



DNA barcoding as a diagnostic tool

DNA barcoding is a generic diagnostic method that uses sequence data of a short standardised genetic marker in an organism's DNA to aid species identification. The chosen marker region should reflect the target species group taxonomy and at the same time provide high variability between species with low levels of differences within the species. The first genetic marker to be described as a 'barcode' was the *mitochondrial cytochrome c oxidase I* (COI) gene which is used for species identification in the animal kingdom.



DNA barcoding is increasingly used as a diagnostic tool in phytosanitary laboratories. For application in the plant health field, the method should allow identification on the taxonomical level provided in national or regional regulation (e.g. the pathovar level for several quarantine bacteria).

Standardisation of DNA barcoding protocols used in the plant health field and linking them to other barcoding initiatives is essential to benefit from publically available data. The objectives of this Euphresco project were to improve and validate the protocols for DNA barcoding of plant pest arthropods, bacteria, fungi, invasive plants, nematodes and phytoplasmas initially developed in the EU-funded project 'Quarantine organisms Barcoding Of Life' (QBOL).

The chloroplast large subunit *Ribulose-1,5-bisphosphate caboxylase-oxygenase* (rbcL) gene and the nuclear ribosomal internal transcribed spacer (ITS) region have been proposed as barcodes for the plant and fungi kingdom respectively. As became apparent from the QBOL project, using a single barcode region does not provide sufficient reliability for the identification of the majority of regulated plant pests. Within QBOL, DNA barcoding protocols for eukaryotic and prokaryotic plant pests were developed and validated that provide sufficient reliability on the desired taxonomical level (Bonants *et al.*, 2010). A test performance study (TPS) was organised to determine the user-friendliness and performance of the protocols, and allowed the identification of possible pitfalls that had to be resolved before the protocols could be accepted as an EPPO Standard (van de Vossenber *et al.*, 2013). These improvements were addressed in the Euphresco project which resulted in the addition of four new tests



covering bacteria, fungi and invasive plants, the guidelines on generating sequence data, creating consensus sequences from raw sequence data, (on-line) data-analysis, and a suggested form for standardised reporting of DNA barcoding analysis results.

An international TPS with 23 participants world-wide was performed to determine the user-friendliness of the draft EPPO standard, and to generate validation data. Overall diagnostic sensitivity was 87%. Analysis of the TPS data showed that neither the amplification/sequencing of loci, nor the creation of consensus sequences, negatively influenced the diagnostic sensitivity. Consensus sequence data generated by the participants was highly similar to the reference sequences. The interpretation of analysis results had the biggest influence on the diagnostic sensitivity, and the majority of incorrect identified samples were the result of conservative identification (i.e. identification on a higher taxonomical level) showing that some participants did not feel confident in assigning a lower taxon level to the sample. Re-analysis of the consensus sequence data provided by TPS participants showed that an overall diagnostic sensitivity of 99% could be obtained. DNA barcoding protocols are typically used in conjunction with other diagnostic tests and (morphological) observations. In this test performance study, DNA barcoding was taken out of the context of a diagnosis which could have led to these conservative identifications.

The DNA barcoding protocols in the EPPO Standard have proven to be fit for purpose, but there is a strong influence of proficiency of the assessors handling data. The Standard has been sent for country consultation and the document could be finalised by the end of the year 2016. Validation data have been uploaded on the EPPO validation database using the EPPO PM7/98 (2) (EPPO 2014) format and the TPS results will be published in a scientific journal.

Project ID: Update and validation of DNA Barcoding protocols by end-users (DNA Barcoding).

References:

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