ID 100

Conventional PCR based semi-quantitative method for quantification of sugarcane white leaf disease phytoplasma

Y.A.P.K. Dayasena[1*] and M. Niranjan[2]

[1]Division of Crop Protection, Sugarcane Research Institute, Sri Lanka
[2]Division of Plant Pathology, Tea Research Institute, Sri Lanka

Abstract

Semi-quantitative PCR technique is applying to indirectly quantify the level of nucleic acid. It is becoming more popular due to the limitation real-time quantitative PCR technology. The present study was done to optimization of a semi-quantitative PCR (semi-qPCR) protocol to quantification of sugarcane white leaf (SCWL) phytoplasma presence in the sugarcane tissues based on the conventional PCR technology. Genomic DNA was extracted from SCWL infected leaves and disease-free seedlings by CTAB protocol. A PCR program was performed with the DNA concentration gradient to find the optimum template DNA concentration for subsequent optimization of other parameters of the program. Well optimized primer pair SPP1/SPP2 and DnhF/DnhR newly designed primer pair were used to molecular detection of SCWL phytoplasma by conventional PCR instead of nested PCR. A PCR cycle gradient 25, 27, 29, 31, 33 and 35 was performed to determine the peak of the linear phase of the amplification for each primer pair. The PCR program was performed at 95^0C for 3 min initial denaturation followed by 25-35 cycles: denaturation at 95 ^{0}C for 45, annealing at 53 ^{0}C for SPP1/SPP2 and 56 ^{0}C for DnhF/DnhR for 1 min and extension at 72 ^{0}C for 45 seconds for SPP1/SPP2 and 30 sec for DnhF/DnhR primers. The endpoint analysis was determined by quantification and analysis of DNA band intensities by ImageJ software of the resolved DNA bands in 1.5% agarose gels. The template DNA concentration 250 ng/µL was given consistent and significant band intensity. The primer pair SPP1/SPP2 had been amplified the target band for all replicates in all PCR cycles and band intensity was gradually increased with the cycle number. Statistical analysis confirmed that cycle number 31 started the plateau of the PCR program of SPP1/SPP2 primer pair. The primer pair DnhF/DnhR was capable of producing the target band, however, poorly amplified the negative control also in the later stage of the program. Hence, this primer pair cannot be used for the semi-qPCR. The SPP1/SPP2 primer pair, 250 ng/µl template DNA with 31 cycles PCR program can be successfully used to quantify the level of SCWL phytoplasma by conventional PCR instrument.

Keywords: Conventional PCR, Semi-quantitative PCR, Sugarcane, White leaf phytoplasma, 16S rRNA gene

**Corresponding Author:* prasannaup@hotmail.com