VARIABILITY IN *Albugo candida* (Pers.) Kuntze CAUSING WHITE RUST OF RAPESEED-MUSTARD

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
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MASTER OF SCIENCE
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PLANT PATHOLOGY
(Minor Subject: Entomology)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **"Variability in *Albugo candida* (Pers.) Kuntze causing white rust of rapeseed-mustard"** submitted for the degree of **Master of science,** in the subject of **Plant Pathology** (Minor subject: **Entomology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Yash Pal (L-2009-A-91-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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(Dr. P. S. Sandhu)
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CERTIFICATE II

This is to certify that the thesis entitled, **"Variability in *Albugo candida* (Pers.) Kuntze causing white rust of rapeseed-mustard"** submitted by **Yash Pal (L-2009-A-91-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of science** in the subject of **Plant Pathology** (Minor subject: **Entomology**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination of the same.

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Place…….. ……..

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ABSTRACT

White rust caused by *Albugo candida* (Pers.) Kuntze is a serious threat to the production of oilseed *Brassica* crops in India and around the world. Variation occurs w.r.t. infection of the pathogen on different species of oilseed *Brassicas* which encouraged us to carry out the studies on "Variability in *A. candida* (Pers.) Kuntze causing white rust of rapeseed-mustard". Survey was conducted in different locations (Bathinda, Mansa, Barnala, Faridkot, Muktsar, Sangrur, Ferozepur, and Ludhiana) of Punjab to estimate disease incidence and severity of the disease. The disease incidence and severity of white rust ranged from 44.39-59.36 and 21.92-27.83 per cent, respectively, in different locations of Punjab. A total of 52 isolates were collected from different locations and varieties. These isolates were classified into three groups (AC-I, AC-II and AC-III) on the basis of pustule size, pustule shape and germination of sporangia. Three representative isolates of each group were tested on twelve differential host cultivars (*B. juncea, B. nigra, B. napus, B. carinata, B. oleracea, B. tournifortii, B. rapa* var. *Toria*, *B. rapa* (Brown *sarson*), *B. rapa* (Yellow *sarson*), *Raphanus sativus, Sinapis alba* and *Eruca sativa*) of rapeseed and mustard. AC-III group has most virulent and AC-II group showed least virulence based on disease reaction, incubation period, latent period, pustule size, shape, number of pustules per leaf and number of sporangia per pustule. Further, within AC-III group, Ac6 isolate was observed as more virulent than other isolates of same group. Hence, it is concluded that morphological and pathological variation exists in *A. candida* population from Punjab.

Keywords: *Albugo candida*, differential hosts, isolates, rapeseed-mustard, variability.

Signature of Major Advisor **Signature of the Student**

CONTENTS

CHAPTER - I

INTRODUCTION

Rapeseed-mustard is a group of cruciferous crops and comprise of *toria* (*Brassica rapa*), *taramira* (*Eruca sativa*), *raya* (*B. juncea*), *gobhi sarson* (*B. napus*) and African *sarson* (*B. carinata*). In trade; *toria*, *taramira* and *gobhi sarson* are categorized as rapeseed while *raya* and African *sarson* are categorized as mustard. Amongst crucifers, genus *Brassica* comprises most of the important oleiferous and vegetable crops. Among oleifers, *B. napus*, *B. juncea* and *B. rapa* are the important oilseed crops containing about 35 per cent oil. *B. napus* is grown in temperate regions, particularly Europe and Canada for oil and fodder, whereas, *B. juncea* (Indian Mustard/*Raya*) is a main source of cooking oil in Asia, Europe and Africa. Asian countries account for more than two-third of the world's area and production under these crops. Rapeseed-mustard comprise important source of cooking oil in India (Cobley and Steel 1976). In India, these crops are grown in 5.59 million ha area with annual seed production of 6.61 million tonnes (Anon 2010a) and rank third in the world next only to China and Canada. Rapeseed and mustard are mainly grown in the north and north-western states of India *viz.* Rajasthan, Utter Pradesh, Punjab, Haryana, Madhya Pradesh, Assam, Gujarat, West Bengal, Orissa and Bihar during winter season.

In Punjab, rapeseed-mustard is grown over an area of 30,000 ha with production and productivity of 39,000 mt respectively (Anon 2010a). This level of productivity is not satisfactory in view of the demand for domestic consumption. Various endeavors including improved methods of cultivation, proper fertilization and use of improved varieties have currently been pursued to boost the productivity of various oilseed crucifers with limited success. Diseases are the important limiting factors in realizing full yield potential of these crops.

Several important diseases like Alternaria blight (*Alternaria brassicae*), white rust (*Albugo candida*), downy mildew (*Peronospora parasitica*) and Sclerotinia rot (*Sclerotinia sclerotiorum*) create a serious threat to successful cultivation of rapeseed-mustard. Among these diseases, white rust caused by an obligate parasitic fungus, *A. candida* (Class: Oomycetes, Order: Peronosporales and Family: Albuginaceae) is one of the most important disease and is a major limiting factor to the successful cultivation of rapeseed-mustard (Saharan and Verma 1992).

This disease was considered to be of little economic importance in the past, but in recent years, it has gained the importance of being the most widespread and destructive disease of rapeseed-mustard in the country particularly in the north and north western regions. From India, white rust was first reported by Butler (1918) and then by Mitter and Tandon (1930). This disease is of regular occurrence on oilseed *Brassica* crops. The first appearance

of the disease is observed on lower leaves of plants in the early January. Thereafter, it rapidly spreads to upper leaves and other aerial plant parts. Two types of infection are observed, local and systemic. Local infection is characterized by the formation of isolated raised creamy-white sporangial pustules on leaves. Systemic infection of white rust is mostly seen in young inflorescence. The pathogen stimulates hypertrophy and hyperplasia resulting in abnormal swelling and malformation of affected organs. Affected stem may become swollen up to a considerable length and show conspicuous bending due to unequal expansion of tissue on both sides. When the inflorescence is affected, it turns into stag-head. Floral organs turn green, become enlarged and distorted. Seed formation is prevented and pods become curved or twisted.

Kolte (1985) reported 47 per cent loss in yield due to white rust. In *toria*, the loss due to white rust may exceed the above figures, if infection of white rust occurs in combination with downy mildew (*P. parasitica*), particularly when *toria* is planted after second fortnight of October in Uttar Pradesh (Kolte 1985, Bains and Jhooty 1979). Staghead incidence is directly correlated with loss in yield. Significant yield losses in the range of 20-60 per cent have been reported in heavily infected crops by various workers (Berneir 1972, Bains and Jhooty 1979, Kolte *et al* 1981, Saharan *et al* 1988, Lakra and Saharan 1989, Saharan 1998, Meena *et al* 2002, 2011, Kaur *et al* 2008). In Canada, the systemic infection of white rust was reported to cause an average loss of 60 per cent in seed yield (Petrie and Vanterpol 1974).

Such a high level of losses can be prevented by implementation of effective disease management practices. Understanding the pathogen population structure and mechanisms by which variation arises within populations leads to successful execution of a disease management programme. Biological specialization in *A. candida* has been noted long back based on morphological, physiological, biophysical and biochemical methods (Biga 1955, Eberhardt 1904) but no specialized races were described. Variation in pathogen populations is detected with standard sets of differential hosts (Flor 1971). While several methods of classification have been proposed, the method described by Pound and Williams (1963) based on host specificity is generally accepted. *A. candida* is a highly specialized pathogen and a number of biological races have been identified and classified based on specificity to different crucifer species (Pidskalny and Rimmer 1985, Hill *et al* 1988, Petrie 1988). Pound and Williams (1963) studied the biological specialization in *A. candida* and reported six races: race 1 on *R. sativus*, race 2 on *B. juncea*, race 3 on *Armoracia rusticana*, race 4 on *Capsella bursa-pastoris*, race 5 on *Sisymbrium officinale* and race 6 on *Rorippa islandica*. Later on, race 7 on *B. rapa* (Verma *et al* 1975, Pidskalny and Rimmer 1985, Petrie 1988) and race 8 on *B. nigra* (Delwiche and Williams 1977) were reported. In addition to eight races, race 9 and 10 on *B. oleracea* and *Sinapis alba* respectively were reported by Hill *et al* (1988) while race 11 on *B. carinata* was reported by Williams (1985).

Although biotypes from *Brassicas* are most virulent on the species from which they were originally isolated, they can also infect some genotypes of related *Brassica* species. Specificity also occurs even among cultivars within a species. This fact helps to monitor the changes in virulence among and within the races and distribution of different biotypes of *A. candida* in different rapeseed mustard growing areas using host differentials.

Since the information on *A. candida* variability is scanty in Punjab, therefore, the present investigations were carried with the following objective:

Objective

- To study variability in *Albugo candida* populations causing white rust of rapeseed-mustard in Punjab.

CHAPTER - II

REVIEW OF LITERATURE

The available literature on white rust of rapeseed and mustard caused by *Albugo candida* (Pers.) Kuntze in respect of geographical distribution, host range, economic importance, symptomatology, survival, pathological specialization, effect of temperature on sporangia germination, biological specialization, host-pathogen interaction, disease scoring scale and epidemiology of disease development has been reviewed as given below:

2.1 Geographical distribution and host range

White rust disease is found to be more prevalent in temperate climate countries such as Canada, France, Germany, India, Japan, Pakistan, Palestine and Turkey (Kolte 1985). In India, the disease appears in varying incidence on rapeseed-mustard crops (Chowdhary 1944, Vasudeva 1958, Bains and Jhooty 1979, Kolte and Tewari 1980, Kolte *et al* 1981, Chaurasia *et al* 1982, Kolte 1982, Lakra and Saharan 1988).

A. candida has a wide host range. It attacks almost all the members of cruciferae family and some of the members of Capparaceae (or Capparidaceae) and Cleomaceae (Walker 1957). It has been reported on *B. rapa*, *B. juncea*, *B. oleracea*, *Capsella bursa-pastoris*, *Cardamine hirsuta*, *Cleome spinosa*, *Lepidium oleraceum*, *L. sativum*, *Malcomia maritime*, *Raphanus sativus* (Baker 1955). Morris and Knox (1980) reported *A. candida* on a weed host (*Raphanus raphanistrum*). Parisi (1924) reported the fungus on non-cruciferous host, *Onobrychis crista* and this fungus was considered to be a strain of *A. candida*.

2.2 Economic importance

White rust of rapeseed-mustard is a major constraint in the successful production of oilseed crucifers. In most of the rapeseed-mustard growing countries particularly in Canada (Downey and Bolton 1961, Petrie 1973, Harper and Pittman 1974) and India (Vasudeva 1958, Kolte and Tewari 1980, Kolte 1981, Kolte 1982, Kolte 1985, Lakra and Saharan 1988) white rust is observed as a serious threat to its productivity. Petrie (1973) reported that the turnip rape (*B. campestris*) in western Canada was affected by white rust incidence in the range of 20-50 per cent. The estimated losses in Saskatchewan (Canada) resulting from hypertrophy of the inflorescence in *Brassica campestris* in 1970, 1971 and 1972 were worth 1.68, 4.13 and 2.43 million dollars, respectively. Petrie and Vanterpool (1974) from Canada reported that systemic stem infection causes an average reduction of 60 per cent in seed yield. Petrie (1975) reported that in Saskatchewan (Canada) the average yield reduction in turnip rape (*B. campestris*) for the year 1970 to 1973 was 6 per cent.

Bains and Jhooty (1979) reported that in the case of mixed infection with *A. candida* and *Peronospora parasitica*, the infected plants yielded 37.2, 47.2 and 40.0 per cent less pods and 22.2, 32.2 and 17.2 per cent less grains during 1975-76, 1976-77 and 1977-78 crop

season, respectively. In Chinese mustard, mixed infection by *A. candida* and *P. parasitica* in Punjab was 29.0, 0.5 and 19.2 per cent in 1975-76, 1976-77 and 1977-78 crop season, respectively. Infected plants produced 37.0-47.0 per cent lesser pods and 17.0-32.0 per cent less seed.

Annual yield losses to the tune of 10 per cent (range 5-10 %) due to stagheads in rapeseed were reported in Western Australia (Barbetti 1981). Kolte (1985) reported that the systemic infection results in malformation of the whole plant or systemic staghead phase of infection causing racemes to develop into malformed floral organs results in considerable damage and the consequent yield loss. Kolte *et al* (1986) reported that the the crop showed disease incidence 10.38 per cent as well as severity 23.43 per cent when sown in November. The disease was successfully managed with the cultivation of resistant cultivars in Saskatchewan, Canada (Petrie 1985). This resulted in a significant decline in the staghead incidence on B. *campestris* over the four years, though a new race of *A. candida* (race 2) became more prevalent on *B. juncea* (Petrie 1985).

2.3 Symptomatology

Two types of infection *viz.* local and systemic were observed by Butler (1918) and Walker (1957). In case of local infection, symptoms of the disease appear on leaves and are characterized by the appearance of white or creamy yellow raised pustules (1 to 2 mm in diameter) which later coalesce to form large patches. The pustules are found scattered on the under surface of the leaves. The upper surface, corresponding to the pustules on the lower surface, become yellow and the prevalence of the disease is easily recognized from the upper surface of the affected leaves. After the complete development of the pustule, it ruptures and releases a chalky dust of spores.

The symptoms of systemic infection are distortion, hypertrophy, hyperplasia and sterility of inflorescence. This phase of infection has been referred to as the staghead (Petrie 1973, Maheshwari *et al* 1985). The affected flowers show malformation. The petals become green sepal like and stamens may be transformed into leaf like or carpelloid structures. The petals and stamens persist in the flower instead of falling early as in the case of normal flowers. The stamens sometimes get changed into thick, club-shaped sterile bodies. The ovules and pollen grains are usually atrophied resulting in complete sterility (Kolte 1985). Yield loss is greater from systemic than local infection (Petrie 1973, Harper and Pittman 1974, Verma and Petrie 1980). At maturity, stagheads are entirely composed of brown thick walled oospores, which can survive in dry storage for a period of over 20 years (Verma and Petrie 1975).

Raabe and Pound (1952) reported that white rust (*A. occidentalis*) symptoms on spinach appear as small chlorotic areas on upper and lower surface of leaves. As these areas enlarge, the chlorotic conditions become more pronounced on the upper surface, while the

conidial pustules begin to appear as small whitish areas on the lower surface. They are blister like, oval, irregularly oval or elongated varying in size from 0.5 to 2.0 mm in diameter and 3 to 4 mm in length.

Ho and Edie (1969) have given description of symptoms of white rust (*A. ipomoeae-aquaticae*) on water spinach and they reported that in addition to foliar symptoms, the host plants are found to react to infection by producing two type of galls on stem, inflorescence and roots.

2.4 Epidemiology

The pathogen, *A. candida* and other *Albugo* species have been reported to survive in the diseased crop debris in the soil (Butler 1918, Takeshita and Linn 1953, Walker 1957, Singh 1978, Petrie 1973, Harper and Pittman 1974, Verma and Petrie 1975, Kolte 1982, Kolte 1985, Petrie 1986).

Melhus (1911) reported that chilling of sporangia is essential for production of zoospores and after chilling, the germination of sporangia occurs over a temperature range of 1 to 20°C. Optimum temperature for sporangial germination of *A. occidentalis* was 12°C. Germination decreased sharply with increase or decrease in temperature above or below 12°C (Raabe and Pound 1952). *A. candida* sporangia germinated at an optimum temperature range of 10 to 15°C but the zoospores germinated at 10 to 20°C (Endo and Linn 1960). The most favourable temperature for germ tube elongation was 15 to 25°C, however, at relative humidity less than 90 per cent, the sporangia lost their viability even at 15 to 20°C. The fungus grows most rapidly through the leaf and sporulated most abundantly at 15 to 20°C.

Sempio (1940) reported the effects of various environmental factors on infection of red radish by *A. candida* during the three main phases of infection (1-3, 4-7 and 7-10 days after inoculation). Most favourable temperature for infection was approximately 16-18°C, though the disease developed readily at 12-21°C. The lowest and highest temperatures at which infection occurred were 6-7°C and 28-29°C, respectively. Temperature above 24°C was critical for the host- parasitic complex and this leads to reduction in the intensity of attack and delay in the appearance of symptoms. At 60-80 per cent relative humidity infection was more severe as compared to 98-100 per cent. Takeshita and Linn (1953) reported that in horse radish white rust caused by *A. candida* survive mostly in the hypertrophied petioles of crops in soil. Vanterpool (1959) observed excellent germination of *A. candida* oospores at 10-12°C within 7-10 days in water.

Edie and Ho (1970) reported that sporangial germination of *A. ipomaeae-aquaticae* could occur between 10 and 35°C. The optimum temperature was about 22°C. Zoospore germination was more sensitive to temperature than sporangial germination and was restricted in the range of 12-30°C with an optimum temperature of 25°C. They further showed that at 20-25°C sporangial germination occurred very rapidly with around 25 per cent germination

take place after half hour and the maximum of over 80 per cent after 3 hrs. The maximum rate of elongation of zoospore germtube was observed at 25°C and ceased after 8-10 hrs at 15 and 28°C. Petrie and Verma (1974) observed that when oospores scattered over the surface of a sintered glass bacteriological filters of ultrafine porosity and sterile tap water dripped slowly through it, the oospore germination starts on 10^{th} day and upto 70 per cent of them germinated by the 2^{nd} week.

Petrie (1986) reported that seed-borne oospores of *A. candida* on radish serves as primary infection. Verma and Petrie (1975) reported that high percentage of *A. candida* oospores from hypertrophied inflorescence of *B. campestris* were germinated by incubating them on moist paper for 21 days and at 10-20°C. Vyalykh and Zheryagin (1977) reported that *A. tragopoganis* infection was highest at 16.7 to 18.4°C with a direct correlation between spore germination of the pathogen and infection at the same temperatures. The number of infected plants reduced when the drops of inoculum remained for less than 4 hrs on the leaves. Germination was rapid at 15°C with the first zoospore emerging after only 30 minutes.

Hartmann and Watson (1980) reported that *A. tragopogi* sporangia germination varied at 5 to 30°C both under light and dark conditions. At optimum temperature of 15°C, 65 per cent germination was recorded after 12 hrs interval. Sporangial germination was substantially reduced at 20°C or more. Leu and Rimmer (1990) reported that *B. napus* showed severe infection of *A. candida* when night and day temperature ranged between 17-22°C as these conditions were found to be more favourable for fungal growth compared to the temperature range of 10-15°C.

Sakai (1981) reported the infection by *A. macrospora* on turnip and Chinese cabbage in Taiwan. In spring crop, the disease occurred first in April increasing rapidly till mid May. In autumn crop, it started in early October increasing gradually till early December. In field temperature for sporangia and zoospore germination was favourable in the range of 10-20°C. The suppressed germination was observed at 5°C, whereas, it was better at 15°C. Singh and Singh (1983) reported that the maximum disease intensity could be obtained when Indian mustard (*B. juncea*) was inoculated with sporangia germinated at 10°C and applied to the lower leaf surface and the plant covered for 48 hrs. Verma *et al* (1983) reported that white rust developed most rapidly at 21°C on detached *B. campestris* leaves infected with *A. candida* race 7. The maximum number of pustules occurred for 14 days after inoculation at 15 to 18°C, although at 14 days after inoculation there was little difference in the per cent infected leaves at incubation temperature of 12 to 24°C

While studying the effect of temperature, leaf age and time of leaf detachment on the development of white rust (*A. candida*) on detached leaves of *B. campestris* cv. Torch, Verma *et al* (1983) found that the best disease development took place at 21°C. Medium aged leaves supported maximum pustules development followed by the younger leaves. Leaves developed

more pustules when detached in dark period than at the end of a light period. Lakra *et al* (1989) investigated the effect of temperature, relative humidity and light on germination of *A. candida* sporangia from mustard. They found that 12-24°C temperature was the most favourable for sporangial germination.

Kumar *et al* (1986) reported that infection occurred on all ages but medium-aged leaves showed the maximum disease followed by the young leaves. They also reported that the mean increase in pustules was the highest in cultivar Prakash (16.0) followed by that in YRT 3 (3.2) and the least in RC 781 (1.1). Barbetti (1981) reported that in Australia when sowing was delayed in field from early June to mid July, there was reduction in the incidence of leaf and staghead infections. The early sowing and the level of oospores contamination with seed contributed to the final staghead incidence. Verma and Bhowmik (1988) reported that the germination was induced in oospores by continuous leaching with water for 15 days at 15°C.

Field experiments carried out at IARI, New Delhi during 1983-86 seasons on Indian mustard (cv. Pusa Bold) indicated that mean temperature (MT) less than 16°C and mean relative humidity (MRH) more than 60 per cent are essential for the development of disease. Infection rate was the highest (0.57) when MT and MRH was 14.7°C and 73.25 per cent, respectively and rainfall was around 230 mm. Infection rate declined with the increase in MT and decrease in MRH. Long sunshine hours for prolonged periods coincided with poor white rust development (Verma and Bhowmik 1989). Incubation period at 8-26°C was longer than at 10-31°C but white rust intensity was higher at low temperature (Verma and Bhowmik 1989). Medium aged turnip rape (*B. compestris*) show maximum infection than younger and older ones. At Pantnagar, high incidence of stagheads due to white rust and downy mildew (*P. parasitica*) infection, was obtained with only 2-6 h sunshine per day associated with mean maximum and minimum temperature of 21-25°C and 6-10°C respectively, MRH of 68-73 per cent and rainfall upto 161 mm (Kolte *et al* 1985). At Hisar, rainfall more than 100 mm, MT 10-18°C, MRH more than 65 per cent, potential evapotranspiration less than 60 per cent, cloudy weather and wind velocity of 2.7 - 3.4 km/h favoured faster increase in the number and size of pustules and epidemic development of the disease (Lakra and Saharan 1991, Saharan *et al* 1988).

2.5 Survival

Butler (1918) reported the survival of the fungus through oospores in soil which serve as source of primary inoculums, while Petrie (1974) emphasized the possibility of survival of the pathogen through infested seeds. They detected *A. candida* oospores in 468 out of 585 seed samples of turnip rape and in 20 out of 25 samples of rape in western Canada during 1967-73. Verma and Petrie (1980) investigated that oospores of *A. candida* race 7 mixed with cv. Torch seeds before sowing resulted in significant increase in both locally and systemically

infected plants over the control. Barbetti (1981) reported the high level contamination of seeds with oospores contributed to final staghead incidence.

Verma and Bhowmik (1988) ruled out the possibility of soil borne oospores as source of primary inoculum. They further reported that samples of six *B. juncea* cultivars and one of *B. campestris* cultivar showed heavy contamination with 12.6-81.0 oospores/gm seed.

According to Kajornchaiyakul and Brown (1976) sporangia of *A. tragopagi* stored in MC Corlney bottle at -18°C remained viable for 6-12 months. Pound and Williams (1963) collected *A. candida* sporangia in number-00 gelatin capsules, using a small mouth operated cyclone separator and stored them at -10^{o}C. Later, Verma *et al* (1975), Fan *et al* (1983) and Fox & Williams (1984) used the same method for the preservation of white rust inoculum.

2.6 Host-pathogen interaction

Albuga candida enters the resistant host through stomata as readily as in susceptible host, but in resistant host, the growth of pathogen usually ceases in the substomatal chamber. The development of intercellular mycelium and production of haustorium in a susceptible host is very fast (Napper 1933). The *A. candida* zoospore produce germtube, which formed appresoria on contact with any epidermal cell and only those appresoria which formed adjacent to stomatal pore produce an infection peg. Germination of sporangia on leaf surface and penetration of the host tissue was studied on artificially inoculated plant by whole-leaf clearing technique (Shipton and Brown 1962).

Verma *et al* (1975) compared the infection process of *A. candida* in susceptible (*B. juncea, B. campestris*), moderately susceptible (*B. hirta*) and resistant (*B. napus*) by inoculating cotyledons with zoospores of the pathogen. They observed similar series of events from encystment of zoospores to the formation of first haustorium in all hosts. The resistance was manifested after the contact between hyphal tip and host mesophyll was established. There was apparently no morphological barrier to zoospore encystment and the subsequent penetration through stomata. Thickness of cuticle-epidermis, palisade and spongy layers of susceptible *B. juncea* (cv. Pusa Bold), moderately resistant (cv. NA38) and immune (cv. NA38 Scl) *B. napus* plants, bear no relationship to their reaction to *A. candida.* In susceptible host-parasitic combination, the hyphal growth rate of the pathogen increases rapidly after the formation of first haustorium, the hyphae develop around palisade mesophyll cells, penetrating individual cells with a variable number of haustoria. In resistant host (*B. napus*), the process is much slower and usually one haustorium is formed after which the hyphae cease to elongate and at 2-3 days after inoculation, a marked encapsulation is formed around each single haustorium. On the other hand, in susceptible hosts at about 3-4 days after inoculation, the first sign of a development of pustule is observed and their cells contain massive amount of fungus thallus emphasizing highly specialized type of parasitism evolved by the pathogen (Verma *et al* 1975).

Resistant reaction is characterized by long incubation period, small discrete, chlorotic or necrotic lesions, low disease intensity and low sporulation in contrast to short incubation period, large spreading lesion, higher disease intensity and higher sporulation exhibited by susceptible reaction (Bhardwaj and Sud 1989).

Verma and Petrie (1980) suggested two possible mechanisms of staghead development: (i) Early infection of young seedlings starting with oospore-infected seeds and infection progressing throughout the development of the plants and (ii) infection of young flower buds by zoospores arising from wind-borne sporangia. According to them, oospores of *A. candida* race 7 mixed with seeds of turnip rape prior to sowing results in high levels of leaf and staghead infections. Over 55 per cent staghead infections were induced by flower bud inoculation. Petrie (1988) reported distinct 4 races of *A. candida* (white rust and staghead) from *Raphanus sativus, B. juncea* cv. Southern Giant curled, *Capsella bursa-pastoris* and *B. campestris* sub. sp. *pekinensis* cultivars. Some *sarson* accessions (*B. campestris* sub sp. *sarson*) were susceptible to both the *B. juncea* and *B. campestris* races.

Lakra and Saharan (1989) although could not initiate staghead infection on *B. juncea* by inoculating flower buds with zoospores-sporangial suspension but were able to get more than 66 per cent stagheads when 5 g/pot of oosporic inoculum was added with the seed at sowing time, along with subsequent sporangial spray inoculation at the seedling, branching and flowering stages of the crop. Systemic infection was found to be directly correlated with the host age. With increase in age, a sharp decline in the ability to induce systemic infection occurs.

Bains (1991) observed development of identical symptoms on the different cultivars and upto 40 per cent of flowers were aborted before pods formed, out of the matured pods 17-30 per cent were deformed. Variation in pod symptoms was attributed to the stage at which ovaries or young pods developed infection, differences in inoculum load and changes in weather conditions.

Awasthi *et al* (1995) reported interaction between *Peronospora parasitica* and *A. candida* in relation to development of stagheads in *B. juncea* and *B. campestris* under field conditions. When susceptible accessions of *B. juncea* were inoculated with *A. candida* and *P. parasitica* under epiphytotic conditions (90% RH, 19°C and 16 hours light) at flower initiation stage, staghead incidence of 64 per cent was observed. But the plant inoculated with *P. parasitica* alone did not show any evidence of the staghead development at the sililar set of inoculation conditions.

2.7 Biological Specialization

Biological specialization in *A. candida* has known from long time ago. Eberhardt (1904) recognized two specialized groups of the fungus; one attacking *Capsella, Lepidium* and *Arabis,* and the other attacking *Brassica, Sinapis* and *Diplotaxis* on the basis of host

specialization.

Hiura (1930) reported three strains of *A. candida* on the basis of cross inoculations on a number of cruciferous plants. Each strain exhibited a different host range and was classified as a distinct biological strain of the fungus. The strains were described as: strain 1 occurring on *Raphanus sativus*, strain 2 on *B. campestris* sub sp. chinensis and strain 3 on *B. juncea,* Chinese mustard. The strain 1 from *Raphanus* is capable of infecting all the varieties of radish but none of the other plants. The strain 2 from *B. campestris* var Chinensis infected herb mustard (*B. japonica*), *B. campestris* sub sp. chinensis var. Komatsuna, turnip *B. campestris, B. pekinensis*, *B. juncea* and its original host with varying degree of severity, but none of the other crucifers. The strain 3 from *B. juncea* was capable of attacking *B. cernua, B. campestris* sub sp. chinensis and its own host with varying severity but failed to infect radish. Napper (1933) described 21 different biological races of *A. candida* on the basis of cross inoculations on 25 species belonging to 13 genera of the cruciferae. All 21 biological races of *A. candida* can infect *B. alba*. However, some races usually the most common and generally distributed (eg. those on *Capsella bursa-pastoris* and *Arabis alpina*) have an overlapping host range which may extend to some six different genera.

Togashi and Shibasaki (1934) found that sporangia of *Albugo* from *Brassica* and *Raphanus* were 20.0 x 18.0 µm in size, while those from *Cardamine, Capsella, Draba* and *Arabis* measured 15.5 x 14.5 µm; these were classified as macrospora and microspora, respectively. They reported five distinct biological forms of *Albugo*. Savulescu (1946) recognized eight different *A. candida* forms specialized on the basis of their reactions on different cruciferous hosts. These were:

1. *A. candida* f.sp. alysii-alyssoidis, 2. *A. candida* f.sp. brassicae-nigrae, 3. *A. candida* f.sp. capsellae-bursal pastoris, 4. *A. candida* f.sp. coronopi-procumbontis, 5. *A. candida* f.sp. hesperidis-matronalis, 6. *A. candida* f.sp. lepidii-perfoliati, 7. *A. candida* f.sp. sinapidis-arvensis and 8. *A. candida* f.sp. cheiranthi-cheiri.

Biga (1955) recognized two morphological taxa: *A. candida* macrospora and *A. candida* microspora, as proposed by Togashi and Shibaskaki (1934), but renamed them *A. candida* microspora and *A. candida* candida, respectively. On the basis of conidial measurements from 63 species, Biga (1955) reported that *A. candida* microspora (15.0-17.5 µm diameter) is restricted to *Armoracia, Brassica, Erucastrum, Raphanus* and *Rapistrum*, whereas, *A. candida* candida (12.5-15 µm diameter) has a wide range of cruciferous hosts. In addition, two intraspecific taxa of *A. ipomoeae-panduratae* and five of *A. tragopogonis* also identified. Endo and Linn (1960) reported one race of *Albugo* on *Armoracia rusticana.*

Lakra and Saharan (1988) reported that the size of sporangia of *A. candida* ranged between 13.55-21.78 µm diameter. The size of sporangia formed on *B. campestris* var. Brown *sarson* from Hisar ranged from 13.55-16.99 µm. While sporangia formed on *B. campestris*

var. *Toria* at the same place yielded only macrospores of 17.54-21.25 µm size. Sporangia from *B. juncea*, under Madhya Pradesh conditions, showed two distinct forms. In first form microsporangia ranging in size 14-16 µm and in second form macrosporangia ranging in size 17.0-21.0 µm. They also recognized that the small sized sporangia were circular to spherical while big sized sporangia were elongated to globular.

Pound and Williams (1963) studied collection of the fungus from six cruciferous hosts representing six different genera. According to them, an isolate from each genus represents a distinct race and in every case, the original host species from which the isolate is taken serves as the best differential host for that race. The races were:

Race	**Host**
Race 1	*Raphanus sativus* var Early Scarlet Globe
Race 2	*Brassica juncea* var Southern Giant Curled
Race 3	*Armoracia rusticana* var Common
Race 4	*Capsella bursa*—pastoris
Race 5	*Sisymbrium officinale*
Race 6	*Rorippa islandica*

Later, race 7 from *B. rapa* (Verma *et al* 1975) and race 8 from *B. nigra* (Delwiche & Williams 1977) were reported. In addition to eight races described earlier three more races described as race 9 and 10 on *B. oleracea*, *Sinapsis alba,* respectively (Hill *et al* 1988) and race 11 on *B. carinata* (William 1985). Petrie (1988) reported two new races 2V and 7V on *B. juncea* and *B. rapa*, respectively.

In Russia, Novotel'nova (1962), while analyzing intraspecific taxa, established that the *A. candida* species consists of separate morphological specialized forms confined to a particular range of host plants i.e. to plants of certain species or groups of genera and species. Within the morphological forms, races can be differentiated, while within heterogeneous populations both races and forms can be differentiated. Geographical and climatic conditions leave their distinguishing mark on the processes of form development so that populations of the fungus encountered by investigators in different countries were not identical.

Pound and Williams (1963) while studying the biological races of *A. candida*, inoculated seedlings of test plants with zoospore suspension obtained by incubating sporangial suspension at 12^{o}C for 3-6 hrs. The same method was used for inoculating cotyledons of four *Brassica* spp. (Verma *et al* 1975) and flower buds (Verma and Petrie 1980).

The concept of races in *A. candida*, as proposed by Pound and Williams (1963), was based on species relationships. Recent studies have, however, clearly demonstrated that cultivars of *Brassica* crops must be included in a set of host differentials to distinguish

isolates of the pathogen within a presently accepted race (Burdyukova 1980, Pidskalny and Rimmer 1985). There is an urgent need to standardize host differentials keeping in mind the homogeneity and purity of species and varieties. A detailed study regarding identification of biological races of *A. candida* occurring on different *Brassica* crops in India using differential hosts described by Pound and Williams (1963) is still lacking.

Using North American races 2 and 7 from *B. juncea* and *B. campestris*, respectively, Petrie (1988) screened accessions of several *Brassica* species including *B. campestris* var. Yellow *sarson*, *B. campestris* var. Brown *sarson*, *B. campestris* var. *Toria* and *B. juncea* from India: both yellow and brown *sarson* were equally and highly susceptible to both races, *toria* only to race 7 and *B. annua* only to race 2. A detailed study is needed to determine whether races of *A. candida* attacking *B. juncea* and several *B. campestris* crops in India are similar to races 2 and 7 from Canada and the U.S.A. In India, using all the differential hosts proposed by Pound and Williams (1963), Verma (1989) reported that the *A. candida* race attacking *B. juncea* does not appear to be distinct from the race attacking *B. campestris* var. *Toria*.

In India, Singh and Bhardwaj (1984) tested 12 *Brassica* species from Himachal Pradesh and identified 9 races from four hosts, *viz*., *B. juncea, B. campestris* var. *Toria*, *B. campestris* var. Brown *sarson* and *B. campestris* var. pekinensis.

Lakra and Saharan (1988) recognized two races of *A. candida*, race 2 and race 3 from small and big pustule types of *B. juncea* respectively and were most virulent on their original host. The five different pustule types were found on infected leaves of *B. rapa* and *B. juncea*. Two distinct types of pustules on *B. rapa* were (i) irregular surrounding by green ring type pustules ranging from 2-5 mm in size and (ii) circular broad pustule raised mass ranging from 5.0-6.5 mm in size. Similarly three different types of pustules on *B. juncea* leaves were (i) circular pin head ranging from 0.5-2.0 mm (ii) circular surrounding green border ranging from 2.0-5.0 mm and (iii) small circular raised mass ranging 5.0-7.0 mm in size. Kolte *et al* (1985) and Gupta and Saharan (2002) described white rust symptoms in details on different crucifers. The size of sporangia ranged from 13.5 to 20.5µm and 15.5 to 20.9µm in the *B. rapa* and *B. juncea,* respectively. Bhardwaj and Sud (1988) reported the reaction of nine isolates of *A. candida* collected from different hosts differed from each other on 26 differential hosts. It was reported that *A. candida* on crucifers exist in the form of nine different biological races.

Verma and Bhowmik (1989) reported an isolate of *A. candida* (race 2) from *B. juncea* (cv. Pusa Bold) that attack the genotype BN-38 Sel of *B. napus*. According to Verma (1990) both *B. juncea* and *B. rapa* (var. *Toria*) isolates of *A. candida* from India, infected a series of cruciferous hosts of both Canadian and Indian origin. In addition to cultivars of *B. juncea*, they infected yellow *sarson*, brown *sarson*, *toria*, *B. nigra* and *B. alba*. Both the isolates gave highest disease severity ratings on *B. juncea* although there were some differences in their

virulence on other hosts, implying their similarity to *A. candida* race 2. Similar view was expressed by Lahri and Bhowmik (1995), when 3 isolates of *A. candida* obtained from *B. juncea*, *B. rapa* and *B. nigra* were tested on host differential set. These isolates, though showed some variations in their reaction to different hosts, readily cross infected the three host species of their origin and consistently produced high disease severity on cultivars of *B. juncea*.

Two new races of *A. candida,* race AC 12 from *B. juncea* and race AC 13 from *B. rapa* var. *Toria* were identified using 14 crucifer host differentials (Verma *et al* 1999). Race AC 12 and race AC 13 showed differential interactions on *B. carinata*. Race AC 12 identified resistant genes from *B. rapa* cvs. Candle and Torch. Except for the differential reaction of Canadian race 2 on *B. rapa* var. Yellow *sarson* cv. R-500 and of Indian race AC 12 on *B. rapa* cvs. Candle and Torch, both races showed a similar reaction on other differentials. Canadian race 7 differed from race 2 and race AC 12 by initiating a resistant reaction in all five cultivars of *B. juncea*. *B. juncea* cv. *A. candida* isolates from different agro-climatic regions of India reacted similarly to *B. rapa* var. *Toria* cv. T-9 and Pusa Bold.

Isolates of *A. candida*, collected from different geographical locations in western Canada, were tested for virulence on a set of differentials from *Brassica* species to determine the variability and distribution of different races and pathotypes. Most isolates were classified as race 7 based on virulence to *B. rapa* accessions and these could be subdivided broadly into two pathotypes, 7A and 7V, on the basis of their virulence to *B. rapa* cvs. Torch and Reward (Rimmer *et al* 2000). Some isolates were classified as race 2 based on virulence to *B. juncea* cvs. Burgonde and Cutlass. The predominant pathotype was 7V. Race 7 was the most prevalent in collections from Alberta and Manitoba. In British Columbia, pathotypes 2A, 2V, 7A and 7V were commonly observed and about 50 per cent of the sample collections were mixtures of races 2 and 7. A few single pustule isolates from the Peace River region of Alberta and British Columbia appeared to be hybrid pathotypes combining virulence characteristics of pathotypes 2A or 2V with 7A or 7V. These isolates were pathogenic on both *B. juncea* and *B. rapa* cultivars (Rimmer *et al* 2000).

Gupta and Saharan (2002) reported four new pathotypes of *A. candida* isolated from cultivars of Indian mustard (AC-14 from RL 1359, AC-15 and AC-16 from Kranti and AC-17 from RH 30) and characterized them based on interaction with 11 host differentials (EC-129126-1, EC-322090, EC-322092, EC-322093, Varuna, EC-287711, ZEM-1, RC 781, RH 30, RH 8113 and Rajat). The symptoms induced were stable and similar to those induced by the isolates under natural conditions. Gupta *et al* (2002) assessed 14 elite genotypes of *B. juncea* under three environments. High disease intensity of white rust was observed under artificial inoculated environment as compared to natural disease environment followed by controlled environment for all the 14 genotypes. Patni *et al* (2005) observed that isolates

collected from two hosts, *B. rapa* and *B. juncea* were found different in morphology, incubation period, latent period, duration for initiation of sporangium germination, number of zoospores per sporangia and size of sporangia (μm). This clearly shows that the isolates are quite distinct from one another in morphological and pathological characteristics.

Kaur *et al* (2008) tested two isolates of *A. candida*, representing strains collected from *B. juncea* and *Raphanus raphanistrum* (wild radish) on cruciferous host differentials to characterize their pathogenic behaviour. It was observed that strains obtained from *B. juncea* and *R. raphanistrum* are different in their host range. The pathotype 2V of *A. candida* was reported for the first time in Australia.

Mishra *et al* (2009) also observed variable number of sporangia per pustule. The number of sporangia per pustule were correlated with size of pustule.The pustules with large size (3-4mm), medium size (1-3mm) and small size (0.5-1mm) have 17500, 9500 and 6500 number of sporangia per pustule after 14 days of inoculation and 20500, 14000 and 8500 number of sporangia per pustule after 21 days of inoculation, respectively. Mishra *et al* (2009) observed disease on different varieties of *B. juncea* inoculated with *A. candida.* Disease incidence at the cotyledonary leaf stage varied from 31-67 per cent, whereas at the true-leaf stage, it ranged from 9-28 per cent at 14 days after inoculation and 6-45 per cent at 21 days after inoculation.

2.8 Disease scoring scale

Pound and Williams (1963) reported a procedure to asses symptoms of white rust on mature cruciferous plants. This procedure has since been modified by different workers. Fox and William (1984) reported that spore production was highly correlated with a visual white rust interaction phenotype rating scale. Plants were rated 0-9 according to the amount of leaf necrosis or area covered by pustules. Means of spore production on plants rated as 1, 3, 5, 7 and 9 were significantly different from one another.

Mayee and Datar (1986) suggested the use of 0-9 scale based on growth stages of the crop for leaf as well as staghead infections. To assess leaf phase and staghead phase infections separately and / or in combination, a 0-5 scoring scale has been given by Lakra and Saharan (1988). Khangura and Sokhi (1991) suggested a new method to calculate area under disease progress curve (AUDPC) in white rust (*Albugo candida*) infecting *Brassica.* In this method an equation was derived for the white rust pathogen using the time interval between two successive observations and number of successive observations.

CHAPTER - III

MATERIAL AND METHODS

The present studies were conducted at the research farm of Department of Plant Breeding and Genetics and Department of Plant Pathology, Punjab Agricultural University, Ludhiana during 2009-2010 and 2010-2011.

Plan of work

The investigations were carried out to study variability in *Albugo candida* (Pers.) Kuntze causing white rust of rapeseed-mustard. The experimental material was sown in pots on 12th February, 2010 and 10th November, 2010. Cultural practices as per required for the crops were followed as per Package of Practices for Rabi crops recommended by Punjab Agricultural University, Ludhiana (Anon 2010b).

3.1 Collection of *Albugo candida* isolates from different locations and their maintenance

3.1.1 Collection of isolates

Rapeseed-mustard growing areas in districts of Punjab *viz.* Ferozepur, Faridkot, Mansa, Muktsar, Barnala, Bathinda, Sangrur and Ludhiana were surveyed during February, 2010. The observations were recorded on white rust symptomatology, disease incidence, severity and varietal reaction of different genotypes of rapeseed-mustard. The diseased samples were collected from different locations and brought to the laboratory for further studies. These isolates were designated as 'Ac'. The data on the disease incidence was recorded by random sampling in each field with the help of square meter quadrate from at least five spots (four corners and center) and per cent incidence was calculated by following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant observed}} \times 100$$

The severity of the disease was recorded by following the 0-9 rating scale (Williams 1985) as given in table 3.1.

Table 3.1: Disease severity scale (0-9) of white rust

Rating	Symptoms	Reaction
0	No lesions	Immune for WR
1	Non-sporulating pinpoint size or small brown necrotic spots, less than 5% leaf area covered by lesions.	HR
3	Small roundish slightly sporulating larger brown necrotic spots, about 1-2 mm in diameter with a distinct margin or yellow halo, 5-10 % leaf area covered by lesions.	R
5	Moderately sporulating, non- coalescing larger brown spots, about 2-4 mm in diameter with a distinct margin or yellow halo, 11-25% leaf area covered by spots.	MR
7	Moderately sporulating, coalescing larger brown spots about 4-5 mm in diameter, 26-50% leaf area covered by lesions.	S
9	Profusely sporulating, rapidly coalescing brown to black spots measuring more than 6mm diameter without margins covering more than 50% leaf area.	HS

Disease severity calculated by using the formula:

$$\text{Disease severity} = \frac{\text{Sum of numerical ratings}}{\text{Maximum rating x No. of samples}} \times 100$$

3.1.2 Maintenance of isolates

Sporangia were derived from the pustules of white rust affected leaves of *B. juncea* by scrapping the single white rust pustule with a blade. The sporangial powder was placed in the test tubes containing sterilized double distilled water for germination of sporangia to produce zoospores at 18°C for 4 hrs. The zoospores were used for inoculation of respective host for the multiplication and maintenance of the isolates. The inoculum as generated by the single white rust pustule on their respective host during crop season was used for inoculation in further studies. The white rust inoculum of respective host was then stored in gelatin capsules at -20°C, for further use.

3.2 Morphological characterization of different *A. candida* isolates.

3.2.1 Sowing in pots

Earthern pots of 25 cm diameter were used. For sowing two kilograms of field silty loam soil mixed with the compost in 3:1 proportion was filled in each pot. Pots were first watered and left as such in the glasshouse for two days to ensure appropriate moisture for seed germination. Ten seeds of test hosts (*B. juncea* var. RLM 1359) were sown in each pot.

The first thinning was done when the seedlings were 10 days old. The number of seedlings per pots was reduced to five by removing weak seedlings. At the age of 20 days urea solution (1%) was applied to the soil using 25 ml urea solution/pot for the better growth of the plants.

3.2.2 Inoculum

Suspension of sporangia from newly ruptured single pustules from freshly infected leaves maintained separately in glass house were gently dislodged with an artist soft brush and allowed to fall into a Petri plate containing double distilled water. Using small glass rod, the contents of the Petri plates were gently stirred to disperse the sporangia. Plates were then incubated at 18 ±1°C in an incubator for 4 hrs for production of zoospore from sporangia. The concentration of zoospores in inoculum suspension (2.5×10^5 zoospores/ml) was prepared using haemocytometer.

3.2.3 Inoculation on test host

Plants were inoculated at 2-3 leaves with the zoospore suspension. The inoculated plants were kept in polythene humid chamber (80-90 per cent RH) for 72 hrs. The pots were then removed from the humid chamber, placed at proper isolation distance and allowed for development of symptoms and observations were recorded.

3.2.4 Size of pustules

Size of pustules on leaves of different isolates was recorded. For this purpose, 5 pustules/leaf were taken randomly. Data was recorded from 10 leaves. The diameter of pustule was measured with the help of scale and then the average size of the pustules based on 50 observations was calculated.

3.2.5 Shape of pustules

Shape of white rust pustules was observed on the basis visible appearance. Pustule shape from 50 pustules was observed and pustules were grouped into different categories as scattered, pin head and ring type.

3.2.6 Colour of pustule

The colour of the pustules on leaves of different isolates was recorded. Colour was observed on the basis of their visible appearance and ascertained with the help of RGB colour chart. All the 52 isolates were classified into three categories *viz*. white, mint cream and ivory.

3.2.7 Size of sporangia

Size of sporangia of different isolates was recorded. Observations were recorded from three replications for each isolate, with 20 sporangia per replication. The size of sporangia was measured by using ocular and stage micrometer.

3.2.8 Shape of sporangia

Shape of sporangia of different isolates was recorded. Average of 50 observations was taken.

3.2.9 Germination of sporangia

Sporangial germination of different isolates was studied by adding sporangial powder in double distilled water in test tubes and incubated at 18°C for 4 and 8 hrs.

Germination of sporangia was calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of sporangia germinated}}{\text{Total number of sporangia observed}} \times 100$$

3.3 Virulence of different isolates

A set of 12 host differentials was used for determining the virulence of different isolates of *A. candida.* These host differentials are given in table 3.2.

Table 3.2: Differential hosts used for studying virulence of *A. candida* isolates

Sr. No.	Differential host	Varitiy/genotype
1.	*B. juncea*	RLM 1359
2.	*B. nigra*	90745
3.	*B. napus*	GSL1
4.	*B. carinata*	PC-5
5.	*B. oleracea*	Broccoli sprouts
6.	*B. tournifortii*	BT-02
7.	*B. rapa* (*Toria*)	TL-15
8.	*B. rapa* (Brown *sarson*)	Reward
9.	*B. rapa* (Yellow *sarson*)	BIOYSR
10.	*Raphanus sativus*	White Icicle
11.	*Sinapis alba*	Kirby
12.	*Eruca sativa*	TMLC 2

The host differentials were grown in 25 cm earthern pots in screen house of the Department of Plant Breeding and Genetics, PAU, Ludhiana by following standard agronomic practices.

To determine the pathogenic variability, single pustule isolates (SPI) of *A. candida* derived from different cultivars of *B. juncea,* were tested on differential hosts. Three representative isolates were chosen from each group. Isolates Ac2, Ac16, Ac46 were taken

from group AC-I, isolates Ac4, Ac21, Ac35 from group AC-II and Ac6, Ac15, Ac47 from group AC-III. For each isolate, spore suspension (2.5 $\times 10^5$ zoospores/ml) was prepared in distilled water with the help of haemocytometer. Fifteen plants (20 days old) of each cultivar were inoculated by drop inoculation method for each isolate. The inoculated plants were kept in polythene humid chamber at RH 80-90 per cent and at 20°C for 72 hrs. The pots were then removed from the humid chamber for the development of symptoms. The observations on disease reaction, incubation period, latent period, disease severity and other characters *viz*. pustule size, pustule shape, number of pustules per leaf and sporangia per pustule were recorded.

3.3.1 Incubation period and latent period

Incubation period was considered as the time difference between the time of incubation to the appearance of disease symptoms and latent period was taken as the time difference between the time of inoculation to the sporulation of the pathogen on the diseased tissue. To record incubation and latent period, 15 inoculated plants for each isolate were observed.

3.3.2 Disease severity

Average disease severity on leaves due to white rust was taken on 45 days old plants. The data on disease severity were recorded on 0-9 scale from 25 leaves of each differential hosts.

3.3.3 Pustule size and shape

Size and shape of randomly selected 5 pustules / leaf were recorded. Average size and shape of white rust pustules were calculated from the 15 plants of each differential hosts.

3.3.4 Number of pustule

Leaf size of different test species showed considerable variation. Therefore, for comparative study of pustule number on leaves per 10 cm^2 leaf area was counted with the help of glass slide by marking 5×2 square cm area. Observations were taken randomly on number of pustules on lower surface of the leaf starting from lower to upper leaves. This system of counting of leaves and number of pustules per unit area was followed in all the successive observations. The numbers of pustule on 50 leaves/isolate were recorded. Average number of pustules per leaf was recorded based on the observation taken from lower to upper leaves.

3.3.5 Sporangia per pustule

For recording the numbers of sporangia, ten leaves were washed off separately in 20 ml distilled water in a glass vial. Number of sporangia was calculated with the help of haemocytometer. Numbers of sporangia per pustule were calculated by following formula:

$$\text{Sporangia per pustule} = \frac{\text{Concentration of sporangia per ml}}{\text{Number of pustules per leaf}} \times 20$$

3.4 Effect of temperature

Temperature ranging from 10, 15, 20, 25 and 30°C was adjusted in different incubators. The sporangial suspension of *A. candida* was prepared in double distilled water. The sporangial suspension was kept for 8 hrs for germination at the respective temperature. One isolate from each group was used for this study. Sporangial germination was observed at 4 and 8 hrs interval. The precentage sporangial germination was calculated as described in 3.2.9.

3.5 Effect of environment

A total of 10 plants for each cultivar were studied under natural and artificially inoculated environment. Under artificial environmental conditions, plants were inoculated with *A. candida* suspension @ 2.5×10^5 zoospores/ml. Response of cultivars under different environmental conditions was observed.

CHAPTER - IV

RESULTS AND DISCUSSION

The results of investigations on "Variability in *Albugo candida* (Pers.) Kuntze causing white rust of rapeseed-mustard" are presented and discussed below:

4.1 Disease survey

The survey was carried out during 2009-10 crop season to record the disease incidence, severity, varietal reaction and the collection of white rust diseased samples from different rapeseed-mustard growing areas of the Punjab.

A perusal of data in table 4.1 showed that average disease incidence was highest at Bathinda (59.36 %) followed by Mansa (56.78 %), Barnala (53.31 %) whereas, lowest disease incidence was recorded at Ludhiana. Disease severity was maximum in Bathinda (27.83 %) followed by Mansa (25.84 %), Barnala (24.75 %), Faridkot (24.17 %), Muktsar (23.79 %), Sangrur (23.30 %), Ferozepur (22.41 %) and Ludhiana (21.92 %).

Table 4.1: Prevalence of white rust (*A. candida*) on rapeseed-mustard in different districts of Punjab during 2009-10

Sr. No.	District	Disease (%)	
		Incidence	Severity
1.	Bathinda	59.36	27.83
2.	Mansa	56.78	25.84
3.	Barnala	53.31	24.75
4.	Faridkot	52.12	24.17
5.	Muktsar	50.55	23.79
6.	Sangrur	49.98	23.30
7.	Ferozepur	48.26	22.41
8.	Ludhiana	44.39	21.92

4.1.1 Disease incidence on different varieties/genotypes at different locations

Table 4.2 and 4.3 illustrate the variable disease incidence and severity of white rust on different cultivars at different locations. It was observed that variety RLM 1359 was the most susceptible having significantly higher disease incidence (58.05 %) and severity (28.91 %) than all other cultivars. Variety RLM 619 was least susceptible and expressed significantly lowest disease incidence (39.42 %) and severity (16.24 %) than other cultivars.

In the survey, it was observed that disease incidence and disease severity on same cultivar varied in different locations. Disease incidence on RLM 1359 cultivar varies significantly with highest value (65.71 %) in Bathinda and lowest (49.86 %) in Ludhiana. Similar pattern was observed for disease severity. It may be associated with the prevalence of different environmental conditions and/or due to different pathogen types.

Raya cultivar PBR 91 was found to be the most predominant variety being cultivated in almost all the rapeseed and mustard growing areas of the state. Other varieties of rapeseed and mustard grown were PBR 97, PBR 210, RLM 619, RLM 1359 and RLC 1. From the data it was evident that RLM 619 and RLC 1 had less disease incidence as well as disease severity in different locations of Punjab (Table 4.2 and Table 4.3).

Table 4.2: Incidence of white rust (*A. candida*) on cultivars of *B. juncea* at different locations during 2009-10 crop season

Districts	Variety						Mean
	PBR 91	PBR 97	PBR 210	RLM 619	RLC 1	RLM 1359	
Bathinda	61.84	63.71	62.14	45.86	56.86	65.71	59.35
Mansa	58.86	59.29	61.43	44.14	54.14	62.86	56.79
Barnala	56.43	53.57	55.57	41.14	52.57	60.57	53.31
Faridkot	56.29	51.86	53.57	40.43	52.42	58.14	52.12
Muktsar	55.43	50.46	52.57	37.14	50.14	57.57	50.55
Sangrur	53.86	52.29	52.14	36.92	48.57	56.14	49.99
Ferozepur	50.14	50.86	51.86	35.43	47.71	53.57	48.26
Ludhiana	46.89	46.71	46.43	34.28	42.29	49.86	44.41
Mean	54.97	53.59	54.46	39.42	50.59	58.05	51.85
CD for varieties (p= 0.05)							**1.25**
CD for districts (p= 0.05)							**1.45**

Table 4.3: Severity of white rust (*A. candida*) on cultivars of *B. juncea* in different locations during 2009-10 crop season

Districts	Variety						Mean
	PBR 91	PBR 97	PBR 210	RLM 619	RLC 1	RLM 1359	
Bathinda	30.86	27.71	28.54	19.57	26.14	34.14	27.83
Mansa	28.84	25.86	27.19	17.14	23.86	32.14	25.84
Barnala	26.93	24.83	26.57	16.86	23.29	30.00	24.75
Faridkot	25.86	24.57	26.14	16.71	23.14	28.57	24.17
Muktsar	25.24	24.43	26.12	16.23	23.12	28.00	23.86
Sangrur	24.94	23.29	25.57	15.71	22.86	27.43	23.30
Ferozepur	23.71	22.19	24.71	14.57	23.29	26.00	22.41
Ludhiana	22.97	24.29	24.14	13.20	22.29	25.00	21.98
Mean	26.17	24.65	26.12	16.25	23.50	28.91	24.27
CD for varieties (p= 0.05)							**0.94**
CD for districts (p= 0.05)							**1.09**

4.1.2 Collection of isolates

Diseased samples were collected from different locations *viz*. Ferozepur, Faridkot, Mansa, Muktsar, Barnala, Bathinda, Sangrur and Ludhiana. Table 4.4 reveals the data of 52 isolates along with their locations, varieties, average disease incidence and severity of white rust on rapeseed- mustard. These isolates were designated as "Ac" from Ac1 to Ac52.

Table 4.4: Isolates of white rust (*A. candida*) from different varieties from different locations in Punjab state during 2009-10 crop season

District	Location	Variety	Incidence (%)	Severity (%)	Isolate code
Barnala	Kattu	PBR 210	56	30	Ac1
	Karamgarh	RLM 1359	62	37	Ac2
	Harigarh	PBR 91	43	25	Ac3
	Jhaloor	PBR 97	52	27	Ac4
	Hamidi	RLM 619	35	17	Ac5
	Kotduna	PBR 210	54	28	Ac6
	Kothe Sran	RLC 1	42	21	Ac7
	Kothe	PBR 91	40	24	Ac8
Bathinda	Gurusar	PBR 210	61	31	Ac9
	Ramuwala	PBR 97	50	27	Ac10
	Kamalu	PBR 91	47	28	Ac11
	Daulatpura	PBR 210	60	30	Ac12
	Gill Khurd	RLC 1	41	25	Ac13
	Rampura	RLM 1359	67	42	Ac14
	Galib Khurad	PBR 91	54	28	Ac15
	Jeond	PBR 210	60	31	Ac16
Mansa	Ahmedpur	PBR 97	58	30	Ac17
	Gobindpura	RLC 1	43	23	Ac18
	Gandhu Khurd	PBR 210	58	29	Ac19
	Khiwa	RLM 1359	64	39	Ac20
	Piplian	PBR 91	52	28	Ac21
	Tamkot	PBR 97	55	27	Ac22
	Khokhar Kalan	PBR 97	62	31	Ac23
	Khatriwala	RLM 619	38	21	Ac24
Muktsar	Arniwal Wajira	PBR 91	56	28	Ac25
	Jand Wala	RLM 1359	58	31	Ac26
	Ghumiara	PBR 210	56	28	Ac27

	Kakhanwali	RLM 619	28	19	Ac28
	Khema Khera	PBR 97	57	30	Ac29
Sangrur	Bhulerheri	PBR 91	54	28	Ac30
	Daulatpur	RLM 619	24	18	Ac31
	Bhojowali	PBR 97	47	27	Ac32
	Banganwali	PBR 210	56	29	Ac33
	Dohla	PBR 91	52	27	Ac34
Faridkot	Sadiq	PBR 91	54	27	Ac35
	Kot Kapura	RLM 619	32	22	Ac36
	Kamiana	PBR 97	44	24	Ac37
	Lamb Wali	PBR 210	44	25	Ac38
	Ahel	RLM 1359	60	34	Ac39
	Butter	RLC 1	49	23	Ac40
Ferozepur	Amarpura	PBR 97	36	21	Ac41
	Chana Khera	RLC 1	36	22	Ac42
	Alias Jhurar Khera	PBR210	52	26	Ac43
	Guru Har Sahai	PBR 91	34	18	Ac44
	Jhandu Wala	RLM 619	29	17	Ac45
	Jiwan Arian	PBR 97	44	23	Ac46
Ludhiana	Baddowal	PBR 91	34	22	Ac47
	Hassanpur	RLC 1	40	24	Ac48
	Hammayaunpur	PBR 210	42	22	Ac49
	PAU Campus	RLM 1359	48	24	Ac50
	Lalouri	RLM 619	28	15	Ac51
	Pamal	PBR 97	43	22	Ac52

4.1.3 Categorization of *A. candida* isolates on the basis of symptomatology

Different types of pustule were observed in the field which categorized on the basis of their shape into three categories *viz.* scattered, pin head and ring type in all the 52 diseased samples collected from different places. Scattered and pin head pustules were further divided into 3 subcategories based on colour appearance of pustules *viz.* white, mint cream and ivory colour whereas third category ring type pustules was divided into two subcategories *viz.* white and ivory colour pustules (Table 4.5).

In the first symptom category, fifteen isolates were placed which showed scattered pustules. The size of pustules was generally large. The subcategory, white colour included five isolates (Ac2, Ac28, Ac31, Ac41 and Ac52), mint creamy colour included six isolates (Ac3, Ac12, Ac18, Ac25, Ac45 and Ac46) and ivory colour included four isolates (Ac16, Ac42, Ac49 and Ac50). Second category comprises twenty four isolates which produced pin head pustules. These types of pustules were generally small in size. The white colour subcategory include ten isolates (Ac4, Ac7, Ac20, Ac23, Ac26, Ac29, Ac33, Ac37, Ac40 and Ac43), mint creamy and ivory colour subcategories included six isolates (Ac8, Ac10, Ac27, Ac30, Ac35 and Ac38) and eight isolates (Ac1, Ac5, Ac21, Ac24, Ac34, Ac36, Ac39 and Ac48) respectively. Thirteen isolates came under third category which produced ring type pustules. Pustules of this type were large in size. White colour subcategory includes seven isolates (Ac9, Ac13, Ac15, Ac17, Ac19, Ac44 and Ac51) and ivory colour subcategory includes six isolates (Ac6, Ac11, Ac14, Ac22, Ac32 and Ac47) (Table 4.5).

In all the cultivars, symptoms first appeared on lower leaves which later on progressed to middle and upper leaves. Pustules were also observed on stem and pods in later stages of the crop growth. Patni *et al* (2005) reported different characteristics of pustules i.e. circular pin head type, irregular surrounded by green ring, circular broad pustule with raised mass, circular surrounding green border, small circular raised mass. Gupta and Saharan (2002) also reported different categories of isolates based on size, shape and colour of the pustule.

Table 4.5: Categorization of different isolates of *A. candida* (white rust) of rapeseed mustard on the basis of symptoms

Symptom category	Sub Category	Isolates
Scattered pustule	White colour	Ac2, Ac28, Ac31, Ac41, Ac52
	Mint cream colour	Ac3, Ac12, Ac18, Ac25, Ac45, Ac46
	Ivory colour	Ac16, Ac42, Ac49, Ac50
Pin head pustule	White colour	Ac4, Ac7, Ac20, Ac23, Ac26, Ac29, Ac33, Ac37, Ac40, Ac43
	Mint cream colour	Ac8, Ac10, Ac27, Ac30, Ac35, Ac38
	Ivory colour	Ac1, Ac5, Ac21, Ac24, Ac34, Ac36, Ac39, Ac48
Ring type pustule	White colour	Ac9, Ac13, Ac15, Ac17, Ac19, Ac44, Ac51
	Ivory colour	Ac6, Ac11, Ac14, Ac22, Ac32, Ac47

4.2 Morphological characterization of *A. candida* isolates

4.2.1 Pustule Size

Leaves inoculated with different isolates of *A. candida* were examined to find out variability in pustule size on *B. juncea* var. RLM 1359. The data are presented in table 4.6. Pustules vary in size from 0.5-7.0 mm. Ac14 isolate has maximum pustule size 7.0 mm while Ac 48 isolate has minimum size of 0.5 mm. Patni *et al* (2005) observed significant correlation in pustule shape and diameter of pustule. The diameter vary with different pustule shape as circular pin head type (0.5-2.0 mm), irregular surrounded by green ring (2-5 mm), circular broad pustule raised mass (5-6.5 mm), circular surrounding green border(2-5 mm), small circular raised mass (5-7 mm). Mishra *et al* (2009) also categorized pustule on the basis of size. Gupta and Saharan (2002) observed varying size of pustules in different isolates. Similar observations regarding variations in sporangial size were also observed by Lakra and Saharan (1988).

4.2.2 Pustule Shape

Different isolates of *A. candida* were studied on *B. juncea* variety RLM 1359 and variation in shape of pustules was recorded. The data are presented in table 4.6.

Three types of pustule shapes were observed from different isolates *viz.* pin head pustules (Ac1, Ac4, Ac21, Ac27, Ac34, Ac35, Ac36, Ac43 and Ac48), scattered pustules (Ac2, Ac3, Ac5, Ac7, Ac8, Ac12, Ac16, Ac24, Ac25, Ac28, Ac29, Ac31, Ac33, Ac38, Ac39, Ac40, Ac41, Ac42, Ac45, Ac46, Ac49, Ac50 and Ac52) and ring type pustules(Ac6, Ac9, Ac10, Ac11, Ac13, Ac14, Ac15, Ac17, Ac18, Ac19, Ac20, Ac22, Ac23, Ac26, Ac30, Ac32,

Ac37, Ac44, Ac47 and Ac51). Similar type of pustule shapes were observed by Patni *et al* (2005), Gupta and Saharan (2002) and Lakra and Saharan (1988).

4.2.3 Pustule Colour

The colour of the pustules of 52 isolates was studied on *B. juncea* variety RLM 1359. These isolates were classified into 3 categories based on colour of pustule *viz*. white, mint creamy and ivory colour pustules. The data summarizing these categories are presented in table 4.6.

Twenty one isolates (Ac2, Ac4, Ac7, Ac9, Ac13, Ac15, Ac17, Ac19, Ac20, Ac23, Ac26, Ac28, Ac29, Ac31, Ac33, Ac37, Ac40, Ac41, Ac44, Ac51 and Ac52) have whitish coloured pustules. Ten isolates (Ac3, Ac8, Ac12, Ac25, Ac27, Ac30, Ac35, Ac38, Ac45 and Ac46) developed pustules with mint creamy colour. Twenty one isolates (Ac1, Ac5, Ac6, Ac10, Ac11, Ac14, Ac16, Ac18, Ac21, Ac22, Ac24, Ac32, Ac34, Ac36, Ac39, Ac42, Ac43, Ac47, Ac48, Ac49 and Ac50) had ivory colour.

The present investigations have shown that different isolates of *A. candida* differ in their pustule colour. Isolates with white, mint cream and ivory colour pustules have been observed. Gupta and Saharan (2002) also observed different colours of pustules of white rust from four isolates (AC-1, AC-2, AC-3 and AC-4) collected form different locations.

Table 4.6: Pustule characters of *A. candida* isolates on leaves

Isolate	Pustule Size (mm)	Pustule Shape*	Pustule Colour**	Isolate	Pustule Size (mm)	Pustule Shape*	Pustule Colour**
Ac1	1.8	PH	I	**Ac27**	1.2	PH	MC
Ac2	3.0	S	W	**Ac28**	5.5	S	W
Ac3	4.6	S	MC	**Ac29**	3.8	S	W
Ac4	1.0	PH	W	**Ac30**	4.8	R	MC
Ac5	4.5	S	I	**Ac31**	4.6	S	W
Ac6	4.6	R	I	**Ac32**	6.5	R	I
Ac7	3.7	S	W	**Ac33**	3.2	S	W
Ac8	5.6	S	MC	**Ac34**	1.5	PH	I
Ac9	5.8	R	W	**Ac35**	1.9	PH	MC
Ac10	6.8	R	I	**Ac36**	0.6	PH	I
Ac11	6.5	R	I	**Ac37**	5.2	R	W
Ac12	2.0	S	MC	**Ac38**	5.4	PH	MC
Ac13	5.4	R	W	**Ac39**	5.3	S	I
Ac14	7.0	R	I	**Ac40**	4.2	PH	W

Ac15	6.2	R	W	**Ac41**	2.5	S	W
Ac16	5.6	S	I	**Ac42**	5.9	S	I
Ac17	4.6	R	W	**Ac43**	1.4	S	I
Ac18	5.9	R	I	**Ac44**	4.2	R	W
Ac19	6.2	R	W	**Ac45**	4.6	S	MC
Ac20	5.4	R	W	**Ac46**	5.7	S	MC
Ac21	0.7	PH	I	**Ac47**	2.0	R	I
Ac22	4.2	R	I	**Ac48**	0.5	S	I
Ac23	6.1	R	W	**Ac49**	5.4	S	I
Ac24	4.9	S	I	**Ac50**	6.5	S	I
Ac25	3.8	S	MC	**Ac51**	3.4	R	W
Ac26	3.4	R	W	**Ac52**	3.8	S	W

Pustule Shape[*]

S = Scattered Pustules

PH = Pin head Pustules

R = Ring type Pustules

Pustule Colour [**]

MC = Mint Cream

I = Ivory

W = White

4.2.4 Sporangia Size

Average size and range of sporangia in different isolates are presented in table 4.7. The data on sporangial size revealed that isolates differed in their sporangial size. The average sporangial size varied from 13.2 to 21.0 µm. Isolate Ac10 had maximum average size (19.7 µm) with range from 18.8 to 20.9 µm whereas Ac40 isolate had minimum average size (14.2 µm) with range from 13.2 to 15.6 µm. Similar observations regarding variations in sporangial size were also observed by Patni *et al* (2005). The earlier as well as present variations in the sporangial size indicated the existence of morphological variability in this pathogen. Lakra and Saharan (1988) also observed variation in sporangial size ranging from 13.55 to 21.78 µm.

4.2.5 Sporangia Shape

Different isolates of *A. candida* were studied for the shape of sporangia. These isolates were classified into two categories based on shape of sporangia *viz.* globose and slightly spherical. The data summarizing these classes are given in table 4.7.

Fourty three isolates (Ac2, Ac3, Ac5, Ac6, Ac7, Ac8, Ac9, Ac10, Ac11, Ac12, Ac13, Ac14, Ac15, Ac16, Ac17, Ac18, Ac19, Ac20, Ac22, Ac23, Ac24, Ac25, Ac26, Ac28, Ac29, Ac30, Ac31, Ac32, Ac33, Ac37, Ac38, Ac39, Ac40, Ac41, Ac42, Ac44, Ac45, Ac46, Ac47, Ac49, Ac50, Ac51 and Ac52) had globular shape of sporangia. Nine isolates (Ac1, Ac4, Ac21, Ac27, Ac34, Ac35, Ac36, Ac43 and Ac48) had sporangia of slightly spherical shape.

Similar results recorded by Patni *et al* (2005) who observed globular and slightly spherical sporangia in *A. candida*. Lakra and Saharan (1988) also observed three type of sporangial shape *viz.* globular, spherical and slightly spherical from different isolates of *A. candida.*

Table 4.7: Dimensions of sporangia of *A. candida* isolates

Isolate	Size of Sporangia (µm)		*Shape of Sporangia	Isolate	Size of Sporangia (µm)		Shape of Sporangia
	Average	Range			Average	Range	
Ac1	17.7	15.8-18.6	SS	Ac27	17.9	16.1-19.0	SS
Ac2	15.4	14.3-16.8	G	Ac28	15.5	14.4-17.8	G
Ac3	14.8	13.6-16.2	G	Ac29	16.4	15.5-17.8	G
Ac4	17.1	16.5-18.0	SS	Ac30	18.1	17.7-19.4	G
Ac5	16.2	15.4-17.9	G	Ac31	15.1	14.1-16.3	G
Ac6	18.7	17.6-20.1	G	Ac32	18.3	16.8-20.0	G
Ac7	14.4	13.5-15.6	G	Ac33	18.2	14.8-16.4	G
Ac8	15.4	14.8-16.5	G	Ac34	16.6	15.8-18.4	SS
Ac9	19.1	18.5-20.6	G	Ac35	16.3	15.5-17.6	SS
Ac10	19.7	18.8-20.9	G	Ac36	16.1	15.5-17.2	SS
Ac11	19.1	18.2-20.2	G	Ac37	15.4	13.7-16.8	G
Ac12	16.4	15.9-17.5	G	Ac38	16.3	15.5-17.4	G
Ac13	18.4	17.8-20.2	G	Ac39	16.1	15.4-17.3	G
Ac14	19.4	18.8-20.6	G	Ac40	14.2	13.2-15.6	G
Ac15	17.6	17.0-18.6	G	Ac41	16.6	15.5-17.5	G
Ac16	15.4	14.2-16.7	G	Ac42	15.1	13.8-17.7	G
Ac17	18.9	17.6-20.5	G	Ac43	16.4	15.0-17.8	SS
Ac18	19.3	18.6-20.5	G	Ac44	18.8	17.7-20.3	G
Ac19	18.6	17.5-19.8	G	Ac45	15.5	14.4-16.8	G
Ac20	17.1	15.6-18.4	G	Ac46	16.4	15.8-17.1	G
Ac21	16.2	15.0-17.4	SS	Ac47	17.7	16.8-18.9	G
Ac22	17.6	16.8-19.2	G	Ac48	17.1	16.2-18.4	SS
Ac23	18.2	17.9-20.4	G	Ac49	16.9	15.5-17.4	G
Ac24	16.9	15.6-17.5	G	Ac50	15.8	13.5-16.2	G
Ac25	16.2	14.8-17.5	G	Ac51	17.5	16.8-18.6	G
Ac26	16.6	15.4-17.9	G	Ac52	16.3	15.5-17.4	G

*** Sporangia Shape**
G = Globular
SS = Slightly spherical

4.2.6 Sporangial Germination

The perusal of data on germination of sporangia of different isolates revealed that all the 52 isolates of *A. candida* varied w.r.t. germination period (table 4.8). These isolates were classified into three categories based on per cent sporangial germination observed at four hours and 8 hours intervals.

Table 4.8: Germination of sporangia of *A. candida* isolates

Isolate	Germination (%) after		Isolate	Germination (%) after	
	4 hrs	8 hrs		4 hrs	8 hrs
Ac1	70.2	81.4	Ac27	72.8	81.5
Ac2	70.5	83.5	Ac28	75.4	81.0
Ac3	72.5	84.6	Ac29	72.8	83.5
Ac4	70.3	82.5	Ac30	75.2	85.3
Ac5	72.5	85.2	Ac31	72.3	85.3
Ac6	75.5	81.5	Ac32	70.5	83.7
Ac7	75.6	83.4	Ac33	73.2	84.1
Ac8	73.8	86.0	Ac34	70.5	78.9
Ac9	71.6	80.4	Ac35	68.5	82.3
Ac10	72.3	83.7	Ac36	76.2	81.6
Ac11	78.4	86.8	Ac37	73.8	80.2
Ac12	74.2	85.2	Ac38	69.5	85.7
Ac13	70.8	80.2	Ac39	73.5	82.4
Ac14	75.8	85.3	Ac40	72.6	83.6
Ac15	74.6	86.4	Ac41	70.2	85.2
Ac16	75.8	84.3	Ac42	75.2	84.8
Ac17	75.6	82.4	Ac43	72.5	79.4
Ac18	76.8	84.6	Ac44	73.5	85.3
Ac19	74.3	86.2	Ac45	70.5	80.3
Ac20	68.5	83.7	Ac46	74.5	85.3
Ac21	68.2	79.2	Ac47	74.5	86.5
Ac22	75.3	82.5	Ac48	70.5	81.6
Ac23	72.8	83.4	Ac49	75.4	85.2
Ac24	69.5	83.5	Ac50	70.5	83.7
Ac25	74.2	82.7	Ac51	73.4	82.0
Ac26	70.2	85.2	Ac52	70.2	84.2

At 4 hours interval; eighteen isolates (Ac1, Ac2, Ac4, Ac9, Ac13, Ac20, Ac21, Ac24, Ac26, Ac32, Ac34, Ac35, Ac38, Ac41, Ac45, Ac48, Ac50 and Ac52) showed 68 -72 per cent germination. Twenty isolates (Ac3, Ac5, Ac8, Ac10, Ac12, Ac15, Ac19, Ac23, Ac25, Ac27, Ac29, Ac31, Ac33, Ac37, Ac39, Ac40, Ac43, Ac46, Ac47, Ac51) had 72.1-75 per cent germination. Fourteen isolate (Ac6, Ac7, Ac11, Ac14, Ac16, Ac17, Ac18, Ac22, Ac28, Ac30, Ac36, Ac42, Ac44, Ac49) had 75.1-78.4 per cent germination. It was observed that Ac11 isolate has maximum sporangial germination (78.4 %) and Ac21 has minimum sporangial germination (68.2 %) (Table 4.8).

At eight hours interval; fourteen isolate (Ac1, Ac6, Ac9, Ac13, Ac21, Ac27, Ac28, Ac34, Ac36, Ac37, Ac43, Ac45, Ac48, Ac51) showed 79-82 per cent germination. Twenty-two isolates (Ac2, Ac4, Ac7, Ac10, Ac12, Ac17, Ac20, Ac22, Ac23, Ac24, Ac25, Ac26, Ac29, Ac31, Ac32, Ac35, Ac38, Ac39, Ac40, Ac46, Ac50 and Ac52 showed 82.1-85 per cent germination. Sixteen isolates (Ac3, Ac5, Ac8, Ac11, Ac14, Ac15, Ac16, Ac18, Ac19, Ac30, Ac33, Ac41, Ac42, Ac44, Ac47 and Ac49) showed 85.1-86.8 per cent germination. Isolate Ac11 had maximum sporangial germination (86.8 per cent) while Ac21 has minimum sporangial germination (79.2 per cent) at eight hours interval.

The present investigations have shown that *A. candida* isolates differ in their sporangial germination. The results were also in agreement with Patni *et al* (2005). They observed germination percentage of sporangia ranging from 85.0 to 87.5 per cent. Lakra and Saharan (1988) also observed germination percentage of sporangia ranging from 72.67 to 82.11 per cent.

4.2.7 Categorization of Isolates

All the 52 isolates have been categorized into different groups on the basis of pustule characters *viz*. pustule diameter, pustule shape and germination of sporangia. These isolates were divided into three morphological groups, designated as AC-I, AC-II and AC-III. Details of these groups are given in table 4.9.

Isolates of group AC-I had scattered pustules with diameter varying from 2.0-6.5 mm. Germination of sporangia varied from 81.0-86.0 per cent. Twenty-three isolates belonged to this group. Isolates of group AC-II had pin head pustules with diameter varying from 0.5-1.9 mm. Germination of sporangia varied from 78.9-82.5 per cent. This group comprises nine isolates. Isolates of group AC-III had ring type pustules with diameter varying from 2.0-7.0 mm. Germination of sporangia varied from 82.0-87.0 per cent. Twenty isolates fall under this group. It was also observed that all the isolates when inoculated at the most susceptible variety RLM 1359, reproduced similar symptoms of the category to which they originally belonged except few isolates which changed their position.

Variation in morphological and physiological characters of different isolates of *A. candida* had been reported by earlier workers. Gupta and Saharan (2002) had also compiled

four groups of different isolates on the basis of their size, shape and colour of pustule, number of concentric rings, with or without halo zones and number of pustules inside the concentric rings on leaves. Patni *et al* (2002) divided different isolates of *A. candida* into five broad categories on the basis of their morphological characters.

Table 4.9: Groups of *A. candida* isolates based on morphological characters

Groups	Morphological characters	Isolates
AC-I	Scattered pustules with diameter varying from 2.0-6.5mm, sporangial germination varying from 81- 86 per cent.	Ac2, Ac3, Ac5, Ac7, Ac8, Ac12, Ac16, Ac24, Ac25, Ac28, Ac29, Ac31, Ac33, Ac38, Ac39, Ac40, Ac41, Ac42, Ac45, Ac46, Ac49, Ac50, Ac52
AC-II	Pin head pustules with diameter varying from 0.5-1.9 mm, sporangial germination varying from 78.9- 82.5 per cent.	Ac1, Ac4, Ac21, Ac27, Ac34, Ac35, Ac36, Ac43, Ac48
AC-III	Ring type pustules with diameter varying from 2.0-7.0 mm, sporangial germination varying from 82-87 per cent.	Ac6, Ac9, Ac10, Ac11, Ac13, Ac14, Ac15, Ac17, Ac18, Ac19, Ac20, Ac22, Ac23, Ac26, Ac30, Ac32, Ac37, Ac44, Ac47, Ac51

4.3 Reaction on host differentials

A perusal of data presented in table 4.10 revealed that variation existed in different *Brassica* spp. w.r.t. disease reaction for all the three groups of isolates. Among the twelve host differentials tested against three groups of *A. candida* isolates, there was no differential response of *B. napus, B. carinata, B. oleracea, B. tournifortii, B. rapa* (Yellow *sarson*), *Raphanus sativus* and *Eruca sativa,* since none of the isolate could infect these species.

Isolates of different groups (AC-I, AC-II and AC-III) of *B. juncea* expressed maximum virulence on five differential hosts *viz. B. juncea, B. nigra, B. rapa* var. *Toria*, *B. rapa* (Brown *sarson*) and *Sinapis alba.* It was observed that even under favourable epiphytotic conditions symptoms produced merely in traces on *B. rapa* var. *Toria* and *Sinapis alba*.

Isolates of group AC-I expressed symptoms on three differential hosts (*B. juncea, B. nigra* and *B. rapa* (Brown *sarson*) and Isolates of group AC-II exhibited symptoms only on *B. juncea.* Group AC-III express symptoms on three differential hosts (*B. juncea, B. rapa* var. *Toria* and *Sinapis alba*). AC-II group has least host range and was less virulent than other two groups (AC-I and AC-III). Further, it was observed that all the groups varied in different host range.

Overall scenario of differential interaction of these isolates tested on 12 host differentials revealed that these were differing among themselves. Similar results were observed by various workers *viz.* Singh and Bhardwaj (1984), Verma (1989), Verma (1990), Lahri and Bhowmik (1995), (Verma *et al* 1999), Rimmer *et al* (2000), Gupta and Saharan (2002), Patni *et al* (2005) and Kaur *et al* (2008).

Singh and Bhardwaj (1984) tested 12 *Brassica* species from Himachal Pradesh and identified 9 races from four hosts, *viz*., *B. juncea, B. campestris* var. *Toria*, *B. campestris* var. Brown *sarson* and *B. campestris* var. *pekinensis*. Bhardwaj and Sud (1988) reported that the reaction of nine isolates of *A. candida* collected from different hosts differed from each other on 26 differential hosts. It was reported that *A. candida* on crucifers exist in the form of nine different biological races. Verma (1989) reported that in India, the *A. candida* race attacking *B. juncea* does not appear to be distinct from the race attacking *B. campestris* var. *Toria*.

According to Verma (1990) both *B. juncea* and *B. rapa* (var. *Toria*) isolates of *A. candida* from India, infected a series of cruciferous hosts of both Canadian and Indian origin. In addition to cultivars of *B. juncea*, they infected yellow *sarson*, brown *sarson*, *toria*, *B. nigra* and *B. alba*. Both the isolates gave highest disease severity ratings on *B. juncea* although there were some differences in their virulence on other hosts, implying their similarity to *A. candida* race 2.

Similar view was expressed by Lahri and Bhowmik (1995), when three isolates of *A. candida* obtained from *B. juncea*, *B. rapa* and *B. nigra* were tested on a set of host differentials. These isolates, though showed some variations in their reaction to different hosts, readily cross infected the three host species of their origin and consistently produced high disease severity on cultivars of *B. juncea*.

Two new races of *A. candida,* race AC 12 from *B. juncea* and race AC 13 from *B. rapa* var. *Toria* were identified using 14 crucifer host differentials (Verma *et al* 1999). Race AC 12 and race AC 13 showed differential interactions on *B. carinata*. Isolates of *A. candida*, collected from different geographical locations in western Canada, were tested for virulence on a set of differentials from *Brassica* species to determine the variability and distribution of different races and pathotypes. Most isolates were classified as race 7 based on virulence to *B. rapa* accessions and these could be subdivided broadly into two pathotypes, 7A and 7V, on the basis of their virulence to *B. rapa* cvs. Torch and Reward (Rimmer *et al* 2000).

Gupta and Saharan (2002) reported four new pathotypes of *A. candida* isolated from cultivars of Indian mustard (AC14 from RL 1359, AC15 and AC16 from Kranti and AC17 from RH 30) and characterized them based on their interaction with 11 host differentials (EC1291261, EC322090, EC322092, EC322093, Varuna, EC287711, ZEM1, RC 781, RH 30, RH 8113 and Rajat). The symptoms induced were stable and similar to those induced by the isolates under natural conditions.

Patni *et al* (2005) observed that isolates collected from two hosts, *B. rapa* and *B. juncea* were found different in morphology, incubation period, latent period, duration for initiation of sporangium germination, number of zoospores per sporangia and size of sporangia (μm). This clearly shows that the isolates are quite distinct from one another in morphological and pathological characteristics.

Kaur *et al* (2008) tested two isolates of *A. candida*, representing strains collected from *B. juncea* and *Raphanus raphanistrum* (wild radish) on cruciferous host differentials to characterize their pathogenic behaviour. It was observed that strains obtained from *B. juncea* and *R. raphanistrum* are different in their host range.

Table 4.10: Reaction of *A. candida* isolates on host differentials

Genotype	Isolates								
	AC-I			AC-II			AC-III		
	Ac2	Ac16	Ac46	Ac4	Ac21	Ac35	Ac6	Ac15	Ac47
B. juncea	+	+	+	+	+	+	+	+	+
B. nigra	+	+	+	-	-	-	-	-	-
B. napus	-	-	-	-	-	-	-	-	-
B. carinata	-	-	-	-	-	-	-	-	-
B. oleracea	-	-	-	-	-	-	-	-	-
B. tournifortii	-	-	-	-	-	-	-	-	-
***B. rapa* (*Toria*)**	-	-	-	-	-	-	+	+	+
***B. rapa* (Brown *sarson*)**	+	+	+	-	-	-	-	-	-
***B. rapa* (Yellow *sarson*)**	-	-	-	-	-	-	-	-	-
Raphanus sativus	-	-	-	-	-	-	-	-	-
Sinapis alba	-	-	-	-	-	-	+	+	+
Eruca sativa	-	-	-	-	-	-	-	-	-

4.3.1 Incubation period (IP) and Latent period (LP)

The observations on incubation period (IP) and latent period (LP) of *A. candida* on the 12 differential hosts were recorded. It was observed that on *B. juncea* cultivar, isolates of group AC-III has least incubation period (6-7 days) and latent period (9-11 days) and AC-II has highest incubation period (7-9 days) and latent period (11-13 days). AC-I group lies in between them with incubation period (7-8 days) and latent period (11-12 days). From this it can be concluded that, AC-III group was more virulent than other two groups (AC-I and AC-II). The results clearly indicate that AC-III group was fast growing group and considered as most virulent group while AC-I as least virulent group (Table 4.11).

Further, within group AC-III, Ac6 isolate showed lowest incubation period and latent period and was more virulent than other isolates of same group. Ac6 showed similar pattern on *B. rapa* var. *Toria* and *Sinapsis alba.* Similarly in group AC-I, Ac16 isolate had lowest incubation period and latent period and was more virulent than other isolates of same group. Ac16 showed similar behaviour on *B. nigra* and *B. rapa* (Brown *sarson*) (Table 4.11).

Gupta and Saharan (2002) reported that the incubation period (IP) and latent period (LP) in different isolates collected from different locations varied from 6-17 days and 11-14 days respectively. Patni *et al* (2005) also reported the incubation period and latent period of *A. candida*, 6 and 12 days respectively on *B. juncea* genotype. Mishra *et al* (2009) observed disease on different varieties of *B. juncea* inoculated with *A. candida.* The incubation period at the cotyledonary leaf stage varied from 10-13 days, whereas at the true-leaf stage, it ranged from 10-12 days.

Table 4.11: Incubation and latent period of *A. candida* isolates on host differentials

Genotype	Isolates																	
	AC-I						AC-II						AC-III					
	Ac2		Ac16		Ac46		Ac4		Ac21		Ac35		Ac6		Ac15		Ac47	
	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
B. juncea	8	11	7	11	8	12	7	11	9	12	8	13	6	9	7	10	7	11
B. nigra	12	15	11	14	13	17	-	-	-	-	-	-	-	-	-	-	-	-
B. napus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. carinata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. oleracea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. tournifortii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***B. rapa* (*Toria*)**	-	-	-	-	-	-	-	-	-	-	-	-	13	16	15	19	14	17
***B. rapa* (Brown *sarson*)**	11	14	10	13	12	15	-	-	-	-	-	-	-	-	-	-	-	-
***B. rapa* (Yellow *sarson*)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raphanus sativus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sinapis alba	-	-	-	-	-	-	-	-	-	-	-	-	11	14	12	16	14	18
Eruca sativa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IP = Incubation period (Days)

LP = Latent period (Days)

4.3.2 Disease severity

The disease severity of white rust (*A. candida*) on 12 differential host plants under epiphytotic conditions was recorded on the basis of 0-9 point rating scale. From the data it is evident that AC-III group has high disease severity ranging from 0-9 as compared to AC-I and AC-II groups, with 0-5 rating scale on *B. juncea*. AC-I group has more disease severity than AC-II group (Table 4.12).

In AC-III group, Ac6 isolate recorded higher severity than other isolates on all the hosts (*B. juncea*, *B. rapa* var. *Toria* and *Sinapsis alba*). Similar behaviour was observed in Ac16 isolate of AC-I group on *B. juncea, B. nigra* and *B. rapa* (Brown *sarson*) (Table 4.12).

Thus, the above study indicates that AC-III group showed high disease severity and more virulence than AC-II group. AC -II group showed least disease severity. Further, it was concluded that Ac6 and Ac16 isolate of AC-III and AC-I group respectively, showed higher disease severity and were more virulent than other isolates of their respective groups.

Gupta *et al* (2002) also assessed fourteen genotypes of *B. juncea* against *A. candida* on 0-5 rating scale and observed that disease severity of white rust varied from 0.00 to 80.2 per cent on different genotypes. Kaur *et al* (2008) also observed disease severity of white rust on 13 *Brassica* genotypes using 0-9 rating scale. They observed that different genotypes inoculated with same isolate exhibited different disease severity (range 0-5.7). Two *B. juncea* cultivars *viz. B. juncea* cv. Vulcan, *B. juncea* cv. Commercial Brown showed higher disease severity when inoculated with *B. juncea.* Mishra *et al* (2009) observed disease on different varieties of *B. juncea* inoculated with *A. candida.* Disease intensity at the cotyledonary leaf stage varied from 31-67 per cent, whereas at the true-leaf stage, it ranged from 9-28 per cent at 14 days after inoculation and 6-45 per cent at 21 days after inoculation.

Table 4.12: Disease severity of *A. candida* isolates on host differentials

Isolates / Genotype	Disease severity (0-9 scale)								
	AC-I			AC-II			AC-III		
	Ac2	Ac16	Ac46	Ac4	Ac21	Ac35	Ac6	Ac15	Ac47
B. juncea	0-5(3.8)*	0-7(4.2)	0-5(3.4)	0-5(3.6)	0-5(2.3)	0-5(2.8)	0-9(4.8)	0-9(4.2)	0.7(3.4)
B. nigra	0-3(1.7)	0-3(1.9)	0-3(1.2)	-	-	-	-	-	-
B. napus	-	-	-	-	-	-	-	-	-
B. carinata	-	-	-	-	-	-	-	-	-
B. oleracea	-	-	-	-	-	-	-	-	-
B. tournifortii	-	-	-	-	-	-	-	-	-
***B. rapa* (*Toria*)**	-	-	-	-	-	-	0-3(1.7)	0-3(1.5)	0-3(1.3)
***B. rapa* (Brown *sarson*)**	0-5(2.4)	0-5(2.9)	0-5(2.0)	-	-	-	-	-	-
***B. rapa* (Yellow *sarson*)**	-	-	-	-	-	-	-	-	-
Raphanus sativus	-	-	-	-	-	-	-	-	-
Sinapis alba	-	-	-	-	-	-	0-3(1.6)	0-3(1.4)	0-3(1.2)
Eruca sativa	-	-	-	-	-	-	-	-	-

* Figures in parentheses indicate mean score for disease severity whereas outside indicate the range of the score based on 0-9 scale

4.3.3 Pustule Size

The perusal of the data presented in table 4.13 revealed that isolates from different groups showed variable size of pustules on particular genotype of differential hosts. On *B. juncea* genotype, AC-I group had pustule size ranging from 2.0-6.5 mm, while in AC-II and AC-III group, it ranged from 0.5-2.0 and 2.0-7.0 mm respectively. Within AC-I group, Ac16 isolate has maximum size (5.6 mm) and Ac46 has minimum size (3.4 mm) on *B. juncea*. Similar pattern was observed for *B. nigra* and *B. rapa* (Brown *sarson*).

Similarly in AC-III group, Ac6 isolate recorded maximum pustule size (6.8 mm) while Ac47 has minimum size (5.4 mm). Ac6 also expressed same behaviour on *B. rapa* (Brown *sarson*) and *S. alba*. In AC-II group, Ac4 isolate has maximum size (1.8 mm) and Ac35 has minimum size (0.7 mm) (Table 4.13).

4.3.4 Pustule Shape

Different groups of *A. candida* isolates show different shapes of pustule. AC-I group showed scattered type of pustules on *B. juncea* and *B. rapa* (Brown *sarson*) but pinhead type of pustules on *B. nigra.* AC-II group showed pinhead type pustules on *B. juncea* while group AC-III expressed ring type pustule on *B. juncea* and pinhead type of pustules on *B. rapa* var. *Toria* and *S. alba* (Table 4.13).

4.3.5 Pustule Number

On *B. juncea* genotype, AC-I group showed 6-12 pustules per leaf while AC-II and AC-III group showed pustules number per leaf ranging from 4-15 and 8-20, respectively. Within AC-I group, Ac16 isolate showed higher number of pustules per leaf (9) as compared to Ac2 and Ac46 each having 8 pustules per leaf. In group AC-II, Ac4 showed maximum average number of pustule (10) and Ac35 had minimum number of pustule (7). In AC-III group, Ac6 isolate recorded maximum average number of pustule (15) while Ac47 showed minimum average number (9) (Table 4.13).

4.3.6 Sporangia Number

The data in table 4.13 revealed that the isolates varied widely in their sporulation potential. Group AC-III observed to be having higher number of sporangia per pustule as compared to group AC-I and AC-III, on *B. juncea*. Within AC-III group, Ac6 has higher number of sporangia per pustule than other isolates of same group, when observed on *B. juncea, B. rapa* var. *Toria* and similar results were observed for Ac16 isolate of group AC-I, when observed on *B. juncea, B. nigra* and *B. rapa* (Brown *sarson*).

Mishra *et al* (2009) also observed variable number of sporangia per pustule. It was observed that numbers of sporangia per pustule were correlated with size of pustule. Large size (3-4mm), medium size (1-3mm) and small sized (0.5-1mm) pustules having 17500, 9500 and 6500 number of sporangia per pustule after 14 days of inoculation and 20500, 14000 and 8500 number of sporangia per pustule after 21 days of inoculation.

Table 4.13: Reaction of *A. candida* isolates on differential hosts

Isolate Group	Representative Isolates	Genotype																			
		B. juncea				*B. nigra*				*B. rapa* (*Toria*)				*B. rapa* (Brown *sarson*)				*S. alba*			
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
AC-I	**Ac2**	4.1	S	8	2.10	1.5	I	6	1.15	-	-	-	-	2.5	S	8	1.95	-	-	-	-
	Ac16	5.6	S	9	2.60	1.9	I	7	1.40	-	-	-	-	2.8	S	10	2.10	-	-	-	-
	Ac46	3.4	S	8	1.70	1.2	I	5	0.98	-	-	-	-	2.2	S	7	1.74	-	-	-	-
AC-II	**Ac4**	1.8	I	10	0.98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ac21	1.4	I	8	1.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ac35	0.7	I	7	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AC-III	**Ac6**	6.8	R	15	3.25	-	-	-	-	1.5	I	5	0.94	-	-	-	-	1.8	I	7	1.05
	Ac15	5.7	R	12	2.80	-	-	-	-	1.0	I	4	0.92	-	-	-	-	1.4	I	6	0.95
	Ac47	5.4	R	9	2.64	-	-	-	-	0.8	I	3	0.90	-	-	-	-	1.0	I	5	0.92

I - Pustule Size (in mm) **II - Pustule shape** **III - Number of pustule/leaf** **IV - Sporangia per pustule (× 10^4)**

S = Scattered Pustules
I = Isolated (Pin Head) Pustules
R = Ring type Pustules

Gupta and Saharan (2002) also observed prevalence of four different isolates on the basis of their pathogenesis and named them as AC-14, AC-15, AC-16 and AC-17. Verma *et al* (1975) and Delwiche and William (1977) added race 7 from *B. rapa* turnip rapeseed and race 8 from *B. nigra* respectively. Singh and Bhardwaj (1984) tested 12 different isolates on *Brassica* species and identified 9 races from four hosts *viz. B. juncea, B. campestris* var. *Toria*, *B. campestris* var. pekinensis. Verma *et al* (1999) reported two new races of *A. candida* in India *viz.*, race 12 from *B. juncea* and race 13 from *B. rapa* var. *Toria* using 14 (including 6 standard) crucifer host differentials.

Overall scenario of differential interaction of 3 different group of *A. candida* tested on 11 host differentials revealed that Group AC-III was the most virulent with wider host range, high disease severity, more pustule size, higher pustule number per leaf and sporangia number per pustule followed by group AC-I and group AC-II.

4.4 Effect of different temperatures on sporangial germination of *A. candida* isolates

The data recorded on sporangial germination of three representative isolates from each group at different temperatures are presented in table 4.14. The data revealed that all the three isolates Ac41, Ac35 and Ac15 (representative isolates of AC-I, AC-II and AC-III respectively) germinated at five temperatures (10±1°C, 15±1°C, 20±1°C, 25±1°C, 30±1°C) when observed at 4 and 8 hrs interval. It was observed that the isolate Ac15 produced maximum sporangial germination 78.46 per cent. While the isolate Ac35 and Ac41 showed sporangial germination of 62.34 per cent and 70.28 per cent respectively at temperature of 15±1°C. As the temperature increases, their germination was reduced and particular shape of pustules was also distorted. Least sporangial germination was recorded at 30±1°C. Similar pattern was observed at 8 hrs interval. It was also observed that Ac15 which is representative of group AC-III shown higher sporangial germination than other isolates Ac41 (AC-I) and Ac35 (AC-II) (figure 1 and 2).

Endo and Linn (1960) observed that *A. candida* sporangia germinated at an optimum temperature range of 10 to 15°C but the zoospores germinated at 10 to 20°C. Germtube elongation was best observed at 15 to 25°C. Sakai (1981) reported that *A. macrospora* infected turnip and Chinese cabbage in Taiwan. In spring crops the disease occurred first in April increase rapidly towards mid May. In autumn crop it started in early October increase gradually to early December. Temperature for sporangia and zoospore germination was favourable in the range of 10-20°C. Germination was suppressed at 10°C but it was better at 15°C.

Verma *et al* (1983) reported that white rust developed most rapidly at 21°C on detached *B. campestris* leaves infected with *A. candida* race 7. The maximum pustules occurred for 14 days after inoculation at 15 to 18°C, although at 14 days after inoculation there was little difference in the per cent infected leaves at incubation temperature of 12 to

24°C. Leu and Rimmer (1990) reported that *B. napus* showed severe infection of *A. candida* when night and day temperature ranged between 17-22°C as these conditions were found to be more favourable for fungal growth in comparison with the temperature range of 10-15°C.

Table 4.14: Effect of temperature on germination of sporangia of *A. candida* isolates at different time intervals

Isolate	Germination at different temperature (%) after									
	10±1°C		15±1°C		20±1°C		25±1°C		30±1°C	
	4 hrs	8 hrs	4 hrs	8 hrs	4 hrs	8 hrs	4 hrs	8 hrs	4 hrs	8 hrs
Ac41	62.63	71.63	70.28	80.28	49.83	58.83	32.62	51.62	15.82	12.82
Ac35	52.24	70.24	62.34	78.34	44.34	57.34	30.31	50.31	12.28	9.28
Ac15	67.92	73.92	78.46	81.46	51.42	60.42	38.67	52.67	18.28	15.28

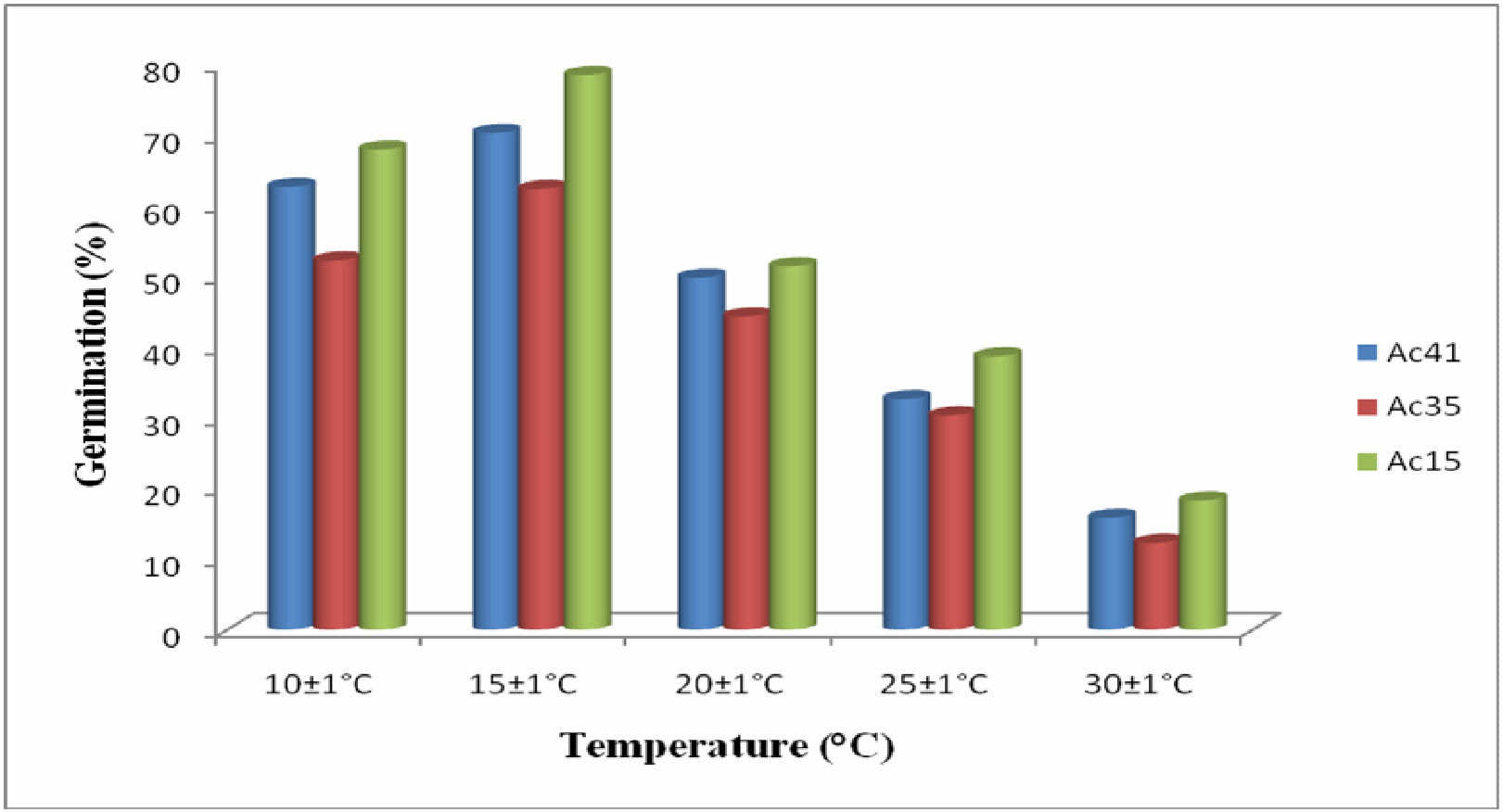

Fig. 4.1 Effect of temperature on germination of sporangia of *A. candida* isolates at 4 hours interval

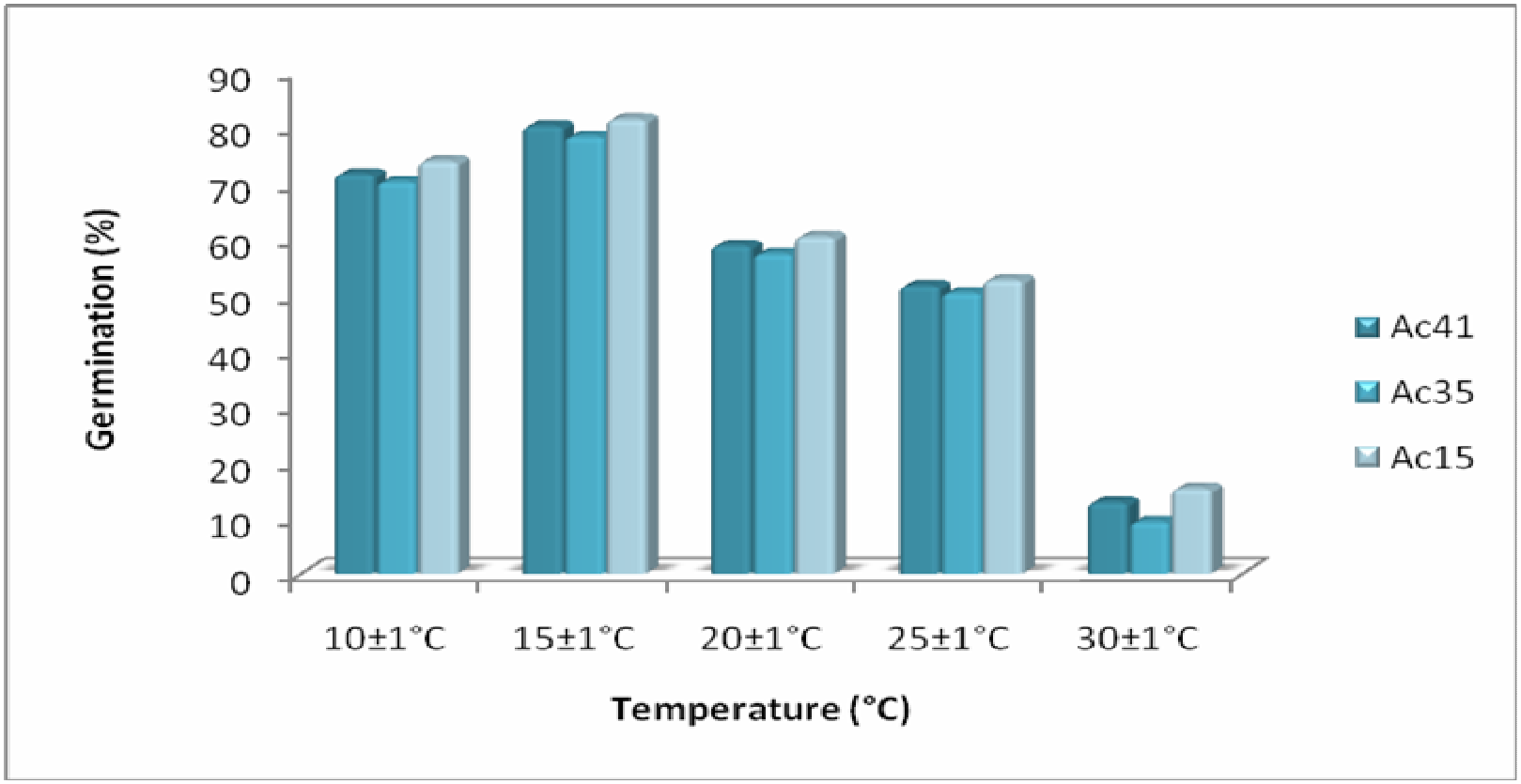

Fig. 4.2 Effect of temperature on germination of sporangia of *A. candida* isolates at 8 hours interval

4.5.1 Disease intensity of white rust at leaf phase stage

Table 4.15 illustrates the disease intensity of white rust (*A. candida*) at leaf phase in different genotypes of Indian mustard (*B. juncea*). The perusal of data reveals that different genotypes exhibit different disease intensity in natural and artificially inoculated environmental conditions. The low mean values of disease intensity were recorded in natural environment whereas higher average disease intensity was observed under artificially pathogen inoculated environment (Figure 4.3). It reveals that RLM 1359 showed significantly higher disease intensity of white rust than all other varities whereas RLM 619 had lowest value under both conditions. Gupta *et al* (2002) also tested different genotypes of *B. juncea* in three environments: natural, artificial and control. It was reported that under artificial environment disease incidence was significantly higher than other environments for all the genotypes.

Table 4.15: Disease intensity of white rust on different genotype of *B. juncea* at leaf phase stage

Genotype	Disease intensity at leaf phase		
	Natural condition	**Artificial condition**	**Mean**
PBR 91	28.73	42.13	35.43
PBR 97	26.93	41.40	34.17
PBR 210	26.47	40.33	33.40
RLM 619	23.87	35.13	29.50
RLM 1359	32.10	47.20	39.65
RLC 1	26.70	41.53	34.12
MEAN	27.47	41.29	34.38
CD for G (p = 0.05)			**0.1876**
CD for E (p = 0.05)			**0.1083**
CD for G×E (p = 0.05)			**0.266**

G = Genotype
E = Environment

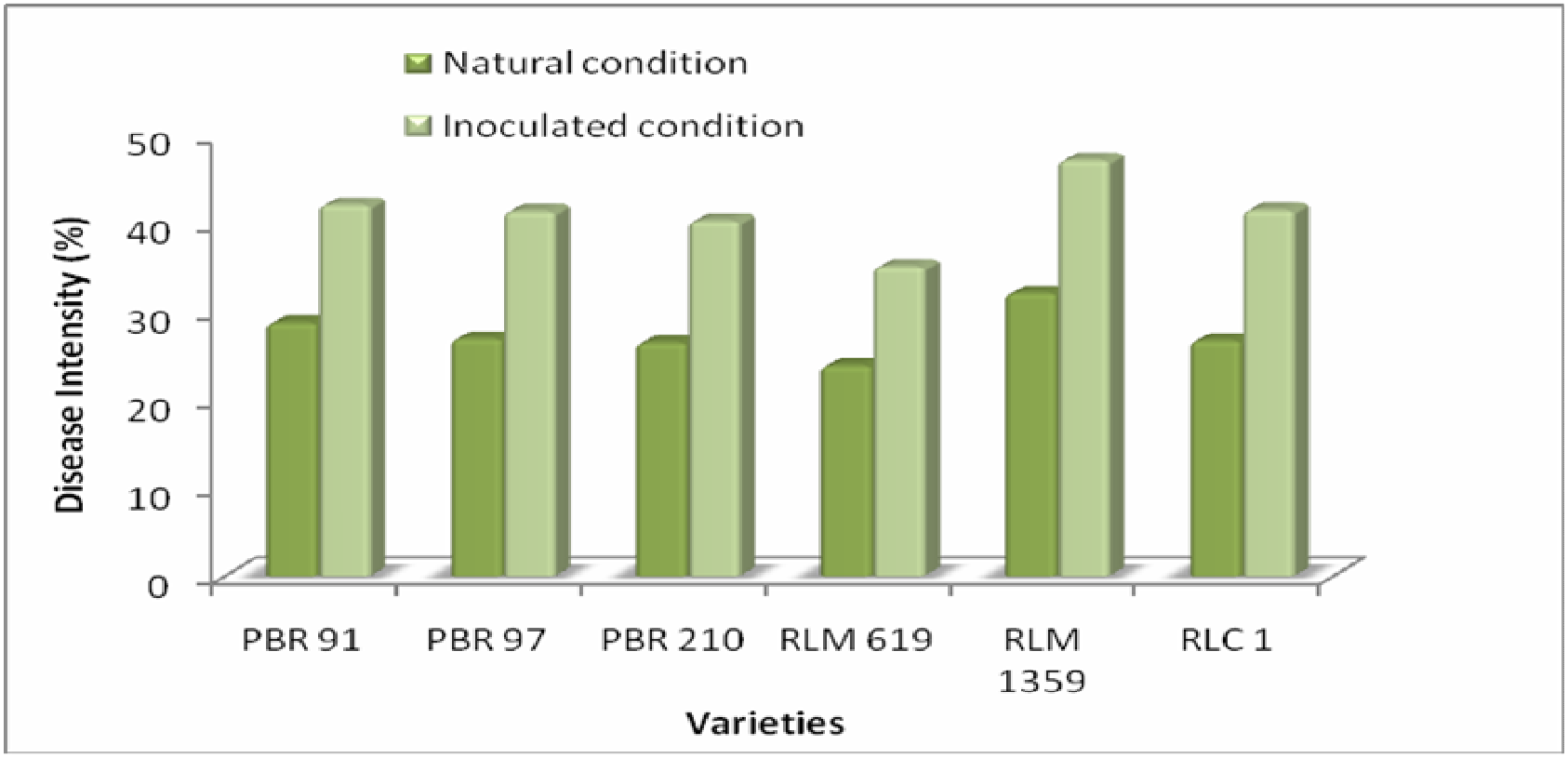

Fig. 4.3 Disease intensity of white rust at leaf phase stage of mustard genotype (*B. juncea*)

4.5.2 Disease intensity (% staghead formation) of white rust at inflorescence phase

The data on per cent staghead formation of white rust (*A. candida*) at inflorescence phase in different genotypes of Indian mustard (*B. juncea*) are presented in table 4.16. It was observed that in artificially inoculated environment per cent staghead formation was considerably higher than natural environment for all the varieties of *B. juncea*. RLM 619 has significantly lowest disease intensity than all other varieties whereas RLM 1359 showed highest disease intensity of white rust (Figure 4.4). These results were also in concordance with Gupta *et al* (2002). They observed that in artificial environment, per cent staghead formation was considerably higher than natural environment followed under controlled environment conditions.

Table 4.16: Disease intensity of white rust on different genotype of *B. juncea* at inflorescence phase

Genotype	Disease intensity at inflorescence phase		
	Natural condition	**Artificial condition**	**Mean**
PBR 91	0.34	0.84	0.59
PBR 97	0.31	0.79	0.55
PBR 210	0.41	0.95	0.68
RLM 619	0.03	0.21	0.12
RLM 1359	0.97	1.47	1.22
RLC 1	0.26	0.46	0.36
MEAN	0.39	0.79	0.59
CD for G (p = 0.05)			**0.0938**
CD for E (p = 0.05)			**0.0542**
CD for G×E (p = 0.05)			**0.133**

G = Genotype
E = Environment

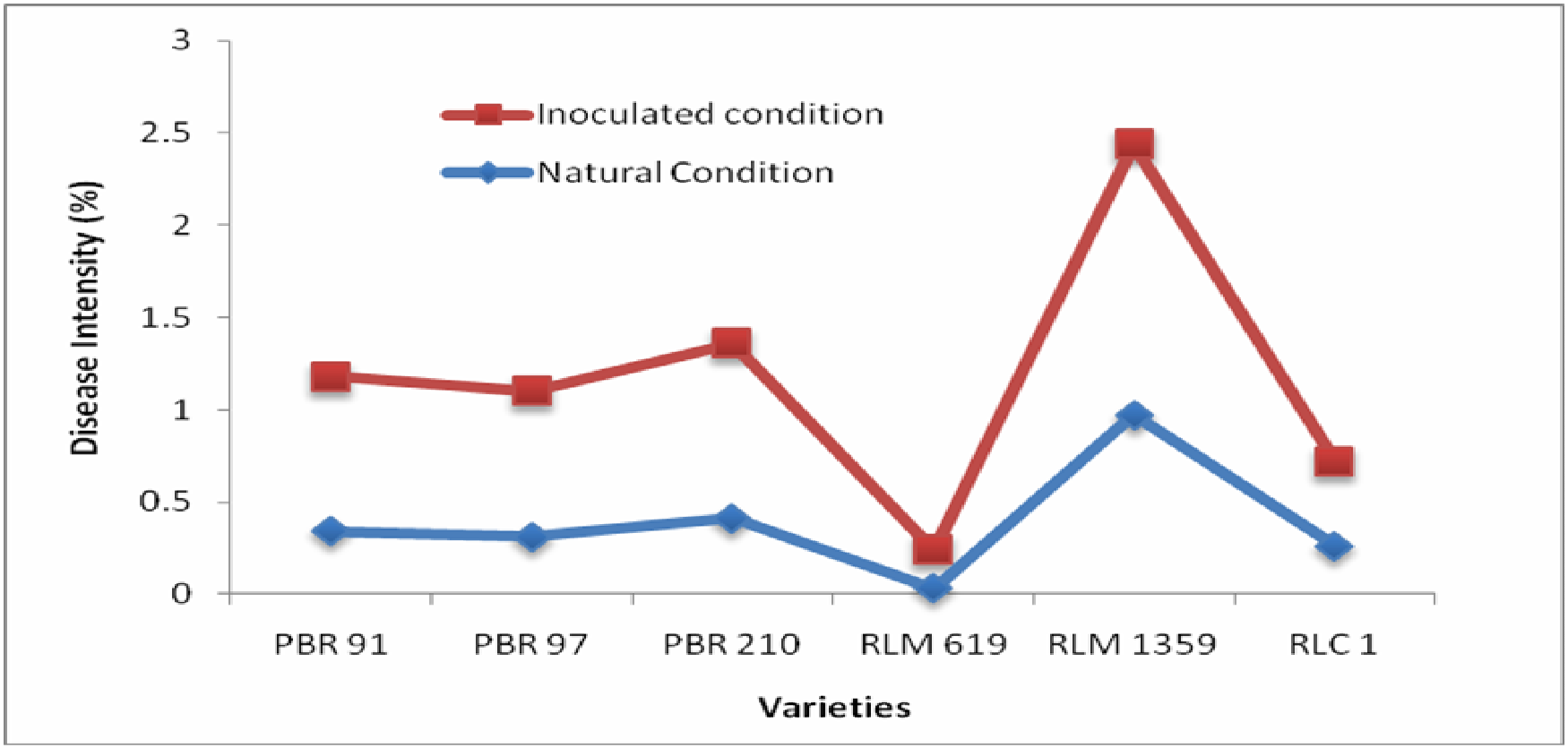

Fig. 4.4 Disease intensity of white rust on inflorescence phase of mustard genotype (*B. juncea*)

CHAPTER - V

SUMMARY

The present investigation entitled "Variability in *Albugo candida* (pers.) Kuntze causing white rust of rapeseed-mustard" was carried out at the Research Farm of Department of Plant Breeding and Genetics and Department of Plant Pathology, Punjab Agricultural University, Ludhiana during 2009-2010 and 2010-2011. During survey of the crop, it was observed that highest disease incidence and severity was in Bathinda district followed by Mansa and Barnala. Whereas in Ludhiana lowest white rust incidence and severity was recorded. The variety RLM 1359 was the most susceptible variety with highest disease incidence (58.05 %) as well as severity (28.91 %), whereas other varieties (PBR 91, PBR 97, PBR 210 and RLC 1) expressed lower disease incidence and severity. Higher incidence and severity in all the varieties was observed at Bathinda district and the minimum was recorded at Ludhiana.

All the 52 isolates of *A. candida* collected from different districts of Punjab were classified into three groups on the basis of their pustule shape *viz.* scattered, pin head and ring type pustules. First two groups were further divided into three subgroups on the basis of colour of the pustules *viz.* white, mint cream and ivory colour. Third group was divided into two subgroups *viz.* white and ivory colour.

Morphological studies revealed that pustule size varied from 0.5 - 7.0 mm in different isolates. Ac14 isolate had maximum pustule size (7 mm) while Ac48 isolate had minimum pustule size (0.5 mm). A total of 23 isolate showed scattered type of pustule, nine isolates showed pin head type pustules and twenty isolates showed ring type pustules. It was observed that 21 isolates have white colour pustule, 10 isolate have mint creamy colour pustule and 21 isolates have ivory colour pustules.

It was observed that sporangial size varied from 13.5-20.9 µm. Ac10 isolate recorded the maximum sporangial size (19.7µm) whereas the minimum size was observed in Ac40 (14.2µm). Sporangia exhibited mainly two shapes i.e. globular and slightly spherical. A total of 43 isolates have globular shape, while 9 isolates showed slightly spherical shape of sporangia. Germination of sporangia was recorded in range from 79.2- 86.8 per cent. Ac11 isolate showed maximum sporangial germination (86.8 %) whereas Ac21 isolate have minimum sporangial germination (79.2 %).

On the basis of pustule characters *viz*. pustule size, shape and germination of sporangia, 52 isolates of *A. candida* were categorized into different groups and designated as AC-I, AC-II and AC-III. Isolates of group AC-I had scattered pustules with diameter varying from 2.0-6.5 mm. Germination of sporangia varied from 81- 86 per cent. Twenty-three isolates belonged to this group. Isolates of group AC-II had pin head type pustules

with diameter varied from 0.5-1.9 mm. Germination of sporangia varied from 78.9- 82.5 per cent. This group comprises nine isolates. Isolates of group AC-III had ring type pustules with diameter varied from 2.0-7.0 mm. Germination of sporangia varied from 82-87 per cent. Twenty isolates fall under this group. It was also observed that all the isolates when inoculated on most susceptible variety RLM 1359, produced similar symptoms of the category to which they originally belonged except few isolates which showed slight variation.

Effect of temperature on sporangial germination was observed at 10±1°C, 15±1°C, 20±1°C, 25±1°C, 30±1°C after 4 and 8 hrs interval. Sporangial germination was maximum at 15±1°C and minimum at 30±1°C at both the time intervals in all the three groups of isolates. Further it was observed that Ac15 isolate which is representative of group AC-III had higher germination percentage than other isolates.

Three representative isolates of each group were tested on twelve differential host plants for their variability. All the groups showed positive reaction on *B. juncea* genotype. Group AC-I showed positive reaction on *B. juncea, B. nigra* and *B. rapa* (Brown *sarson*). Group AC-III showed positive reaction on *B. juncea, B. rapa*, var. *Toria* and *Sinapsis alba*. Group AC-II showed symptoms on *B. juncea* only. This indicates variability in *A. candida* isolates.

Incubation and latent period of the representative isolates of different groups were studied on differential host plants. Incubation period of isolates from AC-I, AC-II and AC-III groups on *B. juncea* varied from 7-8, 7-9 and 6-7 days respectively. Similarly latent period of isolates AC-I, AC-II and AC-III groups on *B. juncea* varied from 11-12, 11-13 and 9-11 days respectively. Thus, AC-III group have less both incubation and latent period than other groups and consequently was more virulent. Isolates of group AC-II had high incubation and latent period followed by group AC-I. Within group AC-III, Ac6 isolate has less incubation period and latent period than other isolates of the same group. Similar behaviour was observed in isolate Ac16 of group AC-I.

Observations on disease severity were recorded for all the groups on 0-9 rating scale. AC-III group has higher disease severity (range 0-9) than AC-I and AC-II (0-5). Within group AC-I, Ac16 isolate has high disease severity than other isolates of same group. Ac6 isolate of group AC-III also exhibited similar pattern.

The pustule size, pustule shape, number of pustules per leaf and number of sporangia per pustule shown that on *B. juncea* AC-III group has bigger pustule size, number of pustules per leaf and number of sporangia per pustule than other groups and was more virulent. Regarding shape of pustule, group AC-I showed scattered type pustules on *B. juncea* and *B. rapa* (Brown *sarson*) and pin head type pustules on *B. nigra*. Group AC-II showed pin head type pustules on *B. juncea*. Group AC-III expressed ring type pustule on

B. juncea and pin head type pustule on *B. rapa* var. *Toria* and *S. alba.* Within group AC-I, Ac16 isolate had bigger pustule size, number of pustules per leaf and number of sporangia per pustule than other isolates of same group. Similar behaviour was shown in group AC-III by Ac6 isolate.

Therefore, it is concluded from the present studies that pathogenic variability exist in *A. candida* isolates from Punjab and they are categorized in three groups *viz.* AC-I, AC-II and AC-III. Among the groups, AC-III group was most virulent followed by group AC-I and AC-II on the basis of host reaction, incubation period, latent period, disease severity, pustule size, shape, number of pustule per leaf and number of sporangia per pustule.

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