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Determination of genetic diversity among *Saccharina* germplasm using ISSR and RAPD markers

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ABSTRACT

Various species of genus *Saccharina* are economically important brown macroalgae cultivated in China. The genetic background of the conserved *Saccharina* germplasm was not clear. In this report, DNA-based molecular markers such as inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) were used to assess the genetic diversity and phylogenetic relationships among 48 *Saccharina* germplasms. A total of 50 ISSR and 50 RAPD primers were tested, of which only 33 polymorphic primers (17 ISSR and 16 RAPD) had an amplified clear and reproducible profile, and could be used. Seventeen ISSR primers yielded a total of 262 bands, of which 256 were polymorphic, and 15.06 polymorphic bands per primer were amplified from 48 kelp gametophytes. Sixteen RAPD primers produced 355 bands, of which 352 were polymorphic, and 22 polymorphic bands per primer were observed across 48 individuals. The simple matching coefficient of ISSR, RAPD and pooled ISSR and RAPD dendrograms ranged from 0.568 to 0.885, 0.670 to 0.873, and 0.667 to 0.862, revealing high genetic diversity. Based on the unweighted pair group method with the arithmetic averaging algorithm (UPGMA) cluster analysis and the principal components analysis (PCA) of ISSR data, the 48 gametophytes were divided into three main groups. The Mantel test revealed a similar polymorphism distribution pattern between ISSR and RAPD markers, the correlation coefficient r was 0.62, and the results indicated that both ISSR and RAPD markers were effective to assess the selected gametophytes, while matrix correlation of the ISSR marker system ($r = 0.78$) was better than that of the RAPD marker system ($r = 0.64$). Genetic analysis data from this study were helpful in understanding the genetic relationships among the selected 17 kelp varieties (or lines) and provided guidance for molecular-assisted selection for parental gametophytes of hybrid kelp breeding.

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1. Introduction

Kelp (Haidai in Chinese) is an economically important brown macroalga, which was introduced in China from Japan in 1927 and widely cultured in Shandong, Liaoning and Fujian Provinces [1]. It was widely used in medicine, in the food and chemical industries among others, occupying an important place in the mariculture industry of China. Up to 2013, the culturing area of *S. japonica* in China reached 37,282 hectares, with a total production of 1,017,737 tons [2]. China has ranked the first in the world for many years in the culture area and annual yield of kelp.

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The life history of kelp consists of a diploid sporophyte generation and a haploid gametophyte generation, alternating with each other. Sporophyte generation could not be conserved as germplasm as its large frond and can not be long-term preserved indoor. Fang et al. [3] found that the male and female gametophytes of kelp can be isolated and propagated in vitro independently under continuous lighting (gametophyte cloning). In the past, gametophytes can only be acquired from mature sporophytes, a process that was limited by the season. The establishment of the kelp gametophyte clone culture technique could provide the gametophytes as laboratory research materials at any time throughout the year [4]. Since then, cloning kelp gametophytes has evolved as an effective way for long-term preservation of the kelp germplasm. The technique has facilitated the preservation of individual genetic strains of *Saccharina* and promoted the subsequent breeding of elite varieties.

Cloning kelp gametophytes has promoted kelp hybridization breeding. The innovation of *Saccharina* gametophyte cloning and hybridizing methods has brought *Saccharina* breeders the opportunity of breeding elite *Saccharina* varieties and *Saccharina* hybrids by crossing gametophyte clones [5,6]. As the earliest country of kelp genetic improvement, more than 20 elite varieties have been bred and applied to production since the late 1950s [7]. In recent years, a set of kelp hybrids and hybrid derived varieties have been bred and applied. The outstanding representatives of kelp hybrids and hybrid derived varieties include, Dongfang No. 2 [8], Dongfang No. 3 [9], Dongfang No. 6 [7], 901 [10,11] and Dongfang No. 7 [12].

At present, cloned gametophytes are the entities of germplasm conserved indoors and the parental resources of the *Saccharina* variety and hybrid breeding. A few germplasm stocks of *Saccharina* gametophyte clones have been constructed. A rich collection of *Saccharina* gametophyte clones were collected and preserved indoors. Various *Saccharina* gametophytes with specific phenotypic characteristics were obtained and applied to breeding. However, little is known about the genetic background of the *Saccharina* gametophytes at the DNA levels. In addition, the performance of hybrids is associated with the differentia between parental gametophytes [13]. Except for those from sporophytes with desirable traits, we have to determine the genetic difference among gametophyte clones so that the differentia can be identified and utilized for parental gametophytes selection of hybrid kelp breeding. In order to effectively manage and exploit *Saccharina* gametophyte clones, the genetic diversity and the phylogenetic relationships among 48 *Saccharina* germplasms were assessed.

Diverse molecular markers have been used widely to evaluate the genetic variation of a wide range of species, which included, for example, simple sequence repeat (SSR), RAPD, ISSR, and amplified fragment length polymorphism – AFLP [14–18]. For *Saccharina*, 18 microsatellites have been developed and used to determine the genetic diversity of gametophyte clones originated from *Laminaria japonica* and *L. longissima* [19,20]; 18 other microsatellites have also been used to determine the genetic distance between parental gametophyte clones of 14 *Laminaria* hybrids and predicting the heterosis of *Laminaria* hybrids [13]. In addition, AFLP, RAPD and ISSR have been adopted in analyzing the genetic distance between gametophyte clones [21], fingerprinting gametophytes [22], and assessing the genetic diversity [23]. These markers are PCR-based and widely used in plant species for identification, phylogenetic analyses, population studies, and genetic linkage mapping.

Of the molecular markers mentioned above, ISSR, which was derived from SSR, amplified the specific region between two microsatellite motifs, and it does not require prior knowledge of the DNA sequence for primer design [23,24,25]. Compared with SSR, ISSR proved to be abundant, highly polymorphic, informative, and efficient, even in closely related genotypes among selected samples in the pre-experimental part of this study. The AFLP technique involves the high-throughput detection marker system of electrophoresis, but enzyme digestion and the detection system of AFLP molecular markers are complex to operate, and high requirements on quality of genome DNA are needed. The RAPD technique has been successfully employed in genetic diversity studies of some species due to the simplicity, low cost and non-requirement of DNA sequence information prior to application [24,26]. Both RAPD and ISSR markers have proved to be reliable, easy to generate, inexpensive and versatile set of markers that rely on repeatable amplification of DNA sequence using single primer.

Overall consideration, ISSR and RAPD were adopted for evaluating the genetic relationship of a selected collection of *Saccharina* in the present study. The aim of the study was to assess the genetic background in order to understand the relationship of the selected varieties (lines), and to identify genetic differentia of selected gametophytes and to screen the candidate parental gametophytes that were used for hybrid kelp breeding test. The two marker systems (ISSR and RAPD) have also been compared for their applicability to the selected *Saccharina* gametophytes population. In addition, the results also indicated that the two marker systems can be used for kelp core germplasm selection.

2. Materials and methods

A total of 48 gametophytes either isolated from 17 varieties (or lines) of *Saccharina* (maintained by independent sporophyte seedling raising) or collected worldwide (Table 1, Fig. 1) were analyzed. These gametophytes preserved in The Germplasm Repository of National Engineering Science Research & Development Center of Algae and Sea Cucumbers of China. At least one female and one male gametophyte were selected to represent a variety or a line except for the two wild lines (6, 9), Rongyuanyuan-1 (3, male) and Yuanza (11, male).

Genomic DNA was extracted from 0.1 g (dry weight) of gametophytes using the plant genomic DNA extraction kit (Tiangen, China). DNA quality and quantity were checked through 1.0% agarose gel electrophoresis and spectrophotometry, respectively. DNA was diluted to 40 ng/μL as templates and stored at −20 °C.

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