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Assessment of genetic stability of micropropagated *Curculigo latifolia* from Indonesia by RAPD and ISSR

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ABSTRACT

Micropropagation of *Curculigo latifolia* is very important because this plant has high economic value including containing Curculin and Neoculin as sweetener and taste-modifying proteins. Micropropagated plants may experience genetic changes like somaclonal variation so it is necessary to assess their genetic stability. The purpose of this study was to obtain a micropropagation method of *C. latifolia* that is more efficient than the previous research and to assess the genetic stability of micropropagated C. latifolia. The plant material used was sterile leaves obtained from sterile seedlings raised from *C. latifolia* seed germination. Leaves were grown on a callus induction medium containing 5 mg l^{-1} indole-3-butyric acid and 3 mg l^{-1} BA in a dark room. Callus formed were subcultured to growth selection medium and shoots formed were subcultured to Murashige and Skoog-free growth regulator medium with the addition of 6 g l^{-1} agar and 3% sucrose. The cultures were incubated at 23ºC ± 2ºC under 1,500 lux white LED (Philip™ TL) on a 16 hour/8 hour light/dark photoperiod. The cultures were incubated for 14 weeks. Genetic stability of *in vitro* propagated *C. latifolia* from Indonesia was assessed by molecular markers, RAPD and ISSR. The micropropagation method produces an efficient protocol because each growth stage occurs in a free growth regulator medium except for callus induction. Based on the results of the assessment using molecular markers, no polymorphism in DNA amplification using RAPD and a high percentage of genetic stability (66.67%) from ISSR analysis were obtained. In sum, the *in vitro* micropropagation protocol created can be used commercially to produce *C. latifolia* on a large scale that is genetically stable.

1. INTRODUCTION

Curculigo latifolia, often known as Lemba or Marasi, is a member of the family Hypoxidaceae. It grows in the Indonesian highlands of Kalimantan Java, Papua New Guinea, Bangka, and Sumatra on well-drained, fertile soils that are rich in organic matter. According to reports, the plant is found in the Philippines, West Africa, India, Myanmar, and Thailand [1,2]. Because of its many applications, *C. latifolia* is a plant with significant economic worth and promising future growth. Fishing nets are made in Kalimantan, Indonesia, and Malaysia using the fibers from *C. latifolia* leaves. The Dayak tribe in East Kalimantan uses fibers from *C. latifolia* leaves to make their traditional clothes, *Ulap doyo*. *C. latifolia* is a significant medicinal plant in Malaysia [1,2]. Furthermore, the plant is well-known for two unique sweet proteins: Curculin which has been demonstrated to be 500–9,000 times sweeter than sucrose by weight [3,4], and Neoculin, a taste-modifying protein that changes sour taste to sweet [5].

Curculigo latifolia is only propagated spontaneously in the wild in Kalimantan, Indonessia. Curculigo grows on well-drained, organic matter-rich soils. Curculigo grows in rain forests in Java up to an elevation of 1,100 m; it has not yet been regularly domesticated [2]. This plant develops slowly, which makes challenging standard propagation. According to Ismail *et al.* [6], seeds are difficult to germinate. The seeds need certain media and attention because they are resistant. An alternate option is the *in vitro* method. Babaei *et al.* [7] reported on the micropropagation of *C. latifolia* using *C. latifolia* shoot tips from Malaysia as explants on Murashige and Skoog (MS) basal medium supplemented with thidiazuron (TDZ: 0, 0.5, 1, 1.5, and 2 mg l^{-1}) and indole-3-butyric acid (IBA: 0, 0.25, and 0.5 mg l^{-1}) for shoot regeneration, Farzinebrahimi *et al.* [8], other researchers, successfully produced callus from the leaves and tuber of Malaysian-origin *C. latifolia* explants. They used MS base medium, which was supplemented with different concentrations of IBA, either alone or in combination with BAP (0.5–4 mg l^{-1}) and IBA (0–4 mg l^{-1}) and contained 3% sucrose that had been solidified using 2.5 g l^{-1} Gelrite. On the other hand, leaf explants with IBA and BAP (4.0 mg l^{-1})

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produced the most callus. Furthermore, callus and shoot induction on medium supplemented with IBA and BAP were also observed by Umar *et al.* [9]. Hormones called auxins and cytokinins are frequently utilized alone or in combination on *in vitro* culture for callus initiation, shoot induction or regeneration, and root induction. Typically, auxin is utilized to promote elongation and cell division as well as the development of callus, roots, and culture suspension. Cytokines are substances that can promote leaf growth, development, and cell division. Combining auxin with cytokines will promote cell division and growth, boost protein synthesis, and have an impact on callus growth [10]. This study, however, provides a more efficient stage for the micropropagation of *C. latifolia* on employing growth regulators.

DNA-based molecular markers have proven to be adaptable instruments and have established themselves in a number of domains, including genetic engineering, plant breeding, and taxonomy. Because they are the most affordable among all the molecular markers available, polymerase chain reaction (PCR)-based RAPD and ISSRs have become the most widely employed markers for assessing genetic fidelity in micropropagated plants [11]. In addition, the RAPD and ISSR markers have the benefit of being fast, easy to use, and requiring very little DNA and require only small amounts of DNA [12].

Many challenges have been well defined on *in vitro* plant research, namely physiological, epigenetic, and genetic quality problems associated with cells, tissues, and organs of *in vitro* plants, absence or loss of organogenic potential (re-calcitrance), hyperhydricity (vitrification) and somaclonal variation. Somaclonal variation is one of the very important problems on *in vitro* culture. It is imperative to determine the genetic stability of *in vitro* regenerated plants [13]. Molecular markers especially RAPD and ISSR have been widely applied to monitor somaclonal variation and verify the genetic stability of micropropagated plants [11–19]. However, genetic stability tests on *in vitro* propagation of *C. latifolia* have never been reported. The objectives of this study were to describe an efficient protocol for micropropagation of *C. latifolia* along with the RAPD and ISSR analysis to determine the genetic fidelity of the regenerated plants.

2. MATERIALS AND METHODS

2.1. Micropropagatin of *C. latifolia*

2.1.1. Callus induction and culture condition

The plant material or explants used in this study were sterile leaves from seedlings grown *in vitro*. Leaves were cut about 1.5 × 2 cm and inoculated on MS solid medium with the addition of 6 g l^{-1} agar and 3% sucrose. Medium pH was adjusted to 5.8. MS medium was enriched with 5 mg l^{-1} IBA dan 3 mg l^{-1} BA according to Umar *et al.* [9]. The cultures were incubated at 23ºC ± 2ºC without lighting (dark room) for 30 days.

2.1.2. Shoot induction and culture conditions

The callus obtained was subcultured on a shoot induction medium. The medium was MS with the addition of a growth regulator with different compositions. They are MS free growth regulator (MS0); 5 mg l^{-1} IBA and 3 mg l^{-1} BA; 1 mg l^{-1} 2,4D and 3 mg l^{-1} BA; 0.5 mg l^{-1} NAA and 5 mg l^{-1} BA. The medium was added 6 g l^{-1} agar and 3% sucrose. The pH of the media was adjusted to 5.8. The cultures were incubated at 23ºC ± 2ºC with 1,500 lux LED lighting (Philip™ TL) on a 16 hour/8 hour light/dark photoperiod.

2.1.3. Growth medium and culture conditions

The shoots were separated from the callus and subcultured onto MS-free hormone medium with the addition of 6 g l^{-1} agar and 3% sucrose. The cultures were incubated at 23ºC ± 2ºC under 1,500 lux white LED (Philip™ TL) on a 16 hour/8 hour light/dark photoperiod. The cultures were incubated for 14 weeks.

2.2. Assessment of Genetic Stability using RAPD and ISSR Markers

2.2.1. Plant material

The materials used for genetic stability testing were callus with 3-level subcultured, leaves of 14-week plantlets from callus and mother plant.

2.2.2. DNA extraction and PCR amplification conditions

Genomic DNA of all material was isolated using *Genomic DNA Mini Kit (Plant) (Geneiaid Biotech Ltd*, Taiwan). Genetic stability tests were conducted using RAPD and ISSR molecular markers. This study used 5 RAPD primer, OPA1, OPA2, OPA3, OPF 4, OPG17 [20] and 5 ISSR primers, BC815, BC823, BC825, BC835, and BC868 [21]. Quantitative assessment of genomic DNA was carried out by NanoDrop™ 2000 (Thermo Fisher Scientific, USA). The final concentration was set to 20 µg $µl^{-1}$ for PCR containing 12,5 µl *My Taq HS Red Mix* 2X (Meredian Bioscience, USA), 1 µl primer RAPD/ISSR (IDT, Singapore), 3 µl template DNA, and 3,5 µl Nuclease free water. RAPD and ISSR reactions were performed in Select Cycler II Thermal Cycler (Select BioProduct, Taiwan). The amplification reaction for RAPD consisted of an initial denaturation step at 95ºC for 3 minutes, followed by 40 cycles of 94ºC for 45 seconds (denaturation), 45 seconds at specific annealing temperatures (Table 2), and 72ºC for 1 minute (extension) followed by a final extension step at 72ºC for 5 minutes. The amplification reaction for ISSR consisted of an initial denaturation step at 94ºC for 5 minutes, followed by 40 cycles of 94ºC for 45 seconds (denaturation), 45 seconds at specific annealing temperatures (Table 2), and 72ºC for 30 seconds (extension) followed by a final extension step at 72ºC for 5 minutes.

2.2.3. DNA visualization

RedSafe (iNtRON Biotechnology, Korea) was used to dye the amplified products after they were electrophoresed in 2% agarose gels using Tris-Borate-Ethylene diamine tetra acetic acid buffer. The amplicons' sizes were measured using a comparison with a 1 kb DNA ladder from Thermoscientific, USA. Using a gel imaging device, the number of amplified products was counted (Digibox 7000, Mbiotech, Korea). To ensure the reproducibility of all PCR reactions for RAPD and ISSRs, significant bands were graded. The presence of a band was indicated by a "1" in the data, and its absence by a "0". Plant samples that included every band were classified as monomorphic, while DNA samples lacking some bands were classified as polymorphic.

3. RESULTS

3.1. Efficient Micropropagation of *C. latifolia*

The characteristics of callus that appeared on the medium of 5 mg l^{-1} IBA and 3 mg l^{-1} BA had a yellowish–white color with a compact texture (Fig. 1).

Furthermore, the callus was subcultured into the medium with different compositions for medium selection for shoot induction. However, the media with an additional growth regulator did not produce shoots but

shoots appeared on media without the addition of a growth regulator (MS0) (80%) (Table 1).

Shoots developed from callus 30–35 days after subculture on MS0 medium. Callus on medium with the addition of auxin and cytokinin did not show a response. In the next step, the shoots that appear are separated and subcultured into MS0 to produce plantlets with roots. Shoot growth on MS0 medium is an efficient technique because it reduces production costs by not using growth regulators. Our micropropagation protocol produced morphologically normal plantlets or seedlings.

In summary, the stages of efficient micropropagation of *C. latifolia* are, callus induction on media containing IBA and BA, then callus that appears is subcultured on shoot induction medium, MS without growth regulator. Shoots that appear are incubated on the same media until they have good performance, namely the appearance of leaves, and sturdy. Furthermore, small plantlets without roots that grow in clusters are separated and subcultured to the MS-free growth regulator. On MS free growth regulator, plantlets grown into good performance, sturdier plantlets, with many leaves and roots (Fig. 2). The development of callus to plantlets in a closed system of *in vitro* culture can be a cycle of development. The resulting plantlets can be used as a supply of seedlings ready for acclimatization or the leaves can be used as sterile explants to produce callus again which will eventually produce seedlings or green stock in larger quantities.

3.2. Genetic Stability

All of the tested primers produced scorable bands although some of the resulting bands are thin under the optimized PCR conditions (Table 2). This study produced a total of 35 bands on RAPD primers and 38 bands on ISSR with an average of 7 bands from each RAPD and ISSR primer.

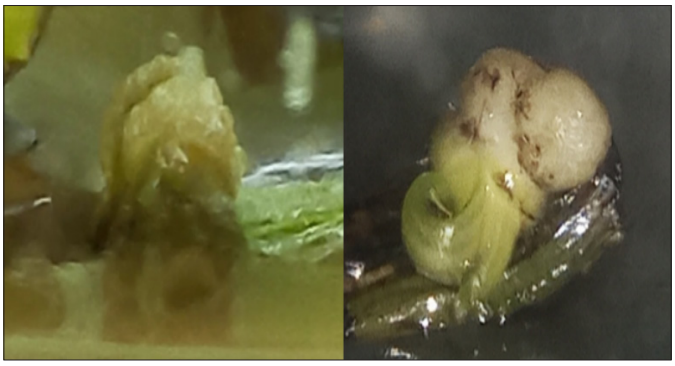

Figure 1. Morphology of callus. (A). Callus emerged from the leaf of *C. latifolia*; (B). Shoot emerged from callus.

Table 1. Effect of growth regulator on percentage of callus emmerged.

No	Composition of growth regulator	Number of sample	Percentage bud emerged (%)
1	MS0	21	80
2	5 mg l^{-1} IBA + 3 mg l^{-1} BA	21	0
3	1 mg l^{-1} 2,4D + 5 mg l^{-1} BA	21	0
4	0.5 mg l^{l} NAA + 5 mg l^{-1} BAP	21	0

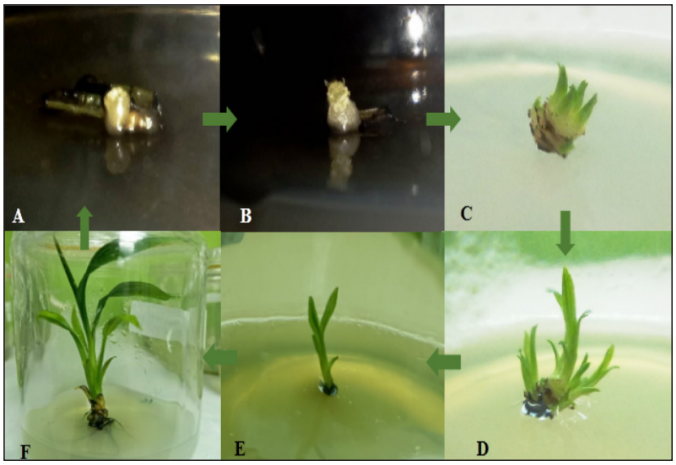

Figure 2. Efficient micropropagation of *C. latifolia*. (A). Callus formed from leaf explants on medium containing IBA and BA; (B). The callus swells and potential shoots begin to appear on MS free growth regulator medium; (C). Shoots on MS free growth regulator medium; (D). The shoots grow into small plantlets on MS free growth regulator medium; (E). Plantlet after being separated from the callus, subculture on MS free growth regulator medium; (F). Plantlets that are ready to be acclimatized or can be used as sterile explants to obtain new callus.

Table 2. List of RAPD and ISSR primers used for assessment of genetic fidelity of *C. latifolia*.

Primers	Sequences (5′- 3′)	Tm (°C)	No. of bands amplified	No of polymorphic bands	% Polymorphism	Size of amplikon (bp)
OPA1	CAGGCCCTTC	36ºC	7	0	0	350–1,500
OPA2	AGTCAGCCAC	36ºC	6	0	0	400–1,000
OPA3	TGCCGAGCTG	36ºC	6	0	0	350–1,000
OPF4	GGTGATCAGG	36ºC	5	0	0	250–700
OPG17	ACGACCGACA	36ºC	11	0	0	300–1,500
BC815	CTCTCTCTCTCT CTCTG	47ºC	6	2	33.33	400–900
BC823	TCTCTCTCTCTCTCTCC	50ºC	7	0	0	400–900
BC825	ACACACACACACACACT	50ºC	7	0	0	300–900
BC835	AGAGAGAGAGAGAGAGYC	50ºC	10	0	0	100–800
BC868	GAAGAAGAAGAAGAAGAA	44ºC	8	0	0	300–750

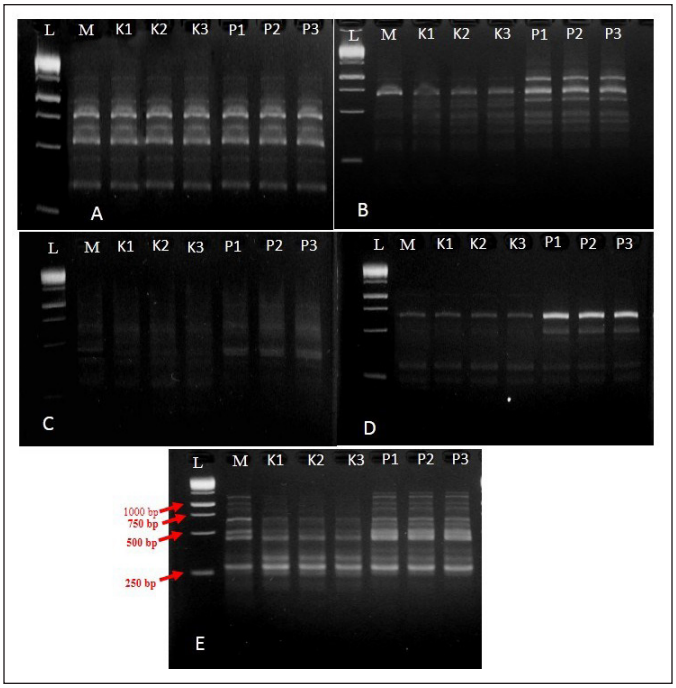

Figure 3. PCR amplification obtained with RAPD primers (A) OPA1; (B) OPA2; (C) OPA3; (D) OPF4 (E) OPG17. Lane L, represents 1 kb ladder (red arrows indicate ladder sizes of 250, 500, 750, and 1,000 bp in all electrophoresis results in A, B, C, D, and E), lane M represents the mother plant, and lanes K1–K3 represent callus and P1–P3 represents *in vitro*-raised clones.

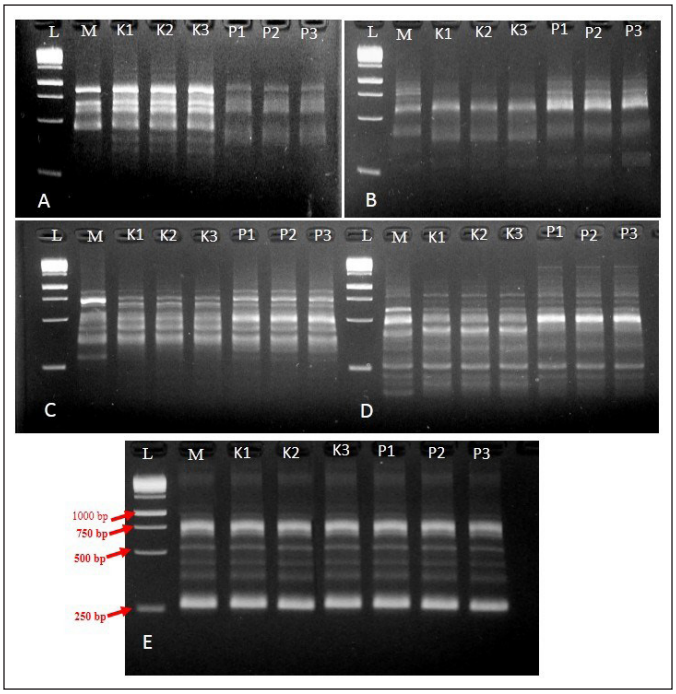

Figure 4. PCR amplification obtained with ISSR primers. (A) UBC815; (B) UBC823; (C) UBC825; (D) UBC835 (E) UBC868. Lane L, represents a 1 kb ladder (*t* red arrows indicate ladder sizes of 250, 500, 750, and 1,000 bp in all electrophoresis results in A, B, C, D, and E), lane M represents the mother plant, and lanes K1–K3 represent callus and P1–P3 represents *in vitro*-raised clones.

The results of DNA amplification using RAPD showed no polymorphism. The size of the band on each primer shows a size that is not much different, namely OPA1 produces 7 bands with range sizes 350–1,500 bp, OPA2 formed 6 bands measuring 400–1,000 bp, OPA3 produces 6 bands with 350–1,000 bp, OPAF4 produces the least number of bands, namely 5 bands with 250–700 bp and OPG17 produces the most number of bands, 11 bands with 300–1,500 bp (Fig. 3).

Amplification using ISSR primers obtained the same results as RAPD, namely no polymorphism, except for primer UBC815 which showed polymorphism of 33.33%. Polymorphism is shown from the results of DNA amplification in plantlets that only detected 4 bands out of 6 bands in the mother plant and callus with a size of 400–900 bp. This indicates a reduction or deletion in plantlet DNA amplification. Primer UBC823 detected 7 bands of monomorphysm of 400–8,900 bp, UBC825 detected 7 bands of 300–900 bp, UBC835 detected 10 bands of 100–800 bp and UBC868 detected 8 bands of 300–750 bp (Fig. 4).

4. DISCUSSION

Not all *C. latifolia* callus are able to multiply cells and develop into shoots because they experience tissue damage due to browning. A serious problem faced in *in vitro* culture of *C. latifolia* is browning as has been reported in according research to Babaei *et al.* [7]. Browning in plant tissue culture refers to the phenomenon in which explants release phenolic compounds into the medium from their own tissues in the process of dedifferentiation and/or redifferentiation. Phenolic compounds are commonly present in healthy tissues and can accumulate in cells and be released as a defense response, especially after tissue injury or stress. Consequently, this natural defense response can lead to the accumulation of phenolic compounds that ultimately damage or kill plant cells and tissues. The process of cutting explants causes the release and accumulation of phenols in the tissue and media. Browning can be seen in almost all explants, especially on the wounded side and the color of the media turns brown. This caused some callus to break down and turn brown.

In some species, it is reported that MS free growth regulator produces better growth compared to growth on medium with the addition of growth regulator. In a previous study Sulaiman *et al.* [22] reported pineapples were cultured on MS medium without a plant growth regulator produced the highest shoot development. In addition, *Nepeta rtanjensis* shoots spontaneously rooted *in vitro* on a hormone-free medium [23]. In addition, Haddadi *et al.* [24] reported that the stunted shoots lengthened and took on a normal look after being subculturing on hormone-free MS media and on the medium MS free growth regulator obtained the longest root.

Callus on medium with the addition of auxin and cytokinin showed no response. In fact, the callus will experience browning when subcultured on media containing auxin and cytokinin. This indicates that the addition of exogenous growth regulators is not efficient or can even inhibit growth. The germinated plants reported by Muslihatin *et al.* [25] and the plantlets raised from callus have the same character of growing well in medium without growth regulators. The growth of *C. latifolia* does not require the addition of exogenous growth regulators. A basic medium called MS is typically used to propagate a wide range of plant species. This foundational media contains a lot of minerals that promote organogenesis [26], This means that plant tissue culture for *C. latifolia* is cost-effective because no growth regulator was required to obtain a mass quantity of *in vitro* clonal *C. latifolia*.

The process of plant micropropagation aims to produce clones (true copies of a plant in large numbers). The use of fewer growth regulators

is thought to reduce production costs of plant micropropagation. Our method of micropropagation resulted in morphologically regular plantlets or seedlings. Nonetheless, one of the most crucial prerequisites for the micropropagation of crop species is true-to-type clonal fidelity. The potential for cryptic genetic defects to arise in regenerates can significantly restrict the broad application of the technology of micropropagation [12].

In general, a total of 10 primers used in this study produced monomorphism, which means that there is no genetic variation from *C. latifolia* callus and plantlets compared to the mother plant except for the ISSR primer UBC815 which shows a low level of polymorphism (33.33%). The absence of genetic variation using RAPD has been reported such as in micropropagation of *Rosa hybrida* [27], *Aloe vera* [12], *Musa sp*. cv. Williams [28], *Rauwolfia tetraphylla* L [16], *Neolamarckia cadamba* [15], *Dendrocalamus strictus* (Roxb.) [29], *Muntingia calabura* [17], and *Dendrobium transparens* Wall. Ex Lindl. [18]. In addition, Patel *et al.* [30] reported no genetic variation was observed on micropropagated *Curculigo orchioides* through RAPD analysis.

Although there is polymorphism in the UBC815 primer, the value is less than 50%, which means that the genetic variation that occurs is low [31]. According to Wibowo *et al.* [32], polymorphisms with a value of ≤50% are still classified in the category of high genetic profile stability. This study is in line with the assessment of the clonal fidelity of the *in vitro* regenerated *Thunbergia coccinea*, *Artocarpus altilis, Orthosiphon stamineus* using ISSR produced polymorphism 1%, 22.2%, and 7.32% [19,33,34]. In contrast, previous study showed no genetic variation in many species using ISSR marker, *Andrographis alata* [35], *Valeriana jatamansi* [36], *Momordica cymbalaria* [37], *Solanum khasianum* [38]. Polymorphism was detected in *C. latifolia* plantlets, DNA bands that appeared less than DNA bands that appeared in control or mother plants and callus. Micropropagated plantlets are frequently exposed to *in vitro* stress, which induces modifications at specific locations, including repetitive DNA, activating transposable elements and potentially leading to genetic diversity through insertion or deletion [39]. Variations in the RAPD and ISSR patterns can be attributed to the loss or gain of primer annealing sites resulting from transposon insertion/deletion and point mutation [34]. Using distinct marker systems increases the likelihood that variation brought about by genetic and epigenetic mechanisms will be reflected in banding profiles. Molecular markers' effectiveness and dependability are regularly questioned, nevertheless. Combining two different types of markers that amplify distinct areas of the plant genomes allows for a more thorough investigation of the genetic stability of micropropagated plants. Earlier reports suggested that using more than one type of molecular marker in genetic fidelity studies of *in vitro* regenerated plants is always useful for reliability [16]. Therefore, it is recommended to use multiple markers or several DNA amplification techniques as useful in evaluating somaclonal variation.

5. CONCLUSION

This work offered a productive technique for *C. latifolia in vitro* micropropagation. By using RAPD and ISSR analysis, genetic stability between the mother plant, callus, and the *in vitro*-raised plants was found. Conversely, the five RAPD primers that were evaluated revealed no genetic variation, whereas the five ISSR primers that were tested revealed high genetic profile stability (66.67%). The *in vitro* micropropagation procedure created in this work can be used commercially to produce *C. latifolia* on a large scale that is genetically stable and free of pathogens.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Raden I, Nugroho CC, Syahrani S. Identification and characterization of morphological diversity of Lemba (*Curculigo latifolia*) in East Kalimantan, Indonesia. Biodiversitas 2017;18:1367–76.
2. Farzinebrahimi R, Taha RM, Rashid KA. Effect of light intensity and soil media on establishment and growth of *Curculigo latifolia* Dryand. J Appl Hortic 2013;15:224–6.
3. Yamashita H, Theerasilp S, Aiuchi T, Nakaya K, Nakamura Y, Kurihara Y. Purification and complete amino acid sequence of a new type of sweet protein with taste-modifying activity, curculin. J Biol Chem 1990;265:15770–5.
4. Nookaraju A, Upadhyaya CP, Pandey SK, Young KE, Hong SJ, Park SK. Molecular approaches for enhancing sweetness in fruits and vegetables. Sci Hortic (Amsterdam) 2010;127:1–15.
5. Shirasuka Y, Nakajima KI, Asakura T, Yamashita H, Yamamoto A, Hata S. Neoculin as a new taste-modifying protein occurring in the fruit of *Curculigo latifolia*. Biosci Biotechnol Biochem 2004;68:1403–7.
6. Ismail MF, Abdullah PNA, Saleh G, Ismail M. Anthesis and flower visitors in *Curculigo latifolia* Dryand. Biol Life Sci 2010;1:13–5.

7. Babaei N, Ashikin N, Abdullah P, Saleh G, Abdullah TL. An efficient *in vitro* plantlet regeneration from shoot tip cultures of *Curculigo latifolia*, a medicinal plant. Sci World J 2014;2014:275028.
8. Farzinebrahimi R, Mat Taha R, Rashid KA, Ali Ahmed B, Danaee M, Rozali SE. Preliminary screening of antioxidant and antibacterial activities and establishment of an efficientc Callus induction in *Curculigo latifolia* Dryand (Lemba). Evidence-based Complement Altern Med 2016;6429652:1–9.
9. Umar AH, Ratnadewi D, Rafi M, Sulistyaningsih YC, Hamim H. Callus of *Curculigo latifolia* Dryand. ex W.T. Aiton: initiation, regeneration, secretory structure and histochemistry. IOP Conf Ser Earth Environ Sci 2021;948:012051.
10. Mayerni R, Satria B, Wardhani DK, Chan SROS. Effect of auxin (2,4-D) and cytokinin (BAP) in callus induction of local patchouli plants (*Pogostemon cablin* Benth.). IOP Conf Ser: Earth Environ Sci 2020;583:012003.
11. Chittora M. Assessment of genetic fidelity of long term micropropagated shoot cultures of *Achras sapota* L. Var. "Cricket Ball" as assessed by RAPD and ISSR markers. Indian J Biotechnol 2018;17:492–5.
12. Khatun MM, Tanny T, Yesmin S, Salimullah M, Alam I. Evaluation of genetic fidelity of *in vitro*-propagated *Aloe vera* plants using DNA-based markers. ScienceAsia 2018;44:87–91.
13. Yadav A, Kothari SL, Kachhwaha S, Joshi A. *In vitro* propagation of chia (*Salvia hispanica* L.) and assessment of genetic fidelity using random amplified polymorphic DNA and intersimple sequence repeat molecular markers. J Appl Biol Biotechnol 2019;7:42–7.
14. Khaled AGA, Motawea MH, Said AA. Identification of ISSR and RAPD markers linked to yield traits in bread wheat under normal and drought conditions. J Genet Eng Biotechnol 2015;13:243–52.
15. Pei-Kieng M, Wei-Seng H. Genetic fidelity assessment of tissue culture derived *Neolamarckia cadamba* plantlets using DNA-based markers. Malaysian Appl Biol 2019;48:29–37.
16. Rohela GK, Jogam P, Bylla P, Reuben C. Indirect regeneration and assessment of genetic fidelity of acclimated plantlets by SCOT, ISSR, and RAPD markers in *Rauwolfia tetraphylla* l.: an endangered medicinal plant. Biomed Res Int 2019;3698742:1–14.
17. Vankudoth S, Dasari R, Pavani C, Gopu C, Jogam P, Kota S, *et al.* Micropropagation of *Muntingia calabura* L. and assessment of genetic fidelity of *in vitro* raised plants using ISSR and RAPD analysis. J Plant Dev 2020;27:33–45.
18. Joshi PR, Pandey S, Maharjan L, Pant B. Micropropagation and assessment of genetic stability of *Dendrobium transparens* Wall. Ex Lindl. using RAPD and ISSR markers. Front Conserv Sci 2022;3:1–11.
19. Sultana KW, Das S, Chandra I, Roy A. Efficient micropropagation of *Thunbergia coccinea* Wall. and genetic homogeneity assessment through RAPD and ISSR markers. Sci Rep 2022;12:1–11.
20. Quang HT, Tan H, Nhan, TN, Duc TM, Giang TV, Thane P. Study on genetic diversity of *Curculigo orchioides* Galertn population from Vietnam, an endangered medical herb. Plant Cell Biotechnol Mol Biol 2018;19(7&8):293–301.
21. Ranjbarfard A, Saleh G, Abdullah NAP, Kashiani P. Genetic diversity of lemba (*Curculigo latifolia*) populations in Peninsular Malaysia using ISSR molecular markers. Aust J Crop Sci 2014;8(1):9–17.
22. Sulaiman S, Yusuf NA, Awal A. Effect of plant growth regulators on *in vitro* culture of pineapple (*Ananas comosus* L. Merr) MD2 variety. Food Res 2020;4:110–4.
23. Mišić DM, Ghalawenji NA, Grubišić DV, Konjevič RM. Micropropagation and reintroduction of *Nepeta rtanjensis*, an endemic and critically endangered perennial of Serbia. Phyt Ann Rei Bot 2005;45:9–20.
24. Haddadi F, Aziz MA, Saleh G, Rashid AA, Kamaladini H. Micropropagation of strawberry cv. camarosa: prolific shoot regeneration from *in vitro* shoot tips using thidiazuron with n6-benzylamino-purine. HortScience 2010;45:453–6.
25. Muslihatin W, Jadid N, Manuhara YSW, Werbrouck SPO. Effect of light on seed germination and growth of *Curculigo latifolia* in *in vitro* condition. Acta Hortic 2023;1359:105–11.
26. Rustikawati, Herison C, Inoriah E, Dwisari V. Effect of BAP (6-Benzyl Aminopurine) on in vitro shoot growth of Curcumas. AGRITROPICA J Agric Sci 2021;4:82–92.
27. Senapati SK, Aparajita S, Rout GR. An assessment of genetic fidelity of *in vitro* grown plantlets of rose (*Rosa hybrida*) through molecular markers. Afr J Biotechnol 2012;11:16532–8.
28. Khatab IA, Youssef MS. Micropropagation and assessment of genetic stability of *Musa sp*. cv. Williams using RAPD and SRAP Markers. Egypt J Bot 2018;58(3):371–80.
29. Goyal AK, Pradhan S, Basistha BC, Sen A. Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers. 3 Biotech 2015;5:473–82.
30. Patel S, Jasrai YT, Adiyecha R. Induction of somatic embryogenesis and genetic fidelity of endangered medicinal herb *Curculigo orchioides* Gaertn. Res Plant Biol 2011;1:48–52.
31. Irsyad AF, Rindyastuti R, Yulistyarini T, Darmayanti AS, Daryono BS. Genetic variation of agarwood producing tree (*Gyrinops versteegii*) from Pongkor, Manggarai District, flores Island, Indonesia using ISSR molecular markers. Biodiversitas 2020;21:485–91.
32. Wibowo WA, Al Rasyid MF, Maharani SE, Daryono BS. Genetic stability analysis based on inter-simple sequence repeat and β-Carotene content analysis in melon (*Cucumis melo* L. 'GAMA Melon Parfum'). Int J Adv Sci Eng Inf Technol 2022;12:1606–12.
33. Teeluck JM, Kaudeer BF, Ramful M, Boodhram I, Sanmukhiya MR, Soulange JG. Genetic fidelity of *in vitro* propagated breadfruit (*Artocarpus altilis*) using inter simple sequence repeat markers. Int J Agric Biol 2016;18:911–6.
34. Ali H, Musa IF, Abu Bakar NA, Karsani SA, Yaacob JS. *In vitro* regeneration and ISSR-based genetic fidelity analysis of *Orthosiphon stamineus* benth. Agronomy 2019;9:1–12.
35. Kadapatti SS, Murthy HN. Rapid plant regeneration, analysis of genetic fidelity, and neoandrographolide content of micropropagated plants of *Andrographis alata* (Vahl) Nees. J Genet Eng Biotechnol 2021;19:20.
36. Purohit S, Rawat V, Jugran AK, Singh RV, Bhatt ID, Nandi SK. Micropropagation and genetic fidelity analysis in *Valeriana jatamansi* Jones. J Appl Res Med Aromat Plants 2015;2:15–20.
37. Chaitanya G, Suvarchala V, Ramakrishna D, Sunitha D, Shasthree T. *In vitro* plant regeneration of *Momordica cymbalaria* fenzl. and assessment of genetic fidelity using ISSR primers. Res J Biotechnol 2020;15:103–10.
38. Chirumamilla P, Gopu C, Jogam P, Taduri S. Highly efficient rapid micropropagation and assessment of genetic fidelity of regenerants by ISSR and SCoT markers of *Solanum khasianum* Clarke. Plant Cell Tissue Organ Cult 2021;144:397–407.
39. Singh P, Singh SP, Tiwari AK, Sharma BL. Genetic diversity of sugarcane hybrid cultivars by RAPD markers. 3 Biotech 2017;7:1–5.

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