CHAPTER-1  INTRODUCTION

In the present world, Fungi are the main causative agent for agriculture crop loss as compared to insects and weeds. Fungi causes upto 100 % crop loss, weeds caused upto 32% crop loss, animal pest caused upto 15-18% crop loss. It causes infection in field crop, harvested crops and leads to the variety of damage with characteristics features such as odour, change in flavor, loss in nutrients and germ layer destruction. It finally results in the reduction of quality and quantity of crops. Infection and loss are higher in the humid tropics than in cold temperate regions.

The genus *Colletotrichum* was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance (Dean *et al.*, 2012). The genus *Colletotrichum* causes anthracnose disease and ultimately causes damage to large number of crops such as: cereals, coffee and legumes (Bailey and Jeger, 1992; lenne, 1992). The high economic loss occurs due to post harvest infection of tropical and subtropical fruits such as avocado, banana and mango (Mordue, 1967; Jeffries *et al*, 1990). In the present century, about 25 plant diseases caused by different species of *Colletotrichum* namely, *C. gloeosporioides*, *C. capsici*, *C. falcatun*, *C. truncatum*, *C. sansevieriae*, *C. acutatum* and *C. coccodes* were reported. Out of all these species, *C. gloeosporioides* found to be more prevalent anthracnose pathogen as compared to others (Gautam, 2014). Some *Colletotrichum* species produce quiescent infections and some are endophytic on their host plant.

*C. gloeosporioides* requires optimum temperature of 25-28°C, pH range of 5.8-6.5 and high humidity for better growth. *C. gloeosporioides* involves hemibiotrophic mode of infection. Various media were employed for the growth and sporulation of *C. gloeosporioides* including Potato dextrose agar, lima bean agar, malt extract agar and oat meal agar. But oatmeal agar was reported to be the best media for sporulation in case of *C. gloeosporioides*. *C. gloeosporioides* is responsible for anthracnose disease. Anthracnose is caused by fungi that produce conidia within black fungal fruiting bodies called acervuli (www.infonet-biodivision.org). The pathogen infects intact, non wounded immature green fruit and leaves in the field. Therefore, spores germinate and form appressoria on the fruit surface. The fungus,
using its appressorium, enzymatically penetrates the cuticle and then remains as sub-cuticular hyphae until the post climacteric stage of growth is attained (Ponte, 1996).

Traditionally, the identification and characterization of *Colletotrichum* spp was done on the basis of morphological features such as colony, color, size, shape of conidia and appressorium, optimal temperature for growth, growth rate and presence or absence of setae (Von Arx, 1957; Smith *et al*., 1990; Gunnel *et al*., 1992; Sutton, 1992). The more conclusive way of identification and characterization of *Colletotrichum* spp was sequencing the ITS1 region (Mills *et al*., 1992; Sreenivasaprasad *et al*., 1992). The diversity within *C. gloeosporioides* was examined by Lu *et al*., 2004 by studying leaf endophyte of native forest trees. The cluster of *C. gloeosporioides* strain from strawberry and non-crop species was compared (Mackenzie *et al*., 2007).

The alternative tool for taxonomic study is molecular technique and it also acts as an important tool in species delimitation (Maclean *et al*., 1993). At present, morphological and molecular data was very helpful in intra specific differentiation in *Colletotrichum gloeosporioides* (Freeman *et al*., 1998; Abang *et al*., 2002).

*Colletotrichum* spp also cause infection to insects such as *C. fioriniae* infection was observed on hemlock scale insects in New England and *C. gloeosporioides* infection was identified on citrus scale insects in Brazil (Marcelino *et al*., 2008).

The complete genome of this pathogen is not yet sequenced but there are various genes identified which were involved in host defence and pathogenesis such as: **host defense:** Shpx2, Shpx5, Shpx6, Shpx12; PepCYP and **pathogenesis:** Cap 20; CgDN3; Pnl-1, Pnl-2; Pel-1, Pel-2.

Various fungicides are used to eliminate the disease/ infection of fungus. Pesticide can be sprayed as pre-harvest such as copper hydroxide, mancozeb, and copper sulfate products (these are routinely used from flowering through to harvest) (Dirou *et al*., 2005) and post-harvest fungicide generally used as spray or dips to those crops which are already infected with *C. gloeosporioides*. This treatment was employed to those fruits and crops which are shipped to overseas market (Dickman, 1993).
There are billions of dollars worth loss due to crop losses occur by various causative agents such as microorganisms (bacteria and fungi). There are various measures employed to control the plant disease. At present various pathogens adapt according to pesticide and chemicals employed to control the various diseases caused by pathogens. So, now there is continual need to develop safer and more effective ways to control the disease or to reduce the infection. In this condition, biotechnology shows great promise. The National Center for Food and Agricultural Policy (NCFAP) reported that genetically enhanced crops (some engineered for disease resistance, and others for insect resistance), increased crop yields by ~2.7 billion Kg in the United States in 2003, and also reduced pesticide use by ~23.2 million Kg and added $1.9 billion to growers incomes was published on Oct 20, 2004. Biotechnological methods for disease control are entirely dependent on basic research into the molecular mechanisms of disease resistance. Among the various components of disease management strategies, use of resistant variety is one of the most important components. Several mango varieties have been documented for susceptibility to anthracnose but none of them known with significant resistance to the disease. Kulkarni et al. (2009), suggested that development of resistant varieties is the most appropriate approach to control the disease. In order to develop more efficient and safer methods of disease control, it is first important to study the genes which are involved in various stages of pathogenesis. So, present study was undertaken to identify the genes involved in various stages of pathogenesis of *C. gloeosporioides* on various susceptible crops (apple, guava, papaya, kiwi, mango, peach, lemon and capsicum). The present study was carried out with the following objectives:

1. Isolation and identification of *Colletotrichum gloeosporioides* from infected plants.

2. Generation of random mutants of *Colletotrichum gloeosporioides*.

3. Pathogenesis assay of mutants on different fruits and detached leaves.

4. Identification of tagged genes in mutants deficient in causing infection.