

# Amplification of 5 Accessions of DNA Binahong (*Anredera Cordifolia* (Ten.) Steenis) By Inter Simple Sequence Repeats as Tool for Molecular Marker

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**Abstract.** Binahong or *Anredera cordifolia* (Ten.) Steenis is a plant with multiple functions for medicine. It was used as anti-hipercholesterolemia, anti-hyperlipidemia, wound healing, antioxidant, analgesic, skin diseases, anti-cancer and anti-diabetic. Binahong origin from Paraguay to Southern Brazil and Northern Argentina but spreads into Africa, Europe, Australia, and Asia, including Indonesia. In Indonesia, binahong distributed to almost in all island that used for traditional medicines. The aim of this research was to amplify DNA from 5 accessions of binahong with 15 Inter-Simple Sequence Repeats (ISSR) primers for the screening of primers. DNA of 5 accessions of Binahong from Tagari-Toraja, Solok-Sumatera Barat, Rante-Langda, Petulu-Bali, and Makale-Toraja were isolated by CTAB modification methods and were amplified by 15 ISSR primers. Amplification was done at PCR machine with the condition: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 45 seconds, elongation at 72°C for 2 minutes, and final elongation at 72°C for 5 minutes for 40 cycles. The results showed that 8 primers were able to amplification of DNA binahong, while 7 primers failed to amplification. The eight of ISSR primers produced polymorphic bands and those are SBLT2, SBLT3, SBLT4, SBLT6, SBLT8, SBLT13, SBLT14 and SBLT15 primers.

**Keywords:** binahong, *Anredera cordifolia*, traditional medicines, ISSR primers

## 1. Introduction

Binahong (*Anredera cordifolia* (Ten.) Steenis), a family member of Basellaceae, is a medicinal plant native from Paraguay to Southern Brazil and Northern Argentina (figure 1) [1]. Binahong is familiar known as Madeira vein or *Boussingaultia cordifolia* Tenore and in Indonesia familiar as gendola, which is often used as a circular gate on a garden [2]. Binahong has been introduced globally, including to China, Japan, India, Israel, parts of Africa, USA, Mexico, the Caribbean, Australia, Argentina, Africa, Europe, New Zealand, and Asia, including Indonesia. It could grow in Mediterranean, sub-tropical and tropical climates, and has become invasive species especially in Oceania and Africa [3].





**Figure 1.** Leaves of *Anredera cordifolia* (Ten.) Steenis familiar known as binahong or gendola

Star *et al.* [4] reported that binahong has become a major problem in some areas where it has been naturalized due to its invasiveness. But actually, binahong grown as an ornamental species or an accessory plant, other nations consume this plant as food and vegetables that consumed freshly in salad mixture, Taiwan, and Indonesia had been used binahong as traditional medicine [5,6].

As a medicinal plant, binahong have multiple functions for traditional medicinal. Almost all parts of the plant, starting from the root, stem, flower, and leave, are beneficial to humans and animals [2-7]. The multiple functions of binahong were reported by researchers, as antioxidant [6], anti-hypercholesterolemia [8], anti-obesities [9], anti-bacteria [10], anti-hyperlipidemia [11], anti-hypertension [12], anti-inflammation [13], anti-diabetic [14], analgesic effect [15], wound healing [16,17], and improving the failure of kidney [7]. Astuti *et al.* [18] reported that chemical contents of binahong are flavonoids, alkaloids, saponins, and triterpenoids. Sukandar *et al.* [7] added that a leaf of binahong contains saponin, flavonoid, quoin, steroid, monoterpene, and sesquiterpene, while a rhizome contains flavanoid, polyphenol, tannin, and steroid.

Molecular marker is a DNA fragments that used for detection of polymorphisms between allele from DNA sequences or different genotype of organisms [19]. Schulmann [20] said that molecular marker is gene whose phenotypic expression and frequently easily discerned and used to detect an individual. Al-Samarai and Al-Kazaz [21] added that molecular marker used as a probe to mark a chromosomes, nucleus, or locus that associated with certain of traits or genes. Inter Simple Sequences Repeat (ISSR) is one of DNA markers that was introduced by Zietkiewicz *et al.* [22], involves to amplification of DNA segment between two identical microsatellite repeat regions [23,24] without knowing the sequences of DNA. ISSR markers are highly polymorphic and used on genetic diversity, gene tagging, phylogeny, evolutionary biology and genome mapping studies [25]. The advantages of ISSR are effective, easy to operated, cheap, quick, stable and repeatable [26-28].

The research activities of binahong plant that already reported, mostly about the effect of the plants for medicinal. Research about micropropagation methods were reported by [29-31], another research about morphology and chromosome number [32] and managing to protecting invasive plants [33,34]. Study for genetic diversity of binahong plant was not reported yet. The aim for this research was to amplify DNA from 5 accessions of binahong with 15 ISSR primers for screening of ISSR primers as tool for molecular marker.

## 2. Materials and methods

### 2.1 Materials

Plant materials used in this research were leaves of binahong from 5 accessions (Tagari-Toraja, Solok-Sumatera Barat, Rante-Langda, Petulu-Bali and Makale-Toraja). Primers used for DNA amplification were 15 ISSR primers (table 1).

## 2.2 DNA isolation

Isolation of DNA was done using CTAB modification method by [35] that contain 3% of CTAB buffer for extraction and added with 1% PVP and 2% mercaptoethanol. 200 mg of young leaves of binahong were cut into small pieces and ground using mortar with addition of 250  $\mu$ L of extraction buffer (3M NaCl, 100mM Tris-HCl, 20 mM EDTA, 1% (PVP), 1% mercaptoetanol, and 3% CTAB) the next procedure following by [35] protocol instruction. Isolated DNA were test for quantitative DNA analysis using Nanodrop spectrophotometer and for qualitative DNA using electrophoresis with 1.5% gel agarose in 50 mL of 0.5 x TAE buffer. The DNA was visualized using UV illuminator after staining with Syber safe.

**Table 1.** 15 primers from ISSR marker used to amplification DNA of binahong.

Primers	Repeats	Number of base	TA (°C)
<b>SBLT1</b>	(AT)8T	17	52
<b>SBLT2</b>	(AG)8T	17	52
<b>SBLT3</b>	(AG)8C	17	50
<b>SBLT4</b>	(GA)8T	17	52
<b>SBLT5</b>	(GA)8C	17	53
<b>SBLT6</b>	(CT)8T	17	50
<b>SBLT7</b>	(CT)8A	17	50
<b>SBLT8</b>	(CT)8G	17	52
<b>SBLT9</b>	(CA)8A	17	52
<b>SBLT10</b>	(GT)8T	17	52
<b>SBLT11</b>	(TC)8G	17	50
<b>SBLT12</b>	(AC)8C	17	52
<b>SBLT13</b>	(GAA)6	18	50
<b>SBLT14</b>	(GACA)4	16	50
<b>SBLT15</b>	(GA)8	16	55

## 2.3 Screening primers

Screening primers were done using 15 primers from ISSR marker (table 1). Amplification process were done at PCR machine with condition: pre denaturation at 94°C for 5 minute, denaturation at 94°C for 1 minute, annealing at 45 second, elongation at 72°C for 2 minute, and final elongation at 72°C for 5 minute with 40 cycles. Temperature used for annealing different from each primer based on temperature annealing (TA) for each primer.

After amplification with each primer, PCR products were visualization on 1.5% gel agarose and running with electrophoresis. After visualization using UV Illuminator, DNA bands that appears into gel agarose was observed and determinate result of screening from each primers.

## 3. Results

According to [36], high purities of DNA was measured based on ratio of absorbance 260:280 ( $\text{\AA}260/\text{\AA}280$ ) at Nanodrop spectrophotometer, if the value of purities between 1.8 and 2.0, DNA has high purify. Abnormal 260/280 ratios usually indicate that a sample is contaminated by residual phenol, guanidine, or other reagent used in the extraction protocol, in which case the ratio is normally low. If the value of purities less than 1.8 was possibility DNA contaminated by RNA, but if more than 2.0 DNA contaminated by protein.

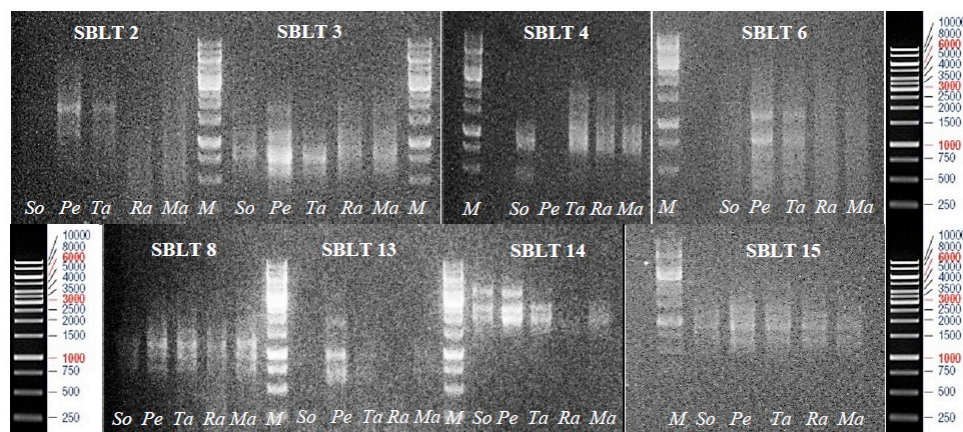
From this research data showed that with 3% CTAB modification method, purity and concentration of DNA binahong had different result based on leaves from binahong accession (table 2). Purity of DNA from 5 accessions with 3% CTAB modification method showed contaminated by protein in 4 accessions (Tagari-Toraja, Solok-Sumatera Barat, Rante-Langda, Makale-Toraja) and for Petulu-Bali accession contaminated by RNA, because none of all accessions had purity value between 1.8 and

2.00. This method was the optimal methods for DNA isolation for binahong if we compared with another method that already used. Concentration of DNA with this method showed that 3 accessions have high concentration, more than 800 ng/μL for 200 mg leaves but for Petulu-Bali accession concentration of DNA was low 36 ng/μL. Treatments for isolation of DNA binahong even were added by RNase or increase concentration of PVP and CTAB, but the optimal result of isolated DNA binahong when used [35] methods (data unpublished).

**Table 2.** DNA isolated by 3% CTAB with modification method [35].

No	Accessions	Purity of DNA	Concentration of DNA (ng/μL)
1	Tagari-Toraja	2,13	915,78
2	Solok- Sumatera Barat	2,08	466,45
3	Rante-Langda	2,12	1615,88
4	Petulu-Bali	1,77	36,28
5	Makale-Toraja	2,18	857,83

Results of 15 primers used for this research showed that 8 primers, i.e. SBLT2, SBLT3, SBLT4, SBLT6, SBLT8, SBLT13, SBLT14 and SBLT15 were capable to amplification DNA of binahong from 5 accessions (figure 2), meanwhile 7 primers SBLT1, SBLT5, SBLT7, SBLT9, SBLT10, SBLT11, and SBLT12 failed to amplified DNA binahong. 8 primers that amplified DNA binahong showed DNA bands and loci bands for each accessions of 5 binahong used. Condition of 7 primers showed that 3 primers (SBLT1, SBLT5, and SBLT7) showed empty bands and 4 primers (SBLT9, SBLT10, SBLT11, and SBLT12) showed smear without appears DNA bands.



**Figure 2.** Polymorphic bands of DNA binahong using 8 primers of ISSR, (M) Marker DNA ladder 1 Kb; (So) Solok-Sumatera Barat; (Pe) Petulu-Bali; (Ta) Tagari-Toraja; (Ra) Rante-Langda; (Ma) Makale-Toraja

#### 4. Discussion

Leaves of binahong have thick shape and slightly fleshy (semi-succulent). The structure of leaves make it difficult to isolated DNA. When grinding the leaves, the presence of sticky mucus causes viscous of leaves and difficult to separation and purification from buffer solution. When DNA visualized with UV illuminator, result showed that smear band appeared at the gel agarose which indicated that DNA contaminated by debris or secondary metabolite. Contaminated of isolated DNA can cause less of purity and concentration of DNA. Explain by Fatchiyah *et al.* [37], purity of DNA obtained based on the DNA character and protein or phenol contaminant.

Optimization of isolated DNA from leaves of binahong were tried over and over to eliminate contaminant (data unpublished). As we know that binahong or mostly of medicinal plants contain

secondary metabolites, like phenol, alkaloid and so on that will become high contaminated problem for DNA isolation.

Screenings of primers become the first step procedure was undertaken when research of genetic variation/distance using molecular marker. These screenings usually done for (1) insuring condition of temperature and primers that used to amplify DNA, and for (2) knowing polymorphisms of DNA binahong when amplified by ISSR primers. 8 primers of ISSR that capable to amplified DNA binahong had the sequences that bound to the DNA binahong as template and they can be extended by polymerase then the region that lies between them will get amplicon.

There are reasons why 7 primers couldn't amplify DNA binahong, (1) the sequence of nucleotide of DNA genome didn't had same sequences with primer sequences, Dinesh *et al.* [38], said that primers with same sequences with DNA genome will complementary to amplified DNA and showed DNA bands; (2) different temperature used for annealing, if annealing temperature is too low, DNA will occur miss-priming and if it is too high, DNA could not amplified, (3) purity and concentration of DNA used, Sulistyaningsih [39] explained that if DNA concentration used was low, primer didn't find the DNA target, but if concentration was high will occur miss-priming. This was confirmed by Weeden *et al.* [40], amplification of DNA band from each primer is strongly influenced by distribution of annealing site of primers.

After screening and found 8 primers capable to amplified DNA binahong, primers that capable will used to amplified another accession of DNA binahong and continue to research about molecular marker of binahong that spreads in Indonesia.

## 5. Conclusion

15 primers of ISSR marker were screening for their capability to amplified DNA binahong. 8 primers that capable to amplified DNA binahong were SBLT2, SBLT3, SBLT4, SBLT6, SBLT8, SBLT13, SBLT14 and SBLT15.

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