Proposal title:

Molecular mechanisms of fungicide resistance in *Alternaria solani* on potato

Target program: PhD

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Project background:

Advances in agriculture and the demand for high yields to fill the gaps in markets have led to grow enormous areas with different crops. And these expansions in growing accompanied by pests and diseases. Some of these diseases are becoming epidemic with the availability of suitable environmental conditions. In the latter type of diseases, the use of chemicals as a sole weapon to combat the disease are inevitable although there are environmental and public concerns. Another concern is the advent of resistance to chemicals especially when several consecutive applications of site specific chemicals are used. In integrated disease management (IDM), the use of chemicals is also play a key component in many plant diseases, nonetheless the appearance of resistance has become a major factor that limit the performance of the chemicals.

Currently, there are some major classes of site-specific fungicides that have been used to combat plant diseases, these include demethylation inhibitors (DMIs) and Quinone outside respiration inhibitors (QoIs). It has been well documented that after a few years of their advent and use, a decline in their performance has been appeared. Fungicide resistance can be conferred

by various mechanisms including: (I) an altered target site, which reduces the binding of the fungicide; (II) the synthesis of an alternative enzyme capable of substituting the target enzyme; (III) the overproduction of the fungicide target; (IV) an active efflux or reduced uptake of the fungicide; and (V) a metabolic breakdown of the fungicide. In addition, some unrecognized mechanisms could also be responsible for fungicide resistance. One mechanism that lead to the resistance towards these chemicals is the alterations and/or mutations in the sequences of the specific genes. Thanks to the molecular studies, the developing of resistance and detection of resistant genotypes of pathogens are become possible and easy to understand.

In our country, Southern Region of Kurdistan, and in Iraq in general, nothing is known about how far the plant pathogens are now resistant to the chemicals in general and to fungicides in particular. Therefore, we found that a study to investigate the fungicide performance and appearance of resistant fungal isolates of different plant pathogens are an imperative and needed to be explored. *Alternaria solani*, the cause of early blight of potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) is a destructive fungal plant pathogen causing severe damage leading to economic losses for growers every year. The pathogen mostly infects the foliage and produces dark brown lesions with concentric rings that enlarge, coalesce and eventually cause leaf death. Foliar application, using fungicides, especially QoI fungicides (strobilurins), is the main method to combat the disease. However, the decline of fungicide efficacy has been recorded in populations of *A. solani*. The resistant phenomenon is mainly related to the genetic diversity and alternations of cytochrome *b* gene in the pathogen's isolates. Due to the lack of any known studies about loss of sensitivity, this study is aimed:

1- To discover the pathogenic variability within *A. solani* populations
2- To explore genetic diversity of the fungal pathogen
3- To study the occurrence of DNA alterations in different isolates of *A. solani*
4- To assess the reduced sensitivity in the pathogen's isolates towards fungicides

Project plan:

Survey and sample collection

Samples of diseased plants will be collected from different locations and then the fungal pathogens will be isolated in pure cultures for later studies. Based on location, fungicides applied in the fields, isolates of each fungal pathogen will be purified and prepared for later use for detection of genotypes.

Diagnosis

The pathogen will be identified using a complimentary diagnostic technique that combine both microscope-based techniques and molecular techniques using PCR. This to discriminate other species of *Alternaria* from *A. solani*, because both potato and tomato are also infected with other species of *Alternaria* such as *A. alternata*.

Genetic diversity and characterizing the fungus

Phylogenetic trees will be generated after generating of multiloci markers using PCR technique such as inter simple sequence repeat (ISSR)-PCR, a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) with the universality of RAPD-PCR.

Sequencing and identification of substitutions in cytochrome *b*

Specific genes that related to chemical resistance will be amplified. This will be done with each single isolate of each pathogen. To compare the isolates and show the differences and for the detection of genotypes, the PCR products of the isolates will be purified and then sequenced and then aligned. Multiple alignment analysis of amino acid sequences will be performed using available softwares and compared with each other and then analyzed.

Detection of cross-resistance

This to assess the possibility of resisting the pathogen to one antimicrobial compound have also resistance to other fungicides within same fungicide group or fungicide in other different compounds.

In vitro sensitivity tests

The isolates of the fungus will be evaluated on agar medium amended with a series of fungicide concentrations. Then EC50 will be measured which to assess the efficacy of each fungicide.

In vivo sensitivity tests

In these trials, plants will be grown, inoculated with the fungal pathogen, and then sprayed with fungicides. The fungicide efficacy will be evaluated in different times and measured by using suitable disease evaluating scales.

References

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