Abstract

Napier stunt phytoplasma (16SrXI and 16SrIII) in eastern Africa is a serious threat to the expansion of Napier grass (*Pennisetum purpureum*) farming in the region, where it is widely cultivated as fodder in zero grazing livestock systems. The grass has high potential for bio-fuel production, and has been adopted by farmers as a countermeasure to cereal stem borer Lepidoptera, since it attracts and traps the insect. Diagnosis of stunt phytoplasma have been largely by nested polymerase chain reaction (nPCR) targeting the 16S rRNA gene. However, the method is laborious, costly and technically demanding. This investigation has developed a simpler but effective phytoplasma diagnostic tool, called; loop-mediated isothermal amplification of DNA (LAMP). The assay was tested on 8 symptomatic and 8 asymptomatic plants, while its detection limit was compared to nested PCR using samples serially diluted from 3 ng/µl to 0.38 pg/µl. Molecular typing of LAMP products was determined by BsrI restriction digestion and Southern blot analysis. The assay sensitivity, positive and negative predictive values were estimated, while the specificity was tested on 11 phytoplasma groups. LAMP was specific to 5 phytoplasma groups: 16SrVI, X, XI and XVI. BsrI restriction digestion produced two predicted fragments, and there was specific binding of probe DNA to the LAMP amplicons in Southern blot analysis. The assay sensitivity was 100%, while the positive and negative predictive values were 63 and 100% respectively. LAMP was 20-fold more sensitive than nested PCR. This study validates LAMP for routine diagnosis of Napier stunt and other closely related phytoplasmas.