



Development of EST-SSR markers in *Larix principis-rupprechtii* Mayr and evaluation of their polymorphism and cross-species amplification

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Received: 2 May 2018 / Accepted: 10 July 2018 / Published online: 21 July 2018
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Abstract

Key message We developed and validated a new set of polymorphic EST-SSR markers across *Larix* species and evaluated genetic diversity in a clonal seed orchard of *Larix principis-rupprechtii* Mayr.

Abstract Prince Rupprecht's larch (*Larix principis-rupprechtii* Mayr) is an important deciduous conifer species that has been widely planted in North China due to its major ecological and commercial value. However, the paucity of genomic data and robust molecular markers has hampered genetic and genomic studies. Here, transcriptome sequencing of *L. principis-rupprechtii* callus was performed using the Illumina platform. By mining 43,753 assembled unigenes, 1418 expressed sequence tag-simple sequence repeats (EST-SSRs) derived from 1300 unigenes were identified. A total of 1065 primer pairs were designed and 240 of these selected at random for validation among 24 *L. principis-rupprechtii* individuals. Of these, 52 primer pairs were scored as polymorphic, and 20 polymorphic EST-SSR markers were further selected to genotype 66 clones deployed in a clonal seed orchard of *L. principis-rupprechtii*; these exhibited a moderate level of genetic diversity, as reflected by the mean values of the number of alleles ($N_a = 3.85$) and polymorphism information content ($PIC = 0.424$). Additionally, all of the 20 EST-SSR markers could amplify clear and stable bands across three related *Larix* species. A neighbor-joining (NJ) clustering tree uniquely distinguished 66 clones and distributed these into three main clusters, which was further validated by principal coordinate analysis (PCoA). The developed EST-SSR markers will serve as valuable tools for future genetics and breeding research in larch species. The evaluation of genetic diversity among 66 clones will provide important information for efficient management and utilization of genetic material in *L. principis-rupprechtii* breeding programs.

Keywords EST-SSR markers · Genetic diversity · *Larix principis-rupprechtii* · Transcriptome · Transferability

Communicated by Carlson.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00468-018-1733-9>) contains supplementary material, which is available to authorized users.

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Introduction

Prince Rupprecht's larch (*Larix principis-rupprechtii* Mayr), a deciduous conifer species endemic to China, is mainly distributed in montane areas at elevations of 1400–2800 m in Shanxi and Hebei Provinces (Di et al. 2014). It plays a vital role in reforestation projects and commercial activities due to

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