Genetic characterization of iler (*Plectranthus scutellarioides* (L.) R. Br.) based on RAPD molecular marker

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**Abstract.** Iler (*Plectranthus scutellarioides* (L.) R. Br.) is an ornamental plant and also used as medicinal plant. The plant is one of medicinal plant for anti hemorrhoids Jamu Saintifik herbal formula in B2P2TOOT Indonesian Ministry of Health. This will lead to commercialization opportunities in herbal drug industry that recently has become increasingly popular. With the emergence of substitution and adulteration of herbal drug and the lack of information on coleus genetic diversity which may result in misidentification, this research was conducted to determine Ille genetic characteristics using RAPD markers. The genomic DNA was extracted from 15 accessions of iler and 3 accessions from genus Plectranthus as an outgroup. Six selected RAPD primers were used in amplification. Dice similarity index was used to calculate similarity index followed by cluster analysis and dendrogram construction using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The results showed 52 DNA fragments were produce within the size range 293-2,667 bp and polymorphism level among accessions at 94.23%. Cluster results results in genetic diversity ranging from 40.45-97.87% and a dendogram that shows clustering trend of Ille accessions based on the similarity of morphological characters such as leaf shape and color.

1. Introduction
Ille (*Plectranthus scutellarioides* (L.) R.Br. are herbaceous a plant that has many variation in leaf character, growth habitus and inflorescence characteristics; more than 500 cultivars of iler were documented [1]. This plant is native to Africa and Southeast Asia and usually grown as an ornamental due to leaves variation. Variation of leaves are consist of leaf shape (narrow or wide; round or ovate, leaf edges shape), foliages coloration (red, pink, purple, green, yellow, orange, brown), and foliar variegation [2].

In addition, as an ornamental plant, Ille is also used as a medicinal plant to treat new wounds, dyspepsia, headaches, asthma, and digestive problems. This plant reported has anti-inflammatory, antioxidant, analgesic, and antibacterial activity [3]. Chemical content in Ille leaves are triterpene, campesterol, α-amirin, β-amirin, essential oils and salvinorin-like compounds [3]. While the main compound in iler leaves is rosmarinic acid [4]. Based on clinical observation research on the efficacy and safety, Iler is one plant for hemorrhoids traditional herbal formula or known as Jamu Saintifik to treat hemorrhoids. This will lead to commercialization opportunities in herbal drug industry that recently has become increasingly popular. In view of its medicinal utilization for Jamu Saintifik, and in order to
ensure the quality of its supply, standardization process is usually needed. Iler standardization deals with its intraspecific variation also adulteration/substitution among species in genus. With the emergence of substitution and adulteration of herbal drug and the lack of information on coleus genetic diversity which may result in misidentification.

To date, it is very lack Information on genetic diversity of *P. scutellarioioides* accession using molecular marker in Indonesia. Molecular techniques help researchers not only in terms of genetic authentication but also estimate and explore genetic variability of plant species [5]. Molecular markers are an alternative method that can be used for genetic diversity analysis in medicinal plants and not affected by age, physiological conditions, and environmental factors [6]. RAPD is one of molecular technique that has been successfully used to study genetic diversity and authentication in plant species which are valuable in agricultural and industrial. RAPD has several advantage due to its low cost, rapid, easy, needs a small sample with no prior sequence information [6]. This research was conducted to determine Iler genetic characteristics using RAPD markers.

2. Materials and methods

2.1. Plant materials
A total of 15 Iler accessions collected from different location (Java, Sumatera and Papua), including iler that cultivated in B2P2TOOT Tawangmangu, and 3 accessions as an out group from genus Plectranthus namely *Plectranthus amboinicus, Plectranthus forsterii, Plectranthus forsterii* variegated leaves (Figure 1).

![Iler accessions and its origin. MD1/ North Sumatera, MD2/ North Sumatera 2, MD3/ North Sumatera, CT/ West Java, TW1/ B2P2TOOT Collection, TW2/ B2P2TOOT Collection, TW3/ B2P2TOOT Collection, ML1/East Java, ML2/ East Java, JP/Papua, 118 H/ B2P2TOOT Collection, 118 UHK/ B2P2TOOT Collection, 118 UHL/ B2P2TOOT Collection, 118 UHV/ B2P2TOOT Collection, 118 UK/ B2P2TOOT Collection, PA= Plectranthus ambonicus, PFL= Plectranthus forsteri variegata, PFP Plectranthus forsteri.](image-url)
2.2. Genomic DNA isolation
Total Genomic DNA was isolated from 0.1 gr fresh leaves using DNA kit isolation (Thermo Scientific gene jet plant genomic DNA purification Mini Kit, Catalogue number K0791). Isolated DNA was quantified by UV-Vis spectrophotometer with absorbance at 260/280 nm and also in electrophoresis using 1% agarose gel.

2.3. PCR-RAPD analysis
Six RAPD primers were selected from 25 primers that produced clear and high polymorphism DNA fragments (Table 1). Component of PCR reaction were DNA template (25 ng μL⁻¹) 2 μL, PCR mix 12.6 μL, primer (20 μM) 0.8 μL, dan nuclease-free water 9.6 μL.

PCR amplifications were performed using thermal cycler (C-1,000 Bio-Rad, USA) with the following program: 95°C for 3 min, followed by 39 cycles of 94°C for 1 min, annealing at 36°C for 60 s, and elongation at 72°C for 2 min, and a final extension at 72°C for 7 min. Amplified products were separated on 2% agarose gels, 50 V for 90 minutes. Visualisation of electrophoresis gel using UV light was carried out and documented using a gel documentation system (Imaging System XR + Bio-Rad, USA).

2.4. Data analysis
Only clear DNA fragments of PCR product were scored as presence (1) or absence (0) of each primer used and in all accessions. Similarity indexes of all accessions were calculated based on Dice similarity [7]. The dendrogram was constructed using Unweighted Pair Group Method Using Arithmetic Mean (UPGMA) cluster analysis. This data analysis was performed by NTSYS software ver. 2.0 [8].

3. Results and discussion
Out of 25 primers screened, 6 primers produced clear and high polymorphism DNA fragments among accessions (Figure 2.). A total of 52 amplified fragments were generated using 6 selected primers, of which 49 were polymorphic and 94.23% of polymorphism (Table 1). DNA fragments size ranged from 293-2,667 bp, with an average number of DNA fragment produced of 8.6 per primer.

Table 1. Polymorphism percentage of 15 iler accessions and Plectranthus spp. as an outgroup using 6 RAPD Primers

| No | Primer | Primer Sequens (5’ — 3’) | Fragment size (bp) | Polymorphism percentage |
|----|--------|--------------------------|--------------------|------------------------|
| 1  | OPA-1  | CAGGCCCTTC               | 479-2,667          | 9/9 (100%)             |
| 2  | OPA-18 | AGGTGACCGT                | 388-2,536          | 7/8 (87.5%)            |
| 3  | OPB-1  | GTTTTCGCTCC               | 315-1,159          | 9/10 (90%)             |
| 4  | OPC-2  | GTGAGGGCCTG               | 364-1,582          | 9/10 (90%)             |
| 5  | OPD-2  | GGACCCAACC                | 293-1,166          | 9/9 (100%)             |
| 6  | OPH-13 | GACGCCACAC                | 645-1,954          | 6/6 (100%)             |

Total 49/52 (94.23%)

Primer OPB-1 and OPC-2 generated the highest number of amplified fragments, meanwhile primer OPA-1, OPD-2 and OPH-13 showed the highest polymorphism (100%). RAPD molecular markers was used to characterized commercially iler accessions from India using 3 selected primers, namely OPD-05, OPH-20, and OPC-12 [9]. Meanwhile, five iler varieties from India were analysis using 6 RAPD primers (OPD-5, OPB-7, OPB-17, OPH-20, OPW-6, and OPW-7) [10].
Figure 2. RAPD Profile of 15 iler accessions and *Plectranthus* spp. as an outgroup using RAPD primers. a. OPD-2, b. OPA-1, c. OPA-18, d. OPB-1, e. OPC-1, f. OPH-13 Primer. MD1/ North Sumatera, MD2/ North Sumatera 2, MD3/ North Sumatera, CT/ West Java, TW1/ B2P2TOOT Collection, TW2/ B2P2TOOT Collection, TW3/B2P2TOOT Collection, ML1/East Java, ML2/ East Java, JP/Papua, 118 H/ B2P2TOOT Collection, 118 UK/ B2P2TOOT Collection, PA= *Plectranthus ambionicus*, PFL= *Plectranthus forsteri* variegata, PFP *Plectranthus forsteri*.

A dendrogram was created using the unweighted pair group method with arithmetic mean (UPGMA) method and the result is shown in Figure 3.
Figure 3. Dendrogram of 15 iler accessions and *Plectranthus* spp. as an outgroup based on RAPD molecular markers.

Table 2. Specific DNA fragments for iler (*P. scutellarioides*) authentication.

| RAPD primer | *P. scutellarioides* (bp) | *P. amboinicus* (bp) | *P. forsteri* (bp) |
|-------------|---------------------------|----------------------|-------------------|
| OPA-1       | 800                       | 950                  | 950               |
|             | 1,246                     | 950                  |                   |
| OPA-18      | 388                       | 582                  |                   |
|             |                          | 696                  |                   |
| OPB-1       | 315                       | 368                  | 940               |
|             |                          |                      |                   |
| OPC-2       | 364                       | 387                  | 364               |
| OPD-2       |                          | 1,050                | 1,050             |
|             | 1,166                     |                      |                   |
| OPH-13      |                          | 645                  | 1,002             |
|             |                          |                      | 1,493             |

Dendrogram showed two major cluster groups that separated iler accessions and its outgroup (Figure 3.) with similarity index 40.45%. Index similarity among accessions ranged from 82.08-97.87%.
indicating low genetic diversity of *P. scutellarioides*. Three accessions of Iler formed a single cluster namely CT, MD1 and MD2. JP accessions and 118 H shared the closest genetic relationships with 97.87% genetic similarity index. Cluster I not reveal for grouping 15 accession based on origin of sample location. It was possible that the grouping based on morphological characters such as shape and colour of leaves. Several sub cluster of *P. scutellarioides* were grouping based on similarity of the colour and/or leaf margin. Similar result was found in clustering of among accessions of iler based on colour and leaves shape using RAPD molecular marker [9].

Amplification using 6 RAPD primers also showed information on specific bands for authentication *P. scutellarioides, P. amboinicus,* and *P. forsteri* (Table 2). Primer OPD-2 only produced 2 specific fragments with a size of 1,050 bp and 1,166 bp. Meanwhile, the highest number (5 fragments) of specific fragments was amplified using the OPB-1 primer, namely 5 bands with a size of 315-1,159 bp (Table 2). Those specific DNA fragments were generated in all accessions of iler and each species of *Plectranthus* spp. Screening and selection of specific DNA fragment based on reproducible in amplification of iler accessions and the outgroups, so it is possible for iler (*P. scutellarioides*) authentication [11].

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