Traditionally the identification of seed-borne pathogens, following the ISTA rules, consists in a 400-seed plating method with microscopic identification of 10-days post-plating rice seeds. The certification of pathogen-free seeds is essential for the control of the pathogens that can be easily spread by this propagation material. However, morphology-based methods may result in mis-identifications of the pathogen because of the high number of shared characteristics between phylogenetically related species. In this study, we developed two LAMP assays for *F. fujikuroi* and *M. oryzae*, designed on the elongation factor 1-alpha and calmodulin genes, respectively. Both assays were validated according to the international EPPO standard (PM7/98) in terms of specificity, sensitivity, repeatibility and reproducibility. The assays were validated using DNA from rice seeds, showing a limit of detection of 100-999 fg of DNA for *F. fujikuroi* and 10-99 pg of DNA for *M. oryzae.* Two different DNA extraction methods combined with the LAMP assays were used to extract the DNA from infected rice seed lots and were compared with the traditional culturing method. The results demonstrated that LAMP assays are fast, specific and reliable for detection of *F. fujikuroi* and *M. oryzae* in seed-testing laboratories, overcoming the drawbacks of traditional identifications.

Characterization of the secondary metabolite profile and phylogenetic analysis of *Alternaria* species isolated from basil

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Alternaria spp. are the causal agents of Alternaria leaf-spot of basil. This disease has recently been reported in Italy. The correct identification of the different species has traditionally been based on morphological features and molecular data. Nevertheless, this fungus underwent multiple reclassifications. In this study, a better characterization of *Alternaria* isolates from basil was sought using morphological characteristics, phylogenetic analysis of seven genomic regions, and secondary metabolite profile. The best-resolute regions allowing the identification of the majority of the isolates as *A. alternata* were OPA 1-3 and OPA 10-2. Morphological characteristics and sporulation groups helped to discriminate *A. tenuissima* from *A. alternata* isolates. All isolates in the *A.* sect. *Alternaria* were demonstrated to be mycotoxigenic and pathogenic to basil with an enhanced production of mycotoxins on this host, as compared with the *in vitro* conditions used in this work. The combined results and, especially, the mycotoxin production allowed differentiating some species within this species group as *A. arborescens* and *A. tenuissima.*

Detection of *Fusarium oxysporum* f. sp. *lactucae* in soil, lettuce seeds and plants by LAMP assay

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Fusarium oxysporum f. sp. *lactucae* (FOL) is a worldwide-distributed soil- and seed-borne pathogen that causes Fusarium wilt of lettuce. Four races have been identified of this *forma specialis*, which is currently spreading to new areas within Europe, with new records of race 1 and race 4 in North Europe. Several molecular tools have been used to identify this pathogen in different propagation material. Due to its harmfulness, it can cause a total crop loss, so the distribution of infected seeds can threat the worldwide production of lettuce. In this study we developed, optimized and validated a LAMP assay for the detection of FOL in lettuce seeds, soil and plants. The LAMP assay was designed on the sequence-characterized amplified region (SCAR) obtained in a previous rapid amplification of polymorphic DNA assay and was validated according to the international EPPO standard (PM7/98) in terms of specificity, sensitivity, repeatibility and reproducibility. The sensitivity of the assay using DNA and artificially inoculated lettuce seeds, in individual lettuce seed testing and batches at different infection rate, reached detection limits as low as 0.004% infected seeds. Thus LAMP was experimentally proved as a reliable laboratory tool for the detection of FOL in lettuce seeds lots, which can assist in avoiding the dissemination of the pathogen to new areas.

Metagenomic analysis of the aerial mycobiome of rice paddies

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Air-borne microbiome is a new topic that has been understudied in comparison with the microbiota present in other environments as soil, rhizosphere or water. Traditional methods as culture-based methods allow the study of only a small fraction of the organisms in the atmosphere. In this study, the aerial composition of fungi in a rice paddy has been examined during the crop production cycle (from June to September) using a DNA-based method (qPCR) to target two important rice pathogens, *Magnaporthe oryzae* and *Cochliobolus miyabeanus,* and using a high throughput sequencing (HTS) targeting the ITS region. The results demonstrated an increase in the alpha diversity analysis (Shannon-Wiener diversity index *H'*, the chao1 and total number of observed species) at the beginning of the trial (June), showing a higher level of complexity than at the end of the trial. The main taxa were identified by HTS where the relative abundance drove the cluster separation as a function of the time and temperature. The OTU core included *Cladosporium*, *Alternaria*, *Myrothecium*, *Epicoccum*, *Davidiella*, *Russulaceae*, *Leptosphaerulina*, *Magnaporthe*, *Aurciculibuller*, *Sporobolomyces*, *Lewia*, *Cochliobolus*, *Hyphodontia* and *Fusarium.* A parallel, oligotyping analysis on the main rice pathogens was performed to obtain a sub-OTU identification. The results revealed the presence of several characteristic oligotypes associated with monitoring time. In addition, changes on mycobiota composition were clearly detected in function of the air temperature. Indeed, temperature variations can drastically affect the mycobiota community structure with a possible impact on the development of rice diseases.