Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.7, No.18, 2017
www.iiste.org

Genetic Variations and Kinship of Coconut Padma (Cocos nucifera, L. 'Padma') in Bali Based on Microsatellite DNA Marker

Eniek Kriswiyanti* I Ketut Junitha Made Ria Defiani
Biology Department, Basic and Natural Sciences Faculty, Udayana University, Bali, Indonesia

The research was funded by Directorate General Strengthening Research, Technology and Higher Education, Ministry of Research, Technology and Higher Education of Republic Indonesia.

Abstract
The *Padma* coconut is unique coconut that characterised by six sulci on epicarpium. There are four trees were found only at Ngis village in Manggis distric, Karangasem Regency, Bali Province. The research were conducted to chracterised genetics variation and relationship between *padma* coconut and other coconuts based on DNA microsatellites markers. Samples were collected from 10 trees from Ngis village (*Padma*, *coklat, hijau biasa, hijau steril, bulan, mulung* and *kapas* coconut). Result showed that genetic diversity was accessed by investigating 10 simple squence repeat (SSR) loci, this provided a total of 53 alleles, 25 alelles 1-5 allel on *Padma* coconut and 38 ranging from 2-7 alleles per locus on the others coconut. Four of 10 loci are monomorphyc (CNZ01, CNZ51, CnCirC3 and WCYZ8635). The mean Polymorphism Information Contents (PIC) ± 0.7035. The mean values of heterozigosity expected (He) and observed heterozigosity (Ho) were 0.461 ± 0.186 and 0.325, respectevely. The trees grouped into three clusters in the dendogram. Cluster 1 consisted of four trees *padma* coconut. Cluster 2 consisted of three trees (coklat, hijau biasa, and hijau steril coconut). Cluster 3 consisted of three trees (*bulan, mulung* and *kapas coconut*).
Keywords: *Padma* Coconut (*Cocos nucifera*, L. 'Padma'), DNA microsatellite, SSR, Heterozigosity, locus

Introduction

Coconut usually used for food and buildings, however, in Bali coconut also used for materials ceremonial in Balinese rituals and medicinal materials. The type of coconut that is used specifically for ceremonial and medicinal purposes is called *Nyuh Madan*, which the name according to its special characteristics (Kriswiyanti, 2013). For example, coconut *Matepat* is a coconut that has four eyes as a food reservation channel from the mother into the ovulum; *Nyuh Buta* (blind coconut) does not have eyes or food reservation channel. Both types of coconuts are found on the island of Nusa Penida (Kriswiyanti, *et al*., 2016).

Padma coconut (*Cocos nucifera*, L. 'Padma') is one of the unique coconut palms in Bali that is characterized by the shape of fruit was not oval (Figure 1), and the other parts are the same as the ordinary coconuts (Sumertayasa and Kriswiyanti, 2016). Its existence is very rare and often used as an alternative medicinal material, therefore the variety of coconut must be conserved.

The study aimed to determine the distribution, variation of DNA microsatellite alleles between individual *Padma* coconut (*Cocos nucifera* L. "Padma") and their kinship relationship with the coconut plant that grows around it. This is done to determine the natural mechanism of *Padma* coconut formation.

Materials and Methods

The exploration of *Padma* coconut plant was done in Karangasem, Bangli, Gianyar, Klungkung, Buleleng, Jembrana, Tabanan, Denpasar and Badung Regencies to know the existence of *Padma* coconut. The samples used as a source of DNA is young leaves from *Padma* coconut and other coconuts that grow around it and from outside the village as a comparison. Other coconut plants belonged to tall coconut with brown, usual green fruit on skin, *Bulan,* sterile green coconut (never produce seed), coconut *Kapas,* and coconut *Mulung* from Pejeng Gianyar. DNA isolation by CTAB method (Doyle and Doyle, 1987). As many as fifteen microsatellite DNA markers, CnCirC3, CNZ05, CNZ09, CNZ01, CNZ21, CNZ40, CNZ51, CAC50, WCYZ8635 and WCYZ7691 were used to amplify the DNA PCR machine. Amplification was performed on a PCR machine with a reaction volume of 13 µl containing: 2 µL genomic DNA, 6.5 µL Master Mix, 3.5 µL sterile water and 1 µL primer. The PCR cycle is as follows: one early predenaturation stage of the PCR process with 94^{o}C for 5 minutes; 30 cycles consisting of denaturation at 94^{o}C for 30 seconds, annealing at varying temperature for 48-52^{o}C 1 minute, elongation at 72^{o}C for 75 seconds; Final extension at 72^{o}C for 5 minutes. Amplicon was electrophoresed on 10% polyacrylamide gel (PAGE) and DNA visualization was used silver nitrate dye (Tagelstorm, 1986). The length of the DNA amplicon band is determined by plotting the migration distance on semilog paper (Hutchinson, 2001). Then performed data analysis include: frequency, heterozigositas, PIC, kinship and making phylogeny tree using Minitab program vis 14.

Results and Discussion

Exploration was conducted in 8 districts and Denpasar city in Bali Province, coconut *Padma* only found in the village of Ngis, district of Manggis, Regency of Karangasem. A total of 4 individual *Padma* coconuts are found in one area and nearby there are other coconut with green and brown fruit skin. Of the 10 primers used in this study, samples of sterile coconut (*Hijau Steril*) were amplified at least, only on two primers namely CAC50 and CnZ01 alone. Sample of amplified and un-amplified samples (null alleles) in Fig. 2. Unsuccessive amplification can be caused by several factors, such as the change of base sequence in DNA so that the primer is not attached, the precision of the reaction mixture, the temperature at each cycle, the primary concentration, and the concentration of DNA (Sambrook and Russell, 2001; Viljoen *et al*. 2005, Rahayu, *et al*., 2006).

According to Callen *et al* (1993), the unfavorable amplification may be caused by a mutation causing a change in nucleotide base sequence on the site of the annealing DNA template so that the primer can not be attached. Not attaching the primer could cause no amplification and no amplicons that produce Null-alleles. In addition, the quality and quantity of template DNA, slippage in the PCR process is also another factor that can lead to the occurrence of null-alleles (Gagneux, *et.al*, 1997; Shinde *et al*., 2003). The result of a thin DNA amplification band on each primer is affected by the purity and concentration of the template DNA. DNA templates containing compounds such as polysaccharides and phenolics often produce thin amplification DNA bands (Poerba and Martanti, 2008). If the concentration of DNA is too high will cause the fragment looks thick so it is difficult to distinguish between one ribbon with another ribbons (Haris, *et al*, 2013).

Variety and frequency of alleles

The entire allele varieties in this study with 10 pairs of primers (locus) were obtained 53 alleles, the average of 5.3 (1-8) allele perprimer. The distribution was not the same, there were alleles found in coconut *Padma* not found in other coconut like 170bp allele CNZ01 locus which was the only allele found in coconut *Padma*. This allele was not found in any other coconut that has 4 different alleles namely Null, 178, 184 and 280bp. Similarly, in other loci such as CnCirC3 locus which had only two kinds of alleles, namely null and 156bp, null alleles were found in *Hijau sterile* and *Hijau Biasa*. *Padma* Coconut was monomorphic with only one allele of 156bp. The *Padma* Coconut had a fewer (1-5) smaller alleles (1-5) averaging 2.5 per cent compared to other coconuts having an average of 2-7 alleles per locus of 3.8 (Table 1). The average allele range per loci of this study was more than that of Dasanayaka *et al* (2009) using 16 loci on 43 coconut accessions in Sri Lanka which received an average of 4.9 mouse alleles. Ribeiro, *et.al* (2010) in 195 individuals from 10 tall coconut populations in Brazil, with 13 loci produced 68 alleles (± 5.23). Kumar, *et.al* (2011) on 14 accessions (4 accessions from Polynesia, 5 accessions from New Guenia and 5 accessions from Solomon Island) examined 8 loci produced 28 alleles (± 3.5 alleles). Similarly, Kriswiyanti and Junitha (2014) research on the parent allele variety and red coconut coconut husk in Bali (16 individuals), with 6 loci generated the mean of alleles 4.16 and 4.5. Various 3.33 and low heterozygosity alleles were found in 12 accessions of coconut dwarf ah from Philippina (Noel *et al*, 2011). The Kriswiyanti *et al* (2013) study showed that a high allele range of 80 alleles, 13.33 alleles of focus on 58 individuals *Nyuh Madan* in Bali Province with 6 loci same with this research.

Polymorphism Information Content (PIC)

The level of informativity (PIC) of the ten primary pairs of this study was between 0.32-0.845 (± 0.7035), indicating that the primer was capable of detecting polymorphism in a population of 32-84.5%, high mean (± 70.35%) except in primary CnCir C3 is only 32% (Table 2). According to Botstein, *et.al* (1980), if PIC value> 0.5 is high or highly informative, while 2.5 <PIC <0.5 and <2.5 are low. According to Anderson *et.al* (1993) the higher the PIC value of a primer the better the primer is used as a marker for detecting polymorphism in the population.

The PIC values of this study were higher than the results of Kumar's research, *et al* (2011) on 19 coconut accessions in Polynisia, New Guinea and Solomon Islands using 8 primers. The value of this PIC depends on the frequency and distribution of the found allele. The small PIC at the CnCirC3 locus is caused by the low number of alleles which only amount to two, namely null and 156bp. Thus this primer required to be tested on many more coconut cultivars or accessions before it was decided whether or not this locus is used in coconut research in Bali.

Genetic Deversity (Heterozygosity)

The mean heterozygosity of *Padma's* coconut (0.461 ± 0.186) was lower than the average of other coconut heterozygosities (0.718 ± 0.183). Padren's lower heterozygosity compared to other coconut palms is caused by fewer alleles of only 25, 1-5 focusing on *Padma's* coconut compared to 38 alleles, 2-7 focusing alleles in coconut instead of *Padma* (other coconut). In addition, four loci from 10 loci on *Padma* coconut were monomorphic which have only one allele ie CNZ01, CNZ51, WCYZ7691 and WCYZ8635 loci. The high heterozygosity of each locus is influenced by the many different alleles and frequency of each allele (Junitha and

Alit, 2011; Junitha and Watiniasih, 2014; Octavia et al., 2015; Arnila et al. 2016). The high value of heterozygosity of expectation is due to the large variety of alleles present in each locus / primer which amounts to between 2 and 8 alleles. Rajesh et al. (2008) found that In-Andaman coconut has high heterozygosity value. High heterozygosity values and a wide range of alleles were also found in In China's Hainan (Liu et al., 2011), and 58 tall coconut individuals in the province of Bali with the same 6 primary heterozygosity 0.856-0.92 (Kriswiyanti et al 2013). On the contrary the observed heterozygosity in this study was obtained on *Padma* coconut is higher than other coconuts ie the average of 0.325 in *Padma* and 0.183 coconut in the coconut is not *Padma*. These results indicate that cross-pollination occurs by other coconuts in the *Padma* coconut because the number of individuals is less than the surrounding non *Padma* coconut. Coconut *Padma* belonged to In coconut so it has cross pollination properties. Devakumar et al (2010); Kumar et al. (2011), Xiao et al (2013) found that relatively high heterozygosity was found in deep coconut compared with early coconut.

Relationship of Padma coconut to other coconut

Dendogram showed the relationship between coconut *padma* (Cocos nucifera L. "Padma") with other coconut plants around it can be seen in Figure 3. Based on 22.5% similarity level, ten individual coconut plants used as research samples are divided into 3 clusters. Group I is coconut *padma* 1, 2, 3 and *padma* 4, group II: coconut with *Coklat* fruit skin, *Hijau sterile* (never produce seed) and coconut with green fruit (*Hijau Biasa*) skin and can produce seeds. Group III consists of *Bulan*, *Mulung* and *Kapas* coconut. Ten individual coconuts used as research samples are present in one population, this may lead to an equality of alleles between individuals on the same or different primary. The similarities and differences of the allele can differ between individual and other individuals into one group or not. For example, group I at the level of 32.9%, coconut *padma* 1 separated with *padma* 2, 3 and 4. This can be seen in the variety of alleles, coconut *padma* 1 has allele on primer CAC50, CNZ21, CNZ09, CNZ05 and WCYZ7691 different with *padma* 2, 3 and *padma* 4. On the other 5 primers else, all of *padma* coconut have the same allele.

CONCLUSIONS

1. Coconut *Padma* (Cocos nucifera L.'Padma ') were found only four trees at Ngis Village, Manggis District, Karangasem Regency, Bali Island, Indonesia.
2. Various alleles using 10 pairs of primers (locus) obtained 25 alleles, ± 2.5 (1-5 alleles), four loci (CNZ01, CNZ51, CnCirC3 and WCYZ8653 are monomorphic) Genetic low genetic diversity average of 0.461 ± 0.186, and Observed heterozygosity of 0.325.
3. Based on the similarity level on 22.5%, ten trees coconut plants are divided into 3 clusters. Group I is coconut *Padma* 1, 2, 3 and 4. Group II: coconut *Coklat*, *Hijau sterile*, and *Hijau Biasa* coconut. Group III consists of coconut *Bulan, Mulung* and *Kapas*.

Acknowledgments

The authors thanks to Directorate General Strengthening Research, Technology and Higher Education for the research funded and Rector of Udayana University for opportunity and trust to conduct this research; Chairman of LPPM Udayana University who has processed this research proposal; Head of laboratory Forensic Udayana University who provided assistance in the use of laboratory facilities. This publication is a part of Fundamental Hibah research funded by Directorate of Research and Community Service Directorate General Strengthening Research, Technology and Higher Education In accordance with Letter of Appointment Agreement Implementation Research No: 486.124 / UN14.2 / PNL .01. 03. 00/2016, May 16, 2016.

REFERENCES

Arnila G.A.P., I K. Junitha., M. Pharmawati. 2016. Genetic variation of Pande Clan in Gianyar Regency Based on Three DNA Mikrosatelit Autosomal Loci. J.Biol. 20(1):1-5.

Dasanayaka, P.N., Everard, J.M.D.T., Karunanayaka E. H. and Nandadasa, H.G. 2009. Analysis of Coconut (*Cocos nucifera,*L.) Diversity Using Microsatellite Markers with Emphasis on Management and Utilization of Genetics Resources. *J. Natn. Sci. Foundation Sri Lanka* 37 (2): 99-104.

Doyle J.J. and Doyle J.L. 1987. A Rapid DNA Isolation Procedure for Small Amount of Leaf Tissue. *Phytochem. Bull*. 19 : 11-15.

Gagneux, P; C Boesch and DS Woodruff.1997. Microsatelite Scoring Error Associated with Noninvasive Genotyping Based on Nuclear DNA Amplified From Shed Hair. *Mol. Ecol* 6: 861-868.

Haris, N; H. Aswidinoor; T.M. Nurita and A. Purwantara.2013. Similarity of Rubber clones (*Hevea brasiliensis* Muell. Arg) based on amplified fragment length polymorphism (AFLP) method. *Jurnal Menara Perkebunan,*71 (1):1-15.

Hutchinson, F.2001. DNA Band Size Semi-Log Plotting: Estimation Size of DNA Band by Semi-Log. *Cancer Research Center: Sci. Educ. Patnership.* International Board for Plant Genetic Resources (IBPGR). 1992

Discriptors for Coconut", Rome, Italy.

Kriswiyanti, E., I Gede Rai Maya Temaja, I Made Sudana, and I Gusti Ngurah Alit Susanta Wirya. 2012. Alleles Variation of Coconut Accessions (*Cocos nucifera* L.) Based on Microsatellite DNA. *Proceeding ICBB* 2012: 235-238

Kriswiyanti, E, 2013. Characters Variation of Coconut (*Cocos nucifera* L.) Used as Materials of *Upakara Padudusan Agung* Ceremonial in Bali. *Jurnal Biologi* XVII (1)2013:15-19

Kriswiyanti, E., I Gede Rai Maya Temaja, I Made Sudana, and I Gusti Ngurah Alit Susanta Wirya. 2013. Genetic Variation of Coconut Tall (*Cocos nucifera* L., Arecaceae) in Bali Indonesia Based on Microsatellite DNA. *Journal of Biology Agriculture and Healthcare* 3 (13) 2013 : 97-101

Kriswiyanti , E. and Junitha, I K. 2014. The Allele Variation Seedling ofsprings and Parent Red Husk coconut (*Cocos nucifera* L.) and Relationship based on Microsatellites DNA. Prosiding Senastek 1, 2014: 743-747.

Kriswiyanti, E; I Ketut Junitha and Sudaryanto. 2016. The variance of coconut (*Cocos nucifera* L., Familia Arecaceae) in Nusa Penida District, regency of Klungkung. Prosiding National Conference, Biosain 2, 55-63.

Junitha I K. and I B. Alit. 2011.Allele Variation on Autosomal Microsatellites DNA of Bali Aga Community in Sembiran Villages, Regency of Buleleng, Bali. *Biota*. 16(1): 63-69.

Junitha I K. and N. L. Watiniasih. Male Genetic Diversity of Siwa Brahmin Clan in Bali Based on Y-Chromosom Microsatellites DNA. *JBAH*. 4(1): 30-35.

Kumar, S. P; Manimekalai, R. and Kumari B.D.R. 2011. Microsatellite Marker Based Characterization of South Pasific Coconut (*Cocos nucifera* L.) Accessions. *Int. J. Plant Breed. Genet.*, 5: 34-43.

Liu, X; Tang,H; Li, D and Hou, L. 2011. Genetic Diversity of Coconut Cultivars in China by Microsatellite (SSR). *Moleculer Plant Breeding* 2 (2):83-91.

Manimekalai, R and Nagarajan, P. 2010 : SSR and ISSR Markers Based Population Genetic Structure of Coconut (*Cocos nucifera* L.) Germplasm Accssion Indian. *J. of Plant Genetic Resources,* 23 (1) : 77-82.

Nei, M.1987. *Moleculer Evolusionary Genetic*. Columbia University Press. New York. USA

Octavia L.E., I K. Junitha, I. Narayani. 2015. Allele Variation of Autosomal DNA Microsatellites Loci D2S1338, D13S317 and D16S539 of Dayak Kaharingan community in City of Palangka Raya. J. Biol 19(2): 69-73.

Poerba, Y S dan D Martanti, 2008. Genetic Variability of *Amorphopallus muelleri* Blume in Java based on Random Amplified Polymorphic DNA. *Jurnal Biodiversitas*, 9 (4): 245-249.

Rahayu, S.; S B Sumitro; T Susilawati, and Soemarno. 2006. Identification of Gen GH (*Growth Hormone*) polymorphism of Bali catle used PCR_RFLP method . *Berk. Penel. Hayati*, 12: 7-11.

Sugiantari, N L P R; I Ketut Junitha and Eniek Kriswiyanti, 2015. Analysis Genetic Variation Of *Rangda* Coconut (*Cocos Nucifera* L.) In Bali Based On Microsatellite DNA Marker. *Simbiosis Journal of Biological Sciences* II (2): 334-337.

Sumertayasa I W. and Eniek Kriswiyanti**,** 2016. Characterization And Distribution Of Madan Coconut In The Manggis District, Karangasem Regency, Bali. *Simbiosis Journal of Biological Sciences* IV (1): maret 2016: 10-15.

Sambrook, J and D.W Russel, 2001. *Molecular Cloning a Laboratory Manual.* 3rd Edition. Cold Spring Harbor Laboratory Press New York.

Shinde, D, Lay Y L., Sun, FZ and Amheim N. 2003. Taq DNA Polymerase Slippage Mutation Rates Measured by PCR and Quasilikelihood Analysis (CA/GT)(n) and Microsatelites. *Nucleic Acid Res.* 31: 974-980.

Tegelstöm H. 1986. Mitochondrial DNA in Natural Population : an Improved Routine for Screening of Genetic Variation Base on Sensitive Silver Staining. *Electrophoresis.* 7: 226-229

Viljoen, G J; L H Nel and J R Crowther. 2005. *Molecular Diagnosis PCR Handbook.* Springer , Dordrecht, Netherland.

Figure 1: *Padma* coconut (*Cocos nucifera* L.'Padma') fruit

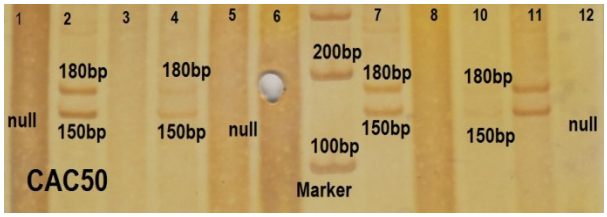

Figure 2 PCR Amplification Results in CAC50

Description: allele lengths on wells /coconuts 1.*Padma* 1, 2.*Padma* 2, 3.*Padma*3 4.*Padma*4, 5.*Coklat*, 6. *Hijau biasa*, 7.*Padma*2, 8. *Hijau Sterile*, 10.*Bulan*, 11.*Mulung* , 12.*Kapas*

Table 1 Frequency of Allele on Ten Primers

CNZ 01 locus			CNZ 05 locus			CNZ 09 locus			CNZ 21` locus			CNZ 40 locus		
Alell	Frequency		Alelle	Frequency		Alell	Frequency		Alell	Frequency		Alell	Frequency	
(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad
Null	0	0.333	Null	0.250	0.334	Null	0	0.334	Null	0	0.500	Null	0	0.334
170	1	0	110	0	0.083	154	0.375	0	220	0.500	0.083	108	0.125	0
178	0	0.333	130	0	0.083	160	0	0.166	230	0.125	0.083	110	0.125	0
184	0	0.167	140	0	0.166	164	0	0.334	240	0.375	0.166	124	0.125	0.083
280	0	0.167	150	0.250	0	168	0.250	0	260	0	0.083	128	0.250	0.417
			154	0.250	0	170	0	0.166	280	0	0.083	134	0	0.166
			164	0.250	0	174	0.375	0				158	0.375	0
			174	0	0.334									

CNZ 51 locus			CAC 50 locus			CnCir C3 locus			WCYZ7691 locus			WCYZ 8635 locus		
Alell	Frequency		Alell	Frequency		Alell	Frequency		Alell	Frequency		Alell	Frequency	
(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad	(bp)	Pad	NPad
Null	0	0.166	Null	0.250	0.166	Null	0	0.333	Null	0	0.5	Null	0	0.5
108	0	0.167	150	0.375	0.417	156	1	0.667	290	0.5	0	220	1	0.167
120	0	0.083	180	0.375	0.417				305	0.25	0	260	0	0.333
124	0	0.083							311	0.25	0.5	280	0	
130	0	0.167												
134	1	0.167												
146	0	0.167												

Description: null allele = no amplicon, bp = base pair of amplicon, Pad = *Padma* coconut
NPad = not Padma coconut as comparison

Table 2. Value of Polymorphism Information Content (PIC) On the Ten Primers

Primer	PIC
CNZ01	0.74
CNZ05	0.825
CNZ09	0.845
CNZ21	0.77
CNZ40	0.79
CNZ51	0.705
CAC50	0.64
CnCirC3	0.32
WCYZ7691	0.7
WCYZ8635	0.7
PIC mean	**0.7035**

Table 3 Genetic Diversity (Heterozygosity) of *Padma* Coconut And the others Coconut .

	Padma Coconut		The other coconut	
Primer	Expected Hetorozigosity (He±SE)	Observed Heterozigosity (Ho)	Expected Hetorozigosity (He±SE)	Observed Heterozigosity (Ho)
CNZ01	0	0	0.788 ± 0.250	0
CNZ05	0.857 ± 0.283	0	0.803 ± 0.249	0.166
CNZ09	0.750 ± 0.313	0.750	0.788 ± 0.250	0
CNZ21	0.678 ± 0.331	0.750	0.757 ± 0.275	0.500
CNZ40	0.857 ± 0.292	1	0.742 ± 0.264	0.166
CNZ51	0	0	0.924 ± 0.198	0.166
CAC50	0.750 ± 0.313	0.750	0.682 ± 0.271	0.833
CnCirC3	0	0	0.485 ± 0.287	0
WCYZ7691	0.714 ± 0.327	0	0.545 ± 0.275	0
WCYZ8635	0	0	0.667 ± 0.277	0
Mean	0.461 ± 0.186	0.325	0.718 ± 0.259	0.183

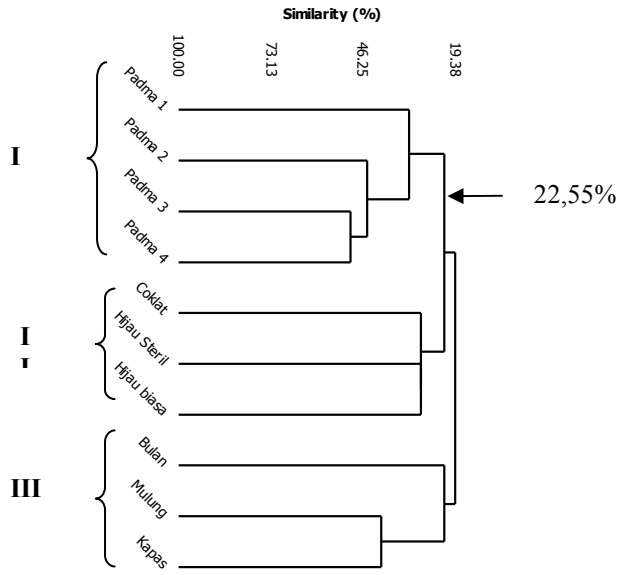

Figure 3 Coconut *Padma* Dendogram with the other coconut