Genetic Variation of Santigi (*Pemphis acidula* J.R.Forst. & G.Forst.) based on ISSR Markers

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Abstract. *Pemphis acidula* J.R. Forst. & G. Forst. is a mangrove-associated plant that grows wild in rocky or sandy coastal areas. Despite being widely exploited due to its high economic value, no genetic diversity study of *P. acidula* has been conducted to date. This study assessed the genetic variation among *P. acidula* populations from three different geographical locations on the southern coast of Gorontalo Province, Sulawesi Island, using ten inter-simple sequence repeat (ISSR) markers. DNA was extracted from leaf samples and used for PCR assays. The presence and absence of bands generated from ISSR analysis were scored. A total of 124 amplicons were generated within a size range of 175–4500 bp, of which 110 were polymorphic. The ISSR analysis of *P. acidula* showed a high polymorphism rate of 88.92%, with a mean polymorphic information content (PIC) value of 0.49, indicating that the ten primers used can effectively assess the genetic diversity of *P. acidula*. Cluster analysis using UPGMA revealed a low similarity percentage of 22.1%, suggesting that the genetic variation of *P. acidula* in Gorontalo is high.

Keywords: genetic variation, *Pemphis acidula*, ISSR makers

1 Introduction

Pemphis acidula J.R.Forst. & G.Forst. widely known as the only member of the genus Pemphis in the family Lythraceae[1]. It is a wild plant associated with mangroves, and in some areas, it can even become a true mangrove [2] [3]. *Pemphis acidula* is primarily found in coastal habitats, particularly on sandy or rocky shores. It is well-adapted to salt spray, strong winds, and the challenging conditions of the coastal environment [4]. The plant is native to various tropical and subtropical regions, including Southeast Asia, the Indo-Pacific, and the western Pacific Ocean. In Indonesia, this plant is known by various names, including Santigi [2], Stigi [4], Cantigi, Sentigi, Mentigi [5], and Pandemor [6].

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Pemphis acidula is a small shrub or subshrub that typically reaches a height of 1-2 meters, but can occasionally grow taller up to 9 meters as reported in Andaman, India [7]. The stem wood is sturdy and stiff with gray to dark brown, and often peels in thin strips [8]. The plant exhibits elliptical to lanceolate leaves with flat edges, dense hair on both surfaces, pale green, thick, and succulent. The leaves are arranged oppositely along the stem with very short petioles. This species produces small, inconspicuous flowers that are usually white or pale pink. The flowers have a tubular shape and are clustered in the axillary [9]. After pollination, they develop into small, spherical fruits that contain several seeds [10]. These fruits play a role in the plant's reproductive cycle and are a potential food source for wildlife [11].

Pemphis acidula is widely known by ornamental plant fans as a plant that can be dwarfed (bonsai) and has a high economic value. Local people in several areas use this plant as traditional medicine. Even though it is not a major timber species, its wood has been used traditionally in some regions for construction materials, traditional ceremonies, and small-scale crafts, such as carving and making utensils. Some local people also believe that this plant has magical powers [12].

In its natural habitat, *P. acidula* contributes to the stability and ecology of coastal ecosystems. Its root system helps prevent soil erosion, making it a valuable species for coastal protection [13]. Even though there are reports in various regions regarding the decline in the number of this species in its natural habitat, this plant is still classified in the IUCN category of least concern. According to Ellison et al [14], this species has received little attention. Its habitat faces potential degradation due to human activities and habitat loss, which highlights the importance of monitoring and conservation efforts.

Molecular markers have demonstrated their effectiveness as robust instruments for assessing relationships and genetic diversity within and between populations, thus playing a significant role in plant conservation and breeding programs. Research conducted using these markers has rapidly provided information on the genetic makeup and lineage of plant species [15]. Inter Simple Sequence Repeat (ISSR) marker is a PCR-based method that amplifies repetitive DNA sequences between two microsatellites [16]]. This method is highly useful for assessing genetic diversity, conducting phylogenetic analysis, mapping genomes, and exploring evolutionary biology [17] [18]. ISSR markers have been widely used in evaluating genetic variation and identifying relationships in various plant species from the families Araliaceae [19], Arecaceae [20], Cucurbitaceae [21] [22], Elaeocarpaceae [23], Lythraceae [18], Sapotaceae [24], and Tamaricaceae [25]. This study aims to assess the genetic variation of *P. acidula* from different geographical locations on the southern coast of Gorontalo Province, Sulawesi Island using ISSR markers.

2 Method

2.1 Sample Collection

Samples of *P. acidula* were obtained from 3 different locations on the southern coast of Gorontalo Province, Sulawesi Island, Indonesia namely Biluhu Beach (Site 1), Dulanga Beach (Site 2), and Olele Beach (Site 3) (Figure 1). The samples used were young, fresh, and healthy leaves from individual *P. acidula* plants which were accessible due to their habitat on steep coral cliffs. Leaf sample of each plants were collected and packed into separate sample bags containing silica gel. Samples were transported to the Integrated Research and Testing Laboratory at Universitas Gadjah Mada, Yogyakarta in a cooling box and stored at -20°C for DNA extraction and further analysis.

Fig. 1. Mapping of sampling site *P. acidula* in the coastal area of Gorontalo, Sulawesi Island, Indonesia.

2.2 DNA Extraction

Genomic DNA from leaf samples was extracted according to the Genomic DNA Mini Kit (Plant) (Geneaid) manufacturer's protocols. DNA quality and quantity were assessed using agarose gel electrophoresis and spectrophotometry. Subsequently, the DNA samples were diluted to a concentration of 40 ng/μl and preserved at -20°C for ISSR analysis.

2.3 ISSR Amplification

DNA segment amplification was performed using 10 ISSR primers selected (Table 1). The PCR reaction was carried out in 25 µL volume contained 2 µl of template DNA (20 ng/µl), 2 µl of each primers (20 µM), 12.5 µl of MyTaq HS Red Mix 1x (Bioline), and adjusted nuclease free water to a total volume of 25 µl. The amplification was performed in thermal cycler with condition of predenaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 45-55°C for 1 min (depend on annealing temperature of ISSR primers), extension at 72°C for 2 min, and post extension at 72°C for 10 min. The PCR products were separated on 2% agarose gel in 1x TBE buffer along with 1 Kb DNA ladder, then visualized on UV light and documented.

Table 1. Details of sequence and optimum annealing temperature of the 10 ISSR primers

Primer	Sequence	Annealing Temperature
UBC 814	(CT)8A	55ºC
UBC 826	(AC)8C	55ºC
UBC 810	(GA)8T	50ºC
UBC 807	(AG)8T	45ºC
UBC 835	(AG)8YC	55ºC
UBC 830	(TG)8G	55ºC
UBC 845	(CT)8RG	50ºC
UBC 817	(CA)8A	50ºC
UBC 834	(AG)8YT	55ºC
UBC 827	(AC)8G	55ºC

2.4 ISSR Data Analysis

The DNA fragments amplified by ISSR markers were recorded as either present (1) or absent (0), then a binary matrix was constructed. The total of DNA bands, monomorphic bands, and polymorphic bands, the percentage of polymorphism, the size of DNA fragments, and the polymorphism information content (PIC) were estimated. Binary matrix data were also constructed into dendrogram with Jaccard's coefficients of UPGMA using MVSP software version 3.22.

3 Result and Discussion

The phenotype of an organism is governed by its DNA. Differences in phenotype, such as those observed among individuals, stem from variations or polymorphisms in DNA sequences. These polymorphisms, which are widespread in nature, play a crucial role in biodiversity, genetic variability, and adaptation. They contribute to preserving a diverse array of forms within a population inhabiting diverse environments [26].

The use of ISSR markers in evaluating genetic variation and identifying relationships among various plant species has been widely reported, including the Lythraceae family [18]. For this investigation, 10 ISSR primers were selected to assess genetic polymorphism in *P. acidula* originating from three distinct sites within the southern coastal region of Gorontalo Province, located on Sulawesi Island, Indonesia.

Visualization of PCR products revealed that all ten ISSR primers employed successfully detected and amplified the genome sequence of *P. acidula*. The size of fragments varied between 175 and 4,500 bp. However, the number of bands generated varied among primers and among specimens using the same primer (Figure 2).

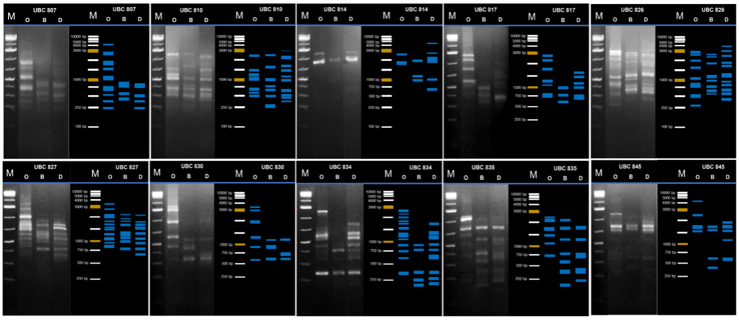

Fig. 2. PCR products visualization of P. acidula from 3 different location using 10 ISSR primer (M = Marker, O = Olele, B = Biluhu, and D = Dulanga).

The ISSR analysis with the 10 primers resulted in a total of 124 bands, out of which 110 exhibited polymorphism, averaging 11 polymorphic fragments per primer. Polymorphic band percentages ranged from 100% to a minimum of 75%, with an average of 88.92% (Table 2). It is suggesting the efficacy of these primers for genetic investigations of *P. acidula*, in line with various studies using ISSR markers that have been reported previously [18].

Table 2. Results of genetic polymorphism analysis using 10 ISSR primers.

ISSR Primer	**Sequence (5′-3′)**	**Total DNA Bands**	**Total Monomorphic Bands**	**Total Polymorphic Bands**	**% Polymorphism**	**Size of DNA Bands (bp)**	**PIC**
UBC 807	$(AG)_8T$	12	0	12	100.00	240-4500	0.49
UBC 810	$(GA)_8T$	15	3	12	80.00	250-3000	0.50
UBC 814	$(CT)_8A$	7	0	7	100.00	625-4500	0.50
UBC 817	$(CA)_8A$	12	0	12	100.00	600-2800	0.48
UBC 826	$(AC)_8C$	19	0	19	100.00	300-4000	0.50
UBC 827	$(AC)_8G$	16	4	12	75.00	650-3500	0.49
UBC 830	$(TG)_8G$	8	1	7	87.50	560-3500	0.50
UBC 834	$(AG)_8YT$	15	3	12	80.00	185-2800	0.49
UBC 835	$(AG)_8YC$	12	1	11	91.67	175-2500	0.50
UBC 845	$(CT)_8RG$	8	2	6	75.00	372-4400	0.50
TOTAL		124	14	110	889.17	-	4.93
MEAN		12.4	1.40	11.00	88.92	-	0.49

The level of informativeness of polymorphism in a molecular marker can be observed through the polymorphism information content (PIC). The PIC of each ISSR primer ranged from 0.48 to 0.50, averaging at 0.49 (Table 2). According to Mateescu et al. [27], PIC values ranging from 0.3 to 0.59 are considered sufficiently informative. The higher the PIC value, the better the primer is at analyzing genetic variation.

In this study, the polymorphism among *P. acidula* from three different locations on the southern coast of Gorontalo based on ISSR analysis showed a high average value (88.92%). Greater polymorphism levels correspond to increased genetic diversity within a species [28]. Nevertheless, significant similarities were observed predominantly between *P. acidula* specimens from the beaches of Biluhu and Dulanga, as opposed to those from Olele and Biluhu, or Olele and Dulanga.

The notable resemblance observed between P. acidula specimens from Biluhu and Dulanga beaches is reinforced by the outcomes of cluster analysis conducted using UPGMA with Jaccard's coefficient. The similarity matrix illustrates the degree of resemblance between P. acidula specimens at the compared locations (Table 3). Specifically, specimens from Biluhu and Dulanga Beaches exhibited a similarity value of 36%, surpassing that observed between specimens from Olele and Biluhu (18.4%), as well as between specimens from Olele and Dulanga (25.7%).

Table 3. Similarity matrix between *P. acidula* at 3 different locations.

Speciments	**Olele**	**Biluhu**	**Dulanga**
Olele	1.000		
Biluhu	0.184	1.000	
Dulanga	0.257	0.360	1.000

The cluster analysis using UPGMA with Jaccard's coefficient revealed a modest degree of similarity, at only 22.1%, among samples collected from the three locations, indicating a high level of genetic variation within the P. acidula species in Gorontalo. Specifically, P. acidula specimens from Biluhu exhibit a closer familial relationship with those found in Dulanga (Figure 3).

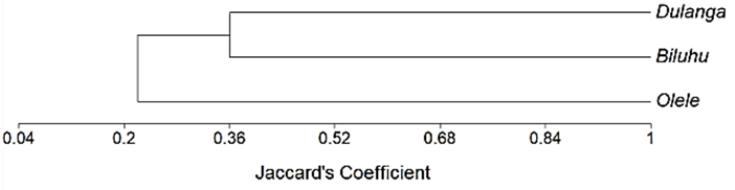

Fig. 3. Clustering results of P. acidula from 3 distinct locations using UPGMA with the Jaccard's coefficient.

As depicted in Figure 3, despite all three specimens belonging to the same species (P. acidula), their similarity is notably low, at 22.1%. Nevertheless, specimens of P. acidula from Biluhu and Dulanga Beach displayed a closer similarity, at 36%. This phenomenon could be attributed to the plant's adaptation to varying habitat conditions, influenced by genetic diversification at the species level. Genetic diversity serves as a measure of the extent of genetic variation within or between populations, predicting whether plants are more likely to exhibit uniformity or diversity, enabling them to potentially adapt and thrive in dynamic environments [25].

Gorontalo, as part of Sulawesi Island, Indonesia, boasts remarkable levels of endemism in its flora and fauna, owing to its prolonged isolation from the Asian and Australian continental shelves. This isolation has facilitated both phenotypic and genotypic changes in individuals, serving as adaptive responses to diverse environmental conditions. The distinct habitat characteristics exhibited across the three study areas have contributed to variations in the adaptive responses of P. acidula. Our research findings indicate the development of unique molecular characteristics among P. acidula populations at these three locations, underscoring the importance of tailored conservation and management strategies for this species in its habitat.

4 Conclusion

The findings from polymorphism analysis utilizing ISSR markers and cluster analysis indicate a substantial genetic variation within the *P. acidula* species in Gorontalo. This is believed to reflect P. acidula's adaptive response to diverse environmental conditions. Consequently, effective management and conservation measures are essential to safeguard *P. acidul*a and its habitat. We would like to extend our gratitude to the Research and Community Service Institution, Universitas Negeri Gorontalo, as well as the Local Governments of Gorontalo Regency (specifically the Chiefs of Bongo and Biluhu Villages) and Bone Bolango Regency (particularly the Chief of Olele Village) for their invaluable assistance during the completion of this research.

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