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Next Generation Sequencing Analysis and Map-based Cloning of RALF Insensitive *Arabidopsis thaliana* Lines.

The Rapid Alkalinization Factor (RALF) peptide family plays a crucial role in plant growth and development. It belongs to a group of related peptide hormones found across the plant kingdom. RALF induces the alkalinization of the apoplast which triggers a signaling cascade that inhibits cell root elongation and growth, however the full mechanism of action is unknown. In this project, a forward-genetics screen was used to identify EMS-mutagenized lines of Arabidopsis thaliana (Ler-0) that were resistant to AtRALF1-induced root growth arrest. 14 of these RALF-resistant mutant (RRM) lines were then re-sequenced using Illumina next generation sequencing technologies in order to find causative mutations and potential mapping polymorphisms. The line RRM30 was found to have 572 exonic SNPs after its genome was mapped to the reference and analyzed with the program Geneious. These SNPs were then used to create a set of dCAPS mapping-markers for a bulked-segregant analysis of RRM30. Many of these markers have been tested and confirmed to be unique to RRM30 using PCR genotyping experiments and gel electrophoresis. Future examination of the bulked-segregant analysis should narrow down the causative mutation in this line and help identify genes required for a RALF response. The causative mutation will be confirmed with an allelism test to knockout lines of A. thaliana in this gene. Identification of genes required for this response will shed more light on the mechanism of growth inhibition by RALF.