



Genome-Wide Discovery of InDel Markers in Pea (*Pisum spp.*) Using Whole Genome Resequencing Data

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Abstract

Pea (*Pisum sativum* L.) is a nutritionally important cool-season crop, has been hindered in genomic and molecular-assisted studies due to its extensive and repetitive genome sequences. Addressing this issue, whole-genome re-sequenced data (WGRS) from two parental lines, ACP 20 (*Pisum sativum* L.), and AWP600 (*Pisum fulvum*), were utilized to develop agarose gel-resolvable InDel markers. A total of 1210 loci were derived through bioinformatics analysis from resequencing and Genbank SRA data. Among these loci, 49 primer pairs, averaging 6-8 primers per chromosome were designed and validated on 31 pea genotypes, including *P. sativum*, *P. fulvum*, *P. elatius*, and *P. abyssinicum* accessions. Furthermore, the markers were also evaluated in a panel of 48-F6 pea RIL population, derived from interspecies crosses between parental lines. Remarkably, 97.96% (48 loci) produced reliable and expected amplification on parental lines. Diversity analysis revealed a total of 101 alleles for 48 InDel loci on 31 pea genotypes, with an average of 2.104 alleles per locus. The mean of polymorphic information content (PIC) was 0.281, with a Shannon diversity index (I) of 0.472. Phylogenetic and principal coordinate analysis (PCoA) distinctly grouped pea genotypes into two main clusters. Evaluation of InDel markers on the RIL population through a Chi-square (χ^2) test resulted in a 1:1 segregation ratio in 33.33 % of the loci tested. On the other hand, allele distribution on the remaining loci skewed towards maternal line ACP20 indicating presence of prezygotic selection due to most likely incompatibility. Furthermore, seventeen marker loci were found primarily associated with enzymatic activities and hypothetical proteins, potentially encoding genes in pea. These newly discovered InDel markers, the first of their kind in the genus *Pisum*, are pivotal for future marker-assisted breeding studies in peas.

Keywords: InDel, Molecular markers, Pea (*Pisum spp.*), RILs, WGRS