Qualitative and quantitative identification of SNPs in plant pathogens

Patrick Wille, Helge Sierotzki, Carole Stanger, Sally Cleere, Judith Burbidge, Alison Hall, John Windass and Ulrich Gisi

Abstract

Single nucleotide polymorphisms (SNPs) account to a considerable degree for the genetic diversity among individuals. Several SNPs are known in the cytochrome b (*cyt b*) gene, one of which has been shown to confer resistance to strobilurin fungicides at the Qo center (QoIs) in a variety of important plant pathogenic fungi. This SNP causes a change in the deduced amino acid sequence from glycine to alanine at position 143 (G143A). For efficient monitoring of fungal populations different molecular techniques are being assessed for their ability to detect and quantify the SNP leading to G143A. Each of the techniques is based on PCR amplification of a DNA fragment that spans the site of mutation. In the first approach, allele unspecific but species specific primers were used for PCR, followed by G143A specific restriction digest of the mutated allele (PCR-RFLP). In the second technique, PCR was again performed with allele unspecific primers in combination with allele specific hybridisation probes known as Molecular Beacons, which are structured oligonucleotides that fluoresce upon hybridisation with their targets (MB-PCR). In a third approach selective amplification of either the wild-type or the mutated *cyt b* was undertaken with allele specific primers (AS-PCR) using the amplification refractory mutation system (ARMS); here detection of the PCR product is accomplished by means of Scorpion primers. These have an integral pathogen specific *cyt b* hybridisation probe which releases a fluorescent signal when annealed to the product. The first approach allows qualitative identification of SNPs, whereas the second two allow their quantitative detection in real-time PCR assays.