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Development of marker for resistance genes by using next generation sequencing technologies

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Abstract

By using high trough-put sequence technologies it is possible to sequence whole genomes in a cost efficient way. Therefore in a current project we aim to index the diversity of all major resistance genes (*R* genes) within the genepool of *Solanum* with emphasis on the common potato (*S. tuberosum*). The goals of the project are to explore and catalog nearly all diverse *R* alleles (*R* haplotypes) conferring disease and pest resistance, at the genetic loci of *R* genes belonging to the NBS-type meta-family in *Solanum* (with emphasis on the common potato), to determine in a large-scale approach *R* allele fragments by their association with the resistance phenotype, to explore evolutionary, structural, and diversification aspects of the plant *R* genes and to set up a method for the development of molecular tools (markers; sets of PCR primers) that can be used in research and by plant breeders, for fast tracking of *R* alleles conferring resistance to pathogens and pests in *Solanum*.

For this study 96 potato samples from different breeding programs and gene banks were collected. For the NBS profiling new primer for the NBS domain were developed and tested. For the amplification six different primers tagging the p-loop motif, 3 primers tagging the kinase 2 motif and 4 primers for the GLPL motif were used. The obtained amplification products were sequences using the HiSeq (Illumina) machine at GATC Biotech (Germany). The obtained sequences were analysed using standard bioinformatics tools and aligned to the potato reference sequence (PGSC_DM_v4.03_pseudomolecules) with NextGenMap 0.4.4. The obtained data will be available in a database.

To prove our concept we selected parents from a breeding program where one parent 'Alegria' carries a new PVY virus resistance gene. To localize the resistance gene on the genetic linkage map microsatellite markers were applied to the population. The raw linkage map was calculated using TetraploidMap. The PVY resistance was linked to SSR markers from chromosome 9. Therefore to find a NBS region linked to the resistance the search for SNPs among the two parents were concentrated on chromosome 9. Several markers based on the NGS data were tested and all markers were placed on chromosome 9 in the right order according to the reference genome. Until now no linked marker to the PVY resistance could be found. New primer for different position on chromosome 9 will be further tested.

Keywords

Microsatellite marker, potato, *Solanum tuberosum*

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