Genetic Diversity of Cogongrass [*Imperata Cylindrica* (L.) Raeusch] in Java Island Based on *psbA-trnH* IGS

Murni Dwiati(✉), Talita Ade Novita Dewi, and Agus Hery Susanto

The Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia
murni.dwiati@unsoed.ac.id

Abstract. Cogongrass [*Imperata cylindrica* (L.) Raeusch] is a wild plant species widely distributed throughout various tropical and subtropical regions. The wide distribution may lead to genetic differences among the species populations, which can be analyzed using particular molecular markers showing high mutation rate. This study was aimed aims to assess the genetic diversity of cogongrass in Java Island, Indonesia, and the genetic relationship among the populations based on *psbA-trnH* IGS. Plant samples were taken randomly from several places in Java, namely Purwokerto, Jetis Beach, Purworejo, Yogyakarta, and Ponorogo. The molecular analysis involved genomic DNA extraction, amplification of *psbA-trnH* IGS, and sequencing of *psbA-trnH* IGS. The results showed that cogongrass populations in Java had a high genetic diversity with respect to haplotype diversity ($h = 1$) but low genetic diversity in terms of nucleotide diversity ($\pi = 0.00658$). This suggested a very close genetic relationship among cogongrass populations in the island, which can also be seen from the low values of genetic distances.

Keywords: cogongrass · genetic diversity · Java Island · psbA-trnH

1 Introduction

Cogongrass [*Imperata cylindrica* (L.) Raeusch] is better known as weed species, although some of its parts, mainly the rhizome, can be utilized as traditional medicinal herbs due to the contents of active chemical substances. The species is widely distributed over various tropical and subtropical regions including Java Island, Indonesia, where it grows well in many types of terrestrial habitats. This is because of the high adaptability to various land conditions, which in long-term may have effects on the level of genetic diversity [1].

Analysis on the genetic diversity can be either morphological- or molecular based. However, the latter has no environmental intervention giving rise to more accurate profiling on a population [2, 3]. Then, molecular markers of higher mutation rate should be employed to assess genetic diversity of a species population. One of them is *psbA-trnH* intergenic spacer (IGS), which is located in the chloroplast genome (cpDNA). This marker has been proven efficient when used as a DNA barcode both solely and in combination with some other markers [4]. As a non-coding region, this site may undergo a

S. B. Sulistyo et al. (Eds.): ICSARD 2022, ABSR 30, pp. 208–216, 2023.
https://doi.org/10.2991/978-94-6463-128-9_22

Table 1. The sampling sites of *Imperata cylindrica* in Java, Indonesia

Sampling site	Site code	Altitude (m asl)	Coordinate	
			S	E
Purwokerto, Central Java	PWT	79	7°24′33”	109°15′14”
Jetis Beach, Cilacap, Central Java	JET	6	7°43′21”	109°23′26”
Purworejo, Central Java	PWJ	21	7°51′17”	109°57′18”
Yogyakarta City, Special Territory of Yogyakarta	YOG	136	7°47′20”	110°19′21”
Ponorogo, East Java	PON	57	7°49′15”	111°32′56”

rapid alteration, so that it is suitable for intra-specific genetic diversity analysis [5]. In addition, the relatively short sequence of *psbA-trnH* IGS enables greater chance of successful amplification. Still another advantage as cpDNA in general is due to the haploid structure avoiding genetic recombination [6].

The objectives of this study were to assess the genetic diversity of cogongrass in Java Island and to demonstrate the genetic relationship among cogongrass populations in the island based on *psbA-trnH* IGS. The data and information thus obtained can be used to enrich database on cogongrass for further studies on its utilization, particularly in support of the development of medicinal herb usages in Indonesia.

2 Materials and Methods

2.1 Plant Collection

Plant samples were collected randomly from five different sites in Java Island representing various altitudes. They were Purwokerto, Jetis Beach, Purworejo, Yogyakarta, and Ponorogo where the altitudes and coordinates are presented in Table 1.

Each plant was totally taken up from the soil where it grew, covered with a wet paper towel, put into a plastic sack, and tangled with a rubber armlet. All the plant samples were then planted in the pots in the screenhouse of the Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia until the genomic DNAs were extracted from the completely expanding leaves.

2.2 Genomic DNA Extraction

Genomic DNA of individual plant was extracted from fresh and completely extended second leaf employing modified CTAB method [7]. The leaf was cut at its base, cleanly rinsed with running water, and then dried with a paper towel. This sample was weighed 0.1 g, sterilized in 70% alcohol, put into a mortar and added with 800μL CTAB buffer previously heated at 65 °C for 30 min in a waterbath. The mixture was homogenized with a pestle and then put into a 1.5 mL microcentrifuge tube for incubation at 65 °C for 1 h in a waterbath while inverting the tube once in every 10 min, after which the mixture was

placed at room temperature for 2 min. As much as 500μL chloroform: isoamyl alcohol (CIAA) was added into the mixture, homogenized using a vortex for 5 min, followed by centrifugation at 12,000 rpm 4 °C for 15 min. The supernatant was transferred gently into a new microcentrifuge tube and recorded for its volume. 3M sodium acetate was added into the supernatant as much as 1/10 volume, homogenized, and then added with cold isopropanol of 2/3 total volume. This was homogenized by inverting the tubes several times and left it in a freezer for 24 h. The mixture was then centrifuged at 12,000 rpm 4 °C for 20 min. After supernatant removal, the DNA pellet obtained was rinsed with 500μL 70% alcohol, followed by centrifugation at 12,000 rpm 4 °C for 5 min. Again the supernatant was removed, while the DNA pellet was air-dried by inverting the tube on a paper towel. When dried, the DNA pellet was dissoved in 100μL TE buffer and incubated at 37 °C for 1 h in a waterbath. The DNA solution was stored at 4 °C in a refrigerator.

2.3 Amplification and Sequencing of PsbA-TrnH IGS

The extracted genomic DNAs were used as PCR templates to amplify *psbA-trnH* IGS using a pair of universal primers, i.e. 5'-GTTATGCATGAACGTAATGCTC-3' as forward primer and 5'-CGCGCATGGTGGATTCACAAATC-3' as reverse primer. Each PCR mixture had a total volume of 50 μL containing 10 μL template DNA (50 ng/ μL), 1 μL forward primer, 1 μL reverse primer, 25 μL Kapa Taq and 13 μL nuclease free water. The PCR was run in an advanced primus 25 thermocyler applying the following condition: pre-denaturation at 94 °C for 5 min, proceeded by 30 PCR cycles consisting of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at72 °C for 1 min 30 s respectively, and terminated by final extension at 72 °C for 5 min.

The PCR products were visualized in a 1.5% agarose gel electrophoresis using 1 x TAE buffer. The electrophoresis was run at 80 V and 399 mA for 50 min. UV transilluminator was used to visualize the amplicons. These were then sent to Firstbase Malaysia for bi-directional sequencing applying automated terminator labelling method [8].

2.4 Measurement on Leaf Chlorophyll Contents

As supporting data, the contents of leaf chlorophyll a, b and total chlorophyll were measured spectrophotometrically. Third leaf was weighed 0.02 g, pounded to soft using mortar and pestle, and added with acetone up to 4 mL. The solution was then filtered and measured for the absorbance at 646 nm and 663 nm. The following formula [9] was employed.

Chlorophyll a (μg/mL) = 12.21 (A663) - 2.81 (A646).
Chlorophyll b (μg/mL) = 20.13 (A646) - 5.03 (A663).
Total chlorophyll (μg/mL) = 17.30 (A646) - 7.18 (A663).

2.5 Data Analysis

The DNA sequences obtained were edited using Bioedit version 7.2 and were checked manually. These were then blasted to those available in NCBI database for validation,

after which sequence alignment was performed using MEGA X. Analysis on haplotype diversity *(h)* and nucleotide diversity *(π)* was carried out using DnaSP, while phylogenetic analysis was made employing MEGA X. Supporting data on chlorophyl contents were analyzed statistically using ANOVA (F test) and proceeded by LSD test when significant difference was observed.

3 Results and Discussion

Genomic DNAs of relatively good quality were successfully extracted from all plant samples. The purities ranged from 1.538 to 1.818 at absorbance ratio of 260/280 nm, while the concentrations varied from 700 to 3,200 ng/μL. When used as PCR templates to amplify *psbA-trnH* IGS, single clear bands of approximately 650 bp were produced from the five samples (Fig. 1). Then, these PCR products resulted in sufficiently readable sequences and after subjected to editing and alignment, 610 bp long sequences were obtained (Fig. 2).

Blasting to NCBI database showed that all the five sequences matched that of *I. cylindrica psbA-trnH* IGS available in the database with considerably high values of query cover, maximum score and percentage of identity. This means that all the sequences obtained are undoubtedly *psbA-trnH* IGS.

(M = 1 kb ladder, PWT = Purwokerto, JET = Jetis,
PWJ = Purworejo, YOG = Yogyakarta,
PON = Ponorogo).

It is shown in Fig. 2 that both insertion-deletion (indel) and base substitution among the five *psbA-trnH* IGS sequences were observed, in which transition occured more frequently than transversion. This is reasonable as transition is an alteration of purine

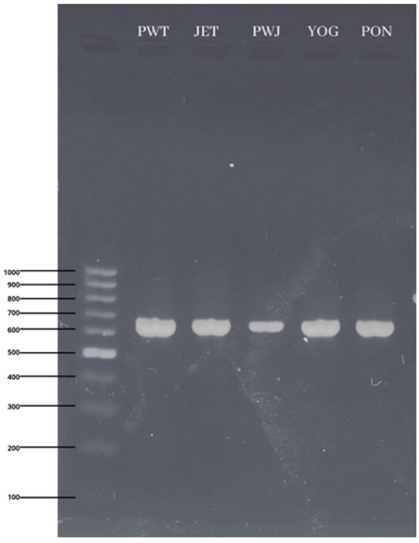

Fig. 1. PCR products of *psbA-trnH* IGS

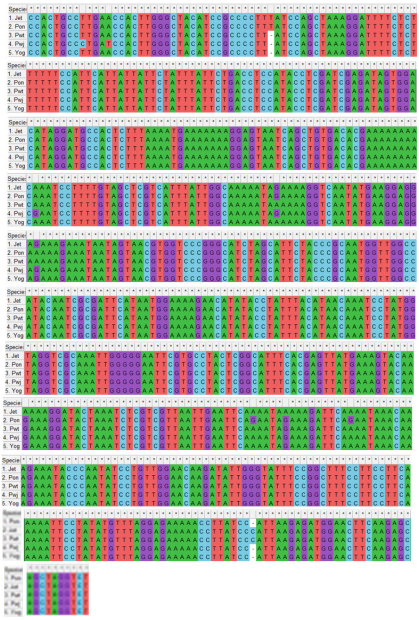

Fig. 2. Sequence alignment of *psbA-trnH* IGS cogongrass from Java Island (PON = Ponorogo, JET = Jetis, PWT = Purwokerto, PWJ = Purworejo, YOG = Yogyakarta, * = homologous for all five sequences).

Table 2. Polymorphic sites among five *psbA-trnH* IGS sequences of cogongrass in Java Island

Location	Sites										
	9	13	38	182	219	242	421	456	461	471	576
Purwokerto	T	A	-	A	A	A	G	A	G	A	C
Jetis	T	A	T	A	G	G	A	A	A	A	-
Purworejo	C	T	-	G	G	G	G	A	G	A	-
Yogyakarta	T	A	-	A	A	G	G	A	G	A	-
Ponorogo	T	A	T	A	G	A	G	G	G	G	C

A = adenine; T = thymine; G = guanine; C = cytosine

into purine or pyrimidine into pyrimidine, which is simpler than transversion where purine changes into pyrimdine and vice versa. Transversion is more energy-consuming in comparison to transition due to the more complicated changes in the nucleotide base molecular structure [10]. Consequently, transversion has larger effects on the regulatory of gene expression than transition [11]. Meanwhile, indels were observed only at sites 38 and 576. Overall, the polymorphic sites are summarized in Table 2.

In general, the base composition of the five psbA-trnH IGS sequences revealed significantly higher A and T contents in comparison to those of G and C as presented in

Table 3. Base composition of *psbA-trnH* IGS sequences of cogongrass in Java Island

Location	A (%)	G (%)	T (%)	C (%)
Purwokerto	34.8	17.6	27.9	19.7
Jetis	34.8	17.5	28.0	19.7
Purworejo	34.2	18.1	27.9	19.7

A = adenine; T = thymine; G = guanine; C = cytosine

Table 3. This is normally the case with non-coding regions, which are not responsible for controlling any protein synthesis thus showing high mutation rate [12].

Analysis on the genetic diversity by the use of DnaSP resulted in five haplotypes represented by every individual in the respective location. In other words, no haplotype-sharing among the individuals occurred. This constitutes a maximum value of haplotype diversity (h), which equals to 1. Genetic diversity values were classified into three categories, i.e. low (0.0 – 0.4), moderate (0.5 – 0.7) and high (0.8 – 1.0) [13].

In contrast to the very high haplotype diversity, considerably low nucleotide diversity value (π) of 0.00658 was obtained. The low nucleotide diversity indicates few number of polymorphic sites [14]. Hence, very small differences in nucleotide sequence among the five haplotypes of cogongrass in Java Island were found. This indicates very close genetic relationship among the five haplotypes, which can probably be caused by the presence of gene flow among the populations. Cogongrass seeds are easily spread by wind over distant areas [15].

The constructed phylogenetic tree (Fig. 3) demostrates that cogongrass samples from Purwokerto and Yogyakarta share the most recent common ancestor, although geographically both populations are not the closest among the others. Jetis is geographically closer to Purwokerto in comparison to Yogyakarta, but it is not the case with respect of genetic distance. Similarly, Purworejo shows the most genetic distance to the other four populations, where it should actually be the case with Ponorogo locating at the most remote distance. This means that no relationship between genetic and geographical distance was observed.

The high level of genetic diversity in a plant species population may correlate with its adaptability to the changes in environmental conditions. This is very important, especially for wild plant species existence [16]. Chlorophyll contents can be used to reflect the adaptability of a plant species to a particular habitat [17].

Measurement on chlorophyll contents reveals that cogongrass from Purworejo has significant difference from those of the other four (Table 4). This corresponds to the value of genetic distance, in which Purworejo population was the most remote one. Despite the close geographical distance and relatively similar habitat conditions between Purworejo and Jetis, cogongrass of both populations exhibit significantly different chlorophyll contents. Nevertheless, environmental conditions have in general substantial effects on both genetic diversity and physiological characteristics [18].

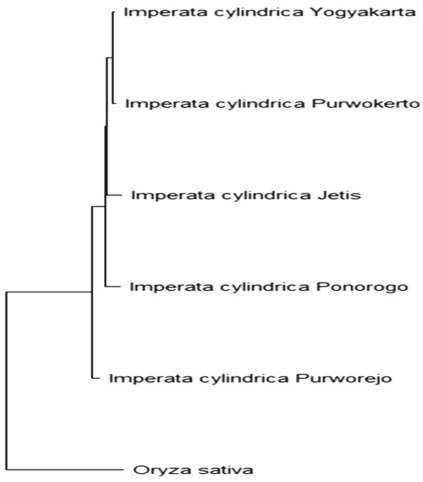

Fig. 3. Phylogenetic tree of cogongrass populations in Java Island based on *psbA-trnH* IGS.

Table 4. Chlorophyll contents of cogongrass in Java Island

Location	Chlorophyll a (μg/mL)	Chlorophyll b (μg/mL)	Total chlorophyll (μg/mL)
Purwokerto	6.688 a	5.017 a	2.478 a
Jetis	7.874 a	5.831 a	2.854 ab
Purworejo	11.946 b	7.796 b	3.464 b
Yogyakarta	7.970 a	5.418 a	2.490 a

4 Conclusion

In conclusion, it could be said that high genetic diversity of cogongrass in Java Island based on psbA-trnH IGS occurred despite the low nucleotide diversity, indicating relatively close genetic relationship among populations. No correlation between genetic and geographical distance was observed.

Acknowledgments. The authors address high appreciation to the Institute for Research and Public Service, Universitas Jenderal Soedirman for funding this project in the scheme of Riset Dasar Unsoed (University Basic Research) with contract number T/585/UN23.18/PT.01.03/2021.

Authors' Contributions. Murni Dwiati designed the research idea and took foremost responsibility for the project, Talita Ade Novita Dewi collected data on both genetic profiling and chlorophyll contents, Agus Hery Susanto analyzed all the data and wrote the manuscript.

References

1. D. Subositi, H. Widodo, Genetic diversity of cogongrass [*Imperata cylindrica* (L.) Beauv.] based on inter-simple sequence repeats (ISSR), Berita Biologi, 17(2), 2018, pp. 115–122. https://doi.org/10.14203/beritabiologi.v17i2.2642 [in Indonesian]
2. A.B. El-Mansy, D.A. El-Moneim, S.M. Alshamrani, F.A. Safhi, M.A. Abdein, A.A. Ibrahim, Genetic diversity analysis of tomato (*Lycopersicum esculentum* L.) with morphological, cytological, and molecular markers under heat stress, Horticulturae, 7(65), 2021, pp. 1–18. https://doi.org/10.3390/horticulturae7040065h
3. D. Bi, D. Chen, M. Khayatnezhad, Z.S. Hashjin, Z. Li, Y. Ma, Molecular identification and genetic diversity in *Hypericum* L.: a high value medicinal plant using RAPD markers, Genetika, 53(1), 2021, pp. 393–405. https://doi.org/10.2298/GENSR2101393B
4. M. Bolson, E.C. Smidt, M.L. Brotto, V.S. Pereira, ITS and *trnH-psbA* as efficient DNA barcodes to identify threatened commercial woody Angiosperms from Southern Brazilian Atlantic rainforests, PLOS ONE, 2015, pp. 1–18. https://doi.org/10.1371/journal.pone.0143049
5. A.H. Susanto, A. Nuryanto, B.S. Daryono, High connectivity among *Synedrella nodiflora* populations in Java Island based on intergenic spacer *atpB-rbcL*, Biosaintifika, 10(1), 2018, pp. 41–47. https://doi.org/10.15294/biosaintifika.v10i1.12038
6. J. Frigerio, G. Agostinetto, V. Mezzasalma, F.D. Mattia, M. Labra, A. Bruno, DNA-based herbal teas' authentication: an ITS2 and *psbA-trnH* multi-marker DNA metabarcoding approach, Plants, 10, 2021, pp. 1–14. https://doi.org/https://doi.org/10.3390/plants10102120h
7. J.J. Doyle, J.L. Doyle, Isolation of plant DNA from fresh tissue, Focus, 12(1), 1990, pp. 13–15.
8. F. Sanger, S. Nicklen, A.R. Coulson, DNA sequencing with chain-terminating inhibitors, Proceeding of National Academic Scence USA, 74(12), 1977, pp. 5463–5467.
9. I. Prabowo, D. Rachmawati, Physiological response and root anatomy of *Amaranthus tricolor* (L.) against NaCl, Jurnal Penelitian Saintek, 25(1), 2020, pp. 36–43. https://doi.org/10.21831/jps.v25i1.27357 [in Indonesian]
10. J. Wang, L. Raskin, D.C. Samuels, Y. Shyr, Y. Guo, Genome measures used for quality control are dependent on gene function and ancestry, Bioinformatics Advance Access, 3, 2014, pp. 1–6. https://doi.org/10.1093/bioinformatics/btu668
11. C. Guo, I.C. McDowell, M.Nodzenski, D.M. Scholtens, A.S. Allen, W.L. Lowe, T.E. Reddy, Transversions have larger regulatory effects than transitions, BMC Genomics, 18(394), 2017, pp. 1–9. https://doi.org/10.1186/s12864-017-3785-4
12. Fitmawati, R. Fauziah, I. Hayati, N. Sofiyanti, E. Inoue, D.D. Matra, Phylogenetic analysis of Mangifera from central region of Sumatra using trnL-F intergenic space, Biodiversitas, 18(3), 2017, pp. 1035–1040 https://doi.org/10.13057/biodiv/d180322
13. M. Nei, Molecular Evolutionary Genetics, Columbia University Press, NewYork, 1987. https://doi.org/10.7312/nei-92038
14. S. Ding, X. Dong, J. Yang, C. Guo, B. Cao, Y. Guo, G. Hu, Complete chloroplast genome of *Clethra fargesii* Franch., an original Sympetalous plant from Central China: comparative analysis, adaptive evolution, and phylogenetic relationships, Forests, 12(441), 2017, pp. 1–17. https://doi.org/10.3390/f12040441
15. A.H. Susanto, M. Dwiati, Short Communication: Assessment of cogongrass (*Imperata cylindrica* (L.) P.Beauv.) genetic variation in Java, Indonesia using *atpB-rbcL* and *trnL-F* intergenic spacer, Biodiversitas, 23(5), 2022, pp. 2760–2767. https://doi.org/10.13057/biodiv/d230558
16. M. Minter, D. O'Brien, J. Cottrell, R. Ennos, J.K. Hill, J. Hall, Exploring the potential for 'Gene Conservation Units' to conserve genetic diversity in wild populations, Ecological Solutions and Evidence, 2e12061, 2021, pp. 1–9. https://doi.org/10.1002/2688-8319.12061

17. M. Pogorzelec, B.Hawrylak-Nowak, B. Banach-Albinska, A. Szczurowska, M. Parzymies, K. Spolna, From *ex situ* cultivation to stands in natural habitats: critical periods for plants during the reintroduction of *Salix lapponum* L. in Eastern Poland, Journal for Nature Conservation, 67, 2022, pp. 1–10. https://doi.org/10.1016/j.jnc.2022.126172
18. M.F. Moustafa, A.E. Hesham, M.S. Quraishi, S.A. Alrumman, Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height, Journal of Genetics Engineering and Biotechnology, 14, 2016, pp. 349–362. https://doi.org/10.1016/j.jgeb.2016.09.001