

DEVELOPMENT AND APPLICATION OF HIGH DENSITY GENETIC MAP OF LIMBER PINE (*PINUS FLEXILIS* JAMES) FOR GENOMICS-BASED BREEDING

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Development and adaptation of genomics-based tools, such as high-throughput genotyping, high-density genetic maps, marker-assisted selection, genome-wide association of genotype versus phenotype, breeding by design, breeding without breeding, and genomic selection is moving conventional breeding into a new era. Next-generation sequencing (NGS)-based RNA-seq, exome-seq, and related technologies provide very effective approaches for detection of genome-wide variations and identification of DNA markers for marker-assisted selection in breeding of forest resistance against pathogens and pests. Genome-wide mapping of functional genes enhances our understanding of genetic resistance and local adaptation to changing climates in living organisms. Limber pine is one of the keystone conifer species of high elevation forest ecosystems in North America, and its populations in many regions are seriously impacted by the invasive, exotic fungal pathogen *Cronartium ribicola*, which causes white pine blister rust (WPBR) on five-needle pines of the subgenus *Strobus*. In the present study, we constructed limber pine high-density genetic maps by exome-seq, and developed TaqMan arrays for marker-assisted selection for breeding programs and field applications. Base on a limber pine reference transcriptome *de novo* assembled previously (Liu et al. 2016), hybridization probes were designed to enrich the exome from the complex genome. Genetic variations and genotypes were explored by exome-seq in two seed families with phenotypic segregation of major gene (*Cr4*) resistance. Single nucleotide polymorphism (SNP) markers were used to construct genetic maps. A total of 9.5K expressed genes, including > 600 NBS-LRR genes and > 200 RLK genes, were mapped on 12 linkage groups for a consensus genetic map. Based on SNP markers at the *Cr4* locus, TaqMan SNP arrays were developed for marker-assisted selection. Using these genomic tools, we revealed genetic relationships of major gene resistance between limber pine seed families originated in Canada and the United States, as well as between limber pine (*Cr4*) and other five-needle pines (including southwestern white pine *Cr3* and western white pine *Cr2*). Genomic resource and practical tools developed here will benefit breeding and genetic conservation programs and facilitate the genome-wide association study and assembly of the full-length genomes in limber pine and related *Pinus* species.

LITERATURE CITED

Liu J-J; Schoettle, A.W.; Sniezko, R.A. [et al]. 2016. Genetic mapping of *Pinus flexilis* major gene (*Cr4*) for resistance to white pine blister rust using transcriptome-based SNP genotyping. *BMC Genomics*. 17: 753.

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