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AASRI Procedia 1 (2012) 305 – 310

AASRI Procedia

www.elsevier.com/locate/procedia

2012 AASRI Conference on Computational Intelligence and Bioinformatics

Distinguishing *Haloxylon persicum* and *H. ammodendron* (*Haloxylon* Bunge, Amaranthaceae) using DNA Marker

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Abstract

Haloxylon ammodendron and *H. persicum* are two closely related species in genus *Haloxylon* of Amaranthaceae. Saxoul trees, designated the King of psammophytic plants, have been playing an important role in sand fixation, wind control and water conservation in the deserts. In recent decades, artificial and natural *Haloxylon* populations have been threatened in China due to environmental degradation. Genetic evaluation on *Haloxylon* germplasm resources has been in urgent need in China. However, the lack of morphological and molecular markers has severely limited the related researches. In this study, a SSR primer pair named QCA58 was found to be transferable and informative for distinguishing the two *Haloxylon* species. Primer QCA58 previously reported by Maughan et al. (2004) produced a DNA fragment of 183bp in length containing a stretch of $(\mathbf{TG})_{16}$ in *Chenopodium quinoa* Willd. (genus *Chenopodium*, Amaranthaceae). Surprisingly, a stretch of a compound repeat motif **TCTTCAGGGTC(T/C)TCTTCAGGGTC** was detected in the PCR product (≈ 970bp in length) of *Haloxylon* species with primer QCA58. Development of SSR markers commonly involve in tandem repeats of short (2–6 bp) DNA sequences. Our results indicated that the longer or compound repeat and its relationship with SSRs are noteworthy for the potential in basic evolutionary applications, such as identification at genus and species

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doi:10.1016/j.aasri.2012.06.047

levels.

Selection and/or peer review under responsibility of American Applied Science Research Institute

Keywords: Haloxylon ammodendron, H. persicum, primer QCA58, DNA marker, identification.

1. Introduction

Plants of genus *Haloxylon* Bunge are psammophytic shrubs or small trees, belonging to Amaranthaceae (formerly Chenopodiaceae) of flowering plants [1-3]. About 13 species have been reported in *Haloxylon* in the world. In China, there are two *Haloxylon* species with a distribution in the deserts of Northwest China, namely *H. persicum* (white saxoul) and *H. ammodendron* (saxoul) [2, 3]. They are designated the King of psammophytic plants and play an important role in sand fixation, wind control and water conservation in the deserts. Distribution of these two species is sympatric in China. *H. persicum* grows mostly at the top of sand dunes, while *H. ammodendron* is widely distributed on sand dunes, clayed deserts, saline or alkaline deserts and Gobi deserts.

However, in recent decades, planted forests and natural populations of *Haloxylon* have been threatened in China, due to the decrease of river water flow, lowering of underground water level, over development in agriculture, and over grazing of animals. Genetic evaluation on *Haloxylon* germplasm resources has been in urgent need [2, 3].

Because of the severe environmental conditions in the deserts, such as sand storms, extreme heats and drought stresses, the habit of *Haloxylon* trees are commonly irregular. The tiny gray-whitish scaly "leaves" on the green assimilating shoots of *H. persicum* are in fact aculeate. The true leaves are quite reduced and the cortex of young annual cylindrical shoots is the major photosynthetic tissue. The assimilating shoots of *H. ammodendron* even lack such scaly "leaves" [2].

RAPD and ISSR analyses revealed that a high level of genetic diversity existed within/among natural populations of *H. ammodendron* in Xinjiang, China [5-7]. Other studies based on DNA ISSR markers demonstrated that there existed genetic diversity and gene flow within/among populations of either *H. ammodendron* or *H. persicum* in Xinjiang, China [8, 9]. Using ITS_1 region of nrDNA, pollen of *H. ammodendron* was detected in surface soil in the centre of Junggar Desert Basin, Xinjiang, China, and the ecological relationship between vegetation characteristics and pollen in the surface soil was discussed [10]. Analysis with 14 ISSR primers did not find genetic difference between individual plants of *H. ammodendron* parasitized and non-parasitized by desertl Cistanche (*Cistanche deserticola*) in the Alxa desert, Inner

Nomenclature

PCR	Polymerase Chain Reaction
SSR	Simple sequence repeat or microsatellite DNA
ISSR	Inter-simple sequence repeat
RAPD	Random amplification polymorphic DNA
ITS	internal transcribed spacer
nrDNA	nuclear ribosomal DNA
TRF	Tandem Repeats Finder

Mongolia Autonomous Region, China [11]. ISSR marker-based analysis indicated that genetic changes at

DNA level occurred in the seeds of *H. ammodendron* exposed to the outer space by "Shenzhou No.4" spaceflight [12]. However, limited success was achieved in detecting genetic diversity of the *Haloxylon* germplasm resources. Lack of morphological and molecular markers has inhibited the advance of the related researches.Among DNA markers, SSR markers have a high level of polymorphism, better stability and reproducibility compared to other DNA markers (such as RAPD and ISSR markers). SSR markers have been widely used in studies on population genetics, cultivar classification, genetic mapping, gene localization and marker-associated breeding programs [13, 14]. However, few SSR markers have been reported for *Haloxylon* species and their genetic background is still poorly understood.

Here we report new insights concerning molecular identification of *Haloxylon ammodendron* and *H. persicum* at species level based on a SSR marker-derived DNA marker. The features and utilization of SSR markers will also be discussed.

2. Materials and Methods

2.1 Sampling

Young fresh assimilating shoots of *Haloxylon ammodendron* and *H. persicum* were collected in July 2008 and May 2009 from Turpan Desert Botanical Garden of Chinese Academy of Sciences, Xinjiang Uygur Autonomous Region and Minqin Eremophytes Botanic Garden, Gansu Province, China, and dried immediately using silica-gels for DNA extraction.

2.2 DNA extraction, PCR amplification and cloned sequencing

Genomic DNA extraction was conducted following the procedure of Plant Genomic DNA Kit (DP305) from Tiangen Biotech (Beijing) Co., Ltd. PCR amplification was conducted following the protocol of TaKaRa Code: DR100B. A primer pair (QCA58-F: 5′—CTCGACCAGCAGGGTCTG—3′, QCA58-R: 5′—CTAGCTAGGCGTTGCCTGAC—3′) [15] was employed. PCR conditions: preheating at 94°C for 4 min.; 34cycles of 94°C for 1 min., 62°C (annealing temperature) for 58 s and 72°C for 1.2 min.; 8 min at 72°C for final extension. PCR amplification was performed in an Applied Biosystems Veriti™ 96-Well Thermal Cycler (Model#: 9902, made in Singapore). The amplicons were resolved simultaneously on 2% agarose gels (Promega, the USA) run in 1 x TAE buffer at 3 $V \cdot cm^{-1}$ for 2 h and were stained with ethidium bromide. Band patterns (Fig. 1) were documented and photographed with the Gel Documentation System of Transilluminator BINTA2020D (Liaoning Langke Business and Trade Co. Ltd., China). PCR products (Fig. 1) were dug out from the gel using a sterilized scalpel for purification and sent to Beijing Tsingke Biotech Co., Ltd. for cloned sequencing. Six to eight independent clones for each sample were randomly taken and sequenced in both directions. The sequences were deposited in GenBank under accession numbers JQ768241-JQ768258.

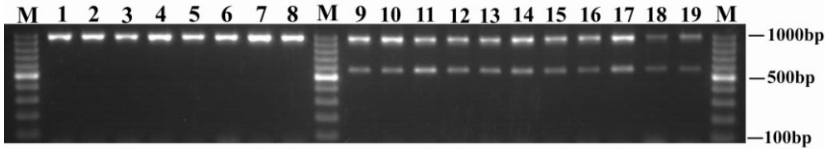

Fig.1. Genomic DNA fingerprinting pattern obtained from 19 individuals of the two Haloxylon species with primer QCA58. Sample numbers: 1 to 8 = H. persicum, and 9 to 19 = H. ammodendron. M is the 100-bp DNA ladder.

2.3 Simple sequence repeats search

The sequence data were searched for simple sequence repeats using TRF program [16]. TRF was run

under the following parameters: 2, 7, 7=weights for match, mismatch and indels used in the Smith-Waterman local alignment (i.e., Match, Mismatch, Delta); 80, 10 = matching and indel probability (i.e., PM, PI); 50= minimum alignment score (Minscore); 500= maximum size of the repeat unit (Max period).

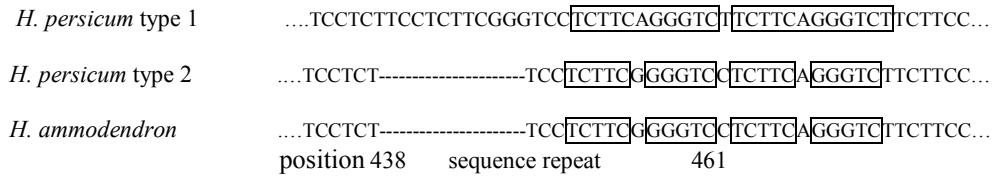

Fig. 2. The sequence repeat (from positions 438 to 461) in the alignment of cloned sequences of the longer band (ca. 956 to 977bp in length) in the PCR product of the two *Haloxylon* species with primer QCA58.

3 Results

By screening 55 SSR primer pairs previously reported for the closely related species [13, 15, 17, 18] of the genus *Haloxylon* in Amaranthaceae, a primer pair named QCA58 was found to be informative in distinguishing *H. persicum* and *H. ammodendron*. A single band was amplified with primer QCA58 from the genome of *H. persicum*, while two bands were amplified from the genome of *H. ammodendron* (Fig. 1). Thus, the two *Haloxylon* species were discriminated successfully with primer QCA58. Primer screening success was 1.8%. A compound sequence repeat motif **TCTTCAGGGTC(T/C)TCTTCAGGGTC** was detected in the PCR product (the cloned sequences of the longer band with a size of 956 to 977bp) of each *Haloxylon* species (repeat type 1, in Fig. 2). The cloned sequence with such repeat motif occupied 16.7% of the investigated sequences (n=6) in *H. persicum*, the rest of the sequences had mutated repeat motif (repeat type 2, in Fig. 2). In *H. ammodendro*n, all of the investigated sequences (n=6) only contained mutated repeat motif without type 1 repeats observed in the longer band. Fig. 2 shows the typical mutated repeat motif (repeat type 2) in *H. ammodendro*n. No repeat was detected in the cloned sequences of the shorter band (ca. 564bp in length) of *H. ammodendron* (Fig. 1). The result of this study was also confirmed using samples collected from the fields.

4 Discussion and conclusion

Cross-genus transferability of primer QCA58 suggested that the binding regions of primer QCA58 may be associated with certain important function in the Amaranthaceae, because of the conservatism. Functional correlations of repeat sequences are frequently reported [19]. Many examples could be given. For example, a $(\mathbf{GT})_n$ repeat could enhance gene activity from a distance independent of its orientation, but more effective transcriptional enhancement resulted from the **GT** repeat being closer to the promoter sequences [19, 20]. In rice, variation in the number of **GA** or **CT** repeats in the 5' UTR of the waxy gene was correlated with amylase content [21, 22]. Microsatellite markers (**CCG**)n in 5' UTRs of some ribosomal protein genes of maize were involved in the regulation of fertilization [23].

A number of EST-SSR primers proved useful in discriminating different genera, species within a particular genus and the different genomes of the tribe Triticeae of the Poaceae [24]. The SSRs derived from the functional portion of the genome of bread wheat may be successfully used in cultivated wheat and its wild relatives belonging to *Triticum-Aegilops* complex for comparative genomics studies such as genome analysis, localization of expressed genes, and discrimination of different species [24].

Six of twenty previously developed Beta SSR primer pairs were found to be useful in classification of five genetically distinct *Salsola* taxa [17]. EST-SSR markers derived from transcribed regions of the DNA produce a higher rate of transferability, but fewer polymorphism [25]. Based on wheat genomic SSR markers, the transferability from wheat to rye was found to be only 17% [26]; however, based on EST-SSR markers, the transferability from wheat to 18 *Triticum-Aegilops* species was found to be as high as 84% [24], and from

Tall fescue (*Festuca arundinacea* Schreb.) to seven grass species was found to be nearly 92% [27]. The transferability (1.8%) of the SSR markers from the other related genera to genus *Haloxylon* was considerably low, suggesting a larger genetic divergence between them.

The two genera (*Haloxylon* and *Chenopodium*) of the Amaranthaceae could be characterized with the length and repeat motif type of the PCR products using primer QCA58. Microsatellites or simple sequence repeats (SSRs) commonly refer to tandem repeats of short (2–6 bp) DNA sequences with a PCR product size ranging from 100—700 bp [13, 14, 19, 23]. Our results indicated that the longer or compound repeat and its relationship with SSRs are noteworthy because of the potential value for use in basic evolutionary applications, such as identification at genus and species levels.

5 Acknowledgements

The study was financially supported by "948 Program" (No. 2008-4-47), State Forestry Administration "Public Welfare Research Foundation" (No. 201004010), the Scientific and Technological Support Project from the Chinese Ministry of Science and Technology (2006BAD26B0101) and the National Natural Science Foundation of China (No. 30972412). The authors thank Linke Yin, Ming Zhao, Yongzheng Tian, Zhiming Zhang, Fufei Chen, Dezhong Chen, Fuhui Chen, Junyan Zhang, Delu Li for their help in material collections.

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