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Quantitative Trait Locus (QTL) analysis and fine-mapping for *Fusarium oxysporum* disease resistance in *Raphanus sativus* using GRAS-Di technology

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Background:

Fusarium oxysporum f. sp. *raphani*, responsible for *Fusarium* wilt (FW) in *Raphanus sativus* is an important pathogen in radish. The best method for its control is breeding resistant varieties because of its spore longevity in soil. A few research has been done on this disease in radish, however, the complete genetic detail of the resistance gene is not yet fully understood.

Objective:

Genotyping by Random Amplicon Sequencing Direct (GRAS-Di) has not been previously reported for generating genetic maps in *Brassica* crops, therefore, the research objectives are (1) Identify QTL responsible for yellows resistance (YR) in radish using the GRAS-Di map, (2) validate the usefulness of GRAS-Di map, and (3) fine-mapping the YR-QTL on R7 chromosome and identification of candidate genes that regulate YR of radish.

Methods:

F_1 plants were produced from a cross between the parents, resistant RK15-1 and susceptible AKM inbred lines. The resulting F_2 and $F_{2:3}$ (F3) populations were all produced by self-pollinations at each subsequent stage. Linkage map was constructed using GRAS-Di technology. For QTL analysis, we collected phenotype data of 126 and 130 $F_{2:3}$ lines in 2021 and 2022, respectively. Ten seedlings per $F_{2:3}$ line were used at each of the two periods of the inoculation tests. QTL analysis was performed using Composite Interval Mapping, after which the detected QTL was fine-mapped to delimit the candidate gene region. Illumina, Nanopore and RNA sequencing were performed to fully elucidate the candidate R-gene sequence.

Results:

Two QTL were detected at R2 (*ForRs2*) and R7 (*ForRs1*) chromosomes, with LOD values of 4.74 and 7.3, respectively, in 2021, while one QTL at R7 (*ForRs1*) chromosome, with LOD value of 11.6, was detected in 2022. These two QTLs were confirmed by segregation analyses. The *ForRs1* QTL accounted for most of the phenotypic variation, 18.1% and 33.0 % in 2021 and 2022, respectively and was designated as a major QTL. Fine-mapping of this *ForRs1* using 7 recombinant $F_{2:3}$ lines (8 graphical genotypes) delimited the candidate gene region to 195kb in between #23 and #31 markers. RNA-seq analysis identified 16 transcripts in this region. Of those transcripts, four genes were identified as encoding Receptor-like protein and Receptor-like kinase (RLP/RLK) domains, which are characteristic of R-proteins with LRR and kinase domains.

Conclusions:

Our study reported the use of GRAS–Di technology for constructing genetic maps in radish for the first time. We successfully detected 2 QTL at R2 and R7 chromosomes, which were responsible for *Fusarium* disease resistance in radish. The major QTL, *ForRs1* on the R7 chromosome was fine-mapped to the candidate gene region of 195kb, and this is the first report of fine mapping on the R7 chromosome of radish. Four candidate genes were identified within this region and specific markers for the four identified candidate genes were designed, to enhance marker-assisted selection in radish YR breeding.