Chapter 1

GENERAL INTRODUCTION
1. **General Introduction**

At present disease control in worldwide is mostly based on the use of toxic chemicals such as fungicides, bactericides and insecticides. Toxic effect of these chemicals on the environment and human health strongly forced researchers or breeders to search for new more efficient plant protection methods and resistant cultivars. Diseases decrease the yield quality and quantity of the crops as well as increase input costs of farmers in fields.

Utilising in particular the plant potentials to combat pathogens, the induced resistance (IR) may be an alternative, non-conventional, non-biocidal and ecologically-friendly approach for plant protection because it minimises the use of toxic chemicals for disease control.

Recently several studies have demonstrated that exogenous application of several biotic or abiotic compounds could trigger a response that mimics pathogen-induced resistance in plants. Commercial development of induced resistance agents and integration of the technology into crop production and protection is underway. Several chemical compounds and plant growth-promoting rhizobacteria (PGPR) have been reported to activate IR in several plants (Nancy et al., 2011; Myresiotis et al., 2011).

The non-protein amino acid β-aminobutyric acid (BABA) has been reported to induced resistance against a wide range of pathogens in a number of plant species (Jakab et al., 2001; Cohen, 2002).

Induced resistance is not based on direct defence activation by the inducing agent, but on a faster and stronger activation of inducible defense mechanism once the plant is exposed to pathogen.
This enhanced capacity to express basal defence mechanisms is called sensitization, potentiation or priming (Conrath et al., 2002).

BABA is known to enhance the activation of defense responses on pathogen encounter, through priming (van Hulten et al., 2006). The majority of research on priming of BABA is still remained unresolved. To further define the role of BABA in expression of resistance against *Alternaria*, work is carried out to better understand the physiological and molecular aspects of priming induced by BABA in *Brassica carinata* against necrotrophic pathogen *A. brassicae*. Following were the aspects focused,

- To investigate changes in oxidants and antioxidative enzyme activities.
- To investigate the expression pattern of defense related genes and WRKY transcription factor genes using semi-quantitative and Q-RT-PCR.

1.1 *Brassica carinata*

The *Brassica* are an important group of oilseed crops, constituting almost 13.2% of the world oil requirement. The genus *Brassica* includes a total of 41 species (Gladis and Hammer, 1990). Six of these are economically important species, namely, *Brassica rapa* (AA), *Brassica oleraceae* (CC), *Brassica nigra* (BB), *Brassica Juncea* (AABB), *Brassica napus* (AACC) and *Brassica carinata* (BBCC). Among these six species *B. carinata* showed better performance under late sowing, irrigated and saline soil conditions (Knowles et al., 1981; Singh et al., 1988; Malik, 1990). The farmer can expect an average leaf and shoot yield of 35 t/ha, but at research stations
leaf yields of 50–55 t/ha have been reported, depending on production season and cultivar. In India and Canada farmers may get seed yields of 1200–1800 kg/ha in a good year.

*Brassica carinata* A. Braun (Ethiopian mustard) is an amphidiploid species (2n = 34, BBCC) derived from the diploid species *Brassica nigra* (L.) Koch (2n = 16, BB) and *Brassica oleracea* L. (2n = 18, CC). It is mainly used as a vegetable, a condiment or salad, green manure. The seed is used in folk medicine to treat stomach-ache. The oil has limitations for cooking because of high contents of glucosinolates and erucic acid. Being a semi-wild species, but domesticated for indehiscent pod, fall or spring seeded in our conditions, with good growth, *Brassica carinata* could be a candidate for biodiesel production and for the preparation of special erucic acid derivatives.

*B. carinata* has good resistance to blackleg, *Leptosphaeria maculans* (Desmaz.) (Gugel et al., 1990), and white rust, *Albugo candida* (Pers.) Kunze (Singh and Singh, 1988) some accessions are also more resistant to alternaria black spot, *Alternaria brassicae* (Berk.) Sacc., than other *Brassica* crop species (Bansal et al., 1990).

1.2 *Alternaria brassicae*: structural features, symptoms and disease cycle.

Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. is an important disease of Brassica spp. which has been reported from all the continents of the world, causing 10-70% yield losses depending on the crop species (Chattopadhyay, 2008). There is no proven source of resistance against the disease reported till date in any of the host (Kumar and Chauhan, 2005; Chattopadhyay, 2008).
*Alternaria brassicae* (Berk.) is a member of the deuteromycetes and has large, multicellular, dark coloured (melanized) conidia with longitudinal as well as transverse septa. Conidia usually single but sometimes produced in chains of up to four when formed in agar cultures. Conidia is obclavate, with a conspicuous beak, smooth, greyish-olive, with 11-18 cross septa, and 0-8 longitudinal ones, slightly constricted at the septa, 39-350 x 9-42 µm. The conidia are called "porospores" or "poroconidia", because they arise from a protrusion of protoplasm through a pore in the wall of the conidiophore. Conidiophore is dark olive, 0-7-septate, geniculate, with a prominent scar at each geniculation, 14-48 x 6-13 µm.

*A. brassicae* can infect virtually any part of the plant with visible symptoms of infection, which includes chlorotic and necrotic lesions on the leaf, petiole, stem, inflorescence, siliqua and seed (Verma and Saharan, 1994; Kolte et al., 1988). *A. brassicae* is considered to be most virulent on oil- yielding brassicas and vegetable crucifers.

Infections on host plants begin directly from infested seed, from spores produced on crop residue, from infections on brassicaceous weeds or possibly from microsclerotia and chlamydospores, if produced, on crop residue. Warm and moist conditions favor spore germination and infection. Stem and leaves are principal sites of infection. Once in contact with wounded host tissue, the conidia start germinating after about 9h by giving rise to a germ tube. Penetration of the undamaged host tissue occurs after about 24h. This germ tube forms spherical or club shaped appressoria which invade the epidermal cells. The mycelium grows through and between mesophyll and palisade cells. The visual sign of infection is formation of dense grey lesions, about 1-2 mm in diameter, showing 2-3 concentric rings which start developing within 48h and are conspicuous at 72h. The lesions contain brown conidiophores and conidia. The
conidia get dispersed by wind and form secondary infection sites when they land on plant tissues. Infection of pods leads to their shattering and infected seeds are released.

In the present study we have studied different parameters to understand the priming of BABA induced resistance in *Brassica carinata car6* (susceptible) against *A. brassicae* such as oxidative and antioxidative enzyme systems, defense related genes and WRKY transcription factor genes.