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Molecular Profile of *Synedrella nodiflora* (L.) Gaertn. from three different altitudes based on *atpB* – *rbcL* IGS

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Molecular Profile of *Synedrella nodiflora* (L.) Gaertn. from three different altitudes based on *atpB* – *rbcL* IGS

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Abstract. Nodeweed (*Synedrella nodiflora* (L.) Gaertn.) is the only member of genus *Synedrella*, which is widely distributed over many tropical countries. It has been reported as potentially having many benefits for human life, but it is also commonly found as broad-leaf weed in several crops. In addition to its wide distribution, this species can also grow in a wide range of altitudes. This study was aimed to assess molecular profile of *S. nodiflora* in three different levels of altitudes, i.e. 0, 130, and 800 m above sea level respectively. Intergenic spacer (IGS) *atpB* – *rbcL* was used as the molecular marker. It was shown that no genetic difference among samples from the three altitudes was observed, indicating that any difference that may appear in the phenotype is merely due to morphological and/or physiological adaptation.

1. Introduction

Nodeweed (*Synedrella nodiflora* (L.) Gaertn.), belonging to the family Asteraceae, is widely distributed over many tropical countries. It is reported to have various potentials, e.g. as medicinal herbs [1, 2, 3, 4, 5], bioinsecticide [6], biofungicide [7] and detoxificant for heavy metals such as Cu and Pb [8]. On the other hands, it is also frequently found as broad-leaf weed in several crops [9, 10].

In addition to its worldwide distribution, this plant species can also grow in various types of terrestrial habitats. It is found not only in fertile soils but also in marginal lands, ditches, and even garbage dumps.

More interestingly, it can grow well in a wide range of altitudes, i.e. from 0 to 1,000 m above sea level (asl), showing slightly different phenotypical performance, especially with respect of leaf color and shape. This leads to an inquiry of whether these phenotypic dissimilarities are related to genetic variation or not. To understand this, a study on the molecular profile of *S. nodiflora* from various altitudes using a particular marker is needed. One of the molecular markers that can be used to analyze genetic variation is IGS *atpB* – *rbcL*, which is a non-coding region in the chloroplast genome [11]. Here we present our study on the molecular profile of *S. nodiflora* from three different altitudes based on IGS *atpB* – *rbcL*.

2. Methods

Three sites of different altitudes were selected for plant sampling, i.e. Jetis Beach (0 m asl), Purwokerto City (135 m asl) and Baturraden Botanical Garden (813 m asl). Individual sample was pulled up to its roots and then put into a plastic bottle previously filled with a little water. This was then grown in the glass house of Fakultas Biologi Universitas Jenderal Soedirman. Molecular analysis was made in the Laboratory of Molecular Genetics of the institution.



The uppermost leaves were used as the sources of genomic DNAs to be extracted following CTAB method [12]. Then, the extracted DNAs served as PCR templates to amplify *atpB* – *rbcL* IGS using a pair of universal primers, i.e. 5' – ACATCKARTACKGGACCA ATAA - 3' as the forward primer and 5' - AACACCAGCTTTTRAATCCAA - 3' as reverse primer [13]. Each PCR reaction was made in a total volume of 10 µl consisting of 2.5 µl genomic DNA; 0.25 µl primers (0.125 µl each primer); 5 µl Gotaq green and 2.25 µl NFW. This reaction mixture was subjected to a PCR condition as follows: pre-denaturation at 94°C for 3 mins, 33 reaction cycles consisting of denaturation at 94°C for 45 secs, primer annealing at 55°C for 45 secs, extension at 72°C for 2 mins respectively, followed by final extension at 72°C for 3 mins and storage at 4°C. The PCR products were visualized in a 1.5 % agarose gel electrophoresis using 1X TAE buffer, which was run at 75 Volt, 400 mA for 40 mins. After being stained with ethidium bromide, the gel was exposed to UV transilluminator for documentation.

The PCR products were purified using QIAquick kit (Qiagen, Germany), and were sequenced following the automated method [14] with terminator labeling. Data on base sequences were edited using Bioedit version 7.0.4.1 [15] and were checked manually. Sequence alignment was carried out using ClustalW [16], which was also implemented in the Bioedit version 7.0.4.1.

3. Results

Only seven of nine samples resulted in PCR products of approximately 900 bp length, as depicted in Figure 1. Two samples which did not show any band were those from Baturraden Botanical Garden, while the other seven have proceeded further to sequencing.

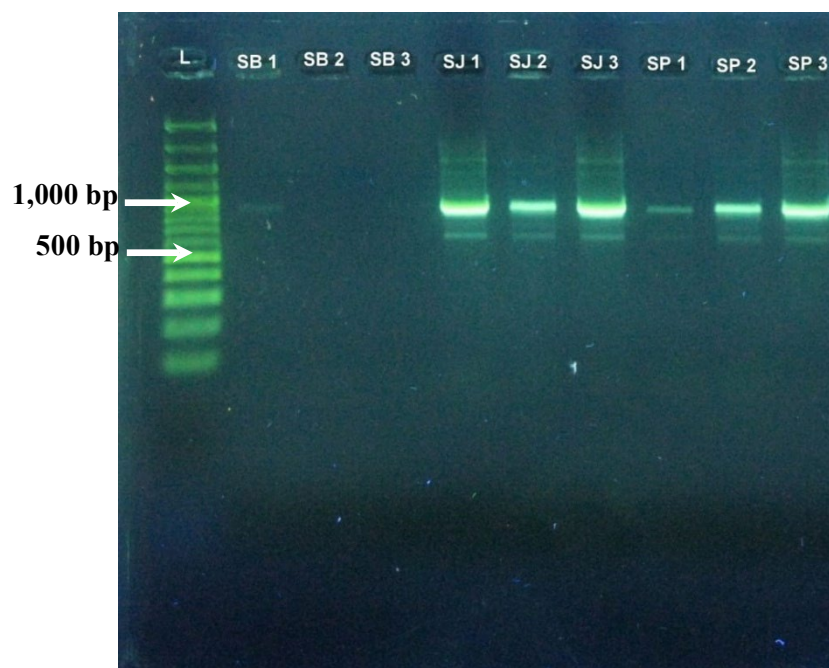


Figure 1. PCR products of *Synedrella nodiflora* (L.) Gaertn. genomic DNAs amplified using *atpB* – *rbcL* IGS primers

L = DNA ladder

SB1 – SB3 = samples from Baturraden Botanical Garden

SJ1 – SJ3 = samples from Jetis Beach

SP1 – SP3 = samples from Purwokerto City

After manual editing, the sequences of the seven samples were pruned into only 856 bp long and showed 100% with each other when subjected to multiple alignments. NCBI blasting revealed that they also show 100% homology with *atpB – rbcL* IGS sequences of *S. nodiflora* available in the database with accession numbers of KX096801.1, KX096802.1, KY983543.1, KY983544.1, KY983545.1 and MF285608.1 [17]. This means that the PCR products are undoubtedly IGS *atpB – rbcL*.

4. Discussion

The absence of variation among *atpB – rbcL* IGS sequences of all samples indicates no genetic difference among *S. nodiflora* populations from different altitudes observed. This corresponds to the results of the population genetic study of the species in Sunda Shelf where no population structuring occurs. In the study the altitudes of the sampling sites were not taken into account [17]. Different finding on *Ceriops tagal* (Rhizophoraceae) populations in Southeast Asia analyzed employing *atpB – rbcL* IGS was reported, in which most sites showed high variation including several long insertion-deletion [18]. This vast different result is presumably due to the more adaptable *C. tagal* as a mangrove species to the newly occupied areas [19], while *S. nodiflora* is genetically more stable in any environmental condition.

Genetic stability of *S. nodiflora* and its capability of adapting to various environmental conditions indicate phenotypical plasticity as commonly observed in invasive plant species. [20] reported that phenotypical plasticity of *Polygonum cespitosum* (Polygonaceae), a very invasive weed, was the characteristics of an ideal weed. In this case, two mechanisms to cause invasive distribution were involved, i.e. tolerance to a wide range of environmental conditions along with high reproduction capacity and high competitiveness against other surrounding species.

High variation of *atpB – rbcL* IGS sequences was shown in several populations of plant species. For instances, it was proved variable in some Alismataceae species in China, i.e. *Sagittaria trifolia* [21], *S. potamogetifolia* [22] and *S. lichuanensis* [23]. This was also the case with the populations of *Hygrophila pogonocalyx* (Acanthaceae) in Taiwan [24] and *C. tagal* (Rhizophoraceae) in Southeast Asia [19]. However, a low variation of *atpB – rbcL* IGS sequences in the population of *S. nodiflora* in Java Island was reported [25].

5. Conclusion

As no difference among *atpB – rbcL* IGS sequences of *S. nodiflora* from the three different altitudes were observed, it can be concluded that some phenotypic variances appearing are merely due to morphological and/or physiological adaptation.

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