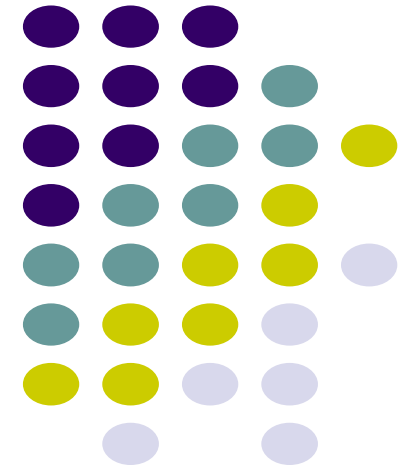
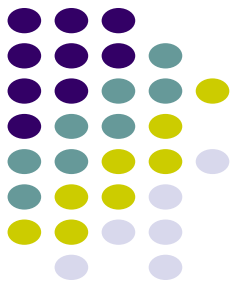


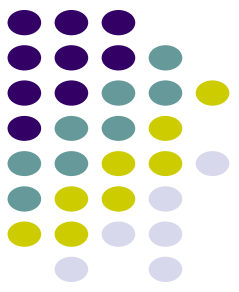
Development and Implementation of Rapid and Specific Detection Techniques



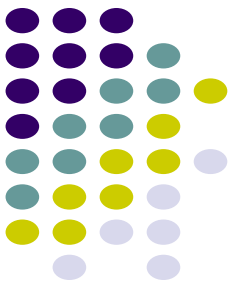
Commercial significance of seeds and seed born pathogens



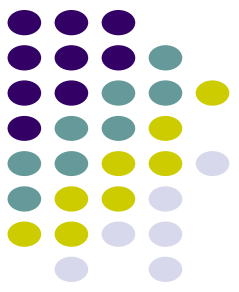
- Fast and sensitive diagnostic tools are necessary to screen seeds and transplants used for commercial purpose. Due to the role of seed transmission, detection methods are also important for the production of pathogen-free seeds and for their certification.
- During the past 15 years, several new diseases were introduced in European countries in the production sector through infected seeds. Molecular methods proved helpful in the detection of several formae speciales of *Fusarium oxysporum*, *Verticillium dahliae* and several causal agents of foliar diseases of leafy vegetables.



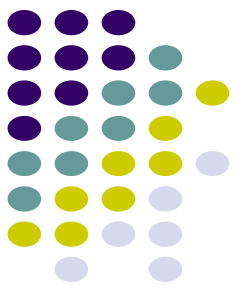
- Fast and sensitive diagnostic tools are needed so that primary inoculum vectors, such as seeds and transplants, can be screened promptly.
- Pathogen's detection on seeds can be a difficult task because in most cases infected seeds can be asymptomatic, making visual detection difficult or impossible.
- Moreover, infected seeds may be present in a limited percent, and non uniformly distributed within a lot. Different detection assays exist for different seed-borne pathogens but only a few respect the minimum requirements for adequate seed tests.



- Traditionally, seed assays have been developed based on
 - visual examination
 - selective media
 - serological techniques
 - Polymerase Chain Reaction

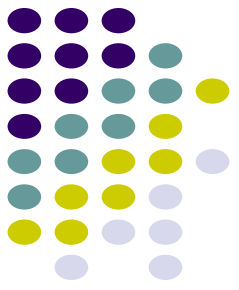


- Several new diseases of leafy vegetables (lettuce, wild and cultivated rocket, lamb's lettuce, cichory, endive, basil, spinach) were introduced in Europe through infected seeds. Some of them were reported for the first time in Europe or worldwide.
- Identifying the source of inoculum is of critical importance for effective disease management. Due to the role of seed transmission, detection methods are also important for the production of pathogen-free seeds and for their certification.



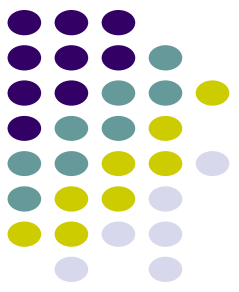
- Among diseases, Fusarium wilts on lettuce (*Lactuca sativa*), wild (*Diplotaxis* spp.) and cultivated rocket (*Eruca sativa*), lamb's lettuce (*Valerianella olitoria*), cichory (*Cichorium intybus*) and endive (*Cichorium endivia*) were newly observed.
- Also several species of Alternaria, for which there is evidence of being seed transmitted, are reported on leafy vegetables. *Alternaria cichorii* is reported on lettuce, endive and scarola, while *Alternaria japonica* has been recently detected on both wild and cultivated rocket.
- Among other pathogens, *Verticillium dahliae*, reported on lettuce, cichory and spinach, *Plectosphaerella cucumerina* on wild rocket, etc.

Fusarium oxysporum



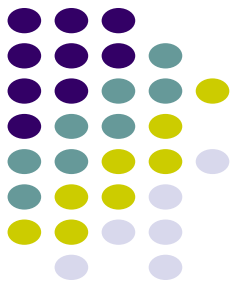
- The search for molecular techniques has been particularly intensive and effective in the case of several formae speciales of *Fusarium oxysporum*. The detection threshold of *F. oxysporum* in seeds and propagation material could be increased by using molecular techniques, such as the PCR.
- In the case of Fusarium wilt of basil and lettuce a nested-PCR-based method allowed fast and unequivocal identification of *F. oxysporum* f. sp. *basilici*. The method permitted to detect 32 conidia/100 seeds and required 4 h. DNA was extracted only from propagules present on the external surface of the seeds.

Verticillium dahliae



- A quantitative real time polymerase chain reaction (qPCR) assay was recently optimized and used for the detection and quantification of *V. dahliae* in spinach seeds, resulting quite reliable and sensitive, permitting to assess values up to 1.3 % of seed infection.
- However, *V. longisporum* has been described as a hybrid species that presents several genomic regions in common with *V. dahliae*. *V. longisporum* is a crucifer pathogen never described in diseases associated with spinach, but in any case the external presence of this pathogen can result into a false positive; then it is important to exclude the presence of this pathogen from the samples.

Alternaria



- Distinguishing *Alternaria* species is a challenge in leafy vegetables.
- PCR-based methods were used for detecting *Alternaria radicina* infections on carrot.
- However, RAPD analysis was helpful to distinguish *A. radicina* from the other *Alternaria* species.
- Further, PCR-RLFP was helpful to identify and distinguish *A. radicina*, *A. dauci* and *A. alternata* from carrot seeds.
- Standard PCR and Real time PCR were useful in identification of seed contamination by *A. brassicae* on cabbage and radish.