MORPHOLOGICAL CHARACTERS AND GENETIC VARIABILITY OF CHAMPACA IN BALI

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ABSTRACT

Bali community utilize champaca flower for offering materials and worship, besides beauty salon purposes, the SPA aromatic ingredients, essential oils, perfumes, cosmetics, and drugs. Various champaca plants in Indonesia have not been studied as one of Indonesia's biodiversity that can be used as excellent genetic resources (germplasm). The objective of the study was to determine the genetic diversity of champaca in Bali. The results revealed that 12 (twelve) champaca accession morphologically was characterized. All of accessions obtained from cultivation centers champaca in Bali. Based on the characteristic was observed by morphological characters i.e.: (a) Cempaka Putih Wilis (b) Cempaka Putih Pateh, (c) Cempaka Putih Patemon, (d) Cempaka Putih Sibang, (e) Cempaka Kuning Muda Petemon, (f) Cempaka Kuning Kecil Patemon, (g) Cempaka Kuning Besar Patemon, (h) (i) Cempaka Kuning Kecil Sibang, (j) Cempaka Kuning Tua Sibang, (k) Cempaka Kuning Muda Sibang, and (l) Cempaka Kuning Punah Sibang. Morphologically, champaca in Bali can be grouped into 4 clusters and therefore, and based on RAPD analysis champaca in Bali could be grouped into 5 clusters.

Keywords: champaca, identification, genetic, diversity, Bali

INTRODUCTION

Champaca plants have been planted in Indonesia, especially in Bali as a commercial commodity, thus it support economically for Bali communities. These plants have been utilized as ceremony purposes and worship for Hindu's people. Others, the champaca flower were used for beauty salon, aromatic ingredients, and SPA. Taxonomically, champaca were planted in Bali are *Magnolia alba* and *Magnolia champaca*. The fragrant aromatic of the flower is to be the specific characters of the plant.

As a tropical plant, champaca has a wide adaptation range in all tropical area from low land to highland. Usually, this family spread in warm subtropical of East Asia, Southeast Asia, and southeastern part of North America, West India, Central America (Kundu, 2009). In Bali, usually champaca was used as landscape element of yard and roadside, even in others part of Indonesia, champaca

timber was used as building material, such as for Balinese Hindu sacred building.

Beside of that uses, champaca flower was utilized for industrial raw materials, such as essential oils (Sanimah *et al*., 2008; Punjee *et al*., 2009; Krisdiana, 2010), parfumes, cosmetics, and drugs (Zumaidar, 2009; Armiyanti *et al*., 2010). The content of essential compounds that existed at plants champaca organs have also been used as an object of research. Research by Normansyah *et al*. (2013) showed that 80% ethanol extract of yellow champaca containing terpenoids and flavonoids compounds. Ahmad *et al*. (2011) analyzed using HPTLC (high performance thin layer chromatography) found that the quercetin compound (a flavonoid) in champaca leaf is higher than in the bark. Flowers of yellow champaca identified to have the ability of antioxidant and antimicrobial activity (Kumar *et al*., 2011), and flowers of white champaca is also identified to has antioxidant and antifungal activity (Bawa, 2011), while Ananthi and Chitra (2013) examined the antioxidants of champaca flower by in-vitro method.

Champaca plant that was planted in Indonesia has many various phenotypic, as exposed by vegetative and generative organs. Vary of champaca accession usually named by character of their organ, such as color of flower. Diversity of champaca plant will continue to increase in accordance with the trait of champaca plants that can be propagated by seed. Propagation by seed causes variations in champaca traits. According to Orwa *et al*. (2009) champaca cross-pollination can be assisted by beetles.

Champaca plant varieties needs to be identified in order to determine their excellence properties that can be used as the basis of selection on further breeding programs. Generally, identification based on morphological traits (shape and structure) easy and fast, however identification by such morphological traits has many constraints, it can be affected by environment, different of plant age, and plant tissue (Khanuja *et al*., 2005). Plant identification that only base on morphological traits is difficult to apply on seedling stage.

Identification is very important to inventory and identify many champaca accessions that found and

cultivated in Bali and it is an effort to genetic germplasm conservation and plant development in the future. First step is morphological identification and genetic identification.

The aim of the study is to find out genetic variability of many champaca accessions and to assess the relationship between morphological and genetic traits of champaca accessions.

MATERIALS AND METHODS

This research was carried out at central of cultivation area in Bali, that are Sibang Gede and Sibang Kaja, Badung Regency and some areas that champaca was found. Morphological identification was done by inventory and observes important plant parts, such as leaf and flower. The plant parts were collected and measured their entire dimension.

After identification of morphological trait was done, genetic identification was carried out by using RAPD method. Young leaves were used as material of RAPD analysis. Centrifuge, digital balance, freezer, micro pipet, PCR machine, electrophoresis machine, UV transilluminator, digital camera, vortex, thermometer, scissors, knife, ice box, and stationary.

Leaves were washed by tap water until clean and dried by tissue, and 0.1 g fresh sample were used. Sample was blended by mortar and added 800 µl CTAB buffer solution. CTAB buffer solution consist of CTAB 2%, 1,4M NaCl, 100 mM Tris HCl pH 8, 20 mM EDTA pH 8, 1% PVP-40 1, 1% mercaptoethnol, and kept under 65^{o}C for 30 minutes. Mix solution incubated under 65˚C for 60 minutes. To homogenize the mixture was mixed for 10 minutes.

After incubated for 60 minutes mixtures were kept for 2 minutes then added for every 500 µl samples by 24 chloroform mixture : 1 isoamil alcohol (CIAA) and vortex for 5 minutes then centrifuged for 15 minutes at 12,000 rpm. Supernatan was taken and added cold isopropanol by 2/3 total (supernatant + sodium acetate), then mixed by shake and kept at -20˚C for minimum 1 hour. Afterward, it centrifuged at 12,000 rpm for 10 minutes. Supernatant was taken and DNA sediment washed by 500 µl ethanol 70% then centrifuged for 5 minutes at 12,000 rpm. Supernatant was taken and DNA sediment was

dried. After dry, DNA sediment was diluted again by 50 – 100 µl TE *buffer* + 1% RNAse in waterbath at 37˚C for 60 minutes. The incubation product was kept at refrigerator at 4^{o}C.

DNA purification was then quantified by *GeneQuant* to detect DNA concentration. Reference was set by used psdH2O. Dilution factor (psdH2O) about 1998 µl and 2 µl DNA poured into cuvette, then put into the *GeneQuant*.

Dilution was carried out to detect the concentration of DNA that suitable for amplification process. To get final volume of dilution, DNA solution was added of psdH2O therefore the DNA concentration suitable for PCR based on primer. Primers were utilized in this experiment as shown in Table 1.

Dilution formula:

$$V1.\ M1 = V2.\ M2$$

M1= concentration of DNA sample

V1= initial volume of sample will be diluted

M2= 2.5 ng/µl

V2= final volume

Dilution volume = V2 - V1

DNA amplification was carried out by PCR to amplify DNA sequent

PCR was carried out at 10 µl total volume on every PCR tube. Each PCR consist of 5 µl PCR *mix* Go Taq® Green (*Promega*), 0.25 µl 100 µM primer (*Sigma-Proligo*), 2.5 µl DNA sample (*template*) and 2.25 *steril aquabidest*.

Tabel 1. Concentration and purity DNA

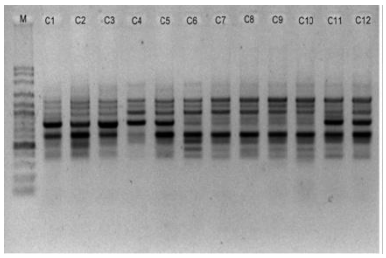
No.	Sample	DNA concentration (ng/ µl)	DNA purity
1.	Putih Wilis	2250	1.731
2.	Putih Pateh	1800	1.333
3.	Putih Patemon	2050	1.206
4.	Putih Sibang	3650	1.352
5.	Kuning Muda Patemon	1850	1.542
6..	Kuning Kecil Patemon	4000	1.455
7.	Kuning Besar Patemon	1800	1.565
8.	Kuning Kecil Sibang	950	1.462
9.	Kuning Tua Sibang	1100	1.692
10.	Kuning Besar Sibang	300	1.200
11.	Kuning Muda Sibang	1400	1.867
12.	Kuning Punah Sibang	1100	2.000

Tabel 2. Primers were used to DNA amplification of champaca

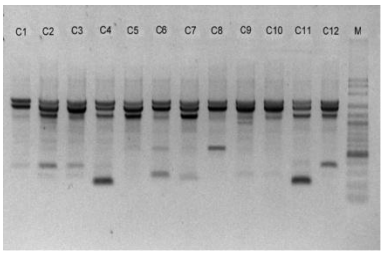
Primer	Nucleotide base sequent
OPA 5	5' AGGGGTCTTG 3'
OPA 7	5' GAAACGGGTG 3'
OPA 8	5' GTGACGTAGG 3'
OPB 3	5' CATCCCCCTG 3'
OPB 5	5' TGCGCCCTTC 3'
OPB 7	5' GGTGACGCAG 3'
OPB 8	5' GTCCACACGG 3'
OPC 1	5' TTCGAGCCAG 3'
OPC 2	5' GTGAGGCGTC 3'
OPC 4	5' CCGCATCTAC 3'

DNA amplification was done by PCR *System* BOECO equipment. First denaturation was done at 95^{o}C for 1 minute, then followed by 45 cycles with temperature at 95˚C for 45 seconds, annealing at 37^{o}C for 1 minute, and elongation at 72˚C for 1 minute and 30 seconds, then followed by final elongation at 72^{o}C for 7 minutes.

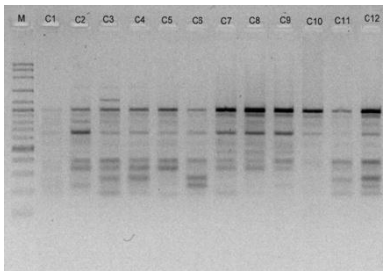
DNA of PCR result then electrophoreses by 1,0 % (b/v) agarosa that was added *ethidium bromide*, in TBE buffer (consist of 0,45 M Tris-HCl pH 8, 0,45 M *Boric acid*, 20 mM EDTA) 100 volt in voltage for 45 minutes. Subsequently, it visualized by UV light, as shown in Figure 1.



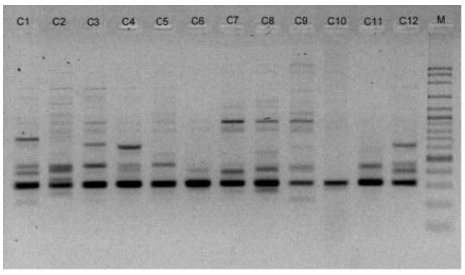
OPA 5



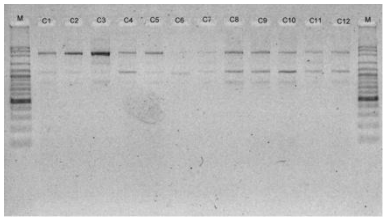
OPA 7



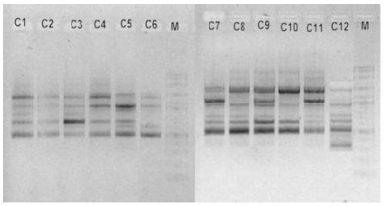
OPA 8



OPA 3



OPB 5



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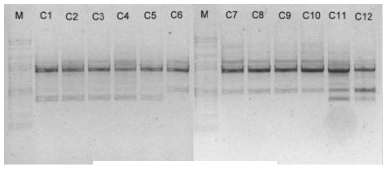

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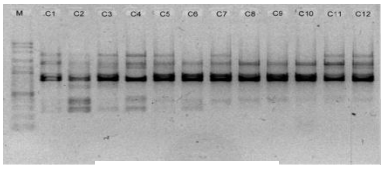
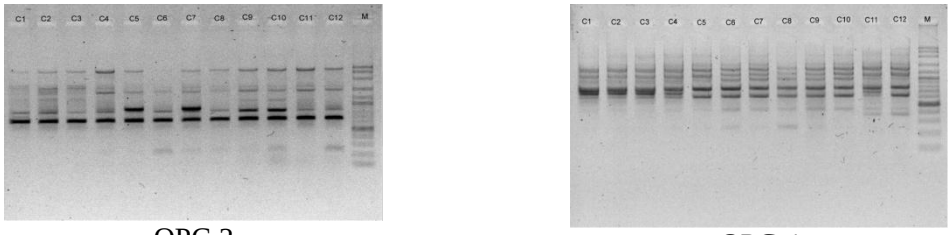


OPC 1



OPC 2 OPC 4

Figure 1. DNA amplification result of champaca by ten primers

Data was found by scoring of electrophoresis result to each sample at certain size, if presence of band was scored 1 and if absence scored 0. Binary data then analyzed by NTSYS-pc ver. 2.2.

RESULT AND DISCUSSION

Based on the results of field observations found that champaca plants cultivated as a cultivation plants are in Sibang Gede and Sibang Kaja, Badung Regency and Patemon, Buleleng Regency. Champaca spread all over Bali, sporadically from low land to highland.

Based on inventory result, champaca plant named based on flower color and their location. According to similarity and difference of champaca characters that were observed, it be discovered 12 champaca variances. Champaca plants grouped into 2 species that depend on color flower that are white champaca and yellow champaca. Twelve variances were found as follows on Figure 2 and Table 3.

White champaca (*Magnolia alba*) has morphology trait as follows: up to 30 meters in high. On the branches of white champaca usually covered with grayish fine hairs. The leaves are oval and green. Petiole is quite long, reaching almost half the length of the leaves. The white champaca also has white flowers that have a distinctive fragrance (Orwa, 2009; Flora of China, 2008).

Morphological trait of yellow champaca plants (*Magnolia champaca*) is as follows (Orwa, 2009; Flora of China, 2008). Yellow champaca trees can reach a height of 15-25 m. Ends of twigs are hairy. The leaves are ovate lanceolate shape, with a pointed tip and base, 10-28 x 4.5 to 11 cm, thin as the skin.

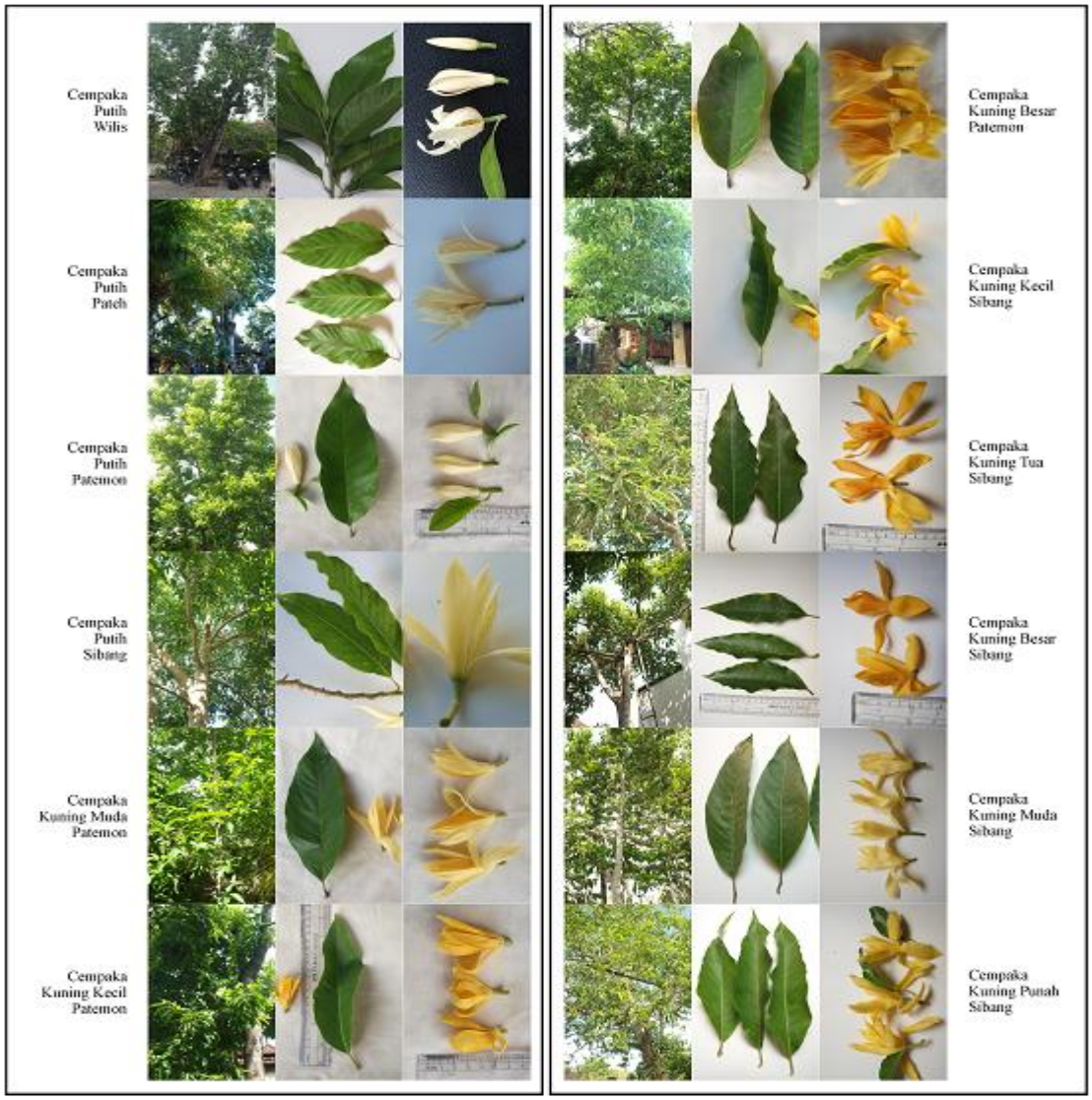


Figure 2. Twelve variances of champaca found in Bali

Table 3. Twelve variances of champaca found in Bali and their traits

No. Variance	Name	Specific Trait
1.	Cempaka Putih Wilis	Flower color white; small sized
2.	Cempaka Putih Pateh	Flower color white; small sized, huge habitus
3.	Cempaka Putih Patemon	Flower color white; medium sized, leaf as Cempaka Putih Sibang
4.	Cempaka Putih Sibang	Flower color white; large sized
5.	Cempaka Kuning Muda Patemon (Putih Cenana)	Flower color yellowish white; smooth tepal
6.	Cempaka Kuning Kecil Patemon	Flower color yellow; small sized
7.	Cempaka Kuning Besar Patemon	Flower color yellow; large sized
8.	Cempaka Kuning Kecil Sibang	Flower color yellow; small sized
9.	Cempaka Kuning Tua Sibang	Flower color dark yellow; large sized
10.	Cempaka Kuning Besar Sibang	Flower color yellow; large sized
11.	Cempaka Kuning Muda Sibang	Flower color yellowish white; smooth tepal
12.	Cempaka Kuning Punah Sibang	Flower color pale yellow; unsmooth tepal

Scared stipule of the petiole length is more than half of the petiole.On the base of the pole-shaped flowers, ovaries and stamens clearly separated by a space. Ovary is more than 20, squeezed, flattened egg shape, hairy, each with some ovules. Fruit shape is spherical elongated, slightly bent, first green then pale gray, covered with acne. Ripe seeds are dark red hanging out on the beam that extends into a slender thread.

Morphologically, champaca can be grouped into 5 clusters. First cluster consist of 1 accession i.e. Cempaka Putih Wilis, Cluster-2 consist of 4 accession i.e. Cempaka Putih Pateh, Cempaka Putih Patemon, Cempaka Kuning Muda Sibang, and Cempaka Kuning Muda Patemon.

Cluster-3 consist of 1 accession i.e Cempaka Putih Sibang. Cluster-4 consist of 4 accessions i.e. Cempaka Kuning Kecil Patemon, Cempaka Kuning Kecil Sibang, Cempaka Kuning Tua Sibang, and Cempaka Kuning Besar Sibang. Cluster 5 consist of 2 accessions i.e. Cempaka Kuning Besar Patemon and Cempaka Kuning Punah Sibang. The dendrogram of morphological trait as shown on Figure 3.

After identify genetically by RAPD method by using 10 primers, champaca found in Bali can be clustered into 4 clusters. Cluster-1 consist of 1 accession i.e. Cempaka Putih Wilis. Cluster-2 consist of 4 accessions i.e. Cempaka Putih Sibang, Cempaka Kuning Muda Patemon, Cempaka Kuning Muda Sibang, and Cempaka Kuning Punah Sibang.

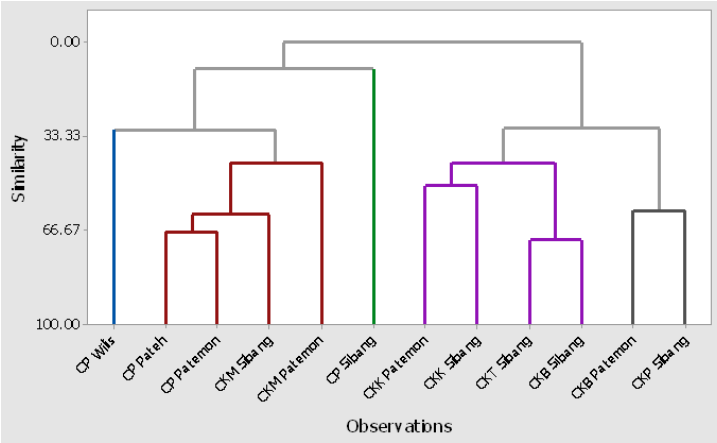


Figure 3. Dendrogram based on morphological trait of champaca found in Bali

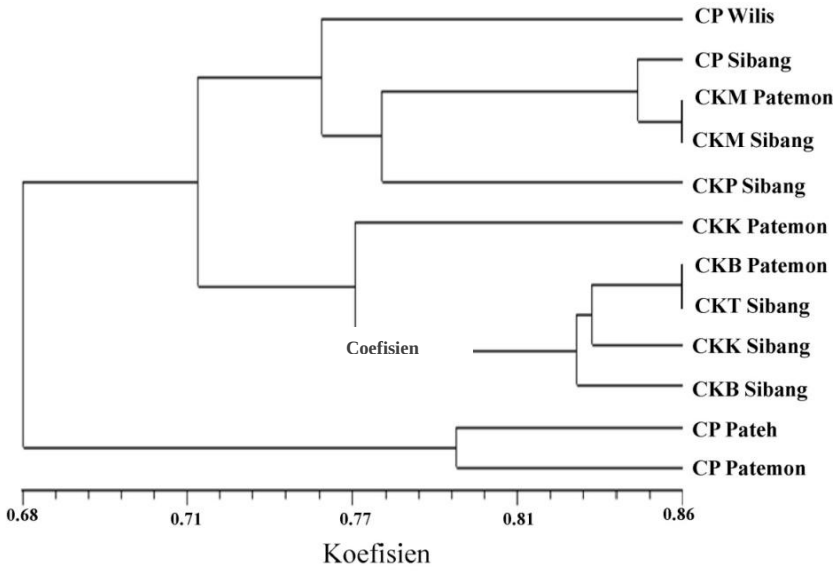


Figure 4. Dendrogram of genetic distance of 12 champaca found in Bali

Cluster-3 consist of 5 accessions i.e. Cempaka Kuning Kecil Patemon, Cempaka Kuning Besar Patemon, Cempaka Kuning Tua Sibang, Cempaka Kuning Kecil Sibang, and Cempaka Kuning Besar Sibang. Cluster-4 consist of 2 accessions i.e Cempaka Putih Pateh and Cempaka Putih Patemon. Dendrogram of genetic analysis by RAPD method shown in Figure 4. Morphologically, champaca in Bali can be grouped into 4 clusters and therefore, based on RAPD analysis champaca in Bali could be grouped into 5 clusters.

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