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BRIEF COMMUNICATION

Development of a sequence characterized amplified region (SCAR) marker associated with high rooting ability in *Larix*

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Abstract

In this study, bulked segregant analysis (BSA) was used on *Larix leptolepis* × *Larix olgensis* hybrids to identify a random amplified polymorphic DNA (RAPD) marker associated with high rooting ability in larch. Two DNA bulks: H (high rooting ability) bulk and L (low rooting ability) bulk were constructed according to the rooting percentages of the stock plants. Among the 328 primers, only S356 could amplify a specific band, named $S356_{445}$, which only existed in the H bulk and was further confirmed following selective genotyping of individual hybrids. Grounded on the border sequences, $S356_{445}$ was converted to a sequence characterized amplified region (SCAR) marker, HRL445, which can be useful in marker-assisted selection (MAS) to screen for larch with high rooting ability. All the results strongly indicated that $S356_{445}$ and HRL445 were closely associated with high rooting ability in larch.

Additional key words: BSA, hybrid, MAS, RAPD, rooting percentages.

Differences in rooting ability of cuttings have been shown to be dependent on the genotype and physiological status of the stock plants in many tree species (Radosta *et al*. 1994, Welander 1995, Hoad and Leakey 1996). Selection for good-rooting larch clones seems to be possible, for a number of studies reporting rooting ability to be under strong genetic control in larch (Farmer *et al*. 1992, Wang *et al*. 1995, Yang and Shen 2002). Given that the processes underlying genetic variation in rooting ability of cuttings remain largely unknown (Greenwood and Weir 1995, Welander 1995), the random amplified polymorphic DNA (RAPD) technology (Williams *et al.* 1990) is the most appropriate and convenient technique in genotype fingerprinting (Williams *et al.* 1990, Kumar *et al*. 2006). However, RAPD technology usually lacks stable amplification patterns (Chakrabarti *et al*. 2001, 2006), so it could be inappropriate for diagnosis purposes. A promising approach to fulfill this requirement is based on the sequence-characterized amplified region (SCAR) technique, which was first applied to the identification of downy mildew resistance genes in lettuce (Paran and Michelmore 1993). The SCAR marker can be easily used in the marker-assisted selection (MAS) program (Jang *et al.* 2004, Zhang *et al.* 2004, Noguera *et al.* 2005) and the implementation of MAS would be advantageous for the identification of superior clones for propagation (O'Malley and McKeand 1994). The objective of this paper is to obtain a reliable and practical molecular marker associated with high rooting ability. By using bulked segregant analysis (BSA; Michelmore *et al* .1991) with *Larix leptolepis* × *L. olgensis* hybrids and RAPD technology we report the development of a SCAR marker associated with high rooting ability trait. This marker could provide an effective and powerful tool for MAS in the breeding program of larch.

Larix leptolepis × *Larix olgensis* F_2 generation and six

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Abbreviations: BSA - bulked segregant analysis; CTAB - cetyltrimethylammonium bromide; MAS - marker-assisted selection; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA; SCAR - sequence characterized amplified region.
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pure species *Larix kaempfeeri* (Lamb.) Carr., *Larix olgensis* Henry var. *koreana* Nakai, *Larix principis-rupprechtii* Mayr., *Larix gmelinii* (Rupr.) Rupr., *Larix deciduas* Mill., and *Larix laricina* K. Koch were planted in Da Gu Jia Plantation in Liaoning Province, China. *Larix leptolepis* line 85, as a female parent, was crossed with four different *Larix olgensis* individuals (lanes 11, 36, 4C, 27) to construct four hybrid groups. The rooting experiments were repeated four times. Analysis of each group revealed that the rooting percentage was high in some plants and low in others. Each group was further categorized into two subgroups according to the rooting percentages (high rooting percentages subgroup and low rooting percentages subgroup). Total genomic DNA was extracted from the leaf tissues using modified CTAB procedure of Semerikov *et al.* (2003). The diluted genomic DNA from 16 hybrids (2 hybrids per subgroup) were used to construct two bulks, H bulk (consisting in 8 hybrids with high rooting ability) and L bulk (consisting in 8 hybrids with low rooting ability).

A total of 328 10-base oligonucleotide random primers (*Sangon*, Shanghai, China) were used in RAPD analysis on H and L bulks. The polymerase chain reaction (PCR) amplifications were performed in a reaction volume of 0.025 cm^3 containing 50 ng of genomic template DNA, 0.5 μM primer, 200 μM of each dNTP (*Sangon*), 1 unit *Taq* polymerase (*Takara*, Shiga, Japan) and *Taq* polymerase 1× buffer (*Takara*). PCR amplification was carried out in a *Mastercycler ep* gradient thermal cycler (*Eppendorf AG*, Hamburg, Germany) under the following conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 0.5 min, annealing at 37 °C for 1 min, extension at 72 °C for 1.5 min and final extension at 72 °C for 10 min. The amplification products were separated on 1.5 % agarose gel and visualized with *GeneGenius* bio-imaging system (*Syngene*, Frederick, USA).

Of the 328 random primers, only primer S356 (5′-CTGCTTAGGG-3′) could amplify a specific band of about 450 bp in H bulk which exhibited apparent association with high rooting ability (Fig. 1). Reactions using primer S356 repeated on different days with different DNA template preparations in three PCR equipments and reproducibility of the specific band verified. To confirm this association, we carried out selective genotyping (Lander and Botstein 1989) of individual plants belonging to the two bulks and the amplification products of individuals were separated on 5 % polyacrylamide gel staining with 0.1 % $AgNO_3$ to obtain more clearly visual amplification patterns. The result showed the stable amplified pattern of the specific band in the individuals.

After cloning and sequencing of the specific band, we obtained the whole sequence of this band in 445 bp, named $S356_{445}$. The sequence had no significant homology with any of the sequences deposited in the public databases on the basis of *BLAST* analysis through the website http://www.ncbi.nlm.gov/BLAST, indicating that $S356_{445}$ is firstly reported in larch genome. Although

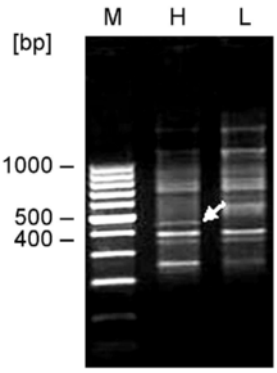

Fig. 1. RAPD agarose gel electrophoresis profile of the H and L bulks using primer S356. *Lane M* - 100 bp ladder marker; *White arrow* pointes the specific band.

the characterization of $S356_{445}$ still needs further investigation, all the results implied that this sequence was closely associated with high rooting ability in larch.

Forward and reverse specific primers were developed based on the border sequences of $S356_{445}$. The primer sequences were: F: 5′-CTGCTTAGGGAGAACCAT GGATGTG-3′ and R: 5′-CTGCTTAGGGGTGGCCTG TC-3′. The components of the PCR reaction were the same as that described in RAPD analysis, except for the primers concentration: 0.2 μM SCAR primers. The optimized protocol was: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 0.5 min, annealing at 61 °C for 1 min, extension at 72 °C for 1.5 min and final extension at 72 °C for 7 min. The SCAR primers succeeded in amplifying a single band SCAR marker, named HRL445, which was identical to $S356_{445}$, and only present in hybrids with high rooting ability.

To check the validity of the SCAR marker HRL445, genomic DNA from other *L. leptolepis*× *L. olgensis* hybrids and six pure species were used as templates in amplification reactions, using the SCAR primers (Fig. 2). The results are in agreement with the phenotypic analyses. All the results strongly suggested that $S356_{445}$ and HRL445 were closely associated with high rooting ability in larch.

In larch, some methods have been attempted to improve the rooting ability. McAfee *et al*. (1993) successfully used suspensions of *Agrobacterium rhizogenes* as rooting agents for the induction of adventitious roots on larch cuttings. The expression of *rol* genes could present traits undesirable for the genetic improvement (Schmülling *et al.* 1988, Zheng *et al.* 1995). Certain amount of growth regulators can improve the rooting percentage of cuttings (Sun *et al*. 2006, Wang *et al*. 2006), but the increased rooting ability can not be inherited. Time-consuming selection based on phenotypic traits would slow down the breeding progress. Therefore, MAS is a promising way to overcome those difficulties. The SCAR HRL445 marker which was proved to be associated with high rooting ability can be used in the marker-assisted selection to facilitate the breeding program of larch.

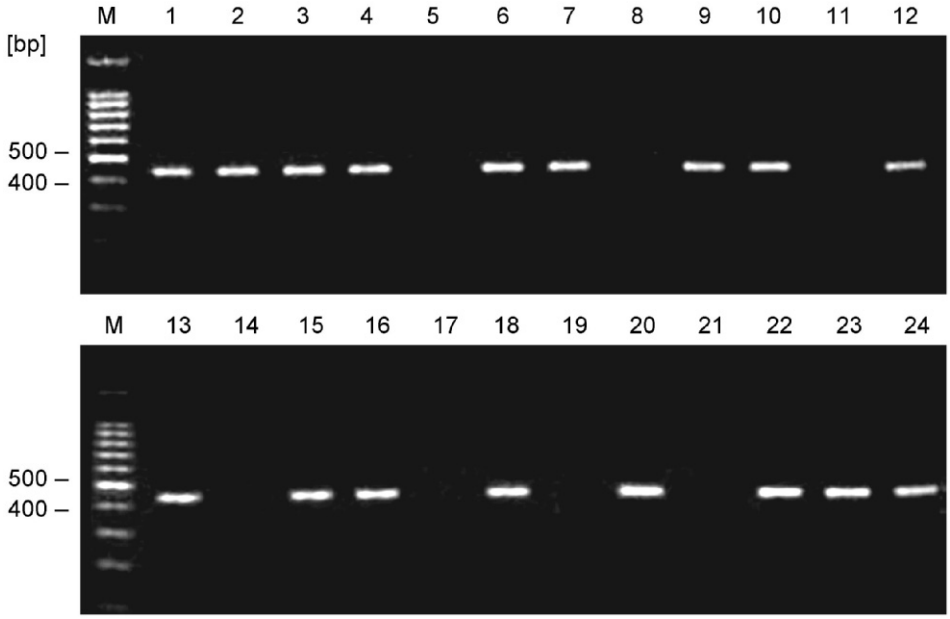

Fig. 2. SCAR agarose gel electrophoresis profile of tested plants. *Lane M* - 100 bp ladder marker, *lanes 1 to 6* - *L. kaempferi, L. olgensis, L. gmelinii*, *L. principis-rupprechtii*, *L. deciduas* and *L. laricina*, *lanes 7 to 24* - tested hybrids.

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