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Species diversity of *Amorphophallus* (Araceae) in Bali and Lombok with attention to genetic study in *A. paeoniifolius* (Dennst.) Nicolson

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ABSTRACT

Kurniawan A, Wibawa IPAH, Adjie B (2011) Species diversity of Amorphophallus *(Araceae) in Bali and Lombok with attention to genetic study in* A. paeoniifolius *(Dennst.) Nicolson. Biodiversitas 12: 7-11. Amorphophallus* belongs to Araceae family that consists of more than 170 species worldwide and distributed predominantly in tropical countries, especially in Asia and Africa. The study of *Amorphophallus* in Bali and Lombok Islands had been conducted to reveal its diversity. Genetic study was also conducted among *Amorphophallus paeoniifolius* species to recognize the variation within the species. The fieldworks showed three species *Amorphophallus* distributed in Bali, notably *A. muelleri* Blume, *A. paeoniifolius* (Dennst.) Nicolson, *A. variabilis* Blume and two species in Lombok, notably *A. muelleri* and *A. paeoniifolius*. Var. *hortensis* and var. *sylvestris* were two varieties of *A. paeoniifolius* that commonly found either in Bali or in Lombok. Genetic study on *A. paeoniifolius* indicated that there was no genetic variation in cpDNA region of *trn*L-F IGS within the species.

Key words: *Amorphophallus paeoniifolius*, diversity, genetic variation, cpDNA.

INTRODUCTION

Amorphophallus is belonging to Araceae family that consists of more than 170 species worldwide and distributed predominantly in tropical countries, especially in Asia and Africa. This genus mostly grow in secondary forests or disturbed spots in primary forests and forest margins, also in tropical humid forest, seasonal forest and sometimes in humus deposits on rocks (limestone). The majority of *Amorphophallus* species seem to be pioneers in disturbed vegetations. Many are found at forest margins, in open savannah forests, on (steep) slopes, in disturbed parts of primary forest. Relatively few species are known to live in dense forest (Hetterscheid and Ittenbach 1996; Mayo et al. 1997; Sugiyama and Santosa 2008).

The genus of *Amorphophallus* has seasonally dormant. The leaves are emerging from tuber, solitary in adult plants; the petiole is long, smooth, conspicuously spotted and marked in a variety of patterns. Blade divided in three main branches, primary divisions pinnatisect, secondary and tertiary divisions approximately regularly pinnatifid to pinnatisect. Inflorescence always solitary, usually flowering without leaves. Spathe variously coloured, inner surface smooth. Spadix sessile or stipitate; female zone shorter, equaling or longer than male zone; subglobose, terminal appendix usually present, erect. Flowers unisexual. Male flower: stamens free, short, filaments absent or distinct. Female flower: stigma variably shaped, entire and sub-globose or 2-4 lobed or stellate or rarely punctiform. Infructescence usually long-peduncled; fruiting part globose or elongate; berries globose or elongate, red, orange-red, white, white-and-yellow, blue. Seeds globose, subglobose, ovate, elliptic (Hetterscheid and Ittenbach 1996; Jansen et al. 1996; Mayo et al. 1997).

Amorphophallus paeoniifolius (Dennst.) Nicolson or *A. campanulatus* Decne is widely distributed and cultivated in Indonesia and other Asian countries. It has large variation in petiole color, such as light to dark green, blackish green, gray, reddish or pinkish white. Petiole structure divide in two varies, notably rough and smooth. Usually classified into two groups *A. campanulatus* (*A. paeoniifolius*) var. *hortensis* (smooth petiole) and *A. campanulatus* (*A. paeoniifolius*) var. *sylvestris* (rough petiole) (Sugiyama and Santosa 2008).

Bali is closely neighboring to Lombok that laid on the Middle to East Indonesia and just separated with 35 km strait but the existence of Wallacea line between these islands make them unique and interesting. Wallace line was known as imaginary line separating the Oriental (Sunda Shelf) and Australian (Sahul Shelf) biotas-extends Bali and Lombok and between Sulawesi and Borneo/Philippines. Alfred Russell Wallace was one of the first to draw worldwide attention to the dramatic change in especially the fauna of central Malesia region between Southeast Asian and New Guinean-Australian fauna (van Welzen et al. 2005). The fauna studies in Wallacea areas are more extensive than flora studies especially in Bali and Lombok. The diversity of *Amorphophallus* never been studied specifically in Bali and Lombok Islands except globally mentioned on Jansen et al. (1996) and Mayo et al. (1997).

Technical progress in the determination of DNA sequence has resulted in an enormous amount of DNA

sequence data, with technical innovations such as the polymerase chain reaction (PCR) accelerating the rate of increase of this data. This combination of data and techniques makes it possible now to exploit sequence differences between individuals. Such studies can answer biological questions, such as genetic variation, allele diversity and hybrid origin. Making comparative studies between individuals inevitably deals with a large number of samples, thus the methods employed in such studies should be simple (Hayasi 1991). One such method is PCR-single-strand conformation polymorphism (SSCP) analysis. SSCP is an accurate method for detecting nucleotide differences among PCR products (Lessa and Appelbaum 1993; Yap and McGee 1994; Sunnucks et al. 2000). This possibility promoted our interest in exploring genetic variation in *Amorphophallus paeoniifolius.*

MATERIALS AND METHODS

Species diversity

Fieldworks conducted at several districts in Bali and Lombok to obtain the recent data of *Amorphophallus* species. Some reports from fieldworks of Bali Botanic Garden staff in both areas are also examined to figure out the *Amorphophallus* diversity in the past. The living *Amorphophallus* collections of Bali Botanic Garden and its herbarium specimens in Herbarium Hortus Botanicus Baliense also checked.

Genetic study

To study intraspecific genetic variation, multiple samples were examined for *Amorphophallus paeoniifolius.* The total DNA was extracted from dried leaf samples from 24 accessions using DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan). The region from *trnL* (UAA)5´exon to *trnF* (GAA), later called *trn*L-F, was amplified with primer *e* and primer *f* of Taberlet et al. (1991). The PCR products were analyzed using the single-strand conformation polymorphism (SSCP) method following the procedure of Watano et al. (2004). The gel was made by using MDE™ gel solution (Cambrex Bio Science Rockhland, Inc., USA) with 2% glycerol concentration. Electrophoresis was carried out at 18^{o} C, and 300 volts run for 7 hours in 0.5X TBE buffer. The DNA bands were then visualized using a DNA Silver Staining method (Pharmacia Biotech, Sweden).

RESULTS AND DISCUSSION

Amorphophallus species

Based on fieldwork activity, there are three species of *Amorphophallus* obtained from Bali and two species from Lombok. *Amorphophallus muelleri* Blume, *A. paeoniifolius* (Dennst.) Nicolson and *A. variabilis* Blume represent the Bali's *Amorphophallus.* The *Amorphophallus* species that recorded in Lombok are *A. muelleri* and *A. paeoniifolius* (Table 1).

Jansen et al. (1996) reported that Indonesian *A. muelleri* is found in Sumatra, Java, Flores and Timor. It is not mentioned in Bali and Lombok Island. This species is cultivated in Java especially in East Java, conversely cultivated *A. muelleri* is never found in Bali or Lombok. This species is still uncommonly for farmer in Indonesia (Soemarwoto 2005). It is still wild and can be found in the natural area, such as forest, in the river sides or in undisturbing area in Bali and Lombok Islands.

Two varieties of *A. paeoniifolius* are found either in Bali or Lombok. *A. paeoniifolius* var. *hortensis* (known as *suweg* in Bali and *lombos* in Lombok) is frequently planted in Bali and Lombok, because its tuber is edible and easily processing before consumed. Another variety is *A. paeoniifolius* var. *sylvestris* (known as *tiyih* in Bali and *lombos babi/lombos sawak* in Lombok). The last variant is almost not cultivated, whereas it's commonly found wild in the forest boundary or in disturbed/unused land. Actually, Jansen et al. (1996) separated wild species of *A. paeoniifolius* into two varieties, notably var. *sylvestris* Backer and var. *paeoniifolius.* The cultivated species also divided into two varieties, such as var. *hortensis* Backer and var. *campanulatus* (Decaisne) Sivadasan.

Table 1. *Amorphophallus Species* in Bali and Lombok Islands

Scientific name	Samples from Bali				Samples from Lombok	
	Buleleng	Jembrana	Karangasem	Tabanan	West Lombok	Central Lombok
A. muelleri	-	-	-	Ag.225	Ag.173	Ag.183, Ag.184, Ag.185
A. paeoniifolius var. *hortensis*	Ag.195, Ag.196*, Ag.197*, Ag.210*, Ag.213*, Ag.216*,	Ag.139*, Ag.140*	Wb.10, Wb.11*, Wb.12a*, Wb.12b*, Wb.13,	Ag.148*, Ag.223*, Ag.224*, Ag.225a, Ag.227*	Ag.153, Ag.155*, Ag.156*, Ag.160*, Ag.162. Ag.171*, Ag.175*, Ag.181, Ag.182	-
A. paeoniifolius var. *sylvestris*	Ag.205*, Ag.207*,	Ag.146*, Ag.147*	Wb.14	Ag.228*	Ag.161,	-
A. variabilis	Ag.200, Ag.202, Ag.203, Ag.204, Ag.206, Ag.208, Ag.209, Ag.211	-	Wb.16, Wb.17,	-	-	-

Note: * = leaves samples of *A. paeoniifolius* were used in genetic test.

Amorphophallus variabilis is only known wild in several Island in Indonesia such as Java, Madura and Kangean Islands (Jansen et al. 1996). In fact, this species also distribute in some districts in Bali Island. It could be a new record for uncommon *A. variabilis* in Bali although it can be recognized easily from its bad smell flowers when come close to the night.

The diversity of *Amorphophallus* that mentioned above represented that the Wallace line is not to be the strict boundaries between Bali and Lombok although each island is separated with this line. This result is probably agreed with van Welzen et al. (2005) that mentioned the Wallacea area comprises Java, the Philippines, Sulawesi, the Lesser Sunda Islands (include Bali and Lombok Island), and Maluku. So the diversity between Bali and Lombok is similar because it's still in the same region, Wallacea.

Amorphophallus descriptions

Amorphophallus muelleri

Diameter tuber 20 cm and up to 28 cm, dark brown, smooth, often without nodes or bud, with densely whitish-ivory root in petiole base. Petiole about 40-50 cm long, diameter 1-5 cm, smooth, pale green and rarely brownish, with numerous whitish green continuous spot. Lamina or blade very dissected, carrying epiphyllar bulbils on the major ramifications on the venation intersections; leaflets lanceolate, 10-35 cm x 4-9 cm. Peduncle 30 cm and up 60 cm (Jansen et al. 1996), diameter 1-3 cm. Inflorescence smaller than *A. paeoniifolius* but wider and bigger than *A. variabilis*, inflorescence is absent during fieldwork. According to Jansen et al. (1996), spathe size is 7.5-27 cm x 6-27 cm, limb semi erect or spreading, brownish-purple or grayish-greenish outside, inside purplish or brownish with greenish or brownish spots; spadix longer than spathe, 8-30 cm long. Inflorescence does not produce unpleasant odors. Infructescence cylindrical to narrowly triangular, berry cylindrical to ovoid, 12-18 mm long, bright red.

Amorphophallus paeoniifolius

Diameter tuber 25 cm and up to 30 cm, dark brown, rough with several nodes and producing seasonal rhizomatous buds. Petiole 100 cm and up to 200 cm height, diameter 5-10 cm; strongly verrucose-echinate until shallowly corrugate surface, dark green to brownish green (wild species), slightly verrucose to smooth surface, pale green to green (cultivated species); with whitish green spots either in wild or cultivated species. Lamina or blade 100-150 in diameter, deeply dissected, rachises winged; leaflets ovate to lanceolate, 3-35 cm x 2-12 cm. Peduncle 3-15 cm in height and elongating when fruiting until 75 cm, peduncle surface is similar with petiole both in wild and cultivated species; peduncle turns brownish green-brown when fruit is ripening. Inflorescence is biggest among two other *Amorphophallus*. Spathe 10-30 cm x 15-50 cm, limb spreading and strongly undulate, pale green to brown with pale green-whitish green spot outside, glossy dark brown to dark red-purple inside. Spadix is longer than spathe, 10-25 cm long. Inflorescence produces very upleasant odors like carcass smell. Infructescens cylindric; berry cylindrical, less than 2 cm, bright red.

Amorphophallus variabilis

Diameter tuber is commonly small, about 10-15 cm, very dark, rough, producing seasonal rhizomatous buds. Petiole 50-75 cm long and up to 120 cm according to Jansen et al. (1996), smooth, sometimes glossy, variegated surface color from pale green, greenish brown, pure green to pale brown, with randomly whitish green to dark green irregular spots. Lamina or blade 50-75 cm in diameter and up to 125 cm (Jansen et al. 1996); almost similar with *A. paeoniifolius* lamina, leaflets elliptical to lanceolate, 4-34 cm x 2-12 cm. Peduncle about 50-60 cm, 1-2 cm diameter. Inflorescence is longest than *A. muelleri* and *A. paeoniifolius*; spathe narrowly short, up to 20 cm or 1/3-1/4 spadix length, limb pale green-green with randomly whitish spots outside, creamy white-ivory inside. Spadix is commonly very longer than spathe. When inflorescence is flowering, it's commonly known with bad smell that appearing early in the evening. Only unripening infructescens found during fieldwork, cylindrical, densely arranged, green. Jansen et al. (1996) reported the ripening fruit is orange-red with 1-3 seeded.

Key to Bali and Lombok of *Amorphophallus* species

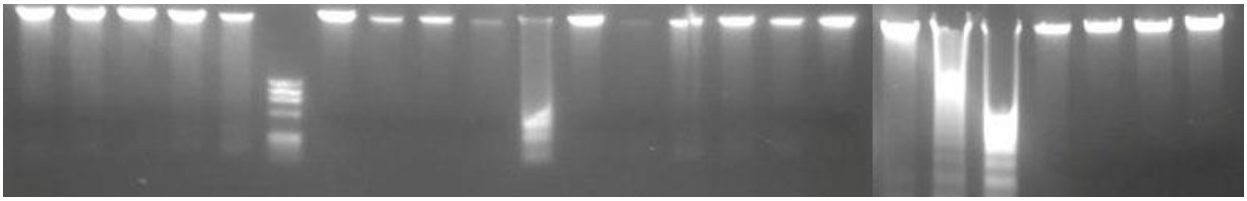
1.a. Leaflets without bulbils on the venation intersections 2
1.b. Leaflets with several bulbils on the venation intersections ..... ***A. muelleri***

2.a. Petiole smooth or slightly glabrous 3
2.b. Petiole strongly verrucose-echinate or corrugate .... ***A. paeoniifolius* var. *sylvestris***

3.a. Inflorescence bigger and wider; limb spreading and strongly undulate, pale green to brown with pale green-whitish green spot outside, glossy dark brown to dark red-purple inside .... ***A. paeoniifolius* var. *hortensis***
3.b. Inflorescence narrowly long, smaller. limb pale green-green with randomly whitish spots outside, creamy white-ivory inside ..... ***A. variabilis***

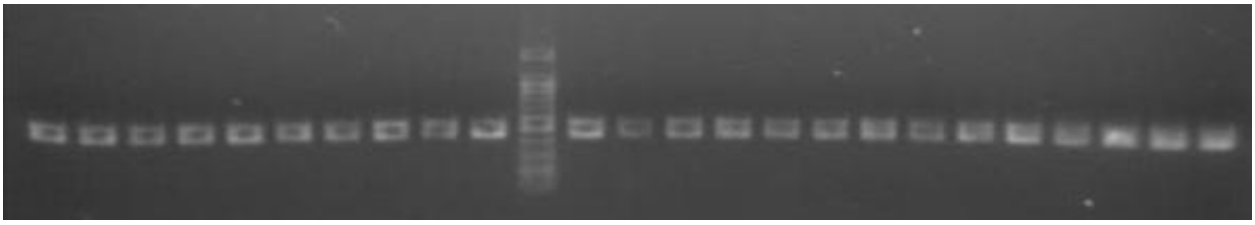
Genetic study on *A. paeoniifolius*

The extracted DNA showed high concentration (Figure 1), and the PCR products were easily amplified about 400 bp of the *trn*L-F IGS region (Figure 2) which is suitable size for SSCP analysis. The SSCP gels showed only single banding pattern for all accessions, this can be interpreted there is no sequence variation among the PCR products. The accessions which include two varieties of *A. paeoniifolius* (var. *hortensis* and var. *sylvestris*); however, the region examined was not differentiated. The cpDNA known to have very slow mutation rate (Clegg et al. 1994) and would be better to use in the study between species. Thus, the placement of two varieties in one species *A. paeoniifolius* may be appropriate. The application of PCR-SSCP method was widely used in various taxa such as ferns (Ebihara et al. 2005; Terada and Takamiya 2006; Adjie et al. 2007; 2008; Adjie and Lestari 2009), gymnosperm (Watano et al. 2004) to human (Orita et al. 1989).



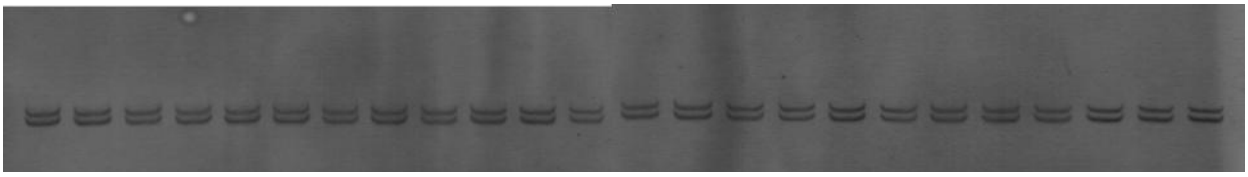
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 1. Total DNA extracted from *Amorphophallus paeoniifolius*. M=DNA marker ϕX174 *Hae*III digest (Takara, Tokyo, Japan), 1=Ag139, 2=Ag175, 3=Ag140, 4=Ag146, 5=Ag147, 6=Ag148, 7=Ag156, 8=Ag160, 9=Ag171, 10=Ag196, 11=Ag197, 12=Ag205, 13=Ag207, 14=Ag210, 15=Ag213, 16=Ag216, 17=Ag223, 18=Ag224, 19=Ag227, 20=Ag228, 21=Wb11, 22=Wb12a, 23=Wb12b and 24=Ag155



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 2. PCR product of *trn*L-F IGS about 450 bp. M=100bp DNA Ladder (Toyobo, Japan).



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 3. PCR-SSCP gels show no sequence variations within accessions.

Using AFLP and SSR markers (Sugiyama et al. 2006; Santosa et al. 2007) also suggest little variation within *A. paeoniifolius* collected from Java. These results suggest that people play important role in the selection and distribution of *A. paeoniifolius*, reducing genetic variation in a region (Sugiyama and Santosa 2008). They also suggest the effectiveness of farmers' participation in the selection of new cultivars in the breeding program.

CONCLUSION

This study concluded that: (i) There are three species *Amorphophallus* in Bali, notably *Amorphophallus muelleri* Blume, *A. paeoniifolius* (Dennst.) Nicolson and *A. variabilis* Blume. Whereas, only two species in Lombok are *A. muelleri* and *A. paeoniifolius. A. paeoniifolius* consists of two varieties, such as *A. paeoniifolius* var. *hortensis* and *A. paeoniifolius* var. *sylvestris*. (ii) There is no genetic variation in cpDNA region of *trn*L-F IGS within the *Amorphophallus paeoniifolius* accessions.

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