PCR Assays for the Detection of *Xanthomonas arboricola* pv. *juglandis*, Causal Agent of Walnut Blight

Young-Hee Noh[1], Jung-Hee Cho[1], Marco Scortichini[2] and Jae-Soon Cha[1]

[1]*Department of Plant Medicine, Chungbuk National University, Cheongju, Republic of Korea*
[2]*C.R.A.-Research Centre for Fruit Crops, Via di Fioranello, 52 I-00134 Roma, Italy*

Abstract

Walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*), is serious disease on English (Persian) walnut worldwide. The development of an efficient detection technique for *Xaj* is very important for plant quarantine services present in different areas of walnut cultivation. Since not many nucleotide sequences of *Xaj* strains useful for PCR primer design has been reported, whole genome sequencing of *Xaj* LMG751 was carried out with the Next Generation Sequencing (NGS). A total of 390 contigs were obtained and the similarity of each ORFs in the contigs was compared to other known genes by BLAST to select candidate genes for primer design. PCR primer set, Xaj-F/Xaj-R, was designed from one of the candidate ORFs to amplify a 489 bp fragment, and nested PCR primer set, Xaj-ne-F/Xaj-ne-R, was designed to increase the detection sensitivity. PCR with Xaj-F/Xaj-R and the bacterial DNA amplified the target 489 bp DNA from all *Xaj* strains tested and representing many countries of walnut cultivation. The target DNA was not amplified from other *Xanthomonas* spp. When nested PCR was performed using the nested PCR primers, the target size DNA was amplified in the all of *Xaj* strains, while it was not amplified in the other bacterial strains. The results presented here indicate that *Xaj* can be reliably detected and identified by PCR assays with Xaj-F/R primer set and Xaj-ne-F/Xaj-ne-R set developed in this study.