A unique two round multiplex PCR based SNP data utilized for parental reconstruction in half-sib family of *Ziziphus jujuba Mill.*

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Abstract

Next-generation sequencing technologies (NGS) have empowered resequencing of genome for investigating genome wide polymorphism between plants, as well as resequencing of targeted parts of genome has enabled for rapid and concomitant spotting of polymorphism in genes related with different biological functions. So, easy and robust way for sequencing of targeted genome parts should facilitate genotyping in broad biological fields. Single Nucleotide Polymorphism (SNP) genotyping is the measurement of genetic variations of single nucleotide between members of a species. It is most commonly used genetic markers for varietal genotyping. For SNP based genotyping, we developed a new method of multiplex PCR amplification in combines with targeted SNP high throughput sequencing and utilized it for genotyping of 334 half-sib plants of *Ziziphus jujuba Mill* that were generated through open pollination. Jujube flowers are small hermaphrodite containing both male and female sex organs on the same flowers. The flowers are protandrous dichogamous having overlap of time of anthesis and stigma receptivity. These characteristics of jujube flowers make self-pollination quite difficult. Artificial pollination in *Ziziphus jujuba Mill* is very hard and percentage of development successful crosses are very low so development of a method for parental identification is very important. For multiplex PCR, 10 different SNP of Jing39 male parents surrounding the mother tree are utilized for parental identification. These 10 different SNPs are distributed on 10 different chromosomes of Jing39 variety. This cost-effective method of multiplex PCR makes as much as possible to utilize the same amount of each pair of various targeted loci primers. In first round of PCR targeted SNP loci are amplified while in second round of PCR specific barcodes are added to differentiate DNA of each half-sib plants during data analysis. After PCR amplification of various targeted genome parts, the mixed products can be directly used in next generation sequencing platform. We concomitantly amplified 10 specific SNP loci of male Jing39 in 334 half-sib plants and sequenced on Illumina Novaseq 6000 platform. Analysis of SNP genotyping accuracy of 334 half-sib plants showed that all 10 SNPs in all 334 plants were correctly called in this multiplex PCR method. Sequencing depth of each SNP for each sample was calculated and sequencing depth of 93.13 % reads of SNPs were more than 800X. Average

sequencing depth of each SNP for all samples were 2300x.

Based on mendelian inheritance of 10 male Jing39 SNPs, we identified 78 full sib plants that have these SNPs DNA in their genomes. These results were further confirmed by whole genome resequencing of 20 randomly selected half sib plants, sequenced were analyzed by Plink software and identity-by-descent values of all full sib plants were between 0.4399 to 0.5652. This study displayed cost effective multiplex PCR method of targeted loci amplification and proper identification of parental reconstruction in *Ziziphus jujuba Mill.* half sib progenies generated through open pollination. This method of parental study is very easy and cost effective for those plants whose flowers are small and artificial pollination is difficult. These finding will be helpful for future parental reconstruction and plants selection in *Ziziphus jujuba Mill*. breeding program through open polycross mating design.

Key words: Two-round multiplex PCR, SNP genotyping, half-sib plants, Parentage analysis.