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Images on front cover: Breeding research work on upland cotton (*Gossypium hirsutum* L.) carried out at the Department of Plant Breeding and Genetics, University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan. Photos contributed by Naqib Ullah Khan, Ph.D.

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The Editor-in-Chief would sincerely like to thank the many reviewers for their time and efforts. An updated list of reviewers is maintained on the website.

Thanks to the publishing team for their countless hours of work for this issue.

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GENETIC PROFILES OF THREE *CINCHONA* SPECIES IN JUNGHUHN NATURAL RESERVE, INDONESIA

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SUMMARY

Cinchona species were widely used as ancient medicines for different diseases because they contain the active component quinine and its derivatives. However, studies on the molecular aspects of cinchona, including its genetic diversity, have not been reported because most previous works focused on the administration of the antimalarial cinchona alkaloid. Quinine is also being tested as alternative compound for the treatment of Covid-19. The Junghuhn Natural Reserve in Indonesia contains three different types of cinchona plants, namely, *Cinchona calisaya*, *Cinchona pubescens*, and *Cinchona* sp. Given that the genetic diversity and kinship of these species have never been studied, collecting data on the cinchona gene pool has become imperative. This study analyzed the genetic diversity of the cinchona species in the Junghuhn Natural Reserve, Indonesia, by using eight RAPD markers, i.e., OPA-2, OPA-9, OPB-02, OPB-03, OPB-04, OPB-05, OPB-7, and OPJ-07, during 2020 at the University of Jenderal Soedirman, Purwokerto-Indonesia. Polymorphic band data were obtained. Then, phenogram analysis was conducted by using UPGMA and maximum parsimony with MEGA7. The RAPD profiles of *Cinchona* species (*C. calisaya*, *C. pubescent*, and *Cinchona* sp.) revealed polymorphism with different markers, i.e., OPA-2 (90%), OPB-2 (75%), OPB-5 (75%), OPB-3 (66.66%), OPB-4 (66.66%), OPB-7 (66.66%), OPJ-7 (66.66%), and OPA-9 (58.33%) sequentially with total polymorphism (70.62%). *C. calisaya* was identified as the most distinctive species. UPGMA yielded a coefficient of 0.200 and two distinctive groups: Group I, which comprised *C. pubescens* and *Cinchona* sp. with the p-distance value of 0.333, and Group II, which contained *C. calisaya. Ixora* sp. was treated as an outgroup plant. The topology of the dendrogram was consistent with that of the UPGMA dendrogram. Results may be used for the further exploration of the genetic diversity of cinchona species.

Keywords: Cinchona species, genetic diversity, RAPD markers, polymorphism, UPGMA analysis

Key findings: Cinchona plants have been explored for their medicinal benefits but not their genetics in relation to their gene pools.

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INTRODUCTION

The *Cinchona* genus is a member of the Rubiaceae family. It was introduced by the Dutch from South America to India, Java, Cameroon, and Vietnam during the early colonial era and was later widely cultivated in Java by Franz Wilhelm Junghuhn to fulfill the increased demand for antimalarial alkaloid compounds in the mid-1900s. However, due to poor management, these countries still import alkaloid compounds from other countries (Hoogte and Pieters, 2016). Alkaloids and organic acids are found in cinchona bark, and their derivatives comprise more than 20 other different compounds, including quinine, quinoline, quinidine, cinchonidine, and cinchonine. In the pharmaceutical industry, these components are used to treat different medical problems, such as stomach problems, blood vessel disorders, leg cramps, mild influenza, cancer, mouth and throat diseases, and enlarged spleen, and are also used as appetizers and digestive-fluid inducers (Achan *et al*., 2011; Kacprazak, 2013; Pratiwi *et al.*, 2020).

Quinine acts as a schizonticide agent to increase erythrocyte numbers in patients with malaria. It is also used to enhance the bitter taste of beverages (Purnaningsih, 2013). Quinidine is used as a cardiac depressant or as an antiarrhythmic agent in heart attacks. Other two major alkaloid quinoline components, namely, cinchonidine (the nonmethoxylated form of quinine) and cinchonine (the nonmethoxylated form of quinidine), act as antisporozoan compounds and antidotes to toxins (Cheng *et al*., 2014). They may also be applied as eye lotions, painkillers, antibacterial precursors, astringents, hemorrhoid and ulcer treatments, and hair growth stimulants (De-Villiers *et al*., 2012). Cinchona species are planted intensively for industrial uses (Goss, 2014).

Cinchona is an upright woody plant with a height of 10–20 m and stem diameter of up to 30 cm (Andersson and Antonelli, 2005). Cinchona plants have dense and either irregular or globular crowns with oval and dark-green leaves in an opposite position with one another completed with a thick central nerve. Their flowers are either white, pinkish to red, with white hair-like structures found on the panicle. Cinchona fruits are dark brown in color with lengths of 2–4 cm and 3–4 seeds. The cinchona bark is 2–6 cm thick with brown color; it contains alkaloids, which are important compounds in the pharmaceutical industry and are used to differentiate the main stem from branches. Gurung and Puspal (2017) recommended using these differences to distinguish and divide cinchona plants into two species, i.e., *Cinchona calisaya*, which is yellow in color, and *Cinchona pubescent,* which is reddish in color.

At least three different cinchona species can be found in Junghuhn Natural Reserve, Indonesia; these cinchona species differs from other species belonging to the Rubiacae family (Gurung and Puspal, 2017). Studies on cinchona using molecular approaches have mainly explored the active compounds, instead of the genetic diversity, of this species. Considering its complexity, profiling the genetic variance of cinchona species at the molecular level might actually help pharmacists identify the fittest species containing the most abundant alkaloid compounds. Since the early 1980s, the application of DNA markers has become important for the exploration of genetic information (Nadeem *et al*., 2018). DNA markers unveil all plant genomes, are unaffected by plant growth phase, and describe the diversity of characteristics among individuals with high specificity. Molecular markers, such as isozyme, RFLP, rapid amplified polymorphism DNA (RAPD), AFLP, and SSR markers, have been used as genetic markers with a high

resolution of genetic variation among individuals and between and within species (Pandin, 2010). RAPD markers, which are based on PCR, are considered to be favorable due to their capability to analyze genetic diversity at the inter- and intraspecific levels (Langga *et al*., 2012; Al-Saghrir and Abdel-Salam, 2015; Uslan and Pharmawati, 2015). The utilization of RAPD markers in molecular breeding, fingerprinting, genetic diversity analysis, phylogenetics, and parthogenesis analysis, has been widely reported. Fei *et al*. (2014) used 180 operon primer-RAPD markers to investigate parthogenesis in *Taxus chinensis var mairei*. OP RAPD primers have been used to reveal the genetic diversity distance of 12 populations of patchouli grown in Bali (Pharmawati and Candra, 2016). Annisa *et al*. (2019) distinguished the genetic diversity of nine bamboo species by using RAPD primers (Zhang *et al*. 2010; Kuluev *et al*., 2018). This method uses random primers to detect polymorphisms at several loci and amplify loci from the genome randomly (Dhaksanamoorthy *et al*., 2017).

Purnaningsih (2013) reported that RAPD markers, namely, OPA-04, OPC-08, OPC-10, OPH-19, and SC10-20, exhibited good reproducibility and consistency in the analysis of somaclonal variations in *C. pubescent* clones. Dimitrijevic *et al*. (2018) utilized various OPB primers, i.e., PB-06, OPA-02, OPA-07, OPA-25, OPB-07, and OPB-18, to explore the genetic diversity of *Aegylops* sp. However, RAPD markers also have several drawbacks, such as low reproducibility while requiring stringent experimental procedures and high-molecular-weight samples. Thus, this technique has some limitations when applied in cases with degraded DNA. This study was proposed to obtain the RAPD profiles of three cinchona species cultivated in the Jughuhn Natural Reserve, Indonesia, to determine their genetic diversity and phylogenetic positions.

MATERIALS AND METHODS

Plant material

This study was carried out during 2020 at the University of Jenderal Soedirman, Indonesia. A survey was performed as a preliminary study to identify the availability of the cinchonas in the Junghuhn area. Three samples of the fresh leaves *C. calisaya*, *C. pubescent*, and *Cinchona* sp. (Table 1) were placed separately in plastic boxes and brought to the laboratory. The local plant *Ixora* sp. was used as an outgroup species. The genomic DNA of each sample was extracted and subjected to PCR-RAPD by using eight different markers, MyTaq PCR master mix, and 100 bp DNA ladder III.

Plant DNA isolation

Genomic DNA was isolated from the fresh leaves of three cinchona species and *Ixora* sp. by using the protocol of the Geneaid™ plant genomic DNA mini kit. The leaves were cleaned with 70% alcohol and macerated by using a mortar and pestle following the addition of GP1 buffer. The sample was then transferred into 1.5 mL microtubes, added with 5 µL of RNAse, and vortexed. The tubes were then incubated in a water bath for 15 min at 60 °C, added with 100 µL of GP2 buffer, and revortexed. In the meantime, cold elution buffer was heated for 30 s. The sample was transferred into a 2 mL collection tube with a filter column and centrifuged for 8 min at 1000 × *g*. The supernatant was collected and placed into a new 1.5 mL tube and mixed with GP3 buffer and isopropanol by as much as 1.5 times the supernatant volume. The mixture was transferred into a new 2 mL collection tube with a GD column and centrifuged at 15 000 × *g*. The GD tube and solution were retained, mixed with 400 µL of W1 buffer, and recentrifuged for 30 s at 15

Table 1. Morphological characters of *Cinchona* species grown in the Junghuhn Natural Reserve, Indonesia.

Character	*C. calisaya*	*C. pubescent*	*Cinchona* sp.
Stem	Woody, 6–10 m height	Woody, 6–10 m height	Woody, 6–10 m height
Leaves	Chartaceous leaf blade with acute to attenuate base; domatia present as small pits on the lower of the leaves; leaf ratio of <2	Glabrous leaf blade with midrib and veins; domatia very indistinct pouch-shaped; leaf ratio of 1:1	Chartaceous leaf blade, acute to attenuate at base; leaf ratio of <2
Seed	Capsules 8–15 mm long; thin chartaceous endocarp, valves wide open; dry seeds 3–7 mm in length (including wing)	Capsules <50 mm long; acropetal dehiscence endocarp; valves remained closed; dry seeds 6.9–8.5 mm in length (including wing)	Capsules 8–15 mm long; thin chartaceous endocarp, valves wide open; dry seeds 3–6.5 mm in length
Flower	White corolla, <8 mm tube	Pinkish to whitish corolla, >8 mm tube	Unacquired
Reference	Tao and Taylor (2011)	Andersson and Antonelly (2005)	Incomplete

000 × *g*, whereas the remaining material, including the flow-through solution, was discarded. The mixture in the GD column was mixed with 600 µL of wash buffer with absolute ethanol and centrifuged for 30 s at 15 000 × *g*. The flow-through solution was discarded. The column matrix was then centrifuged for 3 min at 15 000 × *g* and transferred into a clean 2 mL tube. A total of 100 µL of the preheated elution buffer was poured into the GD column. The mixture was left for 5 min and recentrifuged for 30 s at 15 000 × *g* for the elution of purified DNA.

DNA quality and quantity measurement

Genomic DNA was resolved on 1% electrophoresis agarose gel and visualized by using a UV transilluminator, and its concentration was measured by using a spectrometer (Table 2). Subsequently, the pure obtained genomic DNA was diluted to the final concentration of 5 ng/mL to obtain the PCR-RAPD template.

RAPD marker amplification by using PCR

Eight different RAPD primers that had been previously reported to show polymorphism in different species of plants were used. Table 3 shows the RAPD primers that were used along with their nucleotide sequences and references. The PCR volume was prepared with a total volume of 12.5 µL. It contained 6.25 µL of Mytaq PCR mix, 1 µL of DNA template, 1 µL of 10 µM primer, and 4.25 µL of nuclease-free water. The PCR machine was set with the following cycles: one cycle of predenaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 15 s, followed by annealing at 31 °C for 15 s, elongation at 72 °C for 10 s, and 1 final cycle of elongation at 72 °C for 5 min.

Table 2. DNA concentration and absorbance ratio of *Cinchona* spp. and *Ixora* sp. in duplicate.

Species sample	Concentration (ng/µL)		Ratio 260/280	
	1	2	1	2
C. calisaya	37.0	38.5	1.947	1.918
C. pubescent	84.5	47.0	1.724	1.679
C. sp.	102.0	26.5	1.872	1.893
Ixora sp.	17.5	21.5	1.591	1.792

Table 3. List of PCR primers used in the study.

No.	Primer	Sequence (5'– 3')	Reference
1	OPB-2	TGATCCCTGG	Ardi *et al*. (2012)
2	OPB-3	CATCCCCCTG	Ardi *et al*. (2012)
3	OPB-4	GGACTGGAGT	Ardi *et al*. (2012)
4	OPB-5	TGCGCCCTTC	Ardi *et al*. (2012)
5	OPB-7	GGTGACGCAG	Bahurupe *et al*. (2013)
6	OPA-2	TGCCGAGCTG	Al-Saghrir and Abdel-Salam, (2015)
7	OPA-9	GGGTAACGCC	Al-Saghrir and Abdel-Salam, (2015)
8	OPJ-07	CCTCTCGACA	Malviya and Yadav, (2010)

Visualization

The PCR products were visualized via 1.5% agarose gel electrophoresis. The gel was prepared as follows: 0.45 g of agarose was mixed with 1× TAE buffer to a total volume of 30 mL and heated to dissolve the agar fully. The heated agarose solution was allowed to cool before being mixed with 1 µL of EtBr. Then, it was poured into a tray with an electrophoretic comb and allowed to set for 15 min to form a gel. The tray containing the gel was submerged in an electrophoretic tank filled with 1× TAE buffer. The PCR products were loaded into the gel's wells. Then, 5 µL of the 100 bp DNA ladder was loaded into the first well. The device then was connected to a power supply set at a voltage of 80 V and 250 mA for 45 min. The gel was visualized by using a UV transilluminator. Bands were photographed with a digital camera.

The DNA banding patterns that emerged from each individual sample were used to determine the polymorphism among species by calculating the numbers of alleles that appeared and then scored on the basis of a binary code (1: present; 0: absent) regardless of intensity. These data were then used to determine the genetic diversity among cinchona species by grouping the species in a phenogram on the basis of their similarity or dissimilarity through cluster analysis with UPGMA and maximum parsimony method in MEGA7.

RESULTS AND DISCUSSION

This study, which focused on the RAPD profiles and genetic relationships of the three cinchona species, was conducted through three main processes, i.e., genomic DNA extraction, DNA amplification by PCR-RAPD, and data analysis by UPGMA and maximum parsimony method in MEGA7.

The data showed that the applied DNA isolation method provided genomic DNA yields of 17.5–102 ng/µL, which were sufficient amounts for RAPD analysis as stated by Reinoso and Bettera (2016) and were in accordance with the low concentrations of DNA template needed for RAPD analysis. Furthermore, Hoople (2016) stated that the addition of EtBr could help increase the visibility of a low

Table 4. RAPD bands generated by eight different primers

Primers	Σ bands	Monomorphic	Polymorphic	Polymorphic percentage (%)
OPA-2	10	1	9	90.00
OPA-9	12	5	7	58.33
OPB-2	4	1	3	75.00
OPB-3	3	1	2	66.66
OPB-4	9	3	6	66.66
OPB-5	4	1	3	75.00
OPB-7	6	2	4	66.66
OPJ-7	9	3	6	66.66
Total	57	17	40	70.62

amount of DNA template by staining bands

RAPD profiles of *Cinchona*

The eight RAPD markers applied in this study resulted in 57 bands, of which 70.62% were polymorphic. According to Chesnokov and Artemyeva (2015), this percentage indicated high polymorphism (Table 4). Primer OPA-2 showed 10 bands with lengths of 300–1000 bp and 90% polymorphism (Figure 1). Interestingly, this primer produced three bands for *C. calisaya* but not for the remaining cinchona species (*C. pubescent* and *Cinchona* sp.). Therefore, these bands were specific to this species (Figure 2). These results were in line with the past findings of Khosravi *et al.* (2015), who reported that the major fragment for *Mycobacterium fortuitum* has a length of 280–300 bp. However, *Ixora* sp., as the out-group plant, did not exhibit a single band with this length. Larekeng *et al*. (2019) noted that primer OPA-2 produced 60% polymorphism in Katokkon peppers.

Primer OPA-9 amplified 12 different fragments with lengths of 200–1300 bp, among which 58.33% were polymorphic and 41.67% were monomorphic (Figure 3). Interestingly, *C. calisaya* showed a single marker band in red that was not identified in the two other Cinchona species (*C. pubescent* and *Cinchona* sp.). Two markers in black were found in *C. pubescent* but not in *C. calisaya* and *Cinchona* sp. and were thus interpreted as specific bands for *C. pubescent*. Larekeng *et al*. (2019) applied the OPA-9 primer to analyze Katokkon peppers but failed to obtain clear bands. The red bands of A1 and A2 were suspected to have originated form contamination during extraction (Souza *et al.,* 2012). Primer OPB-2 showed 75% polymorphic bands with lengths of 400–1300 bp that were specific to the said primer only (Figure 4). However, the results of this study were better than those of the work by Suryadi *et al.* (2019), who failed to obtain any polymorphic pattern for three tested peanut cultivars despite amplifying two bands.

Primer OPB-3 exhibited 66.66% polymorphic patterns with the band lengths of 400 bp to 1000 bp (Figure 5). This percentage was slightly larger than the percentage reported by Susilo *et al.* (2018), who obtained only 55.56% polymorphism when they tested banana accessions. Interestingly, the primer OPB-3 also amplified two markers that were specific for *C. pubescent* (red arrow) and another for *C. calisaya* (white) that was absent from *Cinchona* sp.; these bands may thus be concluded as specific to those particular species. The OPB-3 primer, which was used to amplify the long-pod mutant of winged bean (*Psophocarpus tetragonolobus* L.), provided amplicons with lengths of 350–600 bp in the control but slightly lengthened amplicons with lengths of up to 1000 bp after mutation (Maesaroh *et al.,* 2014). However, Neema and Reghunath (2019) reported 60% monomorphic patterns in *Indigofera tinctoria.*

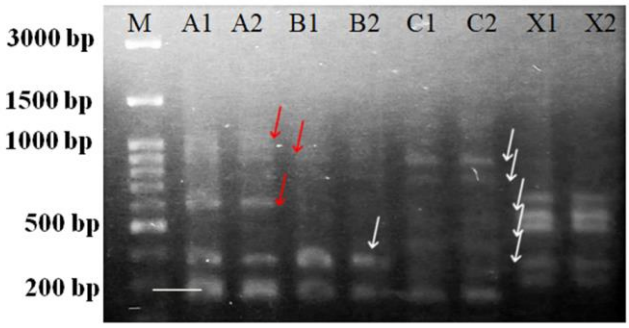

Figure 1. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPA-2 primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

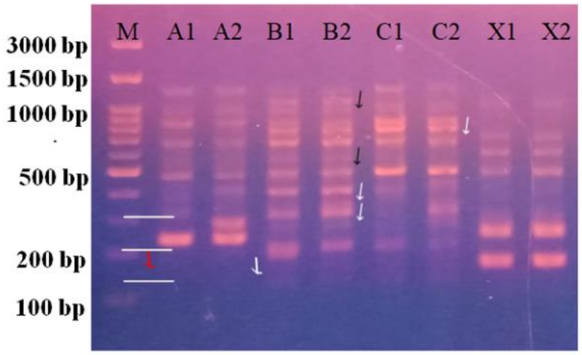

Figure 2. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPA-9 primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

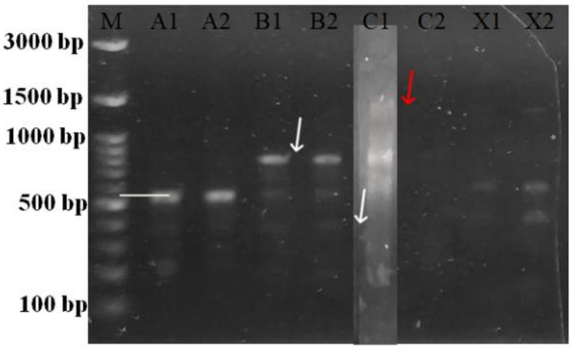

Figure 3. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPB-2 primer. Remarks: M = 100 bp DNA ladder, A = *C.* , B = *Cinchona pubescent*, C = *Cinchona* sp., X = *Ixora* sp.; each sample was tested in duplicate.

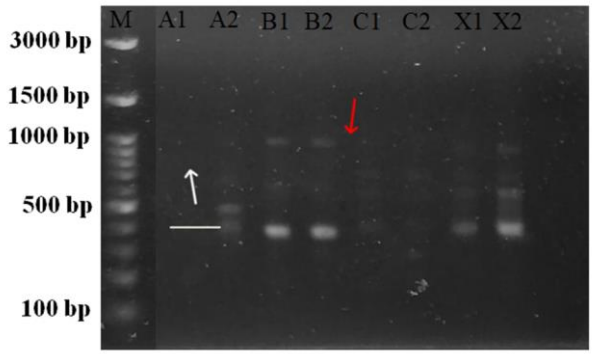

Figure 4. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPB-3 primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

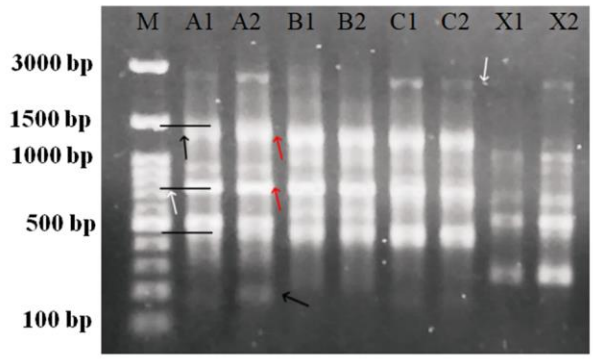

Figure 5. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPB-4 Primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

Primer OPB-4 revealed 75% polymorphism with fragment lengths of 200 bp to 2800 bp with two 900 and 200 bp markers and were to be found specific to *C. calisaya* but were absent from *C. pubescent* and *C.* sp. (Figure 6). Two distinctive markers were shown by *C. pubescent* only and were absent from the others. However, the said primer performed considerably better and revealed 100 polymorphisms when it was used to test nine tea genotypes (Martono and Udarno, 2014). Figure 6 also shows the slanted bands of the OPB-4 primer that might due to uneven electrical current during electrophoresis or the use of TAE buffer that promoted uneven heating and resulted in anomalies (Sanderson *et al*., 2014).

The RAPD primer OPB-5 produced three bands (75% polymorphic) and one monomorphic band with lengths of 200 bp to 1200 bp (Figure 7). A single marker in red color showed specificity for *Cinchona* sp. and was absent from the remaining cinchona species. By using the same primer (OPB-5) to analyze *Ganoderma lucidum*, Rolim *et al.* (2011) found 92.30% polymorphism with 26 bands. However, Bahurupe *et al.* (2013) reported that the OPB-5 RAPD marker produced only 57.14% polymorphism in 23 samples of chili genotypes. Primer OPB-7 showed 66.66% polymorphism with the fragment lengths of 200 and 1100 bp (Figure 8). A single specific marker each was found for *C. calisaya* and *Cinchona* sp.

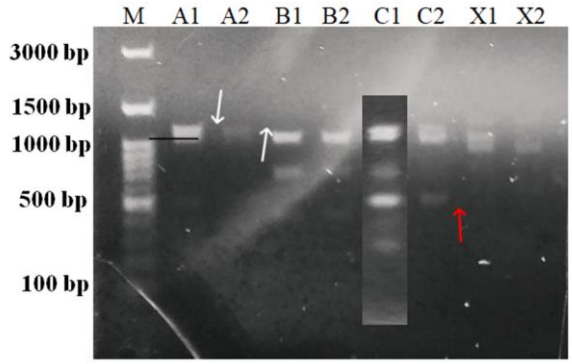

Figure 6. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPB-5 Primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X = *Ixora* sp.; each sample was tested in duplicate.

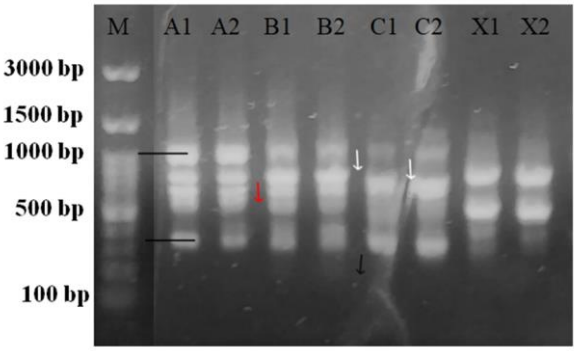

Figure 7. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPB-7 primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

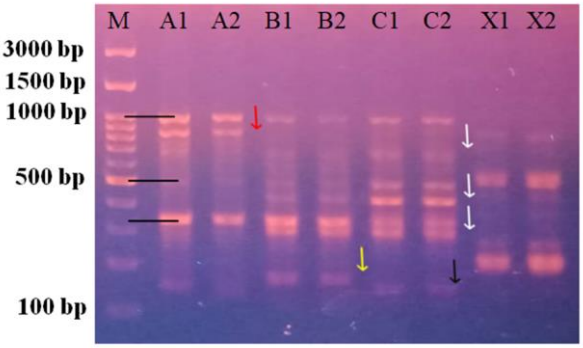

Figure 8. RAPD profile of *Cinchona* spp. and *Ixora* sp. obtained with the OPJ-7 primer. Remarks: M = 100 bp DNA ladder, A = *Cinchona calisaya*, B = *Cinchona pubescent*, C = *Cinchona* sp., X= *Ixora* sp.; each sample was tested in duplicate.

Amplification with the RAPD primer OPJ-7 yielded six polymorphic bands (66.6%) that varied in length from 150 bp to 100 bp and one distinctive band that was absent from other samples (Figure 9). *C. calisaya*, *C. pubescent*, and *Cinchona* sp. had distinct bands with lengths of 850 bp (shown by red arrow), 160 bp (with yellow arrow), and 130 bp (with black arrow), respectively. Chowdhury *et al*. (2014) reported a slightly elevated polymorphism of 71.42% when the primers were used to test Indian fenugreek varieties. The obtained three bands were found to be specific to a particular species likely because of their specific phenetic characters, which can be used for further identification as also suggested by Ellur *et al.* (2016), who identified the plants on the basis of their molecular characters.

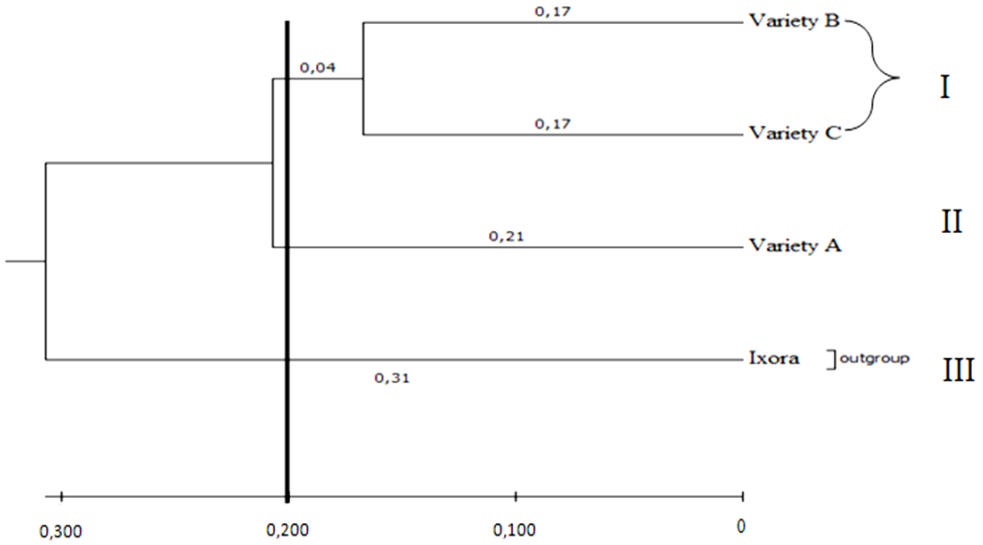

Figure 9. Phylogenetic relationship of *Cinchona* spp. and *Ixora* sp. obtained via UPGMA.

Phylogenetic relationship of *Cinchona*

The current data on the molecular characters of the three cinchona species were then analyzed on the basis of the p-distance value, which represents their genetic distance (Table 5), and later tested by comparing two samples. Furthermore, their genetic relationship was analyzed via UPGMA, which showed that each individual species can be separated from others and provided three different groups (Figure 10) with a coefficient of 0.2 for the closest possible individuals based on phenogram and genetic distances (Gouta *et al*., 2008). Chicorz *et al*. (2014) claimed that molecular performance may be used to reflect genetic diversity and group plants into different orders. The present results further revealed that the first group consisted of monophyletic *C. pubescent* and *Cinchona* sp. units. The second group contained *C. calisaya*, and *Ixora* sp. was located in the third group. The said data were in agreement with the p-distance value obtained from pairwise analysis. The species *C. pubescent* and *Cinchona* sp. had the smallest p-distance value (0.333) and were considered as individuals in contrast to the remaining species (Gouta *et al.*, 2008).

The phenogram of cinchona based on RAPD profiles and further analysis via maximum parsimony assumed that either extreme climatic disturbance or mutation resulted in extended phenogram topology (Tandazo-Yunga *et al.,* 2017). In this

Table 5. Genetic distance matrix of *Cinchona* species and *Ixora* sp.

Sample	*C. calisaya*	*C. pubescent*	*Cinchona* sp.	*Ixora* (outgroup)
Chincona calisaya		0.058	0.059	0.064
Chincona pubescent	0.406		0.056	0.056
Chincona species	0.420	0.333		0.056
Ixora (outgroup)	0.493	0.638	0.710	

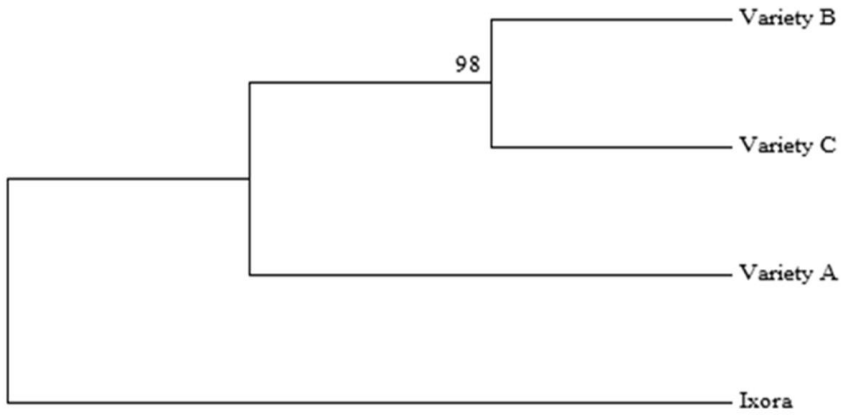

Figure 10. Phylogenetic relationship of *Cinchona* species and *Ixora* sp. identified via maximum parsimony.
Remarks: The maximum parsimony tree was obtained by using the Subtree–Pruning–Regrafting algorithm. Most parsimonious tree length = 70, Consistency index = 0.724138; Retention index = 0.619048.

study, ultrametric data were excluded to minimize the effects of the identical topological positions of the groups. These results were consistent and did not interfere with each other even when the data processing method was changed. However, the present findings contradicted those of Wang *et al.* (2011), who reported a slight change in topology following the change in data processing method by using maximum parsimony.

CONCLUSIONS

The obtained data indicated that RAPD markers can to profile the genetic diversity of three cinchona species in the Junghuhn area. These species were grouped into two groups. *C. pubescent* was closest to *Cinchona* sp. with the p-distance of 0.333.

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