Blackleg

Objective

To determine the presence or absence of ***Blackleg*** in each canola sample and, for positive samples, to identify the ***Blackleg*** race using a panel of avirulence gene markers.

Methodology

1. DNA Extraction
    - DNA will be extracted optimized for fungal DNA from plant tissue.
2. PCR for Presence Detection
    - Species-specific primers will be used to confirm the presence or absence of ***Blackleg*** in each sample.
3. Race Identification
    - For all ***Blackleg***-positive samples, a multiplex or individual PCR panel targeting Avr genes will be used to identify virulence profiles.

Clubroot

Objective

To quantify the spore load of ***Clubroot*** in canola soil samples. Quantification will be reported in spore equivalents per gram of sample based on Ct values obtained from Q-PCR analysis.

Methodology

1. DNA Extraction
    - DNA will be extracted using a method optimized for soil and root matrices.
    - Quality and concentration will be assessed prior to Q-PCR.
2. Q-PCR Setup
    - Q-PCR will be performed using TaqMan probes specific to *P. brassicae*.
    - Each sample will be run in triplicate. The mean Ct value was used for spore load estimation.

3. Standard Curve and Quantification
    - A standard curve will be established using serial dilutions of known *P. brassicae* DNA or quantified resting spores.
    - Results will be translated to resting spore equivalents per gram of root or soil sample.

Verticillium

Objective

To confirm the presence of ***Verticillium*** through PCR-based detection and quantify pathogen load in infected canola tissue using qPCR. Quantification results will be reported in gene copies per gram of tissue, aiding in disease severity estimation and field-level risk assessment.

Methodology

1. DNA Extraction
    - DNA will be extracted from lyophilized or freshly homogenized tissue optimized for fungal pathogen detection.
2. Conventional PCR Detection (Species Confirmation)
    - ***Verticillium***-specific markers will be used for confirmation of identity.
    - PCR products will be resolved on agarose gel (1.5%) and presence of an expected-size band (~230 bp) indicate ***Verticillium*** positivity.
3. Q-PCR Quantification
    - Species-specific markers and probe will be used for qPCR amplification.
    - A standard curve will be generated using known concentrations of ***Verticillium*** genomic DNA to estimate fungal load (copies/g tissue).