, Central of Java	Green, green, transparent, yellow with red spot, orange, green
4.	CI-19	Ganyong	Pager sari, Kalidawir, Tulung agung, East Java	Green, green, transparent, yellow with red spot, orange, green
5.	CI-29	Sebeg hijau	Lembah sari, Batu layar, Nusa Tenggara, East Indonesia	Green purple, green purple, transparent, red, orange, green with purple spot
6.	CI-37	Ganyong	Cibulan, Medangikura, Temanggung, Central of Java	Purple, green purple, purple, red, orange, green purple
7.	CI-43	Ganyong	Lebak, Banten	Purple, purple green, purple, red, orange, green purple
8.	CI-53	Ganyong	Nawungan, Panggang, Yogyakarta	Purple, purple green, purple, red, orange, green purple
9.	CI-66	Ganyong abang	Glenmore, Banyuwangi, East Java	Purple, purple, purple, purple, red, purple
10.	CI-62	ganyong beureum	Cianjur, West Java	Purple, purple green, purple, red, orange, green purple
11.	CI-38	Ganyong abang	Puru, Suruh, Trenggalek, East Java	Purple, purple green, purple, red, orange, green purple
12.	CI-65	Sebeg merah	Bima, Nusa Tenggara Barat	Purple, purple green, purple, red, orange, green purple

DNA isolation

DNA isolation was isolated from 0.2 g dried leaf, using CTAB method with some modification (Doyle and Doyle, 1987). Leaf of each sample was grind into podwer and then mixed with 700 µl preheated extraction buffer (2% CTAB, 5 M NaCl; 1 M Tris-HCl, 0.5 M EDTA, 2% PVP, 2% β-mercaptoethanol). This buffer was preheated to 60 ^{0}C. The solution was incubation for 30 minute and while incubation it was invert every 5 minute. After incubation it was mixed with 700 µl of phenol/chloroform/isoamylalchohol (25:24:1) and centrifuged at 12.000 rpm for 1 minute at room temperature. The DNA was precipited by adding equal volume of cold isopropanol with lysate and incubated for 3 hours at 4 ^{o}C. The pellet was dissolved with 100 µl of high salt TE buffer and mixed with 800 µl ethanol. The solution was incubated for 15 minute at -20^{o}C. The pellets were air-dried and dissolved in 100 µl of TE buffer.

RAPD marker genotyping

Five RAPD primers such as OPA-01, OPA-02, OPA-10, OPD-02, and OPN-05 (Table 2) were used for polymerase chain reaction (PCR-RAPD) amplification. The reaction conditions were as described by Williams *et al*. (1990) with modification. PCR reaction was conducted at 94 ^{0}C for pre-denaturizing 5 minutes, 94 ^{0}C denaturizing 30 second, 36 ^{0}C annealing (temperature melting) 2 minutes, 72 ^{0}C elongation 1 minutes, 72 ^{0}C for post-elongation 5 minute, and it was conducted on 40 cycles. The composition of PCR reaction master mix was shown in Table 3. A total of 10 µl of PCR product (RAPD) were separated by electrophoresis on 1.5% agar gel in 1.0 x TBE buffer mixture with 5 µl staining gel. DNA bands were visualized under ultraviolet light and photographed using a digital camera for data recording. In this article only two pictures of the RAPD markers profiles were shown in Figure 1, that was primer OPA-2 and OPN-5 RAPD markers profiles.

Table 2. Primers and their nucleotides base sequence used in this study.

No.	Primers	Nucleotide sequence	References
1	OPA-01	5'-CAGGCCCTTC-3'	(Tanaka, 2001)
2	OPA-02	5'-TGCCGAGCTG-3'	(Tanaka, 2001; Patra *et al*., 2008)
3	OPA-10	5'-GTGATCGCAG-3'	(Tanaka, 2001; Patra *et al.*, 2008)
4	OPD-02	5'-GGACCCAACC-3'	(Patra *et al*., 2008)
5	OPN-05	5'-ACTGAACGCC-3'	(Patra *et al*., 2008)

Table 3. Composition of PCR master mix.

Reagent	Amount per one reaction (µl)	Concentration
PCR-*grade water*	3	-
KAPA2G™ Fast ReadyMix (2x)	12.5	-
Primer RAPD	2.5	10 µM.
$MgCl_2$	2	25 mM
DNA	5	25 ng
Final volume	25.00	-

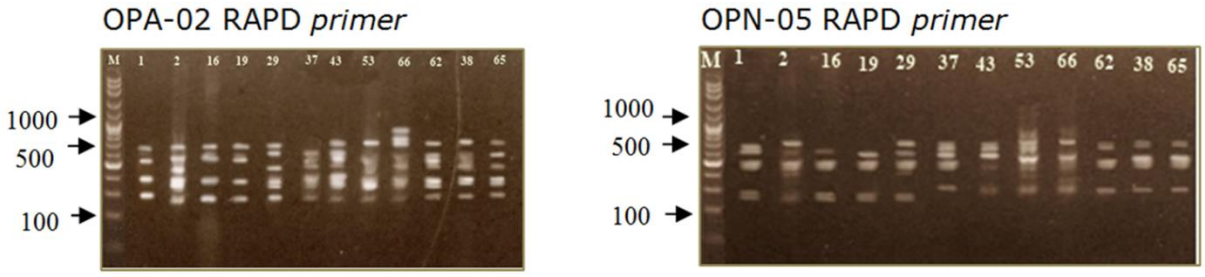

Figure 1. RAPD markers profiles generated by random primer OPA-02, and OPN-05 in 12 Indonesian edible canna accessions. Lane M (marker); lane 1, 16, 19 (accession with green bud, green bract, transparent midrib of leaves, yellow with red spot outside staminode, orange rachis, and green fruit's characters); lane 2 & 29 (accession with green purple bud, green purple bract, transparent midrib of leaves, red outside staminode, orange rachis, and green with purple spot fruit's characters); lane 37, 43, 53, 38, 62, 65 (accession with purple colour of bud, purple green colour of bract, purple colour in the midrib of leaves, red colour of outside staminode, orange colour of rachis, and green purple colour of fruits characters); lane 66 (accession with purple colour of bud, bract, midrib of leaves, outside staminode, rachis, and fruit's characters).

Scoring and analysis

The position of the RAPD bands in each electrophoresis lane was marked in base pairs (bp), by comparing the marker profile with the DNA ladder. Data recording (scoring) was conducted based on presence (1) or absence (0) of the bands.

The binary data matrix of RAPD compiled by the 5 primers of 12 edible canna cultivars. Similarity coefficients were calculated with *Gower General Similarity Coefficient* (Sokal and Sneath, 1963). Based on similarity index data (Table 4), cluster method algoritm Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) was used to construct dendrogram using *Multivariate Statistical Program* (MVSP) *software* versi 3.1 pc (Kovach, 2007). *Principle Component Analysis* (PCA) was also conduct to known role of each characters in grouping (clustering) of samples.

RESULTS

Analysis of polimorphism by RAPD

The perusal of data showed in Table 5 that out of five primers OPA-01, OPA-02, OPA-10, OPD-02, and OPN-05 only OPA-02 and OPN-05 primers produced higher polymorphism. Polymorphic RAPD markers from 5 primers RAPD were listed in Table 5.

Relationship and classification of Indonesian *C. indica* cultivars

Supriyanta *et al*. (2000) was done with 89 accession numbers which has various characters such as leaves colour, flowers colour, bract colours, plants high, rhizomes diameter, and size of leaves. Among all characters, leaves colour, bract colour, and flowers (staminode) colour was the prominent characters which can used as main character for accession classification of edible canna. Based on those 3 prominent characters, 89

Table 4. Similarity matrix between accessions of *C. indica* based on RAPD analysis.

Accession number	CI-01	CI-02	CI-16	CI-19	CI-29	CI-37	CI-43	CI-53	CI-62	CI-66	CI-38	CI-65
CI-01	1											
CI-02	0.75	1										
CI-16	0.929	0.714	1									
CI-19	0.911	0.732	0.982	1								
CI-29	0.714	0.893	0.75	0.768	1							
CI-37	0.536	0.643	0.464	0.482	0.607	1						
CI-43	0.571	0.643	0.5	0.518	0.643	0.929	1					
CI-53	0.536	0.607	0.5	0.518	0.643	0.857	0.893	1				
CI-62	0.518	0.554	0.482	0.5	0.554	0.804	0.839	0.839	1			
CI-66	0.536	0.607	0.5	0.518	0.643	0.714	0.75	0.821	0.696	1		
CI-38	0.571	0.643	0.536	0.554	0.643	0.893	0.929	0.893	0.911	0.75	1	
CI-65	0.571	0.643	0.536	0.554	0.643	0.893	0.929	0.893	0.911	0.75	1	1

Table 5. Polimorphic RAPD markers from 5 primers RAPD.

No.	Primer	Sequence nucleotide	Number of RAPD fragment	Number of polymorphic RAPD fragment	Number of monomorphic RAPD fragment	Fragment sized (*base pair*) RAPD
1	OPA-01	5’-CAGGCCCTTC-3’	12	9	3	270-1700
2	OPA-02	5’-TGCCGAGCTG-3’	12	10	2	290-1000
3	OPA-10	5’-GTGATCGCAG-3’	11	9	2	350-1200
4	OPD-02	5’-GGACCCAACC-3’	9	8	1	450-1500
5	OPN-05	5’-ACTGAACGCC-3’	12	11	1	250-850
Total			56	47	9	

accession number can be grouped in 3 group, that is 'red' group for edible canna with red colour on margin of leaves, red purplish bract, and red staminodes; 'green' group for edible canna with leaves with transparent margin, green purplish bract, and red flowers; and 'green yellow' for edible canna with transparent margined, green bract, yellow labellum, and yellow staminode with red spot. The morphological different between 3 group were not clear. Tanaka (2001) was also classified 4 mophological types of Canna rhizomes. The rhizome of *C. indica* was short creeping, bearing many roots at nodes, and the scaly leaves are brownish. Morphological traits does not always reflect real genetic trait, but based on RAPD analysis can be identified clearly.

Dendogram based on molecular characterization showed that five accessions were divided into 2 clusters i.e. cluster A and B at 0.56 coefficient similarity (Figure 2). Cluster A comprised of 7 accessions (C-37, C-38, C-43, C-53, C-62, C-65, and C-66 accession) and cluster B with 5 accessions (C-01, C-02, C-16, C-19, and C-29 accession). The result showed that the green leaves with purple colour of canna lied in the one group comprising cluster A in dendrogram whereas green leaves, green bud, green bract, green veins form another group of cluster B.

Based on dendogram, cluster A also divided into 2 subcluster, subcluster A1 and A2 with 0,74 0.74 coefficient similarity. The difference of subcluster A1 and A2 can distinguished from OPA-01 (1.700 bp), OPA-02 (370 bp), OPA-10 (400 bp, 700, and 1.000 bp), OPD-02 (500 bp and 1000 bp), and OPN-05 (700 bp) primers. Subcluster A1 was consist only east java sample. It has purple colour of bud, bract, midrib of leaves, outside staminode, rachis, and fruit's. Cluster A2 consist of 6 samples. It was collected from Bima, west java, Central of java, Banten, east java, and Yogyakarta. It has purple colour of bud, purple green colour of bract, purple colour in the margin of leaves, red colour of outside staminode, orange colour of rachis, and green purple colour of fruits.

Cluster B was also divided into 2 subcluster based on dendogram, subcluster B1 (samples from central of Java and East Indonesia) and B2 (samples from west java, central of java, and east java) with 0.73 coefficient similarity. The difference of subcluster B1 and B2 can be seen in primer OPA-01 (300 bp, 600 bp, and 1.700 bp), OPA-02 (830 bp and 1.000 bp), OPA-10 (500 bp and 1.000 bp), and OPN-05 (850 bp). Subcluster B1 morphologically has green purple bud, green purple bract, transparent margin of leaves, red outside staminode, orange rachis, and green with purple spot fruit's. Morphological of subcluster B2 has green bud, green bract, transparent margin of leaves, yellow with red spot outside staminode, orange rachis, and green fruit's.

In this research was also done to known role of each molecular character in grouping of edible canna. Grouping pattern based on PCA (Figure 3) showed equal result with grouping from cluster analysis. There was 4 group of accession which is accession number CI-01, CI-19, and CI-16 become one group supported by OPN-05 (280 bp) and OPA-10 (700 bp) primers, accession number CI-02 and CI-29 grouped in same group supported by OPA-10 (500 bp) and OPN-05 (250 bp) primers, accession

number CI-37, CI-43, CI-62, CI-65, CI-53, and CI-38 was grouped by OPN-05 (300 bp) and OPA-02 (500 bp) primers, accession number CI-66 separated from other accessions and become its own group supported by OPA 10 (1.000 bp and 2.100 bp), OPA-02 (600 bp), and OPN-05 (700 bp) primers.

Each of primer has DNA specific band which supported grouping of cluster. In 56 characters that used to relationship molecular analysis by RAPD, 23 characters can categorized as a characters that has high diagnostic value. Number of characters that has high diagnostic value consist of 6 characters in OPA-01 (300 bp, 600 bp, 700 bp, 720 bp, 800bp, and 1.700 bp) primers, 4 characters in OPA-02 (370 bp, 450 bp, 500 bp, and 600 bp) primers, 6 characters in OPA-10 (350 bp, 400 bp, 500 bp, 700 bp, 950 bp, and 1.200 bp) primers, 3 characters in OPD-02 (500 bp, 900 bp, and 1.000 bp), and 4 characters in OPN-05 (250 bp, 300 bp, 600 bp, and 700 bp) primers.

DISCUSSION

Genetic variability can be caused by environment or internal factors, such as mutation. Based on Figure 2 we known that geographic diversity didn't caused an impact in the grouping of accession. This is clearly seen in the A2 that showed CI-62 which collected from Bima joined with another accession from west Java (CI-62), Yogyakarta (CI-53), central of java (CI-37), East Java (CI-38), and Banten (CI-43). Cluster B1 also grouping not based on geographical of the samples, CI-29 dari NTB joined with CI-02 which is come from central of Java.

Based on Figure 2, also known that grouping of the samples was showed that *C. indica* has stability gene of variability which are not influenced by environment. Tanaka (2001) used 20 *primer* RAPD also clearly divided *C. indica* including their intraspecies taxa belong one cluster. That cluster described that *C. indica* genetically divided from other taxa. Prince (2010) in her research about Cannaceae also clearly grouped *C. indica* cultivar from other *Canna* species which collected from another region in Peru using RAPD. Piyachomkwan *et al*. (2002) in his research in 4 types of *C. indica* in Thailand using 4 *primer* of RAPD also clearly divided 4 types *C. indica* become 3 group without any correlation of their geographical origin. Four canna varieties indigenous to Thailand were examined including Thai-green, Japanese-green, Thai-purple and Chinese-purple and compared with cassava (*Manihot esculenta* Crantz). Using RAPD technique employing ten 10-base primers, four primers implied that at least three types of canna including Thai-green, Japanese-green and Thai/Chinese-purple existed and corresponded to plant characteristics as identified by flower, stem, leaf, and rhizome colors. In this research, based on approximation method of *artificial diagnostic-morphological classification* toward RAPD markers and cluster analysis, *C. indica* can be classified into 2 cultivar, that is 'green' and 'red' group and 4 subcultivar group, that is 'green', 'green purplish', 'red', 'red purplish' as shown in Table 6.

Based on Figure 2 in cluster A2, it is likely *C. indica* cultivars were selected from existing variant through clonal propagation although this accession collected from different

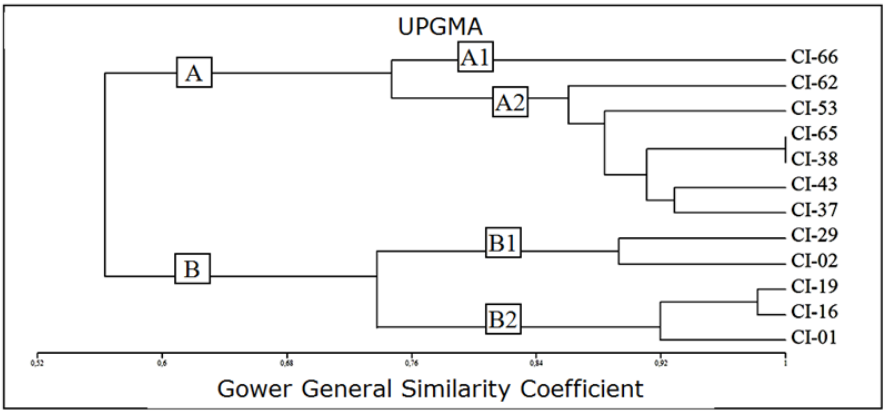

Figure 2. Dendogram illustrating the variability and similarity relationship of *Canna indica* based on RAPD analysis.

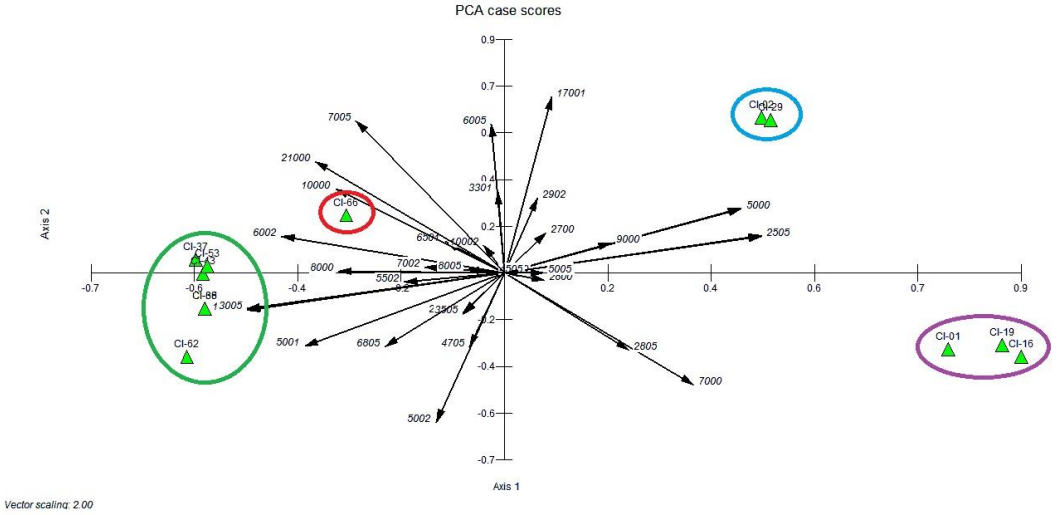

Figure 3. Diagram pattern of grouping accession and spread pattern of molecular characters of *Canna indica* cultivar groups that define grouping accession.

region. Gade (1966) said that the part of the canna plant used for planting is the small corm-like rhizome segment. Peoples influence in the distribution process have a great deal in the process of genetic variation.

Another people influences in variation genetic was selection and hybrid process (Panwar *et al.*, 2013). Furthermore, Indrawan *et al*. (2007) said that individual asexual generation genetically same with the parents and usually has lowest genetic variation. Sources of gen combination in the asexual population are mutation. Mutation can be done spontaneously (mismatch in genetic replication) or by external factors such as UV radiation and another chemistry agent. Another cause of genetic diversity of *C. indica* was *C. indica* produce fertile flower, with the result may occurs hybrid fertilization, although probability of seed forming are small.

Table 6. Intraspecific classification (non-formal), group cultivar, local names, and accession origin from 12 *C. indica* cultivars using RAPD markers.

Group Cultivars	Sub-group Cultivars	Local name of cultivar	Collection sites
Red group	Red purplish	Ganyong abang	Glenmore, Banyuwangi, East Java
	Red	Ganyong	Medangikura, Temanggung, Central of Java
		Ganyong	Lebak, Banten
		Ganyong beureum	Cianjur, West Java
		Ganyong abang	Trenggalek, East Java
		Sebeg merah	Bima, Nusa Tenggara
Green group	Green purplish	Ganyong putih	Wonosobo, Central of Java
		Sebeg hijau	Batu layar, Nusa Tenggara
	Green	Ganyong bodas	Karawang, West java
		Ganyong	Klaten, Central of Java
		Ganyong	Tulungagung, East Java

Edible canna has various advantages. This plant's rhizome can be used as raw materials for flour and fuel because of its high starch contain (Dewi *et al.*, 2011). The starches granules were larger (10-80μm) compared with cassava starch, pastes had a higher peak viscosity (930-1060 BU) than cassava starch, occurring at a higher temperature. Pastes of canna starch were more stable and when cooled, viscosity increased to 1800 BU. Gelatinized pastes of canna starches also rapidly formed good gels on cooling. It is evident that edible canna provides starches with very attractive properties and totally different from cassava and is the greatest promise for the new base starch to be employed complementarily with cassava starch (Piyachomkwan *et al.*, 2002). The plant's rhizomes are also used to make noodles, was conducted in Vietnam (Hermann *et al*., 1997). The edible canna starch was shown to have a large granule size, slightly higher amylase content, longer amylopectin unit-chains, a lower gelatinization temperature, and higher viscosity and retro gradation in comparison with maize starch (Tanaka *et al*., 2006).

Based on Figure 3, grouping pattern based on PCA showed equal result with grouping from cluster analysis. This also shows that primer was a stable primer to be used in determining genetic variation of *C. indica*. Panwar *et al.* (2013) using PCA in his research on 46 *Aloe* accession in India and known that all of the accession divers in equal rates in two axes. Furthermore, it is grouped as a UPGMA dendogram. This was also showed a high of genetic variability.

CONCLUSION

Based on molecular characters edible canna (*Canna indica* L.) in Indonesia was divided in two cultivar group. It was 'green' and 'red' cultivar which green cultivar divided into two sub-cultivar, 'green' and 'green purplish'. Red cultivar was also divided into two sub-cultivar, 'red' and 'red purplish'. Result of PCA analysis showed that used primer was a stable primer to be used to determine genetic variation of *Canna indica* cultivar and grouping of

the cultivar was not influenced by geographical origin.

ACKNOWLEDGEMENTS

Thankful to the head officer of Directorate General of Higher Education for founding this research through grant No: 0094/E4.4/2013 and Indonesian Center for Agricultural Biotechnology Research and Development (BB-Biogen) for lent me to conducted research using their collection plant trough document No: 6124/LB.230/I.11/10/2014.

REFERENCES

Dewi K, Trisunaryanti W, Soetarto ES (2011). Development of Bioethanol Production from Canna (*Canna edulis* Ker.) Rhizome. *Int. Conf. Biol., Environ. Chem.* 1: 237-240.

Dwiatmini K, Mattjik NA, Aswidinoor H, Matius NIT (2003). Analisis pengelompokan dan hubungan kekerabatan spesies anggrek *Phalaenopsis* berdasarkan kunci determinasi fenotifik dan marka molekuler RAPD. *J. Hort.* 13(1):16-27 (In Indonesian Language)

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Gade DW (1966). Achira, the edible Canna, Its cultivation and use in the Peruvian Andes. *Econ. Bot.* 20(4): 407-415.

Gower JC (1971). A general coefficient of similarity and some of its properties. *Biomet.* 27: 857-874.

Hermann M, Quynh NK, Peters D (1997). *Reappraisal of edible canna as a high-value starch crop in Vietnam*. CIP Program Report, Vietnam, pp. 415-424.

Indrawan M, Primack RB, Supriatna J (2007). *Biologi Konservasi*. Yayasan Obor Indonesia, Jakarta, pp. 12-69 (In Indonesian Language)

Julisaniah NI, Sulistyowati L, Sugiharto AN (2008). Analisis Kekerabatan Mentimun (*Cucumis sativus L.)* menggunakan Metode RAPD-PCR dan Isozim. *Biodiversitas* 9 (2): 99-102 (In Indonesian Language)

Laila A, Sulistyaningsih E, Wibowo A (2013). Morphogenetic variation of shallot (*Allium cepa* L. Aggregatumn Group). *Ilmu Pertanian* 16 (2): 1-11.

Kovach WL (2007). *MVSP-A Multivariate Statistical Package, 3.1*. Kovach Computing Service, Pentraeth, Wales, UK.

Matoba H, Tanaka N, Uchiyama H, Koyama T (2011). The origin of Southeastern Asian triploid edible canna (*Canna discolor* Lindl.) revealed by molecular cytogenetical study. *Econ. Bot.* 65(3): 308-314.

Panwar BS, Singh R, Dwivedi VK, Kumar A, Kumari P (2013). Genetic diversity among Indian Aloe accession based on RAPD analysis. *Int. J. Med. Arom.* 3(3): 326-333.

Patra B, Acharya L, Mukherjee AK, Panda MK, Panda PC (2008). Molecular characterization of ten cultivar of Canna lilies (*Canna* Linn.) using PCR based molecular markers (RAPDs and ISSRs). *Int. J. Integr. Biol.* 2(2): 129-137.

Piyachomkwan K, Chotineeranat S, Kijkhunasatian C (2002). Edible canna (*Canna edulis*) as a complementary starch source to cassava for starch industry. *Ind. Crops Prod.* 16: 11-21.

Prince LM (2010). Phylogenetic relationships and species delimitation in *Canna* (Cannaceae). In: O. Seberg. G. Petersen. A. S. Barfod, & J.I. Davis, eds., *Diversity, Phylogeny, and Evolution in the Monocotyledons*, Aarhus University Press, Århus, pp. 307-331.

Purnomo, Daryono BS, Rugayah, Sumardi I, Shiwachi H (2016). Genetic variability and classification of Indonesian Yams (*Dioscorea* spp.)

based on RAPD analysis. *SABRAO J. Breed. Genet.* 48(4): 377-390.

Sari N, Suryadiantina, Setyowati M, Purnomo, Daryono BS (2016). Variation and intraspecies classification of edible canna (*Canna indica* L.) based on morphological characters. *AIP Conf. Proceed.* 1744: 020041-1–020041-8.

Sastrapradja S, Niniek WS, Sarkat D, Rukmini S (1977). *Ubi-ubian*. Lembaga Biologi Nasional. LIPI. PN Balai Pustaka, Jakarta, pp 30-57. (In Indonesian Language)

Sokal RH, Sneath PA (1963). *Principles of Numerical Taxonomy*. W. H. Freeman and Co, San Francisco, pp. 291-303.

Suhartini T, Hadiatmi (2010). Keragaman karakter morfologi tanaman ganyong. *Bull. Plasma Nutfah* 16 (2):118-125 (In Indonesian Language)

Supriyanta, Kurniasih B, Triwitono P, Suharno, Sundari T (2000). *Eksplorasi dan Identifikasi Plasma Nutfah Ganyong (canna edulis) sebagai Sumber Pati*. Laporan Hibah, Lemlit UGM, Yogyakarta, pp. 1-32 (In Indonesian Language)

Susantidiana, Surahman M, Wijaya A, Lakitan B (2009). Identifikasi Beberapa Aksesi Jarak Pagar (*Jatropha curcas* L.) Melalui Analisis RAPD dan Morfologi. *J. Agron., Indonesia* 37(2): 167–173 (In Indonesian Language).

Tanaka N (2001). Taxonomic revision of the family Cannaceae in the New World and Asia. Makinoa 1: 1-70.

Tanaka N, Inouch N, Koyama T (2006). Edible Canna and its Starch: An Under-Exploited Starch-Producing Plant Resource. Foods Food Ingredients J. Jpn 211: 4.

Ugent D, Pozorski S, Pozorski T (1984). New Evidence for ancient cultivation of Canna edulis in Peru. *Eco. Bot.* 38(4): 417-432.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. 18: 6531-6535