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Seed-specific identification of *Larix gmelinii*, *Larix olgensis*, and *Larix principis-rupprechtii* using sequence-characterised amplified region markers

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ABSTRACT

Larix gmelinii, *Larix olgensis*, and *Larix principis-rupprechtii* are the three native and sympatric larch species in North China, and each of these species has a distinctive ecological niche. It is difficult to identify them based only on certain morphological characters, particularly the seed appearance. In this study, the seed endosperms of these three larch species were analysed using the random amplified polymorphic DNA (RAPD) technique to screen for interspecific differences. The following three RAPD markers linked to species-specific segments were observed in the different species: 1475-bp (*Larix gmelinii* and *L. olgensis*), 505-bp (*Larix principis-rupprechtii*), and 1121-bp (*Larix gmelinii*) markers. The three seed-specific fragments amplified by the RAPD markers were sequenced, and the sequences were used to design and synthesise species-specific SCAR markers. The size of the SCAR fragments was concordant with that of the RAPD species-specific fragments. Therefore, these SCAR markers can be used to identify the seeds of different larch species, thereby providing a new molecular tool for the identification of larch seeds that leads to considerable savings in terms of time and economic resources.

1. Introduction

Larix gmelinii (Rupr.) Kuzenneva, *Larix olgensis Henry*, and *Larix principis-rupprechtii Mayr* are the three native and sympatric species of larch in North China (Yang et al., 2005; Liu et al., 2006; Deyi and Sumei, 2008). The range of *Larix gmelinii* is from the Daxinan to the Xiaoxinan Mountain of Heilongjiang Province. *L. olgensis* is primarily distributed in Changbai Mountain of Jilin Province and extends to the southern part of Heilongjiang Province, as well as Liaoning and its neighbouring provinces. *L. principis-rupprechtii* mainly grows in Hebei and Shanxi Provinces and has been introduced to their surrounding areas (see Fig. 1) (Hu et al., 1999; Zheng et al., 2008).

Although the ranges of these three species overlap, each species has a distinctive ecological niche. Because the distribution of these species is consecutive and the interspecific hybridisation among them is technically feasible, their morphological classification is difficult and controversial (Shi et al., 1998). Many morphological characteristics, particularly seed appearance, are too variable within one species to reliably distinguish it from the other. Therefore, heavy economic losses have been

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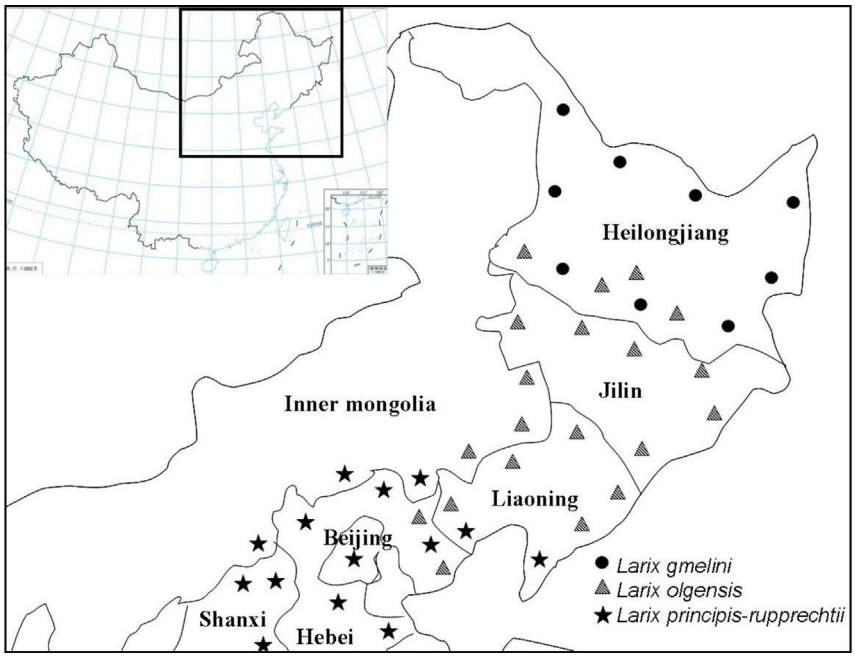

Fig. 1. The distribution of three larch species.

sustained due to the misuse of seeds in larch forestation as a result of the extreme similarity in the seed outlines. To identify the seeds of different larch species, some studies have been conducted at different levels, including morphology, cytology, and biochemistry (Gilmore et al., 1994; Scheepers et al., 2000; Zhang et al., 2008). However, none of these methods was sufficiently accurate to be used for the identification of larch seeds.

The use of a SCAR (sequence characterised amplified regions) marker, which represents an identified loci in a genome, is a novel molecular marker technique developed by Paran that uses the randomly amplified polymorphic DNA (RAPD) technology (Paran and Michelmore, 1993). Specific sequences on the target genome were amplified by a pair of complementary primers designed from the sequence of the original RAPD fragments of interest. These specific amplified regions would maintain the original separation behaviour of dominant RAPD fragments or be converted into co-dominant markers according to Mendelian patterns. Many recent studies have used this technology for the identification of different species, such as *Prunus armeniaca* (Mariniello et al., 2002), *Antheraea mylitta* (Saha and Kundu, 2006), and *Gracilaria changii* (Sim et al., 2007).

In this study, the RAPD fingerprint and SCAR marker technologies were first employed as a tool to identify the differences between the seeds of three Larch species (*Larix gmelinii*, *L. olgensis*, and *L. principis-rupprechtii*) and to identify specific markers for evaluating their genetic differentiation. Based on these techniques, unique species-specific segments of the different species that were amplified by the SCAR primers were obtained, thereby providing an accurate, convenient tool for the identification of larch seeds.

2. Materials and methods

2.1. Seed materials and endosperm separation

The seed samples were obtained from different regions: *Larix gmelinii* seeds were obtained from Wuyiling Forest Bureau (LAT/LONG 48°35′/129°25′) in Heilongjiang Province, China; *L. olgensis* seeds were provided by the Xiaobeihu (LAT/LONG 44°7′/128°42′) and Baidaoshan Forest Bureau (LAT/LONG 45°44′/126°38′) of Heilongjiang Province, China; and *L. principis-rupprechtii* seeds were supplied by the Beijing Forest Bureau (LAT/LONG 39°43′/116°20′), Beijing, China. Each sample from the different larch species was composed of 50 randomly selected seeds, and two replications per sample were used for the RAPD and SCAR detection analyses. Some other seeds were also needed for the preparation and the primer screening experiments.

The seeds were sterilised with 0.5% potassium hypermanganate for 30 min and placed on a wet filter paper in a culture dish. The culture dish was then placed in the testing chamber for 3–5 days at a temperature of 26–28 °C and a humidity of 60% ~80%. The seed coat and embryo were removed when the seeds germinated approximately 1 cm. The endosperm was collected for DNA extraction.