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Molecular approaches for Identification and Management of *Alternaria spp.*

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Alternaria spp. can be identified through molecular approaches like LAMP, Real-time PCR, Conventional PCR, Duplex PCR and Nested PCR. In molecular management approaches, multiple quantitative trait loci (QTL) mapping identified two QTL (qALB-10 and qALB-12) affecting *A. cucumerina* lesion area. From these two QTL several candidate genes were identified. By using tobacco PR-2 protein, β-1, 3-glucanase gene, *A. helianthi* resistant transgenic sunflower can be developed.

Introduction

Alternaria spp. is important pathogens that cause diseases on several economic important crops including cereals, ornamentals, oil crops, vegetables and fruits. They are also well known post-harvest pathogens. The main goal of all diagnosis is to identify disease-causing organism in such a way which is fast, accurate and reliable. Basic methods used to detect the organism mostly rely on microscopic, cultural and morphological approaches that require extensive time, labor and classical taxonomy knowledge. Due to the limitations of conventional methods, molecular techniques came in use for the identification and classification of *Alternaria spp.*

Chemical control for plant disease has its concern on environment at large, thus as alternative concept of molecular Plant Pathology sets techniques for disease management. As an alternative to the application of chemical agents, researchers are altering the genetic composition of plants to enhance resistance to microbial infections (Christou, 2013).

Molecular approaches for identifications of *Alternaria spp.*

- Conventional Polymerase Chain Reaction
- Real Time PCR (quantitative PCR)
- Nested PCR
- Multiplex PCR (Duplex PCR)
- LAMP (Loop-mediated isothermal Amplification)
- RAPD (Random Amplified Polymorphic DNA)
- RFLP (Restriction Fragment Length Polymorphism)

Molecular approaches for management of *Alternaria spp.*

- QTL
- Genetic Transformation (*Agrobacterium*- mediated)

Identification

Kumar *et al.* (2013) developed real-time PCR based method using specific primers (AS1 and AS2). These primers were designed based on β-tubulin gene as a target for the specific detection of *Alternaria solani* (Ellis & Martin) Jones and Grout. The result revealed that the primer pair AS1 and AS2 was highly specific to *A. solani* as well as showing amplification product of 289bp in all 27 isolates of *A. solani*. The lowest detection limit of the real-time PCR assay with designed primer set (AS1 and AS2) was 0.5 pg/µl. The assay was also successfully validated on artificially infested tomato seedlings and able to detect *A. solani* up to 20 days post inoculation.

Qing *et al.* (2017) developed nested PCR based method using specific primers (ptAsQ-F/ptAs-R) based on the ITS region for the rapid, sensitive and specific identification of *A. solani*. They observed that primers ptAsQ-F/ptAs-R allowed the amplification of a product of approximately 251 bp. The lowest detection limit of the nested PCR assay with designed primer set was 10 fg/µl. This PCR assay was also successfully employed to detect *A. solani* in soil with the detection sensitivity of one conidia in 0.5 g of soil.

Khan *et al.* (2018) conducted a LAMP assay as well as conventional polymerase chain reaction (PCR), nested PCR and quantitative real-time PCR (RT-qPCR) assays to determine which of these techniques was less time consuming, more sensitive and more accurate for rapid on-site diagnosis of *A. solani* based on the histidine kinase gene (HK1). They noted that the LAMP assay provided more rapid and accurate results with amplifying the target pathogen in less than 60 min at 63˚C with 10-fold greater sensitivity than conventional PCR but less sensitive than nested PCR and qPCR. Nested PCR was 100-fold more sensitive than the LAMP assay and 1000-fold more sensitive than conventional PCR. The qPCR was the most sensitive among the assays evaluated, being 10-fold more sensitive than nested PCR for the least detectable genomic DNA concentration (100 fg/µl). Despite of the sensitivity, LAMP assay provided higher specificity than qPCR. The LAMP assay also allows detecting *A. solani* from naturally infected young potato leaves.

Management

Kumar *et al.* (2011) generated transgenic in sunflower *cv.* 'DRSF 110' using a tobacco PR-2 protein, β-1,3-glucanase that contributed to plant defences against *Alternaria helianthi* (Hansf.) Tubaki and Nishihara through *Agrobacterium*-mediated genetic transformation. They observed over expression of a tobacco β-1,3-glucanase gene in transgenic sunflower and showed its resistance towards *A. helianthi*. Further, molecular analysis by Southern dot blots and PCR and phenotypic GUS expression (β-glucuronidase) confirmed stable integration of the β-1,3-glucanase gene in sunflower transgenic and its generations.

Daley *et al.* (2017) created a high-density genetic map of a recombinant inbred line (RIL) population of 'MR-1' crossed with the susceptible Israeli 'Ananas Yokneum' and quantitative trait loci (QTL) analysis was conducted to identify QTL affecting *Alternaria cucumerina* (Ellis & Everh.) Elliott lesion area. The result revealed that multiple quantitative trait loci (QTL) mapping identified two QTL (qALB-10 and qALB-12) that explained 33.9 per cent of variation in lesion area. Several candidate genes within range of these QTL were identified using the *Cucumis melo* v3.5 genome.

Conclusion

From the foregoing discussion, it can be concluded that *Alternaria spp.* can be identified through molecular approaches like LAMP, Real-time PCR, Conventional PCR, Duplex PCR and Nested PCR. Early blight pathogen, *A. solani* can be detected by nested PCR with sensitivity of 10 fg/µl. In real-time PCR, the primer pair AS1 and AS2 showing amplification product of 289bp in all isolates of *A. solani* with lowest detection limit of 0.5 pg/µl. Loop-mediated isothermal amplification (LAMP) assay provided more rapid and accurate results with amplifying the target pathogen (*A. solani*) in less than 60 min at 63˚C. In molecular management approaches, multiple quantitative trait loci (QTL) mapping identified two QTL (qALB-10 and qALB-12) affecting *A. cucumerina* lesion

area. From these two QTL several candidate genes were identified. By using tobacco PR-2 protein, β-1,3-glucanase gene, *A. helianthi* resistant transgenic sunflower can be developed.

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