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Genetic variation of *Austropuccinia psidii* in some species of Myrtaceae as host plants in Java, Indonesia based on simple sequence repeats (SSR) markers

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Abstract. *Faradilla FA, Prihatini I , Suranto, Susilowati A. 2022. Genetic variation of* Austropuccinia psidii *in some species of Myrtaceae as host plants in Java, Indonesia based on simple sequence repeats (SSR) markers. Biodiversitas 23: 256-263. Austropuccinia psidii* is pathogenic rust with a wide host and is considered a biosecurity threat to the Myrtaceae family in many countries. The genetic variation of this rust in Java, Indonesia is poorly reported. Therefore, this study aimed to determine the genetic variation among *A. psidii* from different Myrtaceae hosts, namely *Syzygium polyanthum, S. myrtifolium,* and *Melaleuca cajuputi* from three different locations in Java using seven simple sequence repeats (SSR) markers. Data were collected from 28 *A. psidii* samples from three different hosts and locations in Java. The genetic variations of *A. psidii* were found in six isolates in locus USYD_*Pp*168, PpSSR161, and PpSSR195*. The results showed that the expected heterozygosity value among 12 isolates of *A. psidii* is 0.312. The dendrogram illustrates two clusters constructed with cluster I consisting of subcluster IA (S1, S3, S4, S5, K1, K2, K3, K4, and P1) and IB (P2); and cluster II consists of isolates S2 and S6. Principal Coordinate Analysis (PCoA) was used to demonstrate the similarity and dissimilarity among isolates based on microsatellite sites. Axis 1 and 2 explained 87.7 % of the total variations and separated isolate S1, S2, S3, S4, S6 from other isolates, and grouping the rest of isolates together. The dendrogram and PCo analysis demonstrated *A. psidii* isolates tend to have close genetic similarity based on their host.

Keywords: *Melaleuca cajuputi*, microsatellite, myrtle rust, *Syzygium myrtifolium, S. polyanthum*

INTRODUCTION

Austropuccinia psidii is myrtle rust capable of infecting approximately 480 species of Myrtaceae throughout the world (Soewarto et al. 2019). According to preliminary studies, some of the infected genera are economically important, such as *Eucalyptus, Melaleuca* (McTaggart et al. 2016), *Psidium,* and *Syzygium* (Granados et al. 2017). This rust also threatens many native and endemic Myrtaceae species in Australia, such as *Rhodamnia rubescens, Rhodomyrtus psidioides, Eucalyptus amplifolia, Kunzea parvifolia, Austromyrtus inophloia,* and other 370 or more native plants (Winzer et al. 2019). This rust has been reported to infect some Myrtaceae species in Indonesia, such as *E. grandis x E. pellita, M. leucadendra* in the north and south Sumatra (McTaggart et al. 2016), as well as *S. polyanthum,* and *M. cajuputi* in Yogyakarta, Java (Prihatini et al. 2020).

Pegg et al. (2017) stated that *A. psidii* infects young organs of plants, such as young flowers, fruits, and leaves. This rust is an obligate biotoph, and it takes nutrients from the host as a source of carbon to grow (Chock 2020). *Austropuccinia psidii* infection can reduce the growth of the plant and defoliation (Winzer et al. 2018), blighting on the stem, and tree dieback (Pegg et al. 2017), reduces fruit quality, thereby causing premature dropping of fruits and seedling death (Sutherland et al. 2020). Its infection symptoms on mature leaves are necrotic spots and a few uredinia (Beresford et al. 2019).

Studies on genetic variation and population structure of this rust are important aspects to understand the pathogen distribution, origin, and possibility of its mutation to overcome the resistant host (Graça et al. 2011; Machado et al. 2015; du Plessis et al. 2019). Simple Sequence Repeat (SSR) or microsatellite is a highly polymorphic repeated DNA sequence, with one to six base pairs (bp) in repeat (Sandhu et al. 2015). It is commonly used as a marker to study the genetic variation of rust. According to Abdurakhmonov (2016), SSR is a co-dominant marker that usually has a high polymorphism level with small sizes fragments (less than 1kb) therefore, it can be detected even in low quality DNA. SSR markers, such as PpSSR developed by Zhong et al. (2008), have been used widely to study the genetic variation and host specificity of *A. psidii* in different countries, such as Hawaii, Brazil, New Zealand, and Singapore (Zhong et al. 2011; Graça et al. 2013; du Plessis et al. 2019). SSR markers are also used to reveal the unique multilocus genotypes (MLGs) of *A. psidii* from different hosts and areas of infections (Stewart et al. 2017). Another SSR marker developed by Sandhu et al. (2015) has been used to study the genetic variation of *A. psidii* from diverse hosts and locations in Australia.

Genotypes of *A. psidii* studied in limited hosts and locations in Indonesia using seven PpSSR microsatellite markers revealed no genetic variation between the rust from Indonesia and Australia. The alleles in seven loci were identical, therefore, *A. psidii* from both countries are assumed to originate from a single genotype (McTaggart et al. 2016). However, studies on its genetic variation in Java and other islands in Indonesia have not been conducted. Therefore, this study aimed to determine the genetic variation among *A. psidii* from three different hosts, namely *S. polyanthum*, *S. myrtifolium*, and *M. cajuputi* in three different locations in Java using simple sequence repeats markers.

MATERIALS AND METHODS

Research materials

Research materials were collected from different hosts, namely *S. polyanthum* (Wight) Walp., *S. myrtifolium* Walp., and *M. cajuputi* Maton & Sm. ex R. Powell from October 2020 to March 2021. Diseased *S. myrtifolium* were collected from the arboretum of the Center for Forest Biotechnology and Tree Improvement (CFBTI) in Yogyakarta, Indonesia. Furthermore, *M. cajuputi* were collected from the arboretum of CFBTI, and Gunung Kidul, Yogyakarta, while diseased *S. polyanthum* were obtained from three locations, namely the arboretum of CFBTI, a private yard in Sleman, Yogyakarta and Sukabumi, West Java, Indonesia. A total of 28 *A. psidii* samples were collected from three different hosts and locations, as shown in Table 1.

Procedures

Observation of Austropuccinia psidii *symptoms on host plants*

The symptom of *A. psidii* infections, such as yellow urediniospores and lesions in leaves, branches, and twigs of the Myrtaceae tree was observed. Symptom was ranged on susceptible and resistant hosts, where severe attacks mainly occur on young organs of the plants, such as young leaves, branches, fruits, or flowers, and lesions. Furthermore, yellow urediniospores are found on infected organs, while symptoms in the resistant hosts usually present as necrotic lesions or flecks on young leaves with limited or no urediniospores (Glen et al. 2007). Diseased plant organs were taken with scissors, placed on the envelopes, and kept dry at room temperature.

DNA extraction

The DNA extraction was carried out on leaves containing a colony of *A. psidii* using Glen et al. (2002) procedure as follows: One *A. psidii* pustule was placed inside a 2 mL microtube, and 250 µL of SDS extraction buffer was added. It was then shaken by a mini-bead-beater for 5 minutes and incubated for one hour at 65ºC. Samples were centrifuged at 14.000 rpm in 15 minutes, while the supernatant was placed in the new 2 mL microtubes after adding 10 µL silica glass milk and 600 µL NaI 100%. Furthermore, the samples were vortexed, incubated in the icebox for 15 minutes while being shaken occasionally, and centrifuged again with 14.000 rpm in 20 seconds. The supernatant was removed, and 750 µL of wash solutions were added to the pellets. It was vortexed and centrifuged at 14.000 rpm for 20 seconds, followed by 750 µL ethanol to the pellets, and centrifuged at 14.000 rpm for 20 seconds. After removing ethanol, the pellets were kept dry inside a laminar air flow for 1-1.5 hours to make sure the ethanol had completely evaporated. Finally, Tris-EDTA were added to the DNA, then vortexed, and incubated at 45ºC for 10 minutes. The extracted DNA was centrifuged at 14.000 rpm for 2 minutes, then kept at -20ºC.

Amplification of species-specific DNA

Amplification of rDNA ITS was conducted using species-specific primers Ppsi1/Ppsi6 due to its ability to detect the presence of *A. psidii* in extracted DNA (Langrell et al. 2008). This procedure is carried out to select the samples that contain this rust. The PCR reaction for species-specific DNA amplification consisting of 6 µL ddH_2O, 12.5 µL PCR My Taq Red Mix (Bioline), 0.5 µL Ppsi1 primers, 0.5 µL Ppsi6 primers, 0.5 µL BSA, and 5 µL DNA template with the total volume of 25 µL. PCR was performed on the GeneAmp PCR System 9700 machine. The cycle consisted of one pre-denaturation at 94ºC for 3 minutes followed by 30 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC for 1 minute, extension at 72ºC for 1 minute, and a final extension at 72ºC for 10 minutes (Langrell et al. 2008). The product was separated in 1% agarose with 100 V for 30 minutes.

Table 1. *Austropuccinia psidii* samples from different hosts and locations

Host	Number of samples	Location	Isolate code
S. polyanthum	2	Sleman, Yogyakarta	S3, S4
S. polyanthum	3	Sukabumi, West Java	S5, S6, S7
S. polyanthum	6	CFBTI arboretum	S1, S2, S8, S9, S10, S11
M. cajuputi	6	north area of CFBTI arboretum	K1,K2, K5, K6, K7, K8
M. cajuputi	6	south area of CFBTI arboretum	K3, K4, K9, K10, K11, K12
M. cajuputi	2	Gunung Kidul, Yogyakarta	K13, K14
S. myrtifolium	3	CFBTI arboretum	P1, P2, P3

Table 2. Seven SSR markers were used to observe the genetic variation of *Austropuccinia psidii* in three species of Myrtaceae

Primer	Sequences (5' to 3')	References
PpSSR012	F: TTCAATCCCCATAAGGCTTTC R: AAATCCTGAGTCTTCTTCCCC	Zhong et al. (2008)
PpSSR102*	F: TGACTTTAATCATCTTCAAAACCAA R: ACCAATCCCCTTCCTTCATC	Zhong et al. (2008) modified by Graça et al. (2013)
PpSSR161	F: TCGAGGGGTCTCAGTTTTCA R: GAGATCTATCGGACCAACGAA	Zhong et al. (2008)
PpSSR195*	F: GAACGAACCCAAACTTTCCA R: GGAAAGGAATGAGATTGAACACA	Zhong et al. (2008) modified by Graça et al. (2013)
USYD_Pp35	F: ATGAGGGATGATGAAGTGAA R: GCAAGGATGGAGTGATGTGT	Sandhu et al. (2015)
USYD_Pp151	F: GATCCTACTAATCCAATTCT R: CTCGCTCAGATTTTAATTCA	Sandhu et al. (2015)
USYD_Pp168	F: GATATTCTACGCACTACGCA R: GTCTAGTATGATTTGGCCTG	Sandhu et al. (2015)

Polymerase chain reaction (PCR) using selected SSR markers

The isolates that produced positive amplification products using *A. psidii* specific primers were selected for the genetic study using microsatellite markers. A total of 12 isolates from three different hosts were used to carry out this genetic study. These isolates are *A. psidii* found in *M. cajuputi* collected from the south and north areas of CFBTI arboretum, as well as *S. myrtifolium*, and *S. polyanthum* from CFBTI arboretum, Sleman Yogyakarta and Sukabumi West Java. *Melaleuca cajuputi* isolates from Gunung Kidul failed to produce the amplicon, therefore these isolates were not selected for further analysis.

Seven SSR markers were used for this genetic study (Table 2). PCR reaction consists of 11 µL ddH_2O, 12.5 µL PCR My Taq Redmix (Bioline), 0.5 µL forward primer, 0.5 µL reverse primer, and 0.5 µL diluted DNA template with a total volume of 25 µL. PCR was performed on the GeneAmp PCR System 9700 machine, while amplification was conducted using markers PpSSR012, PpSSR102*, PpSSR161 and PpSSR195* with the following cycles: one cycle at 95°C for 5 minutes, followed by three cycles at 95°C for 30 seconds, 40-55°C (depending on the locus) for 30 seconds, and 72°C for 80 seconds, followed by 35 cycles at 94°C for 15 seconds, 40-55°C (depending on the locus) for 15 seconds, and 72°C for 45 seconds, and one final extension at 72°C for 5 minutes (Graça et al. 2013). Amplification using markers USYD_*Pp*35, USYD_*Pp*151, and USYD_*Pp*168 were conducted with the following cycles: Pre-denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56-57°C (depending on the locus) for 30 seconds, extension at 72°C for 30 seconds, and ended with one final extension at 72°C for 7 minutes (Sandhu et al. 2015). MAGE 3% was used to separate the microsatellite DNA fragments on an electrophoresis tank for 3 hours with 100 V and visualize the bands using a gel documentation system (Biorad).

Data analysis

The observation data of *A. psidii* symptoms on host plants were compared to available literature, and descriptions were made for each host tree. Microsatellite data were scored based on the number and sizes of alleles in base-pair (bp), and the data of alleles was manually input into excel (Xls) format. The data in xls was used as an input for analyzing genetic variation using GenAlEx 6.5 (Peakall and Smouse 2012). The parameters, such as expected heterozygosity value, number of alleles, number of effective alleles, and genetic distance were calculated. A dendrogram was constructed in accordance with allele sizes and their presence (1) and their absence (0) using NTSYSpc version 2.02e (Rohlf 1998). Genetic similarity among isolates was measured according to Jaccard's coefficient of similarity to generate a genetic distance matrix using SIMQUAL (Similarity for Qualitative Data). It was further used to construct dendrogram using UPGMA (Unweighted Pair Group Method of Arithmetic Averages) through SAHN (Sequential, Hierarchical, Agglomerative, and Nested Clustering) in accordance with Zhong et al. (2011) procedure. The Principal Coordinate Analysis (PCoA) was performed using GenAlEx 6.5 (Peakall and Smouse 2012) to separate the cluster of individuals based on the genetic distance between isolates.

RESULTS AND DISCUSSION

***Austropuccinia psidii* symptoms on Myrtaceae**

Austropuccinia psidii symptoms were found on *S. polyanthum*, *S. myrtifolium*, and *M. cajuputi* trees (Figure 1). The main symptom of *A. psidii* infections was the presence of yellow urediniospores with reddish-yellow chlorotic spots in diseased organs (Figures 1E and 1I). The urediniospores formed a colony called a pustule. It is often found on the lower surface of the leaves. The infections are mostly located in the young organs of the plants, such as the leaves, shoots, and branches. Severe infections in *S. polyanthum* were found in three different locations, i.e., the arboretum of CFBTI and two private yards in Sleman (Yogyakarta) and Sukabumi (West Java). The infection occurs not only in young leaves but also in the older leaves (Figures 1B and 1C). Brown lesions form in older leaves due to the rust former attacks (Figure 1C). *Austropuccinia psidii* infections in *M. cajuputi* are mostly found in young

shoots, leaves, and branches (Figure 1E). Severe infection of *A. psidii* occurs in most of the *M. cajuputi* trees, which are highly susceptible to rust infections. Shoot dieback was found, and brown lesions occur in older leaves (Figure 1F). A previous study of *A. psidii* attacks in Australia has reported that the presence of urediniospores on the juvenile stem, foliage, and older leaves with blight symptoms in highly susceptible Myrtaceae species such as *M. viridiflora*, *S. eucalyptoides*, and *S. nervosum* (Pegg et al. 2014a).

Different host responses to *A. psidii* infections were found in *S. myrtifolium* (Figure 1G). In this species, only a few parts of young leaves were infected with the rust (Figures 1H and 1I), while the remaining parts, including the older leaves, were uninfected. Limited urediniospores with reddish-yellow chlorotic spots are found in young leaves. *Syzygium myrtifolium* is known for its resistance to disease and pests (Fitra et al. 2013), however the specific relationship of its interaction with *A. psidii* has not yet been properly studied. The tolerant response of *S. myrtifolium* from rust infection is also shown in other Myrtaceae species, such as *S. aqueum*, *M. formosa*, and *Acmena hemilampra* in Australia (Pegg et al. 2014a). The different response of infection on some Myrtaceae species is unclear whether it comes from the host's resistance or other factors such as the environment and the pathogen.

Austropuccinia psidii is a potential threat to Myrtaceae plantations the north and south Sumatra, Indonesia. This rust infects *M. leucadendra* and *E. Grandis* × *E. pellita* with the possibility of infecting more species in the future (McTaggart et al. 2016). In commercial eucalyptus plantations, this rust attacks *E. globulus* and *E. viminalis* in Brazil in July 2000 with severe infections (Alfenas et al. 2003). This study indicates that *M. cajuputi* is one of the most susceptible hosts compared to *S. polyanthum* and *S. myrtifolium*. A preliminary study by Prihatini et al. (2020) showed that diseased *M. cajuputi* comprises shoot death and severe infection on the branch and older leaves. Currently, there is no report on the economic loss caused by myrtle rust in Indonesia, but in New Zealand, this pathogen is predicted to cause real GDP to fall by between $144 million and $411 million relative to baseline by 2030 (NZIER 2017). Meanwhile, a report in Australia predicted the impact on the eucalypt forestry sector to cause a reduction of a total gross value of $170-257 million and emphasized the impact on indigenous culture and stated indirect impact on several sectors (Makinson 2018).

Figure 1. *Austropuccinia psidii* infections on some species of Myrtaceae. A. *Syzygium polyanthum* tree. B. young leaves of *S. polyanthum*. C. old leaves of *S. polyanthum*. D. *Melaleuca cajuputi* tree. E. young leaves of *M. cajuputi*. F. old leaves of *M. cajuputi*. G. *Syzygium myrtifolium* tree. H-I. young leaves of *S. myrtifolium*. red arrow: urediniospores

Cajuput leaves are used as the main ingredients for cajuput oils productions in Indonesia, especially in Java and Maluku. This essential oil, an important non-timber commodity in Indonesia, is mainly found in oil glands on the leaves and other part of cajuputi trees (Brophy et al. 2013). *Austropuccinia psidii* mainly attacks young leaves of three Myrtaceae species as reported in this study. It certainly affects the quality of the leaves because the infection damaged the host tissue, causing dieback, lesion, and likely to have urediniosori when the leaves get older (Figures 1C and 1F). There are some strategies to control and prevent *A. psidii* infections from the traditional approaches, such as applications of fungicides (Glen et al. 2007) to modern approaches, such as selection and breeding for resistance Myrtaceae plants (Silva et al. 2013; Pegg et al. 2014b).

According to Craven et al. (2003), Indonesia has approximately 30 genera of Myrtaceae. All of these native and non-native plants need to be considered as potential hosts for *A. psidii*. One of the most important and studied genus in Myrtaceae is *Syzygium*, with many species that are useful for medicine and herbs, such as *S. polyanthum*. Some studies have indicated that leaves of *S. polyanthum* show antioxidant (Hidayati et al. 2017; Syabana et al. 2021), antibacterial (Ramli et al. 2017), and anti-diarrheal activities (Malik and Ahmad 2013). *Syzygium* has a wide distribution and a great number of species with an estimation of up to 300 species in Indonesia (Sunarti 2015). *Austropuccinia psidii* infection in some native *Syzygium* species, such as *S. polyanthum* and *S. myrtifolium* is a proven example of rust presence in this genus. In this study, rust infections were found on *S. polyanthum, S. myrtifolium* and *M. cajuputi* that grow close in the CFBTI arboretum. This is not surprising because urediniospores can be transported easily through the air (Berthon et al. 2018).

Genetic variation of *A. psidii*

Amplification of fungal DNA extracted from leaf containing fungal spores using specific primers is used to confirm the presence of *A. psidii* (Langrell et al. 2008). A total of 12 out of 28 diseased leaves were confirmed to contain DNA of *A. psidii.* Six isolates were collected from *S. polyanthum* (S1-S6), while the remaining four and two isolates were from *M. cajuputi* (K1-K4) and *S. myrtifolium* (P1, P2). The DNA from those isolates was used for microsatellite analysis for genetic diversity assessment (Table 3). There were no variations in the number of alleles and their size amplified by USYD_*Pp*35, USYD_*Pp*151, PpSSR012, and PpSSR102* among 12 isolates of *A. psidii*. Variations of allele number and size were detected using USYD_*Pp*168, PpSSR161, and PpSSR195* markers.

DNA amplification by USYD_*Pp*168 produced a total of four alleles with sizes of 316 bp, 319 bp, 321 bp, and 324 bp. Isolates S2 and S6 have heterozygous alleles with sizes of 316 bp and 321 bp, while the rest of isolates have alleles with sizes of 319 bp and 324 bp. This marker failed to amplify the DNA of isolate P2. The DNA amplification using PpSSR161 shows the variation of allele number between *A. psidii* isolates and separated P1 which only has one allele (276 bp) compared to others with two alleles (276 bp and 290 bp) (Figure 2B).

DNA amplification by PpSSR195* separated S1, S2 (*S. polyanthum* from the arboretum of CBFTI), S3, S4 (*S. polyanthum* from Sleman) isolates from other isolates by producing heterozygous alleles (214 bp and 234 bp). Meanwhile, the other isolates produce a homozygous allele (214 bp) (Figure 2A). Locus PpSSR195* also showed variation among *A. psidii* isolates in other places such as Australia, where two isolates derived from *Rhodamnia spongiosa* indicated different alleles sizes (215 bp and 221 bp for pustule 1, and 215 bp and 223 bp for pustule 2) with others originating from various Myrtaceae hosts (215 bp and 227 bp). Subsequently, one isolate derived from *Gossia myrsinocarpa* indicates the presence of a homozygous allele with the size of 215 bp, while the other isolates have two alleles (heterozygous) (Machado et al. 2015). The variation in the number and sizes of alleles in the present study and a previous study (Machado et al. 2015) indicate that the locus amplified by PpSSR195* is varied between isolates derived from various hosts and locations. Some different alleles observed in this study are due to mutation, as there is no evidence of sexual recombination (Machado et al. 2015). Graça et al. (2013) carried out research using Microsatellite on *A. psidii*, which demonstrated the highly polymorphic loci among Brazilian isolates due to the random mutation and not sexual recombinations.

The expected heterozygosity value measured by GenAlEx among *A. psidii* isolates averages 0.312, while the number and effective alleles are 1.714 and 1.667 (Table 4). The genetic variation was varied between host trees, *S. polyanthum* has a higher genetic variation (He=0.381) than *M. cajuputi* (He=0.286) and *S. myrtifolium* (He=0.268), as the number of isolates and host trees observed in *S. polyanthum* is higher (Table 4). The differences in genetic variation levels between different host trees found in this study are in accordance with the preliminary study carried out by Graca et al. (2013), which revealed different He values between eucalypt (He=0.355) and guava (He=0.247). Graca et al. (2013) stated that the genetic structure of *A. psidii* populations in Brazil is strongly influenced by host trees. The average of He value in this study reveals lower genetic variations among *A. psidii* isolates compared to the previous study by Stewart et al. (2017), which observed an average of He=0.404, from five clusters of Americas and Hawaii. This is associated with the number of isolates and hosts trees involved in this study, which is lower (3 hosts, 12 isolates) than the previous study (20 hosts, 226 isolates). There is no standard value for heterozygosity of myrtle rust or other rust fungi, but a study on the genetic diversity of *A. psidii* based on Simpson's index (Hi) using SSR markers ranged from 0.23 to 0.33 (Sandhu et al. 2015) and a study on *Puccinia striiformis* f. sp. *tritici* isolates revealed heterozygosity value ranged from 0.14 to 0.57 from various countries (Bai et al. 2021).

Table 3. Alleles sizes of microsatellite loci among *Austropuccinia psidii* present in three species of Myrtaceace

Isolate	Locus (bp)						
	USYD35	**USYD151**	**USYD168**	**PpSS012**	**PpSS161**	**PpSS102***	**PpSS195***
S1	336	208/238	319/324	230/236	276/290	140	214/234
S2	336	208/238	316/321	230/236	276/290	140	214/234
K1	336	208/238	319/324	230/236	276/290	140	214
K2	336	208/238	319/324	230/236	276/290	140	214
K3	336	208/238	319/324	230/236	276/290	140	214
K4	336	208/238	319/324	230/236	276/290	140	214
P1	336	208/238	319/324	230/236	276	140	214
P2	336	208/238	NA	230/236	276/290	140	214
S3	336	208/238	319/324	230/236	276/290	140	214/234
S4	336	208/238	319/324	230/236	276/290	140	214/234
S5	336	208/238	319/324	230/236	276/290	140	214
S6	336	208/238	316/321	230/236	276/290	140	214

Note: *Austropuccinia psidii* isolates derived from S1-S2: *S. polyanthum* in the CFBTI arboretum, S3-S4: *S. polyanthum* in Sleman, S5-S6: *S. polyanthum* in Sukabumi, K1-K2: *M. cajuputi* in the north area of CFBTI arboretum, K3-K4: *M. cajuputi* in the south area of CFBTI arboretum, P1-P2: *S. myrtifolium* in the CFBTI arboretum. NA: Microsatellite locus was not amplified

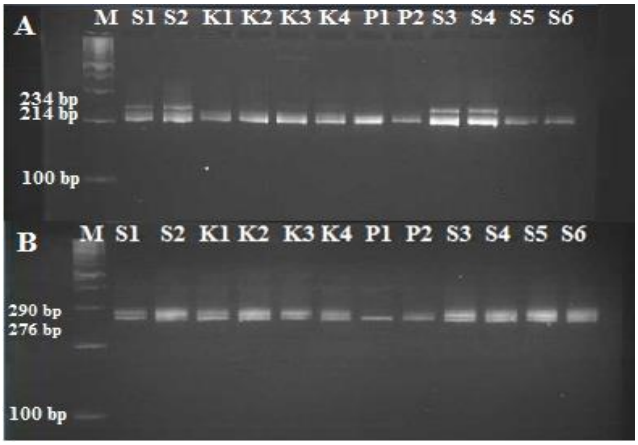

Figure 2. Amplification of *Austropuccinia psidii* using (A) PpSSR195* marker, (B) PpSSR161 marker. Marker 100 bp (M), *Austropuccinia psidii* derived from *S. polyanthum* in the CFBTI arboretum (S1-S2), *S. polyanthum* in Sleman (S3-S4), *S. polyanthum* in Sukabumi (S5-S6), *M. cajuputi* in the north area of CFBTI arboretum (K1-K2), *M. cajuputi* in the south area of CFBTI arboretum (K3-K4), and *S. myrtifolium* in the CFBTI arboretum (P1-P2)

Table 4. Genetic diversity parameter of *Austropuccinia psidii* in three species of Myrtaceae observed using SSR markers

Host	N	Na	Ne	He
S. polyanthum	6	2.000±0.378	1.914±0.329	0.381±0.104
M. cajuputi	4	1.571±0.202	1.571±0.202	0.286±0.101
S. myrtifolium	2	1.571±0.202	1.514±0.190	0.268±0.096
Average		1.714±0.156	1.667±0.142	0.312±0.056

Dendrogram of *A. psidii* derived from different host

Dendrogram constructed based on microsatellite data represents the genetic similarities among 12 isolates of *A. psidii* derived from three different hosts. *Austropuccinia psidii* is clustered into two (I and II) with 69% similarity. Cluster I consists of two subclusters, namely IA (S1, S3, S4, S5, K1, K2, K3, K4, and P1) and IB (P2). Meanwhile, cluster II consists of S2 and S6. Isolates from *S. polyanthum* (S1, S3, and S4) have 100% similarity in their microsatellites loci. Isolates K1, K2, K3, K4, and S5 have 100%, while P1 and P2 have 82% similarity in their microsatellites loci. Furthermore, Isolate S2 and S6 have 92.5% similarity in their microsatellites loci. The dendrogram demonstrated *A. psidii* isolates tend to have close genetic similarity based on their host.

A study by Zhong et al. (2011) demonstrated the high level of genetic diversity among 58 *A. psidii* isolates from diverse hosts and three different locations, Hawaii, Florida, and Brazil. All isolates clustered into three clades at 65% similarity showed that the genetic diversity of *A. psidii* differs in location and tends to have closer genetic similarity based on their location. Isolates from Hawaii only have one genotype even though it comes from diverse hosts, on the contrary, *A. psidii* from Brazil has 11 genotypes and also come from diverse hosts (Zhong et al. 2011). *Austropuccinia psidii* in Australia also show high genetic similarity up to 100% among its isolates in cluster analysis, even though the hosts are diverse, thereby indicating that there is only one *A. psidii* genotype present in there (Sandhu et al. 2015).

Principal coordinate analysis (PCoA)

Based on PCoA, 12 isolates of *A. psidii* construct 2 major clusters, the first was formed by *A. psidii* derived from *M. cajuputi* and *S. myrtifolium*, while the other ones were from *S. polyanthum* (Figure 4). The first two axis explained 87.7 % of total variations. Axis 1 explained 52.4% of the variation and separated most of the isolates derived from *S. polyanthum* (S1, S2, S3, S4, S6) from the other isolates. Axis 2 explained the remaining 35.3% and separated K1, K2, K3, K4 (isolates from *M. cajuputi*), S5 (an isolate from *S. polyanthum* in Sukabumi), P1, and P2 (isolates from *S. myrtifolium*) from the other isolates. PCoA based on the microsatellite data, also shows that *A. psidii* on *S. polyanthum* has 4 genotypes, namely Genotype 1 (S1, S3, S4), Genotype 2 (S2), Genotype 3 (S6), Genotype 4

(S5). Meanwhile, *A. psidii* on *M. cajuputi* only has one genotype, and *A. psidii* on *S. myrtifolium* has two genotypes, namely Genotype 1 (P1) and Genotype 2 (P2). This is in line with the study by Graça et al. (2013) that host trees influence the genetic structure of *A. psidii*. Conversely, Stewart et al. (2017) demonstrated that the C1 pandemic biotype of *A. psidii* is associated with the diverse host and has a large geographic region in USA-Hawaii, Costa Rica, Jamaica, Mexico, and Peurto Rico. Furthermore, another study revealed a unique and different genotype of *A. psidii* in *P. guajava*, which is found in Columbia and Brazil. Even though the host species are similar, the rust can have different genotypes, indicating that locations are a barrier for population mixture (Granados et al. 2017).

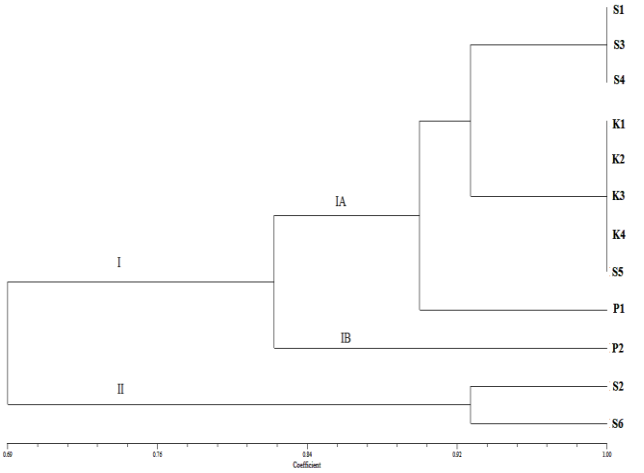

Figure 3. Dendrogram of 12 isolates of *A. psidii* derived from *S. polyanthum* in the CFBTI arboretum (S1-S2), *S. polyanthum* in Sleman (S3-S4), *S. polyanthum* in Sukabumi (S5-S6), *M. cajuputi* in the north area of CFBTI arboretum (K1-K2), *M. cajuputi* in the south area of CFBTI arboretum (K3-K4), and *S. myrtifolium* in the CFBTI arboretum (P1-P2)

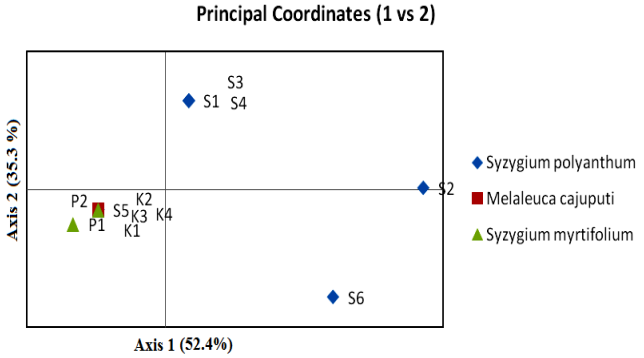

Figure 4. Principal Coordinate Analysis of *A. psidii* isolates derived from *S. polyanthum* in the CFBTI arboretum (S1-S2), *S. polyanthum* in Sleman (S3-S4), *S. polyanthum* in Sukabumi (S5-S6), *M. cajuputi* in the north area of CFBTI arboretum (K1-K2), *M. cajuputi* in the south area of CFBTI arboretum (K3-K4), and *S. myrtifolium* in the CFBTI arboretum (P1-P2)

The lower genetic variation of *A. psidii* in the same host tree is shown in *M. cajuputi* and *S. myrtifolium* might have resulted from a lack of genetic recombination, the same result is shown in Australian and Hawaiian rust populations (Zhong et al. 2011; Machado et al. 2015). The different numbers of alleles and their sizes found in some locus in isolates of *S. polyanthum* are presumably due to random mutations as reported in previous studies conducted by Graça et al. (2013), Machado et al. (2015), and du Plessis et al. (2019), with the possibility of sexual reproductions as reported by McTaggart et al. (2020). The presence of teliospores as sexual spores of *A. psidii* in some hosts such as *Lophomyrtus bullata*, *Eugenia nataliti*, *E. erythrophylla*, and *E. verdoorniae* increases the possibility of rust sexual reproductions (McTaggart et al. 2020), in addition there is possibility of rust sexual reproductions with basidiospores under a controlled environment (McTaggart et al. 2018).

In conclusion, the genetic variation of 12 isolates of *A. psidii* from *S. polyanthum*, *M. cajuputi*, and *S. myrtifolium* were shown in some microsatellites loci such as locus USYD_*Pp*168, by the presence of 4 alleles with different sizes, PpSSR161 locus by the presence of homozygous allele sized 276 bp in isolate P1, and PpSSR195* locus by the presence of heterozygous alleles sized 214 bp and 234 bp in isolates S1, S2, S4, and S5. The genetic structure of *A. psidii* populations was influenced by the host tree. The presence of *A. psidii* can threat Myrtaceae plantations in Indonesia and it is likely to infect other species of Myrtaceae in the future. Therefore, awareness of its infections in Indonesia needs to be significantly increased.

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