# Short Communication: Assessment of cogongrass (Imperata cylindrica (L.) P.Beauv.) genetic variation in Java, Indonesia using atpB-rbcL and trnL-F intergenic spacer

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# Short Communication:
# Assessment of cogongrass (*Imperata cylindrica* (L.) P.Beauv.) genetic variation in Java, Indonesia using *atpB-rbcL* and *trnL-F* intergenic spacer

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**Abstract.** *Susanto AH, Dwiati M 2022. 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: 2760-2767.* Cogongrass (*Imperata cylindrica* (L.) P.Beauv.) is an invasive species commonly found in tropical and subtropical regions worldwide, consequently threatening global plant biodiversity, and sustainable agriculture. Nevertheless, some of its potential as a medicinal herb, phytoremediation agent, and alternative energy have been reported. genetic variation of cogongrass has been studied in certain areas, however genetic variation in Java, Indonesia has not been reported. Therefore, this study aims to assess the genetic variation of cogongrass in Java, Indonesia using *atpB-rbcL* and *trnL-F* intergenic spacer (IGS). Twenty-one plant samples were collected randomly from nine different sites and two pairs of universal primers were employed to amplify the markers. The results showed a much lower genetic difference among subpopulations in comparison to genetic variation within individual subpopulations. This result indicates that the subpopulations have high connectivity, meaning the rapid change in terrestrial ecosystems on the island does not affect the cogongrass population.

**Keywords:** *atpB-rbcL*, cogongrass, genetic variation, Java, *trnL-F*

## INTRODUCTION

Cogongrass (*Imperata cylindrica* (L.) P.Beauv.) is one of the ten most bothersome weed species in the world. It is widely distributed over tropical and subtropical regions, causing serious problems in agriculture and vegetable production in many areas. This plant is considered the reason for much loss of native habitats in the southeastern USA. The invasiveness and persistence are related to the biological features, such as extensive rhizome system, adaptability to poor soils and fire, drought tolerance, easy seed dispersal by wind, and high plasticity (MacDonald 2004). In Indonesia, cogongrass populations cover areas of approximately 8.5 million ha (Irzaman et al. 2021).

Cogongrass is a diploid C4 grass that is known to be harmful in 73 countries, thereby generating a risk to global biodiversity and sustainable agriculture (Burrell et al. 2015). On the other hand, certain potentials of cogongrass for human life have been reported. Some of its parts, either singly or as a mixture have been traditionally used to treat several diseases, such as wounds, sores, aches, back pain, fever, urinary stones, hypertension, and several sexual problems (Hidayat and Rachmadiyanto 2017). The whole plant extract was proven to show effective antibacterial and antiparasitic activities (Lalthanpuii and Zarzokimi 2019), while the roots could accumulate and immobilize copper thereby functioning as a phytoremediation agent in Cu-polluted environments (Vidal et al. 2020). Another possible usage of cogongrass as biofuel or alternative energy has also been intensively explored (Kartikasari et al. 2013;

Oladokun et al. 2016; Hidayat et al. 2018; Loh et al. 2021).

The sufficiently large number of cogongrass varieties is possibly due to the occurrence of natural hybrids with different flowering phenology between both parental ecotypes. The reproductive isolation led to population substructure (Chang and Chou 2006). Nevertheless, no significant genetic differences were observed between *I. cylindrica* and *I. brasiliensis* in the southeastern USA. Instead, population substructure seemed to occur within *I. cylindrica* (Lucardi et al. 2014a, 2014b). This was assumed because of the distinction in the methods of control on *I. cylindrica* in some states. Two lineages of *I. cylindrica* show very different distribution patterns were found (Lucardi et al. 2020).

Genetic diversity and population structure of cogongrass in the USA have been previously assessed using amplified fragment length polymorphisms (AFLPs) marker. However, this study was limited to a particular region of the country. Larger scale analysis of the cogongrass genetic diversity has been performed involving the possibility of anthropogenic influences. As many as 2,507 polymorphic loci were found from 676 cogongrass individuals. No significant relationship between sample size and genetic diversity of cogongrass population in the USA was observed (Lucardi et al. 2020). Other molecular markers such as *atpB-rbcL* and *trnL-F* intergenic spacers (IGS) can be used to assess genetic diversity of cogongrass population. This is because both cpDNA markers have a very high mutation rate as is commonly the case with non-coding sequences (Bi et al. 2018). In combination, both

markers have been employed to discover the evolutionary history of balsams (*Impatiens* spp.) in southern India (Shajitha et al. 2016).

As in many other places in the tropical and subtropical regions, the cogongrass population in Java, Indonesia is also interesting to examine. Java is known as the most populated one in the world (Alsya et al. 2021), causing rapid changes in terrestrial ecosystems condition, which tends to influence cogongrass genetic variation. Therefore, this study aims to assess the genetic variation of cogongrass in Java using *atpB-rbcL* and *trnL-F* IGS as molecular markers. The knowledge of the cogongrass population's genetic variation in Java is useful for providing a better strategy to manage the weed species.

## MATERIALS AND METHODS

### Plant materials

Twenty one plant samples were collected randomly from nine different sites in Java, namely Purwokerto, Banyumas, Kebasen, Kebocoran, Baturraden Botanic Gardens, Jetis Beach, Purworejo, Yogyakarta, and Ponorogo (Figure 1; Table 1).

### Procedures

#### Preparing plant materials

Individual plants were pulled up to the roots, then wrapped using a tissue previously moisturized with a little water, put in a plastic bag, and tied with a rubber bracelet. All the samples were later planted inside pots in the greenhouse of the Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia.

#### DNA extraction

The genomic DNAs were extracted according to the CTAB method (Doyle and Doyle 1990). A 0.1 g leaf was cut into pieces and dropped in a 1.5 mL microcentrifuge tube, then 800 µL CTAB solution previously heated at 65°C for 30 mins was added. The leaf pieces were pounded with a mini-bead beater up to a delicate level and heated at 65°C for an hour. This was left to cool down at room temperature and 500 µL chloroform-isoamyl alcohol (CIAA) was added, then mixed gently and subsequently centrifuged at 12,000 rpm for 5 mins. The supernatant was transferred into another 1.5 mL microcentrifuge and 1/10 volume of 3M sodium acetate was added. Afterward, isopropanol of 2/3 of the total volume was added and mixed gently by inverting the tube several times. This mixture was kept in the freezer for 24 hours before being centrifuged at 12,000 rpm for 10 mins.
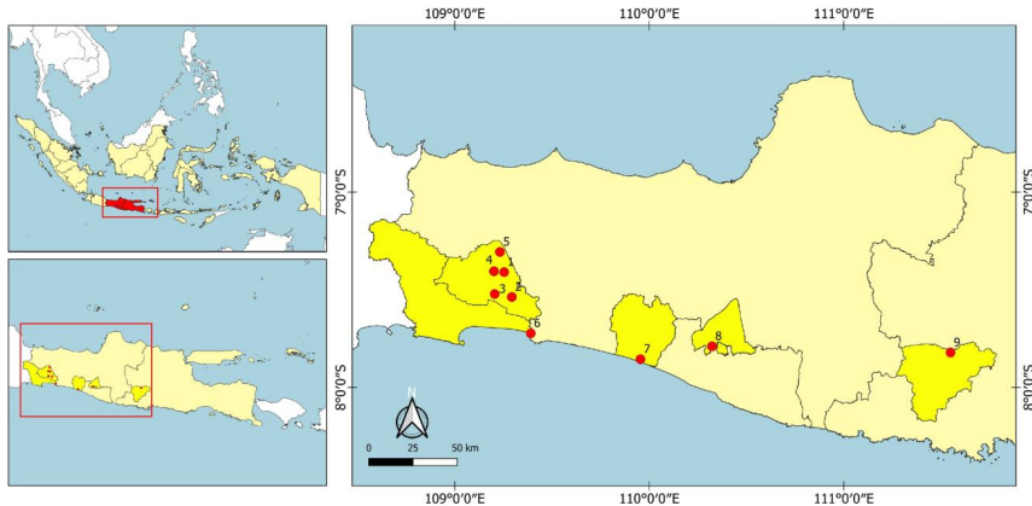


**Figure 1.** Sampling sites of *I. cylindrica* in Java, Indonesia. Note: see Table 1 for sampling location

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

| No. | Sampling site | Site code | Altitude (m asl) | Coordinate | | Sample number |
|-----|---------------|-----------|------------------|------------|------|---------------|
| | | | | S | E | |
| 1. | Purwokerto Utara, Banyumas, Central Java | PWT | 79 | 7°24' 33'' | 109° 15' 14'' | 3 |
| 2. | Banyumas, Central Java | BMS | 110 | 7° 32' 11'' | 109° 17' 37'' | 2 |
| 3. | Kebasen, Banyumas, Central Java | KBS | 68 | 7° 31' 17'' | 109° 12' 19'' | 2 |
| 4. | Kebocoran, Banyumas, Central Java | KBC | 85 | 7° 24' 17'' | 109° 12' 06'' | 2 |
| 5. | Baturraden Botanic Gardens, Banyumas, Central Java | KRB | 644 | 7° 18' 22'' | 109° 13' 56'' | 3 |
| 6. | Jetis Beach, Cilacap, Central Java | J | 6 | 7° 43' 21'' | 109° 23' 26'' | 3 |
| 7. | Purworejo, Central Java | PWJ | 21 | 7° 51' 17'' | 109° 57' 18'' | 2 |
| 8. | Yogyakarta City, Special Territory of Yogyakarta | YOG | 136 | 7° 47' 20'' | 110° 19' 21'' | 2 |
| 9. | Ponorogo, East Java | PON | 57 | 7° 49' 15'' | 111° 32' 56'' | 2 |

The supernatant was removed, followed by adding 500 $\mu$L of 70% ethanol to the DNA pellet and centrifuged again at 12,000 rpm for 5 mins. After removing the supernatant, the pellet was air-dried and dissolved in 100 $\mu$L TE buffer, then the DNA solution was stored at 4ºC before quantification and PCR amplification. Quantification was performed using genequant, while DNA was visualized in a 1.5% agarose gel electrophoresis using 1x TBE buffer.

*Polymerase chain reaction (PCR)*

The extracted genomic DNAs were used as PCR templates to amplify *atpB-rbcL* and *trnL-F* IGS. A pair of universal primers were used to amplify *atpB-rbcL* IGS, namely 5'-ACATCKARTACKGGACCAATAA-3' as the forward primer and 5'-AACACCAGCTTTRAATCCAA-3' as the reverse (Chiang and Schaal 2000). Meanwhile, 5'-GGTTCAAGTCCCTCTATCCC-3' as a forward primer and 5'-ATTTGAACTGGTGACACGAG-3' as a reverse primer, were applied to amplify *trnL-F* IGS (Taberlet et al. 1991). The individual PCR mixture was produced in a total volume of 10 $\mu$L containing 2.5 $\mu$L DNA template of 31 g/$\mu$L, 0.25 $\mu$L primers (forward and reverse) of 1$\mu$M, 5 $\mu$L Gotaq green (Promega), and 2.25 $\mu$L nuclease-free water was prepared. Subsequently, the mixture was subjected to a PCR condition, namely pre-denaturation at 94ºC for 3 mins, followed by 33 cycles which consisted of denaturation at 94ºC for 45 secs each, primer annealing at 55ºC for 45 secs, primer extension at 72ºC for 2 mins, and proceed by a final extension at 72ºC for 3 mins. The PCR reaction was performed in a Bio-Rad-T100™ Thermal Cycler machine. Then, the PCR mixture was stored at 4ºC before visualization in a 1% agarose gel electrophoresis using 1x TBE buffer.

*DNA sequencing*

The PCR products were first purified using QIA Quick kit (Qiagen), which employed the automated dideoxy method (Sanger et al. 1977) with terminator labeling, then DNA sequencing was carried out at the Firstbase Malaysia.

**Data analysis**

DNA sequences were edited using BioEdit version 7.0.4.1 (Hall 1999) and later checked manually. Sequence alignment was conducted with Clustal W (Thompson et al. 1994), which was also implemented in the BioEdit software. Both haplotype (*h*) and nucleotide (*π*) diversities were used as parameters for genetic diversity. These were calculated using Arlequin version 3.5 (Excoffier and Lischer 2010), while to identify the presence of population structure, AMOVA was performed in the Arlequin software as well.

## RESULTS AND DISCUSSION

Genomic DNAs were successfully extracted from all plant samples, yielding concentrations that ranged from 1,100 to 3,200 ng/$\mu$L. Meanwhile, the purities (A260 nm/280 nm ratio) varied from 1.538 to 2.750, meaning the DNAs could be properly used as PCR-templates to amplify the molecular markers.

Two PCR bands were observed in the respective sample, both *atpB-rbcL* and *trnL-F* IGS (Figures 2 and 3). However, it was certain that the correct bands were 912 bp for *atpB-rbcL* and 383 bp for *trnL-F* IGS, since they were more likely of the expected sizes. The amplicons produced by *atpB-rbcL* primers were slightly longer than those obtained in *Synedrella nodiflora* and *Eleutheranthera ruderalis* (Asteraceae), which had 880 bp long *atpB-rbcL* IGS sequences (Susanto and Dwiati 2019). Manual editing on the sequences of the putative *atpB-rbcL* IGS generated only 830 bp length. Meanwhile, the amplicons produced by *trnL-F* primers were also somewhat longer than the counterparts obtained in *Diospyros* spp. (Ebenaceae), showing 367 bp long *trnL-F* IGS sequences (Wanda et al. 2021). After manual editing, the putative *trnL-F* IGS had 372 bp length, which was still longer than the sequences found in *Diospyros* spp.

The blasting carried out to the NCBI database showed that both sequences were undoubtedly *atpB-rbcL* and *trnL-F* IGS. High percentages of identity with high query covers were observed, even the sequence of *trnL-F* IGS had 98.06% identity with total cpDNA genome of *I. cylindrica* (Acc no. MT351433.1) in a query cover of 98% (Table 2). All the sequences obtained in this study have been submitted to NCBI GenBank for accession numbers.

Some variations exist within the 21 sequences of *atpB-rbcL* IGS, in form of either insertion-deletions or base substitutions, and a similar case was also observed in *trnL-F* IGS. Eight haplotypes based on *atpB-rbcL* IGS were obtained, while only two haplotypes were found with *trnL-F* IGS, hence the mutation types and sites are summarized in Table 3.

There are more transversions than transitions obtained in either *atpB-rbcL* IGS or *trnL-F* IGS (Table 3). This is reasonable, since more possibilities with transversions rather than those of transitions exist. There are eight types of transversions, while only four transitions are found. Additionally, transversion seems to have more effects on the amino acid sequence alteration than transition does. Even the larger effects of transversion had also been reported on gene expression (Guo et al. 2017). Nevertheless, transversion is more energy-consuming in comparison to transition because of the more complicated changes in the nucleotide base molecular structure, where purine is replaced with and vice versa (Wang et al. 2015).
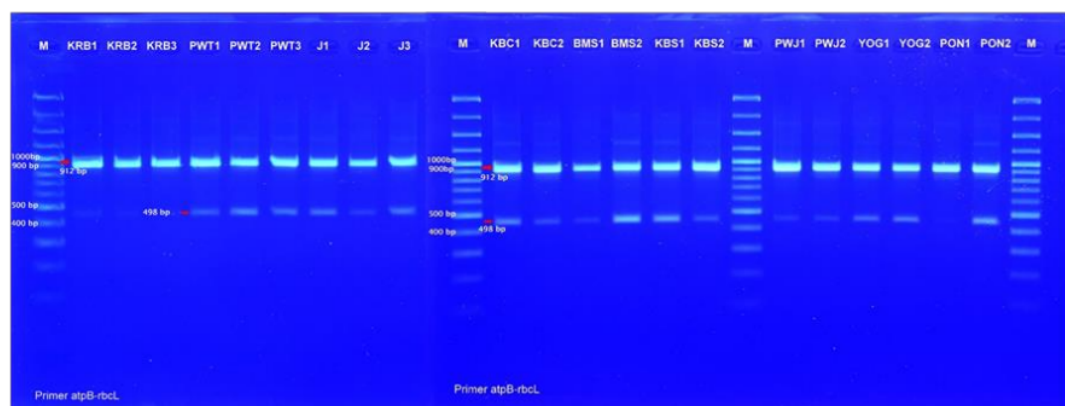
**Figure 2.** Electrophoretic profiles of *atpB-rbcL* IGS. M: 100 bp DNA ladder SMOBIO, KRB: Baturraden Botanic Gardens, PWT: Purwokerto, J: Jetis Beach, KBC: Kebocoran, BMS: Banyumas, KBS: Kebasen, PWJ: Purworejo, YOG: Yogyakarta, and PON: Ponorogo



**Figure 3.** Electrophoretic profiles of *trnL-F* IGS. M: 100 bp DNA ladder SMOBIO, KRB: Baturraden Botanic Gardens, PWT: Purwokerto, J: Jetis Beach, KBC: Kebocoran, BMS: Banyumas, KBS: Kebasen, PWJ: Purworejo, YOG: Yogyakarta, and PON: Ponorogo
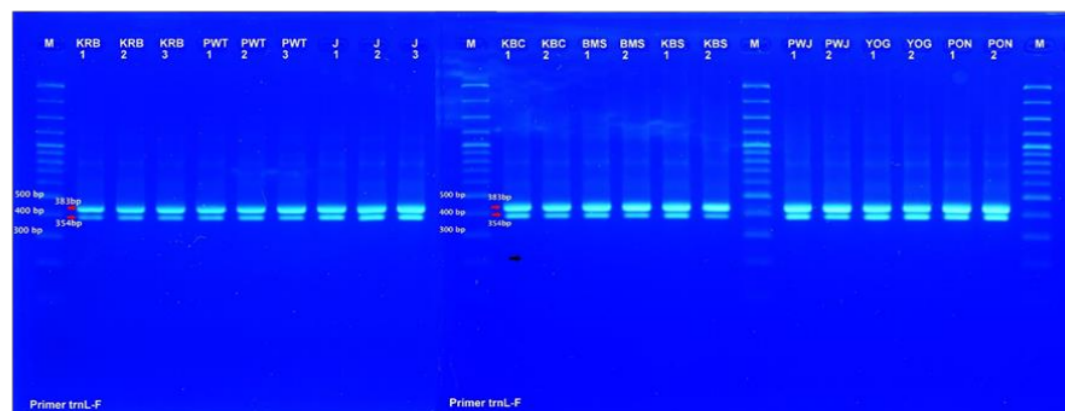
**Table 2.** BLAST results of the *atpB-rbcL* IGS and *trnL-F* IGS sequences to NCBI GenBank

| Sequences blasted | Description of subjects | % Query cover | E value | % Identity | Accession number |
|---|---|---|---|---|---|
| *atpB-rbcL* IGS | *Imperata cylindrica* cpDNA complete genome | 98 | 0 | 97.68 | KU291466.1 |
| | *Imperata cylindrica* cpDNA complete genome | 98 | 0 | 97.47 | MZ351433.1 |
| | *Saccharum rufipilum* cpDNA complete genome | 98 | 0 | 97.27 | LS974679.1 |
| | *Tripidium ravenae* cpDNA complete genome | 98 | 0 | 97.27 | LS974678.1 |
| *trnL-F* IGS | *Imperata cylindrica* cpDNA complete genome | 98 | 0 | 98.06 | MZ351433.1 |
| | *Chionachne kenigii* cpDNA partial genome | 98 | 0 | 97.09 | MT610087.1 |
| | *Saccharum hildebrandii* cpDNA complete genome | 98 | 0 | 96.85 | MF563371.1 |
| | *Sarga versicolor* cpDNA complete genome | 98 | 0 | 96.85 | MT942630.1 |

**Table 3.** Haplotypes of *I. cylindrica* in Java, Indonesia

| Molecular marker | Haplo-type | Sample code | Location(s) | Mutation type(s) | Site(s) |
|---|---|---|---|---|---|
| *atpB-rbcL* IGS | 1 | PWT1, PWT2, PWT3<br>KRB1, KRB2, KRB3<br>KBS1, KBS2<br>BMS1<br>JET3<br>PWJ 1, PWJ2<br>YOG 2<br>PON1 | Purwokerto<br>Baturraden BG<br>Kebasen<br>Banyumas<br>Jetis Beach<br>Purworejo<br>Yogyakarta<br>Ponorogo | Common type | - |
| | 2 | BMS2 | Banyumas | Insertion with A<br>Insertion with G<br>Insertion with T<br>Transition T → C<br>Transversion T → C<br>Transversion T → G | 13, 766, 767<br>585<br>278<br>289, 297, 301, 667, 679, 680<br>670, 688, 692, 696, 701, 706<br>285, 310, 672, 697, 700, 710 |
| | 3 | KBC1 | Kebocoran | Insertion with G<br>Deletion of A | 765<br>75 |
| | 4 | KBC2 | Kebocoran | Insertion with G<br>Insertion with C<br>Deletion of A<br>Transversion T → A | 737<br>736<br>75<br>732 |
| | 5 | JET1 | Jetis Beach | Insertion with C | 13 |
| | 6 | JET2 | Jetis Beach | Insertion with C | 737 |
| | 7 | YOG1 | Yogyakarta | Insertion with A | 43 |
| | 8 | PON2 | Ponorogo | Insertion with A<br>Insertion with C | 762<br>737 |
| *trnL-F* IGS | 1 | PWT2<br>KBC1, KBC2<br>KBS1, KBS2<br>BMS1, BMS2<br>JET1, JET2, JET3<br>PWJ 1, PWJ2<br>YOG1, YOG 2<br>PON1, PON2 | Purwokerto<br>Kebocoran<br>Kebasen<br>Banyumas<br>Jetis Beach<br>Purworejo<br>Yogyakarta<br>Ponorogo | Common type | - |
| | 2 | PWT1, PWT3,<br>KRB1, KRB2, KRB3 | Purwokerto<br>Baturraden BG | Deletion of G,T,A,T<br><br>Deletion of G,T<br>Deletion of<br>G,T,G,A,A<br>Transition A → G<br><br><br><br>Transition G → A<br>Transition C → T<br><br>Transition T → C<br><br>Transversion A → T<br><br><br>Transversion T → A<br>Transversion A → C<br><br>Transversion C → A<br>Transversion G → T<br><br><br>Transversion T → G<br>Transversion G → C<br>Transversion C → G | 84 – 87<br><br>286 – 287<br>311 – 315<br><br>34, 61, 63, 67, 72, 112, 152, 157, 158, 162, 170, 193, 203, 207, 219, 224, 225, 256, 257, 258, 278, 292<br>11, 108, 110, 113, 165, 177, 246, 288<br>4, 8, 9, 28, 90, 121, 122, 127, 139, 176, 184, 188, 189, 194, 196, 234, 275, 276, 279<br>41, 45, 69, 100, 117, 169, 181, 233, 236, 238, 280, 318, 331<br>2, 12, 37, 38, 52, 91, 96, 98, 118, 135, 147, 173, 180, 199, 211, 218, 228, 248, 250, 255, 290, 291, 296, 306, 341, 351<br>30, 33, 55, 59, 92, 151, 154, 192, 195, 254, 259, 295<br>6, 29, 39, 48, 75, 128, 156, 159, 167, 208, 209, 212, 213, 215, 220, 227, 230, 231, 232, 235<br>14, 15, 47, 164, 183, 270, 277, 317, 323<br>1, 3, 22, 40, 46, 64, 102, 103, 111, 114, 115, 116, 119, 126, 131, 133, 134, 137, 138, 143, 153, 179, 187, 190, 265, 267, 273<br>17, 18, 60, 163, 182, 269, 328<br>35, 54, 66, 294, 329<br>19, 94, 252, 324 |

Based on *atpB-rbcL* IGS, the haplotype diversity (*h*) was 0.3809±0.0165, while the nucleotide diversity (*π*) was only 0.0201±0.0103. Lower *h* was obtained with *trnL-F* IGS, i.e. 0.0952±0.0322, however, the *π* was higher than the value found with *atpB-rbcL* IGS, i.e. 0.2277±0.1139. The relatively low genetic variation of cogongrass in Java is presumably related to the high invasiveness of the species, hence most samples used in this study are of the same origin. Similarly, no hybridization among four different clonal lineages of cogongrass in the USA occurred despite geographical overlap, indicating that the plant showed limited evolutionary potential to adapt to novel environmental conditions. Even selection for favorable alleles from a broad genetic base seemed likely not to occur after arrival to a new environment, causing a very low genetic diversity (Burrell et al. 2015).

The limited genetic variation did not correlate with the phenotypical performance of cogongrass. Both morpho-anatomical and physiological characters, show significant differences among altitudes (Ahmad et al. 2020). Moreover, soil water stress had been experimentally proven to reveal a stronger impact on the species distribution in comparison to nutrient deficiency (Zhang et al. 2021). Phenotypic plasticity is a common phenomenon in invasive plants for adaptation to various conditions, such as sunny, shaded, moist, or dry sites, that makes the species as ideal weeds (Sultan and Matesanz 2015). Allelopathy substances from cogongrass root exudates, such as ferulic acid, had been shown to initiate its successful invasiveness (Shen et al. 2020).

AMOVA on the cogongrass in Java revealed that variation within populations was much higher than among populations, which applied to both *atpB-rbcL* and *trnL-F* IGS (Tables 4 and 5).

**Table 4.** AMOVA on cogongrass population in Java based on *atpB-rbcL* IGS

| Source of variation | df | Sum of squares | Variance components | Percentage of variations |
|---|---|---|---|---|
| Among populations | 8 | 4.286 | 0.02735 | 5.47 |
| Within populations | 12 | 5.667 | 0.47222 | 94.53 |
| Total | 20 | 9.953 | 0.49957 | |
| Fixation index ($F_{ST}$) = 0.05475 | | | | |

**Table 5.** AMOVA on cogongrass population in Java based on *trnL-F* IGS

| Source of variation | df | Sum of squares | Variance components | Percentage of variations |
|---|---|---|---|---|
| Among populations | 8 | 3.881 | 0.01154 | 2.46 |
| Within populations | 12 | 5.500 | 0.45833 | 97.54 |
| Total | 20 | 9.381 | 0.46987 | |
| Fixation index ($F_{ST}$) = 0.02456 | | | | |

The much higher variations within populations in comparison to among populations led to low fixation indices, indicating high connectivity or gene flow among members of cogongrass species in Java, meaning no population structure was observed. Correspondingly, high connectivity was also reported among populations of *Synedrella nodiflora* (Asteraceae), a weed species in this area (Susanto et al. 2018). The cogongrass gene flow among populations was reported as mainly assisted by wind dispersal of seeds and spikelets. In addition, a non-wind-borne dispersal mechanism might as well occur for the vegetative parts of the plant. Dense woody vegetation had been shown to slow down the wind dispersal of cogongrass seeds, but it was a less effective barrier for the non-wind-borne dispersal mechanism (Ruger et al. 2011). Biological characteristics, such as extensive rhizome system, adaptability to poor soils and fire, drought tolerance, readily wind dispersal of seeds, and high plasticity had been contributing to the invasiveness and persistence of cogongrass (MacDonald 2004).

Regarding problems with agricultural productivities, cogongrass invasiveness was potentially overcome with earthworm application. In experiments, using the plant's root extracts up to a concentration level of 80% mixed with soil from cogongrass land showed that earthworms tend to cover the soil surface and improve its quality. Subsequently, when these engineered soils were used to grow upland rice seedlings, significant differences in many vegetative parameters were observed in comparison to soils without earthworm application (Kilowasid et al. 2021). On the other hand, glyphosate applications seemed ineffective to control cogongrass. Instead, other abiotic factors such as drought and shade need to be studied for effectiveness (Zaccaro 2016; Enloe et al. 2018). Most recently, an integrated control system involving cultural, mechanical, and chemical approaches was introduced. However, biological control and revegetation were recommended for the long-term management of the weed species ( Lebrun 2020; Rusdy 2020). Bioherbicides, such as the type causing wilt disease in cogongrass could also be introduced (Tamur et al. 2019). However, invasive plants like cogongrass in forest regeneration ought to be considered in the management strategies assisting the persistence of native forest communities (Lázaro-Lobo et al. 2021).

The difficulty in controlling the invasiveness is mainly due to its dispersal through both extensive rhizome systems and wind-assisted seeds. Cogongrass rhizomes make more than 60% of the total biomass and these support rapid growth after mowing or burning. Meanwhile, the seeds are very prolific and capable of traveling over a long distance without significant loss in viability and germination rate (Minogue et al. 2018). The changes in soil microbial and chemical composition because of the occupation by cogongrass affect the growth of succeeding plant communities (Radunzel-Davis 2019). Nevertheless, cogongrass presence was reported to be suppressed by creeping rhizomes of other grass species, such as *Sorghum halepense* (Yamada and Nemoto 2020).

Different altitudes ranging from 0 m asl. in Jetis Beach to approximately 800 m asl. in Baturraden Botanic Gardens

were used as sampling sites in this study. Also, various conditions of terrestrial ecosystems among the sites existed as the consequences of rapid physical development on Java. However, no significant genetic differences among populations were observed, meaning the changes in ecosystem conditions did not affect cogongrass on the island. Conversely, variation among several cogongrass ecotypes in Taiwan was detected, thereby supporting the molecular data previously reported (Chang and Chou 2006). Different phenotypic characteristics, especially concerning foliar anatomy were observed among cogongrass ecotypes naturally grown in the Botanic Gardens of Rajshahi University Bangladesh. This was presumably related to adaptation against drought and saline stress (Sima et al. 2018).

In conclusion, based on both *atpB-rbcL* and *trnL-F* IGS much lower genetic differences among subpopulations of cogongrass in Java, Indonesia in comparison to genetic variation within individual subpopulations were observed. This indicates that the subpopulations show high connectivity, or in other words, the rapid changes in terrestrial ecosystems on Java do not affect the cogongrass population.

## ACKNOWLEDGEMENTS

## REFERENCES

Ahmad KS, Wazarat A, Mehmood A, Ahmad MSA, Tahir MM, Nawaz F, Ahmed H, Zafar M, Ulfat A. 2020. Adaptations in *Imperata cylindrica* (L.) Raeusch. and *Cenchrus ciliaris* L. for altitude tolerance. Biologia 75: 183-198. DOI: 10.2478/s11756-019-00380-2.

Alsya VD, Triwahyuningtyas N, Murtatik S. 2021. Analysis of factors affecting poverty level in Java Island. Intl J Soc Service Res 1 (2): 150-161. DOI: 10.46799/ijssr.v1i2.29.

Bi Y, Zhang MF, Xue J, Dong R, Du YP, Zhang XH. 2018. Chloroplast genomic resources for phylogeny and DNA barcoding: A case study on *Fritillaria*. Sci Rep 8: 1184. DOI: 10.1038/s41598-018-19591-9.

Burrell AM, Pepper AE, Hodnett G, Goolsby JA, Overholt WA, Racelis AE, Diaz R, Klein PE. 2015. Exploring origins, invasion history and genetic diversity of *Imperata cylindrica* (L.) P. Beauv. (Cogongrass) in the United States using genotyping by sequencing. Mol Ecol 24: 2177-2193. DOI: 10.1111/mec.13167.

Chang IF, Chou CH. 2006. Ecotypic variation of *Imperata cylindrica* populations in Taiwan: II. Physiological and biochemical evidence. Bot Stud 47: 175-184.

Chiang TY, Schaal B. 2000. Molecular evolution and phylogeny of the atpB – rbcL spacer of chloroplast DNA in the true mosses. Genome 43: 417-426. DOI: 10.1139/g99-116.

Doyle JJ, Doyle JL. 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus 12 (1): 13-15. DOI: 10.2307/2419362.

Enloe SF, Lucardi RD, Loewenstein NJ, Lauer DK. 2018. Response of twelve Florida cogongrass (*Imperata cylindrica*) populations to herbicide treatment. Invasive Plant Sci Manag 11: 82-88. DOI: 10.1017/inp.2018.12.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564-567. DOI: 10.1111/j.1755-0998.2010.02847.x.

Guo C, McDowell IC, Nodzenski M, Scholtens DM, Allen AS, Lowe WL, Reddy TE. 2017. Transversions have larger regulatory effects than transitions. BMC Genomics 18: 394. DOI: 10.1186/s12864-017-3785-4.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuc Acids Symp Series 41: 95-98.

Hidayat S, Rachmadiyanto AN. 2017. Utilization of alang-alang (*Imperata cylindrica* (L.) Raeusch.) as traditional medicine in Indonesian Archipelago. Proc 1st SATREPS Conf 1: 82-89.

Hidayat S, Abu Bakar MS, Yang Y, Phusunti N, Bridgwater AV. 2018. Characterisation and Py-GC/MS analysis of *Imperata cylindrica* as potential biomass for bio-oil production in Brunei Darussalam. J Anal Appl Pyrolysis 134: 510–519. DOI: 10.1016/j.jaap.2018.07.018.

Irzaman, Yustaeni D, Aminullah, Irmansyah, Yuliarto B. 2021. Purity, morphological, and electrical characterization of silicon dioxide from cogon grass (*Imperata cylindrica*) using different ashing temperatures. Egypt J Chem 64: 4143-4149. DOI: 10.21608/ejchem.2019.15430.1962.

Kartikasari SD, Nurhatika, S, Muhibuddin A. 2013. Potential of cogongrass (*Imperata cylindrica* (L.) Beauv.) in ethanol production using *Zymomonas mobilis* bacteria. Jurnal Sains dan Seni Pomits 2: 1-11. [Indonesian]

Kilowasid LMH, Hasmar LOJ, Afa LO, Sutariati GAK, Namriah, Rakian TC. 2021. Effect of cogongrass (*Imperata cylindrica* L.) root extract on earthworms, arbuscular mycorrhizae fungi spore, and growth of upland rice (*Oryza sativa* L.) for local Kambowa variety. IOP Conf Series Earth Environ Sci 807. DOI: 10.1088/1755-1315/807/3/032034.

Lalthanpuii PB, Zarzokimi KL. 2019. Chemical profiling, antibacterial and antiparasitic studies of *Imperata cylindrica*. J Appl Pharm Sci 9: 117-121. DOI: 10.7324/JAPS.2019.91216.

Lázaro-Lobo A, Lucardi RD, Ramirez-Reyes C, Ervin GN. 2021. Region-wide assessment of fine-scale associations between invasive plants and forest regeneration. For Ecol Manag 483: 118930. DOI: 10.1016/j.foreco.2021.118930.

Lebrun A. 2020. Cogongrass Control Program in the Southeastern United States – Alabama, Georgia, Mississippi and South Carolina. US Department of Agriculture, Riverdale Maryland.

Loh SH, Yee W, Lee SWY, Tan YT, Phua PSP, Cha TS. 2021. Cogongrass (*Imperata cylindrica*) leaf extract counteracts the effects of nitrogen limitation and induces changes in biomass, lipid, and fatty acid profile in *Chlorella vulgaris* UMT-M1 and *Scenedesmus* sp. KS-2. Bioenerg Res 14: 61-69. DOI: 10.1007/s12155-020-10182-z.

Lucardi RD, Wallace LE, Ervin GN. 2014a. Invasion success in cogongrass (*Imperata cylindrica*): A population genetic approach exploring genetic diversity and historical introductions. Invasive Plant Sci Manag 7: 59-75. DOI: 10.1614/ipsm-d-13-00029.1.

Lucardi RD, Wallace LE, Ervin GN. 2014b. Evaluating hybridization as a potential facilitator of successful cogongrass (*Imperata cylindrica*) invasion in Florida, USA. Biol Invasions 16: 2147-2161. DOI: 10.1007/s10530-014-0654-9.

Lucardi RD, Wallace LE, Ervin GN. 2020. Patterns of genetic diversity in highly invasive species: Cogongrass (*Imperata cylindrica*) expansion in the invaded range of the southern United States (US). Plants 9 (423): 1-13. DOI: 10.3390/plants9040423.

MacDonald GE. 2004. Cogongrass (*Imperata cylindrica* L): Biology, ecology, and management. Critical Rev Plant Sci 23: 367-380. DOI: 10.1080/07352680490505114.

Minogue PJ, Brodbeck BV, Miller JH. 2018. Biology and control of cogongrass (*Imperata cylindrica*) in Southern Forests. IFAS Extension University of Florida, Gainesville. 2018: 1-6 DOI: 10.32473/edis-fr411-2018.

Oladokun O, Ahmad A, Abdullah TAT, Nyakuma BB, Bello AAH, AlShatri AH. 2016. Multicomponent devolatilization kinetics and thermal conversion of Imperata cylindrica. Appl Therm Eng 105: 931-940. DOI: 10.1016/j.applthermaleng.2016.04.165.

Radunzel-Davis LA. 2019. Cogongrass (*Imperata cylindrica* (L.) Beauv.) in Louisiana: Cause and Consequence. [Thesis]. Louisiana State University, Louisiana.

Rusdy M. 2020. *Imperata cylindrica*: Reproduction, dispersal, and controls. CAB Rev 15 (038): 1-9. doi: 10.1079/PAVSNNR202015038.

Sanger F, Nicklen S, Coulson A. 1977. DNA sequencing with chain-terminating. Proc Natl Acad Sci USA 74: 5463-5467. DOI: 10.1073/pnas.74.12.5463.

Shajitha PP, Dhanesh NR, Ebin PJ, Laly J, Aneesha D, Reshma J, Augustine J, Linu M. 2016. A combined chloroplast *atpB–rbcL* and *trnL-F* phylogeny unveils the ancestry of balsams (*Impatiens* spp.) in the Western Ghats of India. 3 Biotech 6: 258. DOI: 10.1007/s13205-016-0574-8.

Shen Y, Tang H, Wu W, Shang H, Zhang D, Zhan X, Xing B. 2020. Role of nano-biochar in attenuating the allelopathic effect from: *Imperata cylindrica* on rice seedlings. Environ Sci Nano 7: 116-126. DOI: 10.1039/c9en00828d.

Sima S, Roy A, Akther M, Joarder N. 2018. Cross-sectional anatomy of leaf blade and leaf sheath of cogon grass (*Imperata cylindrica* L.). J Bio Sci 25: 17-26. DOI: 10.3329/jbs.v25i0.37494.

Sultan SE, Matesanz S. 2015. An ideal weed: Plasticity and invasiveness in *Polygonum cespitosum*. Ann New York Acad Sci 1360: 101-119. DOI: 10.1111/nyas.12946.

Susanto AH, Nuryanto A, Daryono BS. 2018. High connectivity among *Synedrella nodiflora* populations in Java Island based on intergenic spacer *atpB-rbcL*. Biosaintifika 10 (1): 41-47. DOI: 10.15294/biosaintifika.v10i1.12038.

Susanto AH, Dwiati M. 2019. Genetic difference between two phenotypically similar members of Asteraceae by the use of intergenic spacer *atpB-rbcL*. Biosaintifika 11 (3): 393-399.

Taberlet P, Gielly P, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non coding regions of chloroplast DNA. Plant Mol Biol 17: 1105-1109. DOI: 10.1007/BF00037152.

Tamur HA, Al-Janabi HJ, Abood Al-Janabi JK, Mohsin LY, Al-Yassiry ZAN. 2019. Characterization and antagonistic activity of new causal agent of wilt disease in *Imperata cylindrica* (*Marasmius palmivorus*). J Pure Appl Microbiol 13: 1525-1536. DOI: 10.22207/JPAM.13.3.24.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc Acids Res 22: 4673-4680. DOI: 10.1093/nar/22.22.4673.

Vidal C, Ruiz A, Ortiz J, Larama G, Perez R, Santander C, Ademar P, Ferreira A, Cornejo P. 2020. Antioxidant responses of phenolic compounds and immobilization of copper in *Imperata cylindrica*, a plant with potential use for bioremmediation of Cu contaminated environments. Plants 9: 1397. DOI:10.3390/plants9101397.

Wanda IF, Djuita NR, Chikmawati T. 2021. Molecular phylogenetics of Malesian Diospyros (Ebenaceae) based *trnL-F* spacer sequences. Biodiversitas 22: 4106-4114. DOI: 10.13057/biodiv/d220959.

Wang J, Raskin L, Samuels DC, Shyr Y, Guo Y. 2015. Genome measures used for quality control are dependent on gene function and ancestry. Bioinformatics 31: 318-323. DOI: 10.1093/bioinformatics/btu668.

Yager LY, Miller DL, Jones J. 2011. Woody shrubs as a barrier to invasion by cogongrass (*Imperata cylindrica*). Invasive Plant Sci Manag 4: 207-211. DOI: 10.1614/ipsm-d-10-00052.1.

Yamada S, Nemoto M. 2020. Effects of weed abundance and frequency of hand weeding on the establishment of transplanted *Imperata cylindrica*. Landscape Ecol Eng 16: 1-9. DOI: 10.1007/s11355-019-00398-3.

Zaccaro MLM. 2016. Cogongrass (*Imperata cylindrica* (L.) Beauv) Control using Chemical Treatments with Cover Cropping Systems. [Thesis]. Mississippi State University, Mississippi.

Zhang B, Yuan Y, Shu L, Grosholz E, Guo Y, Hastings A, Cuda JP, Zhang J, Zhai L, Qiu J. 2021. Scaling up experimental stress responses of grass invasion to predictions of continental-level range suitability. Ecology 102 (8): e03417. DOI: 10.1002/ecy.3417.

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