ABSTRACT

Black rot is a destructive disease of cabbage throughout the world caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson. It is a major disease for vegetable brassica production by small-holding farmers which causes substantial crop loss especially during warm, humid seasons. The Black rot pathogen is seed-borne and spreads in the vascular system of the leaf and stem. In the present study, the cabbage seeds obtained from various seed agencies have been tested in the greenhouse conditions for the presence of pathogen, incidence of disease. The collected seed samples were subjected to seed health testing methods like direct plating and liquid assay methods. The pathogen has been isolated and subjected to biochemical characterization and molecular characterization. DNA was extracted from isolates of *X. campestris* pv *campestris* followed by amplification with 16S rRNA, *est*A and *hrpF* genes specific primers**.** An amplicon of 730 bp, 777 bp and 619 bp was obtained specific to *X. campestris* pv *campestris*. The PCR assay described here is suitable for the rapid detection of *X. campestris* pv. *campestris* from *Brassica* seed, infected leaf and stem tissue, and for complementary testing of bacterial colonies.

The present study concentrated on the role of POX, SOD and CAT enzymes in the host-pathogen interaction of cabbage and *X. campestris* pv. *campestris* model. Temporal pattern of the enzymes were estimated in resistant (R) and highly susceptible (HS) cultivars after challenge inoculation with the pathogen using standard procedures. The temporal pattern of defense-related enzyme activities in R, and HS cultivars showed significant induction after pathogen infection when compared to their respective controls. The results of Native-PAGE analysis showed the change in isozyme pattern and the intensity of isoforms in resistant and highly susceptible cultivars. The suppression subtractive hybridization (SSH) was used to elucidate the resistance mechanism in *Brassica oleracea* var. *capitata* upon infection with *X. campestris* pv. *campestris*. A cDNA library was constructed enriched in differentially expressed transcripts in the resistant cultivar *Pusa mukta*. The transcriptional diversity in *B. oleracea* var. *capitata – X. campestris* pv. *campestris* interaction in resistant cultivar *Pusa mukta* was also elucidated. The 150 unigenes were obtained from the 500 randomly picked positive clones. The cDNA sequences obtained by performing SSH are termed as expressed sequence tags and submitted to

the dbEST database with the library name LIBEST_028420, *Brassica oleracea* var. *capitata* SSH Library with the accession numbers JZ585251 to JZ585260 and JZ585519 to JZ585523 (www.ncbi.nlm.nih.gov/dbEST).

The technique of PCR-SSCP is being exploited only to detect and diagnose human bacterial pathogens. The present study exploits this technique for the detection and diagnosis of *X. campestris* pv. *campestris* in crucifer seeds/planting materials. A 730-bp region amplified using 16S rRNA primers was subjected to restriction digestion followed by SSCP analysis (RFLP-SSCP) to identify polymorphisms, and the results were compared with the RAPD profile obtained using the phage M13 core sequence as a single primer. SSCP is a useful tool for the detection of mutations but large amplicon size can hinder secondary structure formation. Therefore, RAPD using an M13 primer is more efficient for phylogeny detection. The use of a novel PCR-based DNA fingerprinting method using both RAPD and SSCP to detect DNA sequence diversity is reported.

Black rot disease management with the identification of potential disease resistant sources like use of biotic or abiotic elicitors and utilizing an Integrated Pest Management (IPM) strategy including host resistance, planting disease free seed, avoiding transmission of the disease and proper sanitation will lead to reduction in the disease incidence. In the present study abiotic inducer *viz.,* 2, 6-dichloroisonicotinic acid (INA) and *Pseudomonas fluorescens* was used to study the efficacy to induce resistance against black rot of cabbage. The SSH was used to elucidate the differentially expressed genes in cabbage upon the application of *Pseudomonas fluorescens.* A total of 140 ESTs showed that, many defense related genes like peroxidase, catalase, superoxide dismutase were upregulated. Many transcripts related to signalling pathways and pathogen recognition were identified. The important finding of the study is the identification of the unigene belonging to the *SWEET* protein family in cabbage. The study is first of its kind where the resistant cultivar *Pusa mukta* in India has been assessed for its resistance to the bacterial pathogen. The cDNA sequences obtained by performing SSH are termed as expressed sequence tags and submitted to the dbEST database with the library name LIBEST_028425 *Brassica oleracea* var. *capitata* - Pfu SSH Library with the accession numbers JZ585524 to JZ58533.