

Microsatellite markers from expressed sequence tags (ESTs) of seaweeds in differentiating various *Gracilaria* species

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Abstract *Gracilaria* is a red seaweed that has been cultivated worldwide and is commercially used for food, fertilizers, animal fodder, and phycocolloids. However, the high morphological plasticity of seaweeds often leads to the misidentification in the traditional identification of *Gracilaria* species. Molecular markers are important especially in the correct identification of *Gracilaria* species with high economic value. Microsatellite markers were developed from the expressed sequence tags of seaweeds deposited at the National Center for Biotechnology

Information database and used for differentiating *Gracilaria changii* collected at various localities and two other *Gracilaria* species. Out of 33 primer pairs, only one primer pair gave significant results that can distinguish between three different *Gracilaria* species as well as *G. changii* from various localities based on the variation in repeated nucleotides. The unweighted pair group method using arithmetic mean dendrogram analysis grouped *Gracilaria* species into five main clades: (a) *G. changii* from Batu Besar (Malacca), Sandakan (Sabah), Bintulu (Sarawak), Batu Tengah (Malacca), Gua Tanah (Malacca), Middle Banks (Penang), Sungai (Sg.) Merbok (Kedah), Teluk Pelandok (Negeri Sembilan), Pantai Dickson (Negeri Sembilan), Sg. Kong-Kong (Johore), and Sg. Pulai (Johore); (b) *Gracilaria manilaensis* from Cebu, Philippines; (c) *G. changii* from Morib (Selangor); (d) *Gracilaria fisheri* from Pattani, Thailand; and (e) *G. changii* from Pantai Dickson (Negeri Sembilan), Gua Tanah (Malacca), Sg. Merbok (Kedah), Sg. Kong-Kong (Johore), and Sg. Pulai (Johore). This result shows that this primer pair was able to distinguish between three different species, which are *G. changii* from Morib (Malaysia), *G. fisheri* from Pattani (Thailand), and *G. manilaensis* from Cebu (Philippines), and also between different genotypes of *G. changii*. This suggested that the simple sequence repeat primer we developed was suitable for differentiating between different *Gracilaria* species due to the polymorphisms caused by the variability in the number of tandem repeats.

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Introduction

Gracilaria is one of the largest genera in number of species of red seaweeds that has been cultivated worldwide and is