

# Synedrella nodiflora (L.) Gaertn Populations in Sumatra Island Showed Low Genetic Differences: A study based on the intergenic spacer atpB – rbcl

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# ***Synedrella nodiflora* (L.) Gaertn Populations in Sumatra Island Showed Low Genetic Differences: A study based on the intergenic spacer *atpB* – *rbcl***

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**Abstract.** Previous study on *Synedrella nodiflora* (L.) Gaertn populations in Java Island showed both very low haplotype and nucleotide diversity, and at the same time revealed high connectivity among the populations. Sumatra Island, which is like Java Island located in Sunda Shelf, has been subjected to relatively increasing human population and overexploitation of natural resources in a few last decades. This condition put the island of being vulnerable to terrestrial ecosystem changes that potentially influence the existing populations of *S. nodiflora*. Hence, this study aimed to assess genetic differences among *S. nodiflora* populations in Sumatra Island using intergenic spacer (IGS) *atpB* – *rbcl*. This molecular marker has been used in the population genetic study of some plant species. In this study we collected randomly 20 individuals from four different locations in Sumatra. The results showed, based on IGS *atpB* – *rbcl* sequences of 860 bp length, that only two haplotypes were found. One of them was the same haplotype mostly found in Java Island, and the other showed some base substitutions. Low genetic differences indicating high connectivity among populations of *S. nodiflora* in Sumatra Island is observed.

## **1. Introduction**

*Synedrella nodiflora* (L.) Gaertn is a wild plant species widely distributed over approximately 50 tropical countries [1]. Interestingly, it has been known as the only member of genus *Synedrella*, family Asteraceae [2] that shows significantly high reproductive ability and wide range of altitudes [3]. Its potentials as a useful plant and as a sufficiently important weed in some crops have been reported. Most references note its potentials as medicinal plant [4-12], and others reveal its possibility as bioinsecticide [13], biofungicide [14] and heavy metal detoxificant [15]. On the other hands, as broad-leaf weed frequently present in several crops, it may lead to reduce yield [16-19].

Due to its taxonomical status as a member of a monotypic genus, it is strongly assumed that *S. nodiflora* populations worldwide have extremely low genetic diversity. It has been proven in the case of Java Island, where *S. nodiflora* populations show both very low haplotype and nucleotide diversity when analyzed using intergenic (IGS) *atpB* – *rbcl* partial sequence as the molecular marker. Correspondingly, low genetic differences among populations indicating high connectivity were also reported. In other words, no population structure in the island was observed [20].

Sumatra Island, which is also located in Sunda Shelf, has been separated from Java Island since the end of Last Glacial Maximum. Consequently, the terrestrial ecosystems in both islands may have evolved differently. Then, Sumatra Island has undergone a relatively increasing human population and



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overexploitation of natural resources since many years ago. This condition makes it vulnerable to ecosystem alteration, which potentially influences the existing populations of *S. nodiflora*. Hence, population genetics of this species in Sumatra Island, especially in terms of genetic differences among populations, is interesting to study.

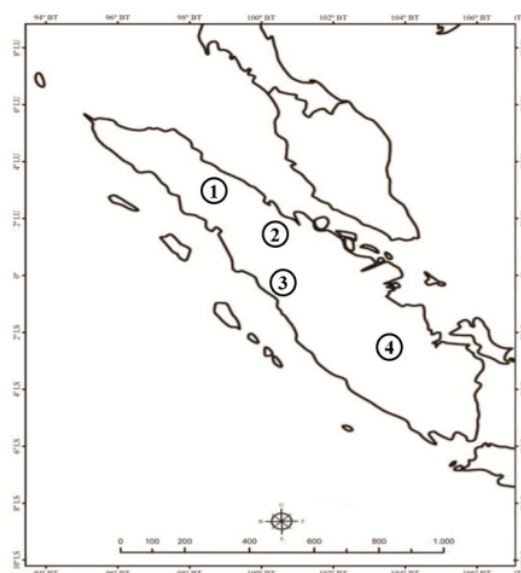
Genetic differences among populations can be analyzed by a particular molecular marker, e.g., intergenic spacer (IGS) *atpB* – *rbcL*. This marker has been shown highly variable in several populations of Alismataceae species in China, i.e., *Sagittaria trifolia* [21], *S. potamogetifolia* [22] and *S. lichuanensis* [23]. Besides, it has been reported to have high variation in the populations of *Hygrophila pogonocalyx* (Acanthaceae) in Taiwan [24] and *Ceriops tagal* (Rhizophoraceae) in Southeast Asia [25]. The high variation of IGS *atpB* – *rbcL* sequences is probably because this chloroplast genome region is not responsible for any protein synthesis [26-29].

This study aimed to assess genetic differences and the level of connectivity among *S. nodiflora* populations in Sumatra Island based on IGS *atpB* – *rbcL*. It was expected from this study that molecular data could be obtained to compare with the existing taxonomical status of the species, which has been based merely on phenotypical characters.

## 2. Methods

### 2.1. Plant Materials

Twenty samples of *S. nodiflora* were collected randomly from four different locations in Sumatra Island, i.e., Medan, Pekanbaru, Padang and Palembang, each of which was represented by five plant individuals (Figure 1). The plants were taken up with the roots and put into plastic bottles which have previously been filled with some little water. They were then planted in polybags in a glass house.



**Figure 1.** Locations of sampling  
1 = Medan, 2 = Pekanbaru, 3 = Padang 4 = Palembang

### 2.2. Genomic DNA Extraction

Genomic DNAs were extracted from the uppermost leaves following the CTAB method [30]. As much as 0.1 g of the leaf pieces of the individual plant was used as a sample. The extracted DNAs

were dissolved in 100 µl TE buffer and were kept at 4°C. Measurement of DNA quantity and quality was carried out using genequant.

### 2.3. IGS *atpB* – *rbcL* Amplification and Sequencing

The genot<sup>3</sup>c DNAs were used as PCR templates to amplify IGS *atpB* – *rbcL* employing universal primers, i.e., 5' – ACA TCK ART ACK GGA CCA ATA A – 3' as forward primer and 5' – AAC ACC AGC TTT R<sup>10</sup>A TCC AA – 3' as reverse primer. Amplification was performed using thermocycler Boeco in a total volume of 46 µl reaction mixture containing 20 µl Kapa, 17 µl nuclease free water; 8 µl geno<sup>3</sup>c DNA; 0.5 µl of the respective primer. The mixture was applied to a touch down PCR condition as follows: pre-denaturation at 94°C for 4 mins, followed by 10 reaction cycles (94°C 45 secs, 49°C 45 secs, 72°C 2 mins), 10 reaction cycles (94°C 45 secs, 48°C 45 secs, 72°C 2 mins), 10 reaction cycles (94°C 45 secs, 47°C 45 secs, 72°C 2 mins), 10 reaction cycles (94°C 45 secs, 46°C 45 secs, 72°C 2 mins), terminated by final extension at 72°C for 10 mins and stored at 4°C. The PCR products were put onto a 1.5% electrophoretic agarose gel using 1x TBE buffer. Then, the electrophoresis was run in 100 V, 87 mA for 45 mins after which the fluorescence stained gel was exposed to UV transilluminator for documentation.

Purification of the PCR products was carried out using QIAquick kit (Qiagen, Germany), and the purified PCR products were then sequenced employing automated dideoxy method [31] with dye terminator. Sequencing was performed in Firstbase Malaysia.

### 2.4. Data Analysis

Sequences were edited using Bioedit version 7.0.4.1 [32] and were checke<sup>4</sup>d manually. Sequence alignment was performed using ClustalW [33], which was also implemented in Bioedit version 7.0.4.1 [32]. Haplotype diversity  $h$  [34] and nucleotide divers<sup>4</sup> $\pi$  [35] were calculated using Arlequin 2.0 [36]. Phylogeography analysis using AMOVA [37] was to investigate the genetic population structure.

## 3. Results

All samples produced amplicons of 860 bp length, which were then aligned using BLAST. The alignment showed 88% to 96% homology to various *atpB* – *rbcL* sequences available in the NCBI database, ensuring that the amplicons are undoubtedly IGS *atpB* – *rbcL*. Two haplotypes of some base substitutions in various positions were observed (Table 1). Haplotype 1 is entirely the same sequence as the haplotype 1 from Java Island already available in the NCBI database with an accession number of KX096801. At present, haplotype 2 have also been registered in the NCBI database with accession numbers of MF285608.

**Table 1.** Parts of the IGS *atpB* – *rbcL* sequences of <sup>1</sup>*Synedrella nodiflora* (L.) Gaertn from Sumatra Island

Haplotype	Origin of sample	Number of individuals	Sequences showing mutation (5' – 3')	NCBI accession number
1	Medan, Pekanbaru, Padang, Palembang	19	tccctccctacaaCtcatgaa (bases 10 to 30) tttttatcGaaatacctaaaa (bases 230 to 250) tgTactgtgatatgTtgTa (bases 295 to 314) GtgaaaatattgtggaatattT (bases 328 to 349) GgtaaaaaaagaacaacagactaG (bases 361 to 384)	KX096801

Haplotype	Origin of sample	Number of individuals	Sequences showing mutation (5' – 3')	NCBI accession number
2	Palembang	1	gaagagtcGatgatatagaaa (bases 420 to 440)	MF285608
			ttcGtctactt (bases 540 to 550)	
			tcctccctacaaAtcatgaa (bases 10 to 30)	
			ttttatcCaaatacctaaaa (bases 230 to 250)	
			tgGacgtgatatatgGtgGa (bases 295 to 314)	
			AtgaaaatatgtggaatatttA (bases 328 to 349)	
			AgtaaaaaaagaacaacagactaA (bases 361 to 384)	
			gaagagtcAatgatatagaaa (bases 420 to 440)	
			ttcAtctactt (bases 540 to 550)	

Extremely low haplotype diversity ( $h$ ), i.e.  $0.1000 \pm 0.0880$ , and nucleotide diversity ( $\pi$ ), i.e.  $0.001409 \pm 0.001046$ , were obtained. Analysis of molecular variance (AMOVA) showing a very low fixation index of 0.02343 is presented in Table 2.

**Table 2.** AMOVA of *Synedrella nodiflora* (L.) Gaertn populations in Sumatra Island

Sources of Variation	df	Sum of Square	Variance Components	Percentage of Variation
Among populations	3	1.933	0.01017	2.34
Within populations	16	10.600	0.42400	97.66
Total	19	12.533	0.43417	

Fixation index ( $F_{ST}$ ) = 0.02343  $p$  = 1.00000

#### 4. Discussion

Similar results showing considerably low molecular diversity of *S. nodiflora* (L.) Gaertn populations in Java Island based on IGS *atpB* – *rbcL* have been reported. The haplotype diversity and nucleotide diversity values were of  $0.0345 \pm 0.0330$  and  $0.000040 \pm 0.000127$  respectively [20]. As in the case of Java Island, haplotype 1 is also found as the most dominant one in Sumatra Island, while haplotype 2 consists of only one individual in Palembang.

Different results using IGS *atpB* – *rbcL* in the population of a mangrove species *Ceriops tagal* (Rhizophoraceae) in Southeast Asia was reported. Much higher calculated  $h$  and  $\pi$  values were found, i.e., 0.667 and 0.0031, respectively. Also, most of the mutations involved not only base substitutions as in the case of *S. nodiflora* population in Sumatra Island, but also several long insertion and deletion [25]. The vast difference between the two results is probably due to the highly adaptable *C. tagal* to any mangrove ecosystem condition of the newly occupied areas [38], while *S. nodiflora* shows merely phenotypic plasticity without alteration in the genetic constitution. Phenotypic plasticity is very common to observe in invasive plant species, such as in the case of *Polygonum cespitosum* (Polygonaceae), which is an ideal weed species showing two mechanisms of invasive distribution, i.e.



high tolerance to a wide range of environmental conditions supported by high reproductive ability and competitiveness against other surrounding plants [39].

The low fixation index indicated high connectivity among *S. nodiflora* populations in Sumatra Island, or in other words, no population structure occurred. This is because of high gene flow among populations, through pollen/seed dispersal either naturally or artificially due to crop seed transportation. Natural pollen/seed dispersal can be mediated by wind, water, or pollinating insects, while dispersal through crop seeds may occur at harvest time if *S. nodiflora* grows in or near cropland.

*S. nodiflora* seeds are contained in a characteristic structure known as cypsela [40], especially in central cypselas which are longer and lighter than peripheral cypselas, enabling considerably remote dispersal. Then, cypsela germination occurs more readily under higher light intensity rather in shaded areas. Nevertheless, *S. nodiflora* can grow well in a wide range of environmental conditions, since other factors than light intensity have less influence on cypsela germination [41].

In addition to wind and water, *S. nodiflora* seed dispersal may be mediated by insects, particularly those belonging to the order of Thysanoptera. This group of insects usually have a developmental period of stage from egg to larvae, which is in accordance with the development of the host flowers. Sticky pollen surfaces enable the insects to carry pollens in a large quantity, either in legs, wings, or setae in the abdominal segments. The capacity of Thysanoptera in carrying *S. nodiflora* pollens can reach 5,536 to 5,716 per capitulum [42]. Commonly, Thysanoptera has only about 1 mm body length, and by the wind can spread across the sea as far as 1,600 km [43].

Although pollens can be distantly dispersed, the more possible mechanism is through seed dispersal, since this is the only way of gene flow among terrestrial plant species populations located in farther places in which pollen dispersal cannot sufficiently support connectivity among the populations [44, 45]. One of the most effective seed dispersal mechanisms is sea-dispersal, which can disperse seeds up to more than 100 km [46]. For instance, *Ipomoea pes-caprae* has been reported to be globally distributed by sea-dispersal [47].

In the case of *S. nodiflora*, sea-dispersal is constrained by the low viability in high salt concentration. The seeds can only survive in a moderate salt concentration, i.e., 40 mM NaCl [1], while most seawater has approximately 600 mM NaCl. *S. nodiflora* seed has been found in the dung of Galapagos turtle (*Chelonoidis nigra*) known to mobile among islands. The seed was found intact and could germinate. Hence, *S. nodiflora* sea-dispersal can seemingly occur through diet and soft digestion of particular vertebrates such as turtles [48].

The absence of *S. nodiflora* population structure in Sumatra Island resembles that reported in *Sagittaria lichuanensis* (Alismataceae) population in central China. Based on I<sub>11</sub> atpB – rbcL, high connectivity among *S. lichuanensis* populations was also observed. As well, no significant correlation between genetic and geographical distance was obtained [23]. Similarly, by the use of IGS atpB – rbcL, high connectivity among *S. trifolia* [20] and *S. potamogetifolia* populations in China [22] have been reported. In contrary, there were no genetic differences between *C. tagal* populations in the west and east of the Malay Peninsula. This big land was considered as the cause of genetic differences between *C. tagal* populations in the Indian Ocean and China Sea coastal areas based on IGS atpB – rbcL and IGS trnL – trnF [25]. Population structure due to the land barrier was also reported in other mangrove plant population, i.e., *Avicennia germinans* (Avicenniaceae) showing the genetic differences between that in the west coast of Atlantic and the east coast of Pacific [49]. Genetic differences have also been reported between *Hygrophila pogonocalyx* (Acanthaceae) populations in the west and east Taiwan employing IGS atpB – rbcL as the molecular marker [24].

## 5. Conclusion

Supported by the low value of fixation index indicating the absence of population structure, it is concluded that high connectivity among *Synedrella nodiflora* (L.) Gaertn populations in Sumatra Island occurs. It leads to the low genetic differences among the populations based on IGS atpB – rbcL partial sequence as the marker. Studies on population genetics of *S. nodiflora* in more extensive areas

are needed to confirm this finding, either employing IGS atpB – rbcL or other highly variable molecular markers.

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