Pathogenic Variability and Identification of Resistance for Ascochyta Blight of Chickpea in Pakistan

By

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In the name of Allah, The Compassionate, the merciful

Dedicated to my late parents

DECLARATION

It is to certify that this dissertation entitled "Pathogenic Variability and Identification of Resistance for Ascochyta Blight of Chickpea in Pakistan" submitted by Sh. Muhammad Iqbal, is accepted in its present form by the Department of Biological Sciences, Qaid-I Azam University, Islamabad, Pakistan, as satisfying the dissertation requirements for the degree of Ph.D. in Biological Sciences (Plant Pathology).

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List of Acronym

%	Percentage
µg	micro gram
µ lit	micro liter
µm	micrometer
^{0}C	degrees Celsius (Centigrade)
AARI	Ayub Agricultural Research Institute
ANOVA	Analysis of variance
APS	Ammonium persulphate
AZRI	Arid Zone Research Institute
cm	centimeter
cv	cultivars
Dia	Diameter
et al.,	and others
g	gram
ha	hectare
HCl	Hydrochloric acid
ICARDA	International Centre for Agriculture Research in Dry Areas
ICRISAT	International Crop research Institute for Semi- arid Tropics
Kg	kilogram
Km	Kilometer
m	meter
Ml	milli liter
MW	Molecular weight
MW-SDS 70	Molecular marker by Sigma Chemical
NARC	National Agricultural Research Centre

NIAB	Nuclear Institute for Agriculture and Biology
NIFA	Nuclear Institute for Food and Agriculture
PARC	Pakistan Agricultural Research Council
PH	Proportionate hydrogen ions
psi	Pressure per square inch
RH	Relative humidity
RT	Room temperature
SDS	Simple distilled water
SDS-PAGE	Sodium dodecyl polyacramide gel electrophoresis
SPSS	Statistical package for social science
t/ tons	tones
UAF	University of Agriculture, Faisalabad
UPGMA	Un-weighted pair group average
V/V	Volume by volume
W/V	Weight by volume

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ABSTRACT

Chickpea (*Cicer arietinum* L.) belongs to the family *Fabaceae* is the most important pulse crop of Pakistan, which is grown as a post-monsoon cool-season crop. It is infected by many diseases but blight caused by *Ascochyta rabiei* (Pass.) Lab. is considered to be the most devastating disease. During field survey, it was observed that blight was a common disease in the major chickpea growing areas of Punjab and North-West Frontier Province (NWFP). The maximum disease prevalence, incidence and severity was found in the districts of Rawalpindi in Punjab whereas in the district of Karak in NWFP. The lowest disease prevalence was recorded in Jhang (Punjab), and in Dera Ismail Khan (NWFP). Disease prevalence was the highest in the northern sub-mountainous region where rainfall and temperature were conducive for disease development during crop growing season.

Forty two isolates of *A. rabiei* representing all areas of chickpea of Punjab and NWFP were collected for pathogenic variability. The isolates exhibited variation in morphological and cultural characteristics. Two clusters were observed using UPGMA (unweigthed pair group method average) that was able to separate *A. rabiei* isolates on the basis of aggressiveness. The virulent isolates gave same intensity of infection, whereas others were observed with varying degrees of infection. Multivariate analyses were able to distinguish isolates on the basis of virulence rather than origin or morphological/cultural characterization. The susceptible differentials (C727, ILC 263, C 44 and CM 72) were identified but no variety could be established as resistant that might be due to complex nature of *A. rabiei*. Clustering pattern indicated the exchange of breeding material and disease cultures among the researchers or high heterogeneity in the isolates.

Two isolates of *A. rabiei* representing the most aggressive and the least aggressive revealed variations in their pathogenic reaction. Significant differences among chickpea genotypes were observed for their response to isolates regarding disease development. Five

varieties, C 727, C 44, Noor 91, Punjab 91 and ILC 263 exhibited high degree of susceptibility. Two genotypes, Dasht and Balkasar showed resistance to both the isolates when applied either alone or mixed in 1:1 ratio. The aggressiveness of the mixture of these two isolates was reduced to the level of the least aggressive isolate.

The relationship of disease factors such as inoculum potential, plant age, effect of leaf wetness and latent period with blight pathogenesis, revealed a linear relationship between disease severity and inoculum concentration. Inoculation of chickpea cultivars; Punjab 91 and C 727 at different physiological stages from 2 to 12 weeks (the seedling to the reproductive stage) revealed that 2-weeks old seedlings were more susceptible to disease than the adult plants. This suggests to screen large nurseries at seedling stage and then only tolerant lines to screen under field conditions. Effect of leaf wetness and incubation period on the disease development revealed that 2-3 days incubation period coupled with 1-2 sprays a day had a pronounced effect on disease development.

In order to identify the sources of genetic resistance to blight, 824 chickpea germplasm accessions were obtained during 1994 to 1996 from national and international research sources and were evaluated under greenhouse and field conditions for three years. None of the genotypes was resistant at pod formation stage during all the three years except thirty seven during 1994, seventy two during 1995 and twenty one during 1996, which were moderately resistant. Chickpea genotypes, ILC 72, ILC 3279, ICC 3996 and NIFA 88 were found highly resistant both under field as well as greenhouse conditions. Disease at seedling and pod formation stage exhibited high association although level of infection was higher at pod formation stage.

A relationship of morphological traits viz; number of hairs on dorsal and ventral sides of leaves, number and size of stomata, guard cells and stomatal aperture with blight resistance of six chickpea cultivars having different level of blight tolerance was studied. No relationship of these morphological traits with resistance was found.

An experiment was conducted to determine the relationship of chickpea genotypes towards blight disease reaction on the basis of biochemical markers (seed protein) of

chickpea. Seed proteins were analyzed through slab type SDS-PAGE using 11.25% Polyacrylamide gel and 6 µl of sample quantity. Most of the genotypes were grouped on the basis of disease reaction and in-vitro fungus growth but no association between disease and SDS-PAGE was observed. Out of twelve SDS-PAGE markers, 6 were polymorphic. The genotypes with similar banding patterns can to be tested by 2-D electrophoresis and DNA markers. Cluster analysis revealed mixed grouping of susceptible and tolerant genotypes that indicated no response for classifying chickpea for disease reaction on the basis of SDS-PAGE. A low level of genetic diversity was observed among 57 genotypes although those originated from diverse sources. As SDS-PAGE alone did not exhibit high level of variation and disease rating was more reliable than protein peptides, but simultaneous study for both aspects (disease and biochemical analysis) suggested.

Seven cultivars of chickpea (C 44, C 727, CM-72, Dasht, Parbat, NIFA 88 and Punjab 91) were tested to study the genotypic response to Ascochyta blight and yield losses. Disease severity index of the cultivars ranged from 44 to 82%. Maximum disease at vegetative stage was recorded on C 727 followed by C 44 and Punjab 91. Minimum disease at vegetative stage was observed on Dasht. Pod infection varied from 17 to 90% and was the highest on C 727 and least on Dasht. Minimum (2%) and maximum (42%) seed infection was in NIFA 88 and C 727, respectively. Comparison of data on pods per plant, seeds per plant, 100- seeds weight, yield per plant and yield per ha. from healthy and diseased conditions revealed that the disease caused more losses to C 727, C 44 and Punjab 91, whereas Dasht and NIFA 88 were moderately resistant to blight with minimum loss of yield and yield components.

CHAPTER-I

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a self-pollinated crop, belongs to family Fabaceae and monogenic tribe *Cicerae*, is a major pulse crop of Pakistan. It is grown on an area of 971,800 hectares with annual production of 464,500 metric tones of dry seed (Anonymous, 2001). Pakistan ranks second in the world in area and third in production of chickpea. It is an important source of protein-enriched human food and animal feed particularly for the population of Southeast Asia, besides helping in the management of soil fertility of dry lands (Suzuki and Konno, 1982). It has also multiple functions in the traditional farming system in developing countries (Saxena and Singh, 1987). It is a subtropical crop, which is drought-resistant and grows most successfully in cooler and dry climates. In Pakistan and other countries of Indo-Pak subcontinent, it grows best as a post-monsoon cool-season crop. Depending upon the varieties and environment, it takes 90-180 days to mature.

Two groups are recognized within cultivated chickpea. Kabuli cultivars which are common in the Mediterranean region and in the North East. They are tall with white flowers and produce large rounded seed usually pale cream in colour. Desi (local) cultivars are relatively short, some times prostrate, commonly with anthocyanin pigmentation in flowers and stems. They produce small, irregularly shaped seeds of various colours (Allen, 1983). Production of chickpea has either remained static or declined over the past decade (Fig. 1.1). Among various factors contributing towards its low production, biological constraints, particularly diseases, are the most important. Of several diseases affecting this crop, blight caused by *Ascochyta rabiei* (Pass.) Lab., teleopmorph *Didymella rabiei* Kovacheveski is the most serious. It is known to occur in almost all countries where chickpea is grown (Nene, 1982).

Epidemics of ascochyta blight of chickpea have been recorded since the early 1900s in many countries. However, the knowledge pertaining to epidemic outbreak is

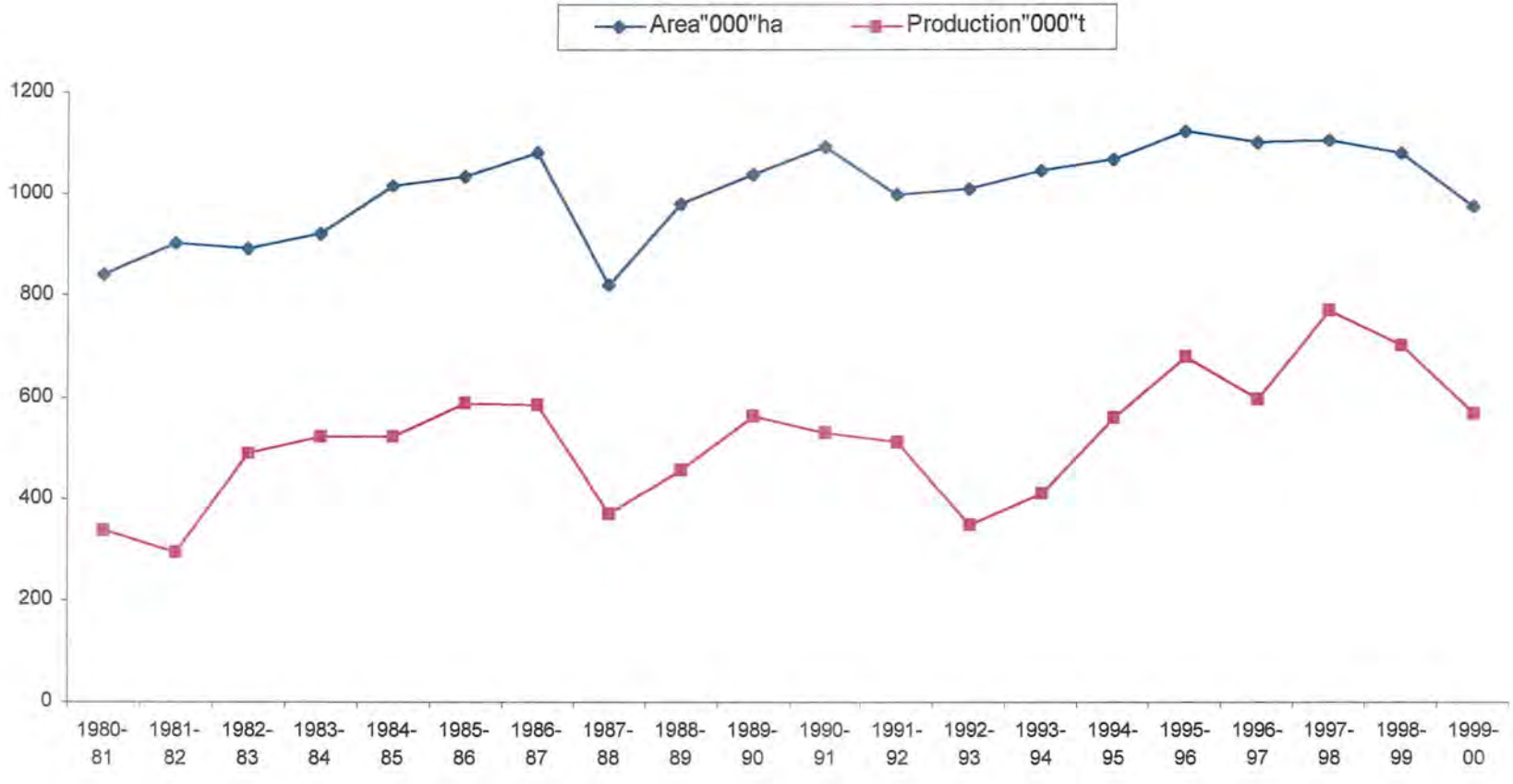


Fig. 1.1: Area and production of chickpea in Pakistan

lacking. Several factors are required for epidemics, including, the presence of numerous susceptible host plants, enough inoculum of virulent isolates of the pathogen and climatic conditions favouring for disease development over a period of time. A number of epiphytotics of Ascochyta blight have been reported in Pakistan (Aslam, 1984). The disease caused heavy losses to the chickpea crop during the epiphytotic years of 1979-1980, 1980-81 and 1981-82 reducing chickpea production by 48, 46 and 46% respectively, causing loss of US$ 158 million to chickpea growers (Malik and Bashir, 1984; Malik, 1986). The shortfalls in domestic supply of chickpea during the blight years were met through import of 282,000 metric tones of pulses worth US$ 88.9 million from 1980 to 1983 (Malik, 1986).

Chickpea is a self-pollinated crop, therefore, its resistance seems to be simple but due to complex nature of genes involved in breeding for disease resistance, has not been fully explored. A number of research studies have been undertaken on multilateral aspects of the disease in various parts of the world in order to understand and manage the disease. Different parameters have been explored including severity, pathogenicity, life cycle, disease cycle, epidemiology, breeding for resistance as well as cultural and chemical control of chickpea blight (Kaiser, 1973, 1992, 1984). A few studies on the role of organic acids, supposedly the contributors of host resistance were also made (Pandey *et al.*, 1986, 87). Investigations on the production of phenolic compounds by chickpea cultivars were conducted by Alam *et al.* 1989) and Hohl *et al.* (1990). Role of mineral elements in determining the magnitude of host resistance to Ascochyta blight was determined by Randhawa (1994).

In Pakistan, resistant varieties contributed to some extent to the improvement and stability of chickpea production. Since 1972, C 44, CM 72, NIFA 88, Pb 91, Piadar 91, Karak 1, CM 68, Dasht, Parbat and Buksar and Bittle have been released as resistant varieties. Most of these varieties became susceptible within a short span of time. The breakdown of genetic resistance may be attributed to the genetic variability in the pathogen (Qureshi and Alam, 1984).

Although a lot of efforts have been done to manage Ascochyta blight by various means yet serious gaps in knowledge are to be filled regarding the genetic manipulation

of host plant for resistance against blight and pathogenic variability in *A. rabiei*. Hence, the study was initiated with the following objectives;

- Survey of chickpea production areas of Pakistan to assess blight prevalence, incidence and severity.
- To study the pathogenic variability among isolates of *Ascochyta rabiei*.
- To determine the factors favouring blight development.
- To evaluate the response of chickpea breeding materials and cultivars against disease under greenhouse and field conditions.
- To determine the effect of morphological and biochemical characters towards disease resistance.
- To investigate the effect of blight on yield of chickpea.

CHICKPEA PRODUCTION AREAS OF PAKISTAN

Chickpea is grown under three cropping systems:(i) rainfed system which constitutes 88% of the total chickpea area, where it is grown as a pure or mixed with other crops, (ii) rice-based system constitutes 11% of the total area and is grown on residual moisture after rice harvest, and (iii) irrigated system which constitutes only 1% of the total area. Eighty seven percent of the chickpea is grown on sandy to loam soil and 13% on clay to clay loam soils (Haqqani *et al.*, 2000). Major part of chickpea production comes from rainfed system, with frequent fluctuation from year to year.

Major chickpea producing area in Pakistan is Thal that contributes about 80% of total chickpea production (Fig. 1.2). Thal includes Khushab, Mianwali, Bhakhar, Leiah and Jhang districts in the Punjab Province and Dera Ismail Khan, Bannu and Karak districts of North-West Frontier Province (NWFP). The climate of these districts is hot and windy during summer and mild in winter. On the basis of long term data (more than 20 years) obtained from Khushab, Dera Ismail Khan and Multan districts located in the vicinity of Thal, average annual rainfall varies from 261 mm to 385 mm in the northeast

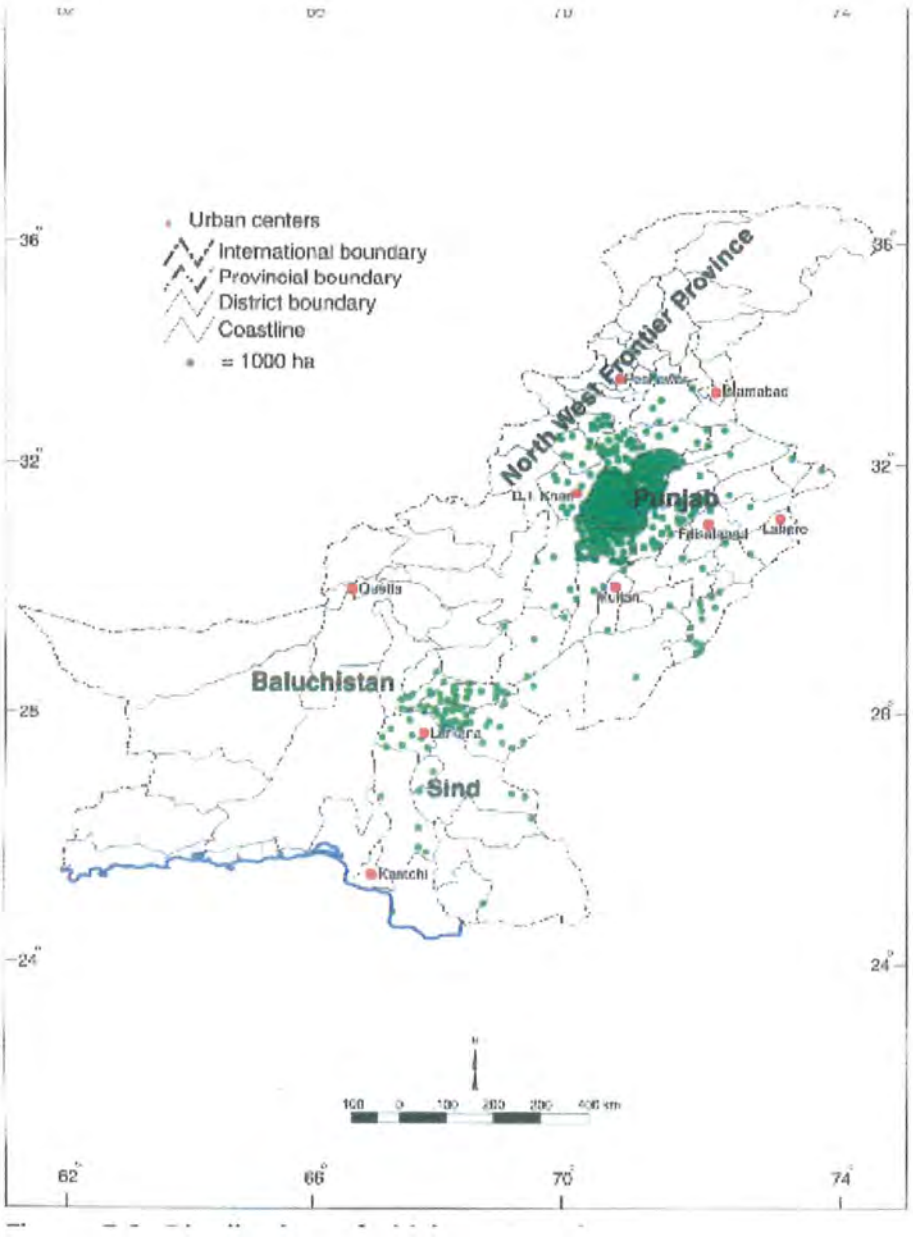


Fig. 1.2: Chickpea growing areas in Pakistan (Source; Government of Pakistan 1999-2000

and around 169 mm in the south. The annual rainfall follows a bi-model pattern, with about 70% of total rainfall occurring in summer (Khan *et al.*, 1991).

Soils of Thal area in Pakistan are mixed calcareous alluvium, which were deposited by the Indus, Chenab and Jhelum rivers. Almost the entire area is undulating sand dunes with interdunal valleys. The soil on dunes is sandy and in the valleys is loamy. There are flood plains near the banks of Jhelum and Indus rivers (Directorate of Soil Survey, 1968). In Sindh Province, chickpea is mainly grown after rice in Shikarpur, Jacobabad, Sukhar, Larkana and Nawabshah districts. The climate of this area is very hot and arid. The soils are sandy, loamy and clayey floodplains. Maximum temperature during chickpea growing period is 20-37^{0}C and minimum is 6-17^{0}C. In Pothohar region (Rawalpindi, Chakwal, Jhelum and Attock districts) farmers have abandoned chickpea cultivation since 1980 due to susceptibility of the existing cultivars to Ascochyta blight. Since 1994, chickpea cultivation in Pothohar region has been restored and the area is increasing every year due to introduction of blight resistant cultivars. Annual precipitation in this region varies from 400 mm to 1000 mm. During chickpea crop season maximum temperature ranges from 19 to 35 C and the minimum 2-18^0 C.

CHAPTER-2

REVIEW OF LITERATURE

2.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important cool season food legume crop in the world after dry peas (Robertson *et al.*, 1995). Most probably, it originated in the area of Southeast Turkey and adjoining Syria (van der Maesen, 1987). Large-seeded chickpea was prevalent around the Mediterranean basin, whereas the small-seeded ones predominated eastwards (Vavilov, 1949). Chickpea is a self-pollinated crop belongs to family Fabaceae and to a monogenic tribe *Cicerae*.

More than 50 pathogens have been reported so far from different chickpea growing countries (Nene and Reddy, 1987). The most important diseases of chickpea include, Ascochyta blight (*Ascochyta rabiei* (Pass) Lab.), wilt (*Fusarium oxysporum* Schlecht. emend. snyder. and Hans. f.sp. *ciceri* (Padwick) Synd. and Hans), dry root rot (*Rhizoctonia bataticola* (Taub) Butler)= {*Macrophomina phaseolina* [Maub]. (Ashby)}, black root rot (*Fusarium solani* Mart. (Apple & Wr.), grey mould (*Botrytis cinerea* Pers. ex Fr.), Phytophthora root rot (*Phytophthora meqasperma* Drenchs.), Pythium seed and seedling rot (*Phythium ultimum* Trow.) and stunt (Pea leaf roll virus). Among all the diseases, Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. is considered to be one of the most devastating disease (Nene, 1982). This disease has been reported from 35 countries of the world (Haware, 1998). It has been reported from Afghanistan, Algeria, Australia, Bangladesh, Bulgaria, Canada, China, Colombia, Cypress, Egypt, Ethiopia, France, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Kenya, Libya, Mexico, Morocco, Pakistan, Portugal, Romania, Spain, Sudan, Syria, Tanzania, Tunisia, Turkey, USA and the former USSR (Nene and Sheila, 1992; Nene *et al.*, 1996; Khan *et al.*, 1997 a). This disease blight is also known as chickpea blight, gram blight, ascochytosis, anthracnose, rabia or scorch of chickpea. It affects above ground-parts of the host plant. *Didymella rabiei* (Kovachevski) vs. Arx (syn. *Mycosphaerella rabiei* Kovachevski) is the

teleomorph stage of this fungus (Wilson and Kaiser, 1995).

2.2 PRODUCTION LOSSES

Ascochyta blight can cause serious yield loss in chickpea (Benloch, 1941; Biggs, 1944; Kaiser, 1972; Kauser, 1965; Malik and Tufail, 1984; Porta-Puglia and Crino, 1993; Radulescu *et al.*, 1971; Singh and Reddy, 1991; Zalpoor, 1963). In Morocco, the disease has caused up to 100% yield loss in wet conditions in 1929 and the whole crop was destroyed in 3 days under optimum condition (Labrousse, 1930; Neergard, 1977). In Azerbaijan, *A. rabiei* attacked all cultivars and yield loss of 15 to 83% occurred in conducive climatic conditions (Askerov, 1968). In India, 25-50% of the crop loss had been reported (Sattar, 1933).

In Pakistan, the blight appeared in epidemic form during 1978-79 and reduced production by 17% (Malik and Tufail, 1984). It appeared again in 1979-80 and resulted in 48% reduction in total chickpea production. According to Kovachevski (1936) 20-50% of the crop was lost annually in Bulgaria. In Dnepropetrovsk region of USSR, blight was severe in 1956, sometimes causing 100% loss (Nemlienko and Lukashevich, 1935). In Greece, 10-12% damage was reported during 1957-58 (Demetriades *et al.*, 1959). Puerta Romero (1964) found that in different provinces of Spain, the reduction in yield varied from 25-100% due to blight. Georgiou and Papadopoulos (1957) found significant economic losses in Cyprus. Mlaiki and Hamid (1984) reported that in Tunisia Ascochyta blight reduced yield by 40%. Benloch (1941) and Grewal (1975) have reported epidemics of disease. Yield losses caused by Ascochyta blight are inevitable with susceptible cultivars, and may go up to 100% on a worldwide basis (Haware, 1998). Under epidemic conditions (high humidity, windy and rainy weather), 100% yield losses can occur within three weeks.

The incidence and severity of Ascochyta blight varies from crop to crop, year to year, and from one geographical area to another, depending on host, pathogen and environmental conditions. In wet seasons, substantial yield losses are likely to occur, whilst in dry season losses will be minimal.

2.3 SYMPTOMS OF BLIGHT DISEASE

Descriptions of disease symptoms reported in different countries are remarkably similar (Nene, 1982). Primary infection results in dark brown lesions at the collar region, which vary in size depending on climatic conditions, and results in damping-off (Nene and Reddy, 1987). Infection by airborne inoculum results in small, necrotic specks in the young developed leaves. Under optimum conditions, the specks rapidly enlarge and coalesce, resulting in necrosis of young leaves and shoots with numerous pycnidia in the infected area (Nene and Reddy, 1987). Necrosis progresses downwards most rapidly in susceptible cultivars and kills the whole plant. In cases of severe foliar infection, the whole plant may become dry. Under conditions adverse for disease development, the symptoms are restricted to circular spots with grey centres and brown margins (Nene and Reddy, 1987).

Generally symptoms include circular, brown spots on leaflets and pods, elongated, irregular lesions on stems and petioles and stem breakage at the point of infection. Pycnidia on the lesions are often concentrically arranged. Characteristic concentric rings of black pycnidia are common on infected pods. The fungus penetrates the pod wall and infects seed. Infected seeds have irregular patches of brown discoloration (Nene and Reddy, 1987). Symptoms of chickpea blight caused by *A.rabiei* are shown in Fig-2.1.

2.4 BIOLOGY OF THE PATHOGEN

The causal fungus of Ascochyta blight of chickpea was first named as *Zynthia rabiei* by Passerine (pre-1890), based on unicellular and hyaline pycnidiospores (Khune and Kapoor, 1980). Subsequent researchers disagreed with Passerine's findings: Comes (1891) identified the fungus as *Ascochyta pisi* Lib., and Prillieux and Delacroix (1893) named it *Phyllosticta cicerina* (Khune and Kapoor, 1980). Trotter (1918), after studying Saccardo's material, concluded that the fungus was not a species of Ascochyta and then proposed the name *Pyhllosticta rabiei* (Pass.). Later, Labrousse (1931a) suggested that the fungus should be called *Ascochyta rabiei* because 2-4% of conidia recovered from inoculated plants were 2-celled. However, Luthra and Bedi (1932) and Aujla (1960) used

Fig. 1.1: Ascochyta blight symptoms on foliage and pods

the name *Phyllosticta rabiei* and Khune and Kapoor (1980) suggested that the fungus should be named *Phoma rabiei* (Pass.) because *Phoma* species can have 5% of the pycnidiospores 2-celled. However, *A. rabiei* (Pass.) Lab. is now accepted by the majority of researchers because the fungus produces 2-4% single septate spores (Haware, 1998) and by the International Mycological Institute (IMI). The *A.rabiei* is the anamorph and *Diymella rabiei* was the preferred name for the teleomorph (Trapero-Casas and Kaiser, 1992).

The mycelium of *A. rabiei* is hyaline to brownish and septate (Reddy *et al.*, 1992). The fungus is characterized by pycnidia produced on infected tissues and on artificial media (Sattar, 1934). The pycnidia, which are visible as dark-brown, pin-head-like structures in infected plant tissues, are immersed, amphigenous, spherical to subglobose, and vary from 65-245 µm in size (Sattar, 1934). The pycnidial wall is composed of 1 to 2 layers of elongated pseudo-parenchymatous cells and the ostiole is 30-40 µm wide. Pycnidiospores (conidia) are hyaline, oval to oblong, straight or slightly curved at one or both ends, non or one septate, constricted at the septum when bi-celled, rounded at both ends, 3.5 x 10-16 µm and formed on hyaline, ampulliform phialides (Haware *et al.*, 1986).

Colonies on artificial media are flat, submerged, with sparse mycelium, white at first, becoming dark and fumaeceous on oat-meal agar (OMA), while on potato dextrose agar (PDA) at 20-25^{0}C they are creamy to pinkish at first, darkening with time (Nene, 1982; Nene, 1984). Pycnidia of *A. rabiei* are formed within 4-5 days and appear in concentric rings on artificial media such as oatmeal agar (Reddy *et al.*, 1992), chickpea seed meal agar (Jan and Wiese, 1991), potato dextrose agar (Porta-Puglia *et al.*, 1996) and seed meal dextrose agar (Reddy and Kababeh, 1985).

Bedi and Aujla (1970) reported that on OMA medium pycnidia developed best at pH 7.6 to 8.6 at 20^{0}C. Besides oatmeal agar medium, chickpea seed meal agar medium has been found to be a good medium for the growth and pycnidial production (Kaiser, 1973; Tripathi, 1985). Kaiser (1973) reported that maximum spore production occurred on 8% chickpea seed meal agar (CSMA), while mycelial growth was greatest on CSMA

or OMA at 15-20^{0}C. Khalil and Khan (1986) developed a new medium, which supports better growth and pycnidial production. Under continuous light, mycelial growth and conidial production increased but zonation occurred in alternating light and dark periods (Kaiser, 1973). At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nene (1984) confirmed these observations. However, Chauhan and Sinha (1973) found reduced sporulation on infected plants in a glass house under continuous light. The incubation period varies between 5 and 7 days depending on the temperatures provided (Zachos *et al.*, 1963; Chauhan and Sinha, 1973). It also varies with genotypes inoculated. Spore germination is improved in the presence of N/50 and N/25 malic acid and carbon food.

Ascochyta rabiei penetrates chickpea directly through the cuticle and hydrothodes, and spreads mainly in the apoplast (Kohler *et al.*, 1995). When the mycelium of *A.rabiei* grows in the leaflets towards the petioles, the mycelium is mainly found in the apoplast and in the cells of phloem, but rarely in the xylem. The infection process leads to a total collapse of the chickpea plant tissues with extensive formation of pycnidia in concentric rings near the vascular tissue (Kohler *et al.*, 1995).

A number of studies have demonstrated that the optimum temperature for growth, pycnidial production and spore germination is around 20^{0}C (Bedi and Aujla, 1970; Chauhan and Sinha, 1973; Kaiser, 1973; Maden *et al.*, 1975; Zachos *et. al.*, 1963). Temperatures below 10^{0}C and above 35^{0}C are unfavourable to the fungus (Chauhan and Sinha, 1973; Kaiser, 1973; Luthra and Bedi, 1932). Maden *et al.* (1975) reported that pycnidia did not form at 4^{0}C nor at 28^{0}C and above, and that the colonies were pinkish-brown with zonation and maximum pycnidial formation in near UV light but light pink, fluffy, without zones and pycnidia in darkness. The optimum temperature for growth, pycnidial production and spore germination is 20-22^{0}C, whereas continuous light increases sporulation (Haware, 1998).

2.5 PYSIOLOGICAL RACES

The term "race" describes a group that is genetically and often geographically distinct within a pathogen species; each race infects a different set of plant cultivars in a

consistent pattern. On the international level "races" are used to distinguish differences in pathogenicity (aggressiveness and virulence) among isolates of the fungus (Porta-Puglia, 1992). Researchers have classified *A.rabiei* isolates into pathogenic groups (Udupa *et al.*, 1998; Porta-Puglia, 1992; Khan *et al.*, 1999) and races (Porta Puglia, 1992; Gowen, 1982; Reddy and Kababeh, 1985a; Reddy and Kababeh, 1985b; Dolar and Gurcan, 1992; Kaiser *et al.*, 1992; Singh and Reddy, 1993a; Jamil *et al.*, 1995). After the release of resistant cultivars such as ILC 482 and ILC 3279, which is susceptible to only the most virulent *A.rabiei* isolates, it is speculated that the most aggressive pathotype evolved through the process of mutation in response to a change in host resistance in the field and was subsequently selected by the deployed host resistance (Udupa *et al*, 1998). Udupa *et al.* (1998) demonstrated that RAPD and microsatellite markers reliably indexed the genetic pathotype diversity in Syrian population of *A.rabiei*. These markers can be used to monitor geographical distribution of pathogen diversity and to monitor changes in pathogen variability over time.

The presence of *Didymella rabiei* is important to a chickpea-breeding programme because it is responsible for generating variability of the pathogen, creating potentially more virulent genotypes of *A.rabiei*. In studies to determine the worldwide distribution of the teleomorph, chickpea debris from Canada was incubated under environmental conditions that favoured development of the teleomorph (Kaiser, 1997). Both mating types MAT-1 and MAT-2 were present and fertile speudothecia developed on naturally infested chickpea residue from Canada, indicating the potential for sexual recombination of *A.rabiei*. Chongo *et al.* (2000) reported that the teleomorph of *A.rabiei* was the indication of development of new races.

Luthra *et al.* (1939) reported six different forms of *A.rabiei* namely A, B, C, D, E and F. Forms B, D, E and F which were biologically identical, differed form C morphologically while form A was nonpathogenic. Aujla (1964) reported differences in cultural characters and pathogenic behaviors of 11 isolates on different varieties of chickpea. Later, Bedi and Aujla (1969) suggested that the possible existence of physiologic races must be kept in view while testing breeding material for resistance. Kaiser (1973) found that isolates of *A.rabiei* from India, Iran, Turkey and Pakistan varied

greatly in growth rate, sporulation, colony appearance and pathogenicity.

Out of 392 lines tested by Luthra *et al.* (1941) three lines namely Pois chick Nos. 4732, 199 and 281 showed a high degree of resistance to *A.rabiei* under varying environmental conditions. These lines were named F-8, F-9 and F-10, respectively. Ahmed *et al.* (1952) released a cultivar C 12-34 (progeny of a cross between F-8 x Pb-7) as resistant to blight. It lost its resistance to blight in 1950-51. A new cultivar C-235 was developed which also lost its resistance in the epiphytotic year 1968, probably due to the appearance of a new race. The studies carried out at New Delhi, India (Satyavir and Grewal, 1974; Grewal, 1984) indicated that 13 morphological forms out of 268 could be distinguished as two pathogenic races on the basis of disease reaction produced on three chickpea cultivars. Resistant reaction produced by cultivars I-13, EC-26435 and C-235 against race-1 represented by fast growing and less sporulating isolates. These cultivars were, however, moderately susceptible to slow growing and more sporulating isolates representing race-2, except isolate J-101 which indicated resistant type of reaction on cultivars I-13 and C-235 and was distinguished as a biotype of race 2. Pathogenicity test with 25 well-known blight resistant lines against race 2 showed that all of them except the highly resistant line 1528-1-1 from Morocco were moderately susceptible to race 2. The observations that cultivar C-235, previously thought to be resistant in India, was susceptible to race 2 indicates that race 2 is a newly evolved or introduced race of the pathogen. It is also probable that the highly sporulating isolate of *A. rabiei* reported from Iran may belong to race 2 of the pathogen (Kaiser, 1973).

In studies on pathogenic variability of *A. rabiei* conducted in Pakistan by Qureshi and Alam (1984), it was reported that isolate 4 from a farmer's field in Attock district was most virulent. Two cultivars (CM 72 and ILC 195) showed resistant reaction to this isolate. Isolate 13 from Islamabad, originally isolated from cv. CM 72, showed susceptible reaction on cvs. CM-72, C-44, Punjab-1 and C-727. The least virulent isolate was from ILC-200 to which only cvs. Punjab-1 and C-727 were susceptible. Cultivars C-727 and Punjab-1 were susceptible and cvs. ILC-195 and ILC-200 were resistant to all the eight isolates. Porta-Puglia *et al.* (1985) tested six isolates of *A.rabiei* from different Italian regions on 21 chickpea land races of International Centre for Agricultural

Research in Dry areas (ICARDA)/ Italian region and observed the existence of complex behaviuor of the pathogen. Bashir *et al.* (1985) studied disease reaction of 46 chickpea genotypes and one local susceptible check against 6 races of *A.rabiei*. Majority of chickpea genotypes were susceptible to all the isolates and not a single line was resistant.

Numerous studies have been done in several countries to show that the morphology and pathogenicity of *A.rabiei* is highly variable (Reddy and Kababeh, 1985a; Reddy and Kababeh, 1985b; Jan and Wiese, 1991; Porta-Puglia, 1992; Chaube and Mishra, 1992; Jamil *et al.*, 1995; Porta-Puglia *et al.*, 1996; Khan *et al.*, 1999) and the loss of crop by Ascochyta blight is a frequent event in chickpea (Reddy and Kababeh, 1985b; Porta-Puglia, 1992).

2.6 EPIDEMIOLOGY

Epidemiology of blight is favoured by temperature of 9-24^{0}C for 10 or more hours per day where the humidity is greater than 60% (Haware, 1998). Windy, wet conditions accelerate disease spread. The frequency and success of epidemics of *A.rabiei* are related to its efficient mechanism of survival from season to season. The pathogen survives in infected plant debris and seed (Kaiser, 1997). Under controlled conditions, *A.rabiei* remained viable for more than 2.5 years in the debris of infected chickpea plants kept at 4-35^{0}C with relative humidity of 30-40% (Kaiser and Hannan, 1987). The pathogen loses viability rapidly under high relative humidity (60-100%) or at soil depth of 10-40 cm, but apparently survives well in debris at the soil surface if conditions are dry (Kaiser, 1973). *Didymella rabiei* can grow saprophytically on infected chickpea tissues left on the soil surface and can remain viable for at least two years (Navas-Cortes *et al.*, 1995). When the debris was buried, *D.rabiei* was restricted to the original lesions and remained viable for only 2-5 months.

Sattar (1933) showed that when chickpea seeds were smeared with conidia of *A. rabiei* and then incubated in batches at 25^{0}C and 35^{0}C for 5 months, 50% of conidia germinated at 25^{0}C and 30^{0}C while only 5% germinated after incubation at 35^{0}C. In comparison, Kaiser (1973) demonstrated that optimum production of pycnidia on dried chickpea stem pieces occurred over the range from 10-30^{0}C, with the optimum

temperature of 20^{0}C. Pycnidia matured in 46 h in continuous light, 50 h in alternating light and dark, and 68 h in continuous darkness at 20^{0}C.

Khachatryan (1963) found that RH of over 60%, with 350-400 mm rainfall during the fallow season and a daily temperature $\geq$ 15^{0}C were the ideal conditions for disease development in Armenia. Chauhan and Sinha (1973), in a glass house study, demonstrated that 85-98% RH and 20^{0}C for at least 46 hours were optimum for disease development, and there was a 6 days incubation period. It has been demonstrated that a minimum of 6 h wetness at 9-27^{0}C is required for disease development in chickpea, but more than 10 h of wetness at these temperature is required for severe disease, moreover, there was no infection of chickpea plants below 6^{0}C nor above 30^{0}C (Weltzien and Kaack, 1984).

Similarly, Trapero-Casas and Kaiser (1992) reported that the optimum temperature for infection was 20^{0}C and severe disease development required a leaf wetness period of 17 h. Relatively little information is available on the effects of temperature and leaf wetness period on disease development, so further research is needed to understand these aspects of epidemiology.

2.7 SURVIVAL OF *A. RABIEI*

As common with most foliar pathogens, *A. rabiei* survives in infected crops debris and seeds (Nene, 1982). These materials act as a reservoir of primary inoculum, which can cause infection in favorable conditions.

2.7.1 INFECTED SEED

Infected seed is an important mean of survival for *A. rabiei* and allows dissemination from one geographical area to another. It plays an important role in the epidemiology of the disease, ensuring a random distribution of the pathogen in a field, which provides many primary infection-courts from which the pathogen can spread and produce secondary infection. Butler (1918) was probably the first scientist to report the infection of chickpea seed by *A. rabiei,* and those pathogens were transmitted from infected seed during germination. Luthra and Bedi (1932) reported that *A. rabiei*

penetrated from the ovary wall into the testa at its contact point and finally colonized the cotyledons. Luthra and Bedi (1932) were the first to demonstrate the seed-borne nature of the pathogen. They showed that the seed coat and cotyledons of infected seeds contained mycelium. Sattar (1933) demonstrated surface contamination of seed with fungus spores and their role in causing infection. He found that 50% of such spores survived on seed for 5 months at 25-30^{0}C, but only 5% of spores survived for 5 months at 35^{0}C. Later other workers (Kaiser, 1972, 1973; Zachos, 1952; Zachos *et al.*, 1963; Morrall and McKanzie, 1974) also confirmed the seed borne nature of the pathogen. Lukashevich (1958) showed that the fungus could behave as a saprophyte and spread to non-infected tissues if the harvested material is stored for some time before threshing. Tripathi *et al.* (1988) observed that in infected seeds stored at low temperature (0-10^{0}C), *A.rabiei* survived for 14-15 months at 20-30^{0}C, the survival was reduced by 2 to 4 months and at room temperature, the survivability of the fungus declined sharply as an initial seed infection of 30-32% dropped down to complete elimination after 12 months.

Research conducted by Luthra and Bedi (1932), Halfon-Meiri (1970), Maden *et al* (1975) and Vishunavat *et al.* (1985) has provided valuable information on the location of the pathogen in seed. Infection of seed may occur during cool, wet weather while immature seeds are still in the pod, or during the harvesting and threshing operations. Halfon-Meiri (1970) observed that 50-80% of the seed from chickpea pods with Ascochyta lesions were infected with *A.rabiei*, but the pathogen could not be detected in seeds from apparently healthy pods collected from diseased plants.

Infected seeds may or may not show signs of infection (Maden *et al.*, 1975, Halfon-Meiri, 1970). On seeds, lesions are light to dark brown (Tripathi, 1985; Maden *et al.*, 1975, Halfon-Meiri, 1970) and range in size from 1-4 mm diameter. Black pycnidia containing mature spores are observed in several lesions, some of which form concentric zones (Maden *et al.*, 1975, Halfon-Meiri, 1970). In seeds with lesions, the fungus frequently penetrates the seed and could be isolated from cotyledonary tissues (Maden *et al.*, 1975, Halfon-Meiri, 1970; Vishunavat *et al.*, 1985). The pathogen has not been detected in embryo (Maden *et al.*, 1975). According to Dey and Singh (1994), *A. rabiei* naturally occurs both externally and internally in seed and external infection is dominant,

the pathogen being located on the seed coat and occasionally penetrating the cotyledons and embryo. Internally and externally seed-borne inocula were found to be equally responsible for the transmission of disease and the disease was transmitted to the aerial parts in a non-systemic manner (Dey and Singh, 1994).

2.7.2 INFECTED CROP DEBRIS

Researchers in various countries have stressed the importance of infected crop debris in the survival of *A. rabiei* from one growing season to another (Askerov, 1968; Kaiser, 1973; Khachatryan, 1963; Kovachevski, 1936; Anonymous, 1973; Lukashevich, 1958; Luthra *et al.*, 1935; Navas-Cortes *et al.*, 1995; Weltzien and Kaack, 1984; Zachos *et al.*, 1963). Sattar (1933) could not determine the absolute importance of infected crop debris in survival of the fungus. However, Luthra *et al.* (1935) considered diseased plant debris as an important source of primary infection. Lukashevich (1958), in the former USSR, found that *A. rabiei* grew saprophytically on dead, infected chickpea plant parts and in the subsequent spring, saprophytic activity on this material increased manyfold. Similarly, Trapero-Casas *et al.* (1988) and Zachos *et al.* (1963) found that the pathogen colonized chickpea stubbles after harvest. Luthra *et al.* (1935) found that *A. rabiei* remained alive for more than 2 years in the infected tissues, but did not survive more than 1 month if the infected debris was buried 5 cm deep in moist soil.

Kaiser (1973) confirmed that the fungus survived for more than 2 years in naturally infected tissues at 10-35^{0}C and 0-30% relative humidity (RH) at the soil surface but rapidly lost viability at 65-100% RH and at 10-40 cm deep in soil. Pandey (1984) reported that the fungus survived for over one year in infected debris stored at room temperature. In diseased debris stored at room temperature, the length of survival was for about 2 months at 40^{0}C, while at 20-35^{0}C, the survival was recorded for a period of 6 to 8 months. When infected stem pieces were buried in moist sterilized and natural soils and incubated at 20-40^{0}C, the fungus did not survive beyond 2-3 months. In infected crop debris in soil under natural conditions at the depth of 0-20 cm, the length of survival was to the extent of 2 to 3 months only.

Kaiser *et al.* (1987) studied the survival of the anamorph and teleomorph stages of

the pathogen in chickpea in field soil, in a weather station shelter at 4-6^{0}C with RH 30-40%. Only brief details were published but it appeared that conidia lost viability after 10 and 15 weeks in infected stem tissues and pods, respectively, when buried in soil, but remained viable for 57 and 81 weeks, respectively, when placed on the soil surface. Some conidia in the infected tissues remained viable after 120 weeks at 4-6^{0}C. Discharge of viable ascosporous from pseudothecia ceased after 8 weeks if buried in the soil and after 27 weeks in infected tissues placed on the soil surface.

In Spain, Navas-Cortes *et al.* (1995) observed that *D. rabiei* grew saprophytically on infected chickpea tissues lying on the soil surface and remained alive for at least 2 years but lost viability within 2-5 months if the infected debris was buried in the soil. Weltzien and Kaack (1984) also found that infected plant debris is a soil-borne form of inoculum. However, in Syria it has been reported that the pathogen survived for only 8 months (ICARDA, 1993).

2.8 DISEASE CYCLE

Available evidence suggests that *A.rabiei* neither produces resistant/resting structures nor has any alternative or collateral hosts as it infects only *Cicer* spp. Information on its saprophytic survival is very limited. Lukashevich (1958) reported that the fungus could behave as a saprophyte and spread to noninfected tissues if the harvested material is stored for some time before threshing. The data do not explain saprophytic survival of the fungus outside the host.

Two major sources of its survival are therefore, diseased crop debris left in the field after harvesting and /or infested or infected seeds. As described previously, infested and /or infected seeds are undoubtedly the most vital source of primary inoculum (Fig-2.1). How far diseased debris left in the field plays role in the perpetuation of the fungus and thereby, serving, as source of primary inoculum is uncertain. Available literature reveals that in areas where hot summer and rainy season follows the chickpea crop season, the perpetuation of the fungus is of very short duration. It appears so because the crop debris left in the field decomposes due to rains and the fungus as such cannot withstand the onslaughts of environment and microbial antagonism. Contrary to it, in

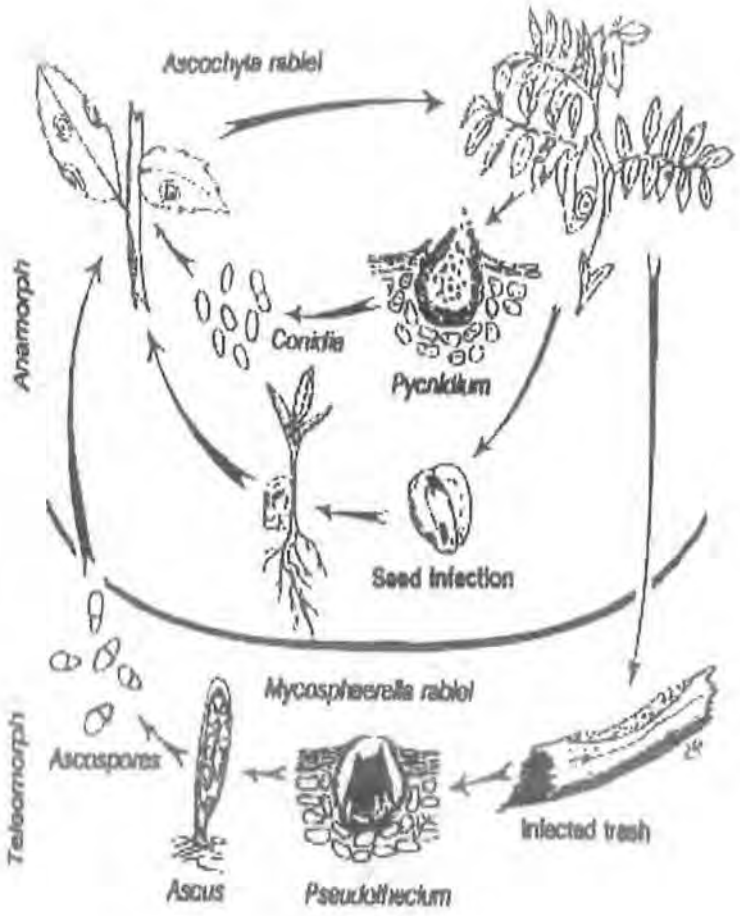


Fig 2.2: Disease cycle of Ascochyta blight of chickpea caused by *Ascochyta rabiei* in the Pacific Northwest, USA (Diagram by R.M. Hannan)

areas where a climatic condition between the two chickpea seasons is dry, the survivability of the fungus will be enough to serve as a source of primary inoculum. In addition, in regions like Eastern Europe and Western Asia, the fungus, by producing perithecial stage, ensures its survival for the next season. Obviously, cold winter following the chickpea season ensures of *A.rabiei* and in these areas crop debris definitely play role in carry over of the inoculum.

2.9 PATHOGENESIS OF *A. RABIEI*

Pandey *et al.* (1986; 1987) studied the mechanism of fungal penetration, invasion and subsequently development of *A.rabie* in leaf and stem tissues. The conidia began to germinate 12 hr after inoculation. The germ tubes elongate and some times form a swelling at the tip. Penetration of stem occurs 24 hr after inoculation directly through the cuticle. For a short distance the hyphae push forward subcutically along the junction of epidermal cells before proceeding inward. Near stoma, the hyphae penetrate through the juncture of guard and subsidiary cells even when the stoma is open. The penetrating hyphae invade the cortical cells resulting in excessive damage to cortex. Till the stage (3 days after inoculation) epidermal cells appear intact.

Pith cells damage 5 days after inoculation coincides with necrosis initiation. By the seventh day most of the nonlignified tissues are destroyed and necrosis is very much evident. There is no effect on lignified tissues, particularly treachery elements. Nature of damage, excessive breakdown and necrosis, of cellulosic tissues in advance of invading hypahae, indicated involvement of wall degrading enzymes and/or toxins (Pandey *et al.*, 1986 and; 1987). Alam *et al.* (1989) identified two toxins (solanpyrones A and B) from *A.rabiei*. Recent studies have also demonstrated pronounced cutinase (Hohl *et al.*, 1990) and other hydrolytic exoenzymes activities in the culture filtrate of *A.rabiei*. More work is needed to demonstrate the role of these toxins/enzymes in pathogenesis.

Hohl *et al.* (1990) performed detailed histopathological study of development of *A.rabiei* in leaf tissues of susceptible and resistant cultivars. Their observation of infection process was more or less similar to one described by Pandey *et al.* (1987) for the stem except they noticed the formation of appresoria and secretion of mucilaginous

exudate, which presumably provided for the fungal cells a tight contact to the host surface, from young germ tubes. Fungal development on the leaf surface (spore germination, germ tube growth, secretion of mucilaginous exudate, and appresoria formation) was essentially identical for both resistant and susceptible plants. Leaves of the susceptible plants were invaded and subepidermally colonized by the fungus and leaf spots and fungal pycnidia could be observed 6-8 days after inoculation. Whereas the resistant cutivars rapidly responded to fungal penetration by rapid cell necrosis and accumulation of phenolic compounds resulting in cessation of fungal colonization (Hohl *et al.*, 1990).

2.10 DISSEMINATION OF INOCULUM

The spread of the disease has been attributed to the pycnidiospores produced at the foci of primary infection, either through crop debris or infected seeds. Mature conidia of *A. rabiei* ooze out from pycnidia in a gelatinous matrix under wet conditions. The matrix dissolves to release the spores, which are then washed or splashed to other plants or scattered in droplets of driving rain. In dry conditions, the extruded conidia dry as hard masses on the infected tissues and, subsequently, are splash-dispersed over short distances only (Kaiser, 1992). Sattar (1933) had concluded that the aerial infection of seedlings raised from infection from infected seed is systemic and compared it with smut infection of cereals. However, Maden *et al.* (1975) failed to locate the fungus in whole mount preparations from green and healthy looking tissues of the seedlings below or in between the lesions. Chaube and Pandey (1986) observed that the fungus starts sporulating on the seeds placed in soil even before the emergence the radicle and plumule. Plumules that initially form "Crozier" often touch pycnidiospores and in the process of their growth, carry the inoculum to the shoots at the crown region.

It is accepted fact that the disease spreads rapidly. This indicates the existence of adequate means of transportation of inoculum in nature. Though experimental evidence is lacking, the circumstantial evidence suggests that air currents and to some extent rain splashes play vital role in the spread of inoculum. Sattar (1933) and Luthra *et al.* (1935) found that infected tissues could be blown by wind for hundreds of meters to act as a source of secondary infection when detached from infected plants by heavy rains and

wind. Zachos *et al.* (1963) had previously reported that the disease developed in circles by means of rain-splashed conidia but wind-driven rain spread the disease in the direction of the wind. Khachatryan (1961) working in America reported over 60% relative humidity, with 350-400 mm rainfall and an average daily temperature of not less than 15^0C to be congenial for the spread of disease.

The teleomorph contributes to long distance dissemination of the pathogen. The ascosporous are forcibly discharged into air through the opening of the pseudothecium in wet conditions (Kaiser, 1997). More than 70% of the ascospores were discharged from mature pseudothecia on naturally infected chickpea debris during a 2-h wet period at 15-25^0C (Trapero-Casas *et al.*, 1988). It has been reported that airborne ascospores served as primary inoculum to establish new infections in chickpea fields that were located 10-15 km from the nearest infected fields (Trapero-Casas and Kaiser, 1992).

2.11 EFFECT OF PLANT AGE AND INOCULUM DENSITY ON BLIGHT

Sattar (1933) studied the effect of plant age on disease development and found the plant is more susceptible at pod formation than at the seedling stage. The period of greatest susceptibility coincided with maximum secretion of malic acid from glandular hairs on the leaf, which was assumed to favour the pathogen. These results were also confirmed by Reddy and Singh (1984) and, later, by Singh and Reddy (1993b), but contradicted by Hafiz (1952), who claimed that resistant cultivars secreted more malic acid than did susceptible cultivars. However, Trapero-Casas and Kaiser (1992), using controlled conditions, did not find any significant difference in disease severity between plants inoculated at 2 and 8 weeks old. Tripathi (1985) observed that with the increase in age of plants, there was increase in incubation period and increase in the incidence and severity of the disease. He advocated that the increased toughness of the tissues and age based biochemical changes might be responsible for such decrease in disease severity. It is not clear whether the apparent greater susceptibility of chickpea plants to Ascochyta blight at maturity is due to the age of the plant or favorable environmental conditions for disease development.

Simon (1982) observed that inoculum concentrations have little role to play in the

initiation and severity of the disease. However, Tripathi (1985) concluded through glasshouse experiments that under congenial conditions even a small amount of inoculum would be enough for initiation and spread of the disease.

2.12 HOST-PATHOGEN INTERACTION

There are reports in the literature that spores of *Phoma medicaginis*, *P. medicaginis* var. *pinodella*, *Mycosphaerella* pinodes, *Ascockhyta pisi*, *Ascochyta rabiei* and *Botrytis cinerea* produce germ tubes which are able to penetrate the cuticle directly or through stomata (Ludwing, 1928; Brewer, 1960; Blackman, 1969; Punithalingam and Holliday, 1972a, b; Rijkenberg *et al.*, 1980; Hohl *et al.*, 1990; Angelini *et al.*, 1993; Dey and Singh, 1994). Pandey *et al.* (1987) studied, by light microscopy, the process of infection and histological changes in susceptible genotypes of chickpea infected by *A. rabiei*. Germ tubes from conidia penetrated the stem tissues at the juncture of two epidermal cells and form subepidermal aggregates until the fourth day after inoculation. On the sixth day, yellowing and necrosis of host tissues coincided with formation of mature pycnidia. There was extensive damage to cell walls of parenchymatous cortical and pith tissues in advance of invading hyphae, indicating involvement of cell wall degrading enzymes.

2.13 DISEASE RATING SCALES

Many rating scales have been used by various researchers for assessing chickpea material in glass house and field conditions, but there are no universally accepted disease assessment scale. Vir and Grewal (1974) used a 5-point scale based on severity of stem and foliar infection, for glass house and field screening, whereas Morrall and McKenzie (1974) used a 6-point scale based mainly on foliar infection, for field screening. Reddy and Nene (1979) suggested a 9-point scale for glass house screening. Later, Singh *et al.* (1981) suggested a scale with five defined categories of severity, for the evaluation of large-scale breeding programs in field conditions. Subsequently, Reddy and Singh (1984), Reddy *et al.* (1984) and Gowen *et al.* (1989) modified previous screening systems and developed 9-class scale for glass house and field screening and these scales have been accepted by the majority of researchers.

The methods used by researchers throughout the world to study the variability of *A. rabiei* and establish differential lines are not standardized (Porta Puglia, 1992). Differences in the studies include different inoculum concentrations, chickpea cultivars used as host-differentials, inoculation technique, age of the plant, medium used for isolation and conidial production, humidity, temperature, time between inoculation and rating, and criterion for discriminating between resistant and susceptible (Porta-Puglia, 1992).

Porta-Puglia *et al.* (1994) reported two challenges in rating Ascochyta blight infection precisely: 1) variation in disease severity among plants of the same line and 2) variation in lesion size and sporulation on the same plant. Factors such as extent of defoliation, stem blighting, pod infection, lesion size on the stems and pods and extent of sporulation in lesions have been used in rating scales. The most important factors are breaking of branches and pod infection because they have the largest impact on yield and quality (Reddy and Singh, 1990). Sing and Reddy (1993) selected lines that showed less than 5% yield loss under three years of disease pressure. They also reported that chickpea lines with as much as 20% pod infection suffers little yield (less than 5%) due to superficial or very late pod infection. The most recent scale is a 1-9 rating scale reported by Singh and Reddy (1993). This method takes into account the percentage of broken branches and infected pods: 1= no infection; 2:highly resistant (1-5% infection); 3= resistant (6-10% infection); 4= moderately resistant (11-15% infection); 5= tolerant (16-40% infection); 6= moderately susceptible (41-50% infection); 7= susceptible (51-75% infection); 8= highly susceptible (76-100% infection) and 9= very highly susceptible (plants killed). Lines with score 2-4 were considered resistant, rating of 5 was considered tolerant and ratings of 6-9 were considered susceptible.

2.14 HOST RESISTANCE

The first known source of resistance to Ascochyta blight was F4, later renamed F8 (Luthra *et al.*, 1938). Subsequently C-12/34, that was identified as resistant cultivar (Ahmad *et al.*, 1949), lost its resistance around 1950 and was replaced by another resistant cultivar C-235 (Anonymous, 1963). Grewal and Vir (1974) identified P.1528-1-1 as immune from Morocco and 1-13 as resistant line from Israel. Ganeva and Matsov

(1977) also indicated resistance in chickpea to blight. Gama irradiated chickpea mutants (CM 72 and CM 68) were identified in Pakistan as resistant sources (Haq *et al.*, 1981).

There have been many reports of resistant genetic materials from India, Pakistan, Iran, Turkey, Syria, Morocco, North Africa, France, Bulgaria and USSR. A comprehensive list of resistant sources by Singh *et al.* (1984) is reproduced in Table-2.1 with certain additions/ modifications.

Chickpea breeding programs at ICRISAT and ICARDA have screened huge germplasm and have supplied resistant lines to national programs for further screening against local isolates of *A. rabiei* in chickpea growing-countries. In general, it has been observed that the frequency of resistance is higher in the kabuli genotypes than in the desi types, based on studies of the inheritance of resistance to Ascochyta blight (Tewari and Pandey, 1986; Verma *et al.*, 1981; Verma *et al.*, 1987; Singh *et al.*, 1992). Singh *et al.* (1992) identified 12 kabuli and 3 desi resistant lines 5107 kabuli and 10,203 desi types during screening of the world germplasm collection at ICRISAT and ICARDA.

Singh *et al.* (1984) evaluated 112 chickpea lines in Algeria, Greece, India, Jordan, Morocco, Pakistan, Spain, Syria, Tunisia and Turkey. Four lines, ILC 72, ILC 191, ILC 3279 and ILC 3856, were found to be resistant to Ascochyta blight in eight of these 11 countries. In addition, Reddy and Singh (1985) reported that chickpea lines ILC 191, ILC 194, ILC 200, ILC 202, ILC 2548, ILC 2956, ILC 3279, ILC 340, ICC 3996, ICC 4107 and ICC 3375 were resistant to four isolates of *A. rabiei* in a multi-location trial conducted in Syria and Lebanon.

Singh *et al.* (1981) reported some resistant accessions in chickpea at ICARDA. Sandhu *et al.* (1990) identified 8 resistant lines and 2 tolerant lines (BG-257) and BG-261) from a collection of 406 varieties screened at 3 localities, under artificial infection and natural epiphytic conditions. They also tested chickpea germplasm lines resistant or tolerant to *A. rabiei* isolates prevalent in Syria and Lebanon. Shukla *et al.* (1984) screened 1000 lines for ICRISAT under a combination of natural and artificial infection. Three lines i.e. ICC 6270, ICC 8160 and ICC 8189 showed only traces of infection while many lines displayed tolerant reaction. Kalia (1984) screened 60 chickpea lines and

Table 2.1 Studies undertaken between 1931 and 2000 to identify sources of resistance to *Ascochyta rabiei* in *Cicer arietinum* L.

Researcher	Year	Country	Total lines	Resistant lines
Labrousse	1931a	Morocco and/or France	36	3
Labrousse	1931b	Morocco and/or France	167	11
Pavlova	1935	USSR	-	6
Luthra *et al.*	1938	Indian	187	4
Luthra *et al.*	1941	Indian	392	3
Luthra *et al.*	1943	Indian	-	1
Padwick	1948	-	-	2
Ahmed *et al.*	1949	Pakistan	-	2
Hafiz	1952	Pakistan	-	3
Enken	1954	USSR	-	2
Aziz and Kainth	1960	Pakistan	700	1
Bushkova	1960	USSR	273	1
Bedi and Athwal	1962	India	-	1
Puerto-Romero	1964	Spain	-	4
Solel and Kostrinski	1964	-	-	1
Vedysheva	1965	USSR	184	4
Vedysheva	1966	USSR	352	17
Aujla and Bedi	1967	India	189	11
Scharif *et al.*	1967	Iran	-	2
Kojnov and Redkin	1970	Bulgaria	-	2
Redkov	1970	Bulgaria	-	1.5%
Kaiser	1972	Iran	-	1
Sandhu	1972	India	600	11
Soho and Singh	1972	India	-	1
Vedysheva	1972	USSR	584	17
Zhelokov	1973	USSR	-	2
Golube	1974	USSR	500	4
Grewal and Vir	1974	India	-	2
Khico	1974	USSR	200	4
Ramanujam	1974	India	-	1
Korsakov	1975	USSR	-	5

Eser	1976	Turkey	-	1
ICRISAT	1976	India	-	7
ICRISAT	1977	India	1200	40
ICRISAT	1978	India	2000	5
ICRISAT	1982	India	-	60
Iqbal *et al.*	1989	Pakistan	759	1
Iqbal *et al.*	1994	Pakistan	467	7
Redkov	1976	Bulgaria	50	3
Ganeva and Matsov	1977	Bulgaria	220	48
Religh and Lehrer	1977	-	-	2
Singh	1978	India	262	4
Okhovat	1979	Iran	729	1
Bejiga	1980	Ethiopia	1086	2
Haq *et al.*	1981	Pakistan	208	2
Singh *et al.*	1981	Syria	9385	57
Pandey *et al.*	1982	India	76	2
Gaur *et al.*	1983	India	47	25
Jalali *et al.*	1983	India	150	7
Okhovat	1983	Turkey	5000	36
Singh *et al.*	1984	Syria	6005 Desi	655
Reddy and Singh	1984	Syria	9574 Desi	6
Malik	1986	Pakistan	4000	34
Verma *et al.*	1987	India	1258 Desi	12
			174 Kabuli	1
Singh *et al.*	1992	Syria	5107 Kabuli	12
Singh *et al.*	1992	Syria	10203 Desi	3
Singh and Reddy	1993	Syria	19000	5

identified the line ICC 1527, ICC 1069 and ICC 2160 as resistant; other lines with satisfactory performance were NEC 1256, ICC 1722, ICC 7520 and PCU 15. Maheshwari *et al.* (1984) proved resistance to blight in various trials in India.

Acikgoz and Demir (1984) studied the reaction of 35 chickpea lines to 25 isolates of *A. rabiei* and reported that 72012, ILC-195, ILC-200, ILC-201, ILC 202 and Nec-138-1 were resistant to all the isolates. Analysis of the reaction of IC 72012 x ILC 9129, ILC-195 x IC 460, ILC 200 x IC 460, ILC 201 x IC 460 and NEC 138-1 x ILC 1929 in the F 1 x F 2 and back crosses showed that one gene was responsible for resistance which could be recessive or dominant. Porta-Puglia (1984) reported that line ILC 191 from ICARDA was a suitable source of resistance against *A. rabiei.* Reddy and Kabbabeh (1984) reported a genotype ILC 1929 was susceptible and ILC 32996 as resistant. Bashir and Haware (1986) at ICARDA tested the response of chickpea lines to six races of the fungus. They observed that none of the lines was resistant to any of the six races. However ICC 3996, ICC 4324, ICC 4475, ICC 6981, ICC 6988, ILC 202, IL 2380, ILC 2467, ILC 2469, ILC 4421 and NEC 138-2 were found to be tolerant while some of the lines such as ICC 4324, ICC 4475, ICC 6981, ILC 4421 showed differential reactions against the races. Bashir *et al.* (1985) have screened 3360. *C. arietinum* lines from ICRISAT under artificial epiphytic conditions. No line was found to be highly resistant.

Crino *et al.* (1985) screened 500 chickpea lines derived from ICARDA and evaluated them under natural infection. ILC 3279 was found to be the most promising for combined resistance and productivity than the local standard cultivar. Porta-Puglia *et al.* (1985) inoculated 18 lines of ICARDA and 3 Italian land races in a greenhouse with 6 isolates from different regions of Italy. Line ILC 191 was resistant to most isolates while all other lines were resistant to only 1 or 2 isolate. Singh and Kapoor (1986) conducted screening in field and pot tests and found that 3 lines showed low pod infection as compared with foliage infection. Five kabuli lines showed more resistance against pod infection than that of foliage.

Gaur and Singh (1987) evaluated 58 cultivars for resistance under artificial conditions and found that five lines were resistant. Kinaci and Dalkiran (1987) in field trials of screening at 2 sites evaluated 1100 samples of chickpea to blight under natural

and artificial infection conditions. In Ankara, 6 highly resistant and 18 resistant lines and in Cankiri 4 highly resistant and 6 resistant lines were identified. ILC 183 and 82-11 were resistant at both locations. Sandhu *et al.* (1990) reported a mutant E100Y (M) resistant to blight which had a thick stem dwarf compact habit, bold seeds. Pal and Singh (1990) screened a large number of chickpea lines in pot tests and found that three lines, namely; ILC 3864, ICL 3870 and ILC 4421, were resistant to blight. Ten chickpea lines showed tolerant reaction while remaining lines were found susceptible to highly susceptible. Ilyas *et al.*, (1991) screened 60 germplasm lines for the sources of resistance against blight, none was found to be immune or high resistant, however 15 lines exhibited moderate resistant reaction, while other expressed moderate to highly susceptible reaction.

2.15 MARKER ASSISTED BREEDING

Until the early 1970's, most of the single gene markers genetic studies of higher plants were those affecting morphological characters, such as dwarfism, chlorophyll deficiencies or altered leaf morphology (Tanksley, 1983). The use of morphological markers has been limited because of properties such as dominance, late expression, deleterious effects, pleiotrophy, epistasis and rare polymorphisms (Arus and Moreno-Gonzalez, 1993). Many reports have been published on the association of traits with foliage shape, corolla colour or seed coat colour in *Cicer*. However, these associations are often due to pleitrophy and do not represent useful linkages (Muehlbauer and Singh, 1987). Studies on the interaction between chickpea and *A.rabiei* have resulted in a list of possible biochemical markers to include in screening programmes (Barz *et al.*, 1993): 1) high level of pre-inflectional secondary constituents (isoflanones), 2) hypersensitive reaction, 3) rapid accumulation of phytoalexins, 4) extensive expression of pterocarpin phytoalexins biosynthetic enzymes (isoflavone 2-hydroxylase, 5) reinforcement of cell walls around infection sites with hydroxyproline-rich glycoproteins, callose, lignin and polyphenols, 6) rapid accumulation of pathogenesis-related proteins, 7) extensive accumulation of ß-1,3-glucanases and chitinases 9) high levels of proteins that inhibit fungal polygalacturonases, 10) insensitivity to fungal toxins, and insensitivity to fungal suppressors. Among the defence reactions, the hypersensitive response is the most important response to follow.

The hypersensitive response is one of the most effective anti-microbial mechanisms of plants that are induced by the infection itself (Barz *et al*., 1993). Genetic incompatibility between the host and the pathogen resulting from a resistance gene in the host and leading to resistance against the pathogen or one of its specific physiological races is characterized by a hypersensitive response. The hypersensitive response is characterized by rapid cell death and necrosis at and around each point at which the leaf tissue was infected and localization of the parasite to the area of each initiated infection.

Molecular markers, such as isozymes, RAPD, and RFLP, are based on the identification of protein or DNA polymorphisms and have most of the properties of an ideal marker. They have more potential to improve selection efficiency than morphological markers because they can be detected early in the development of the plant, and there is a minimal interaction among molecular markers, which allows the use of many simultaneously. The total number of isozyme-gene linked pairs detected so far is small, hence, the probability of finding out one linked to an important gene, such as Ascochyta resistance, is small because of the small size of the segregating population normally used (Arus and Moreno-Gonzalez, 1993). Isozyme segregation has been investigated in several chickpea populations that were segregating for resistance to *A.rabiei*, but no linkages were found among the isozyme loci and the *A.rabiei* resistance loci (Kusmenoglu *et al*., 1992). RAPD markers represent a valid alternative to isozymes because of the nearly unlimited number of primers that can be designed for the RAPD technique, allowing access to virtually any polymorphism that differentiates the two parents of a cross (Mayer *et al*., 1997).

CHAPTER-3

MATERIALS AND METHODS

For the purpose of present investigation, chickpea germplasm, breeding materials and cultivars evaluated against ascochyta blight of chickpea were obtained from national and international sources listed in appendix (Table 3.3). Detail of germplasm screened from 1994 to 1996 is given in the appendix IV to VI. Field and Laboratory facilities of Pulses Programme were used for most of the experiments. However, greenhouse and laboratory facilities of Plant Genetic Resources Institute (PGRI) were used. Laboratory facilities of Crop diseases Research Institute (CDRI) were used for the study of pathogenic variability through the courtesy of Dr. Iftikhar Ahmad, Depty Director General, National Agricultural Research Centre, Islamabad. The data were analysed with the cooperation of Dr. Abdul Ghafoor, Senior Scientific Officer (PGRI).

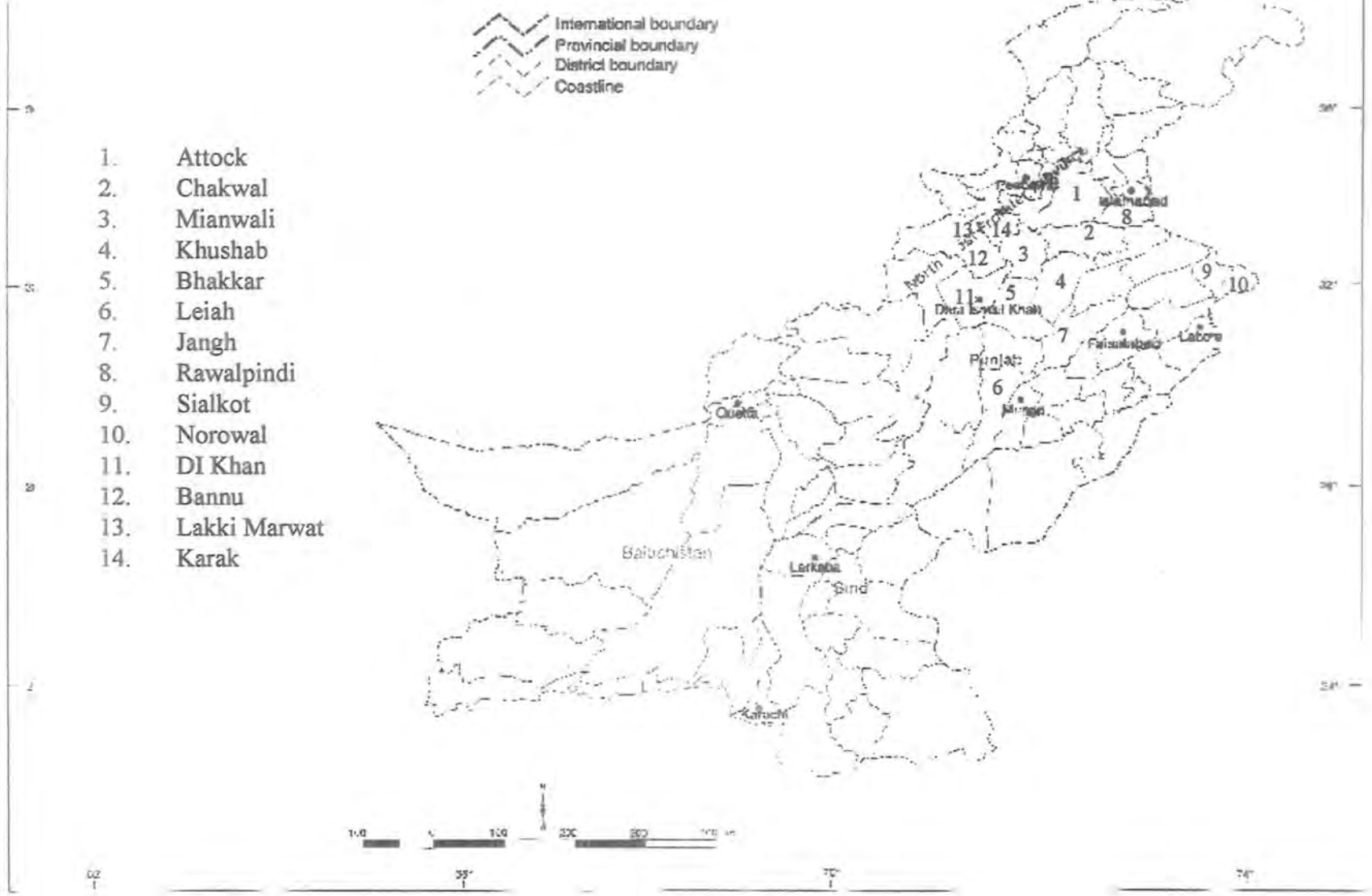
3.1. SURVEY FOR THE ASSESSMENT OF BLIGHT

A detailed survey of chickpea was conducted in the month of March 1996 when the crop was at the flowering to pod-forming stage. Prevalence and distribution of blight in the chickpea production areas of North-western Frontier Province (NWFP) and Punjab was assessed. Major chickpea growing areas of districts Attock, Chakwal, Khushab, Mianwali, Bhakkar, Leiah, Jhang, Rawalpindi, Sialkot and Norowal (Punjab), Bannu, Karak and Dera Ismail Khan (NWFP) were selected for survey (Table-3.1). Location of the areas, which were surveyed, is given in Fig 3.1.

Observations of disease prevalence, incidence and severity in each field were recorded on the samples comprising ten plants from five random places across a diagonal in each of the selected field following the hierarchical. The district average was worked out on the basis of disease prevalence recorded at various locations in each district. Disease severity was assessed on a visual 1-9 rating scale (Singh *et al.*, 1981).

Table 3.1: Chickpea growing areas surveyed during the crop season of 1995-96

Province	Districts	Locations
Punjab	Attock	Bahtar, Fateh Jangh, Hasanabdal, Jand, Nara, Pindi gheb
	Bhakkar	Basti Shaw Alam, Bhakkar, Darya khan, Dullewala, Gauharwala, Kalurkot, Mankera, Nawan Jandanwala Punjgran
	Chakwal	Bhaun, Bulkasar, Chakwal, Dhudial, Rupowal
	Jhang	Atharan hazari, Dager, Daultana, Sanyasiwala, Tremun, Ueh
	Khushab	Adhikot, Hemoka, Girot, Mithatiwana, Nurpur, Rangpur, Roda, Ukhli mohla
	Leiah	Chaubara, Fatehpur, Karor, Kharewala, Kot Sultan, Nawan Kot
	Mianwali	Dab, Mianwali, Piplan, Wanbhachran
	Norowal	Chak Amru, Darman, Mansoorwali, Naina Kot, Zafarwal
	Rawalpindi	Doltala, Jatli, Tarnol,
	Sialkot	Chowinda, Kingra, Sahowali
NWFP	Bannu	Domeli, Ghoriwala, Sarai Gambila, Sarai Naurang
	D.I. Khan	Akalghar, D.I. Khan, Kafir Kot, Kot Jai, Saggu, Yarik
	Karak	Ahmadwala, Bahadar Khel, Banda Daud Shah, Karak, Lachi, Shanwala
	Lakki Murwat	Abahkhel, Isakhan, Lakki, Pezu, Shahbaz Khel


International boundary
Provincial boundary
District boundary
Coastline
1. Attock
2. Chakwal
3. Mianwali
4. Khushab
5. Bhakkar
6. Leiah
7. Jangh
8. Rawalpindi
9. Sialkot
10. Norowal
11. DI Khan
12. Bannu
13. Lakki Marwat
14. Karak
Islamabad
Lahore
Quetta
Balochistan
Sind
Karachi

3.2 FACTORS AFFECTING BLIGHT DEVELOPMENT

Before initiating the study on the pathogenic variability and screening of chickpea against blight, it is important to determine the factors affecting blight. Thus, the studies reported in this section were undertaken to address the following objectives,

- Effect of inoculum concentration on disease development,
- Effect of plant age on disease development,
- Effect of different incubation and wetness period on disease development,

3.2.1 Effect of inoculum concentration on disease development

Seedlings of five chickpea cultivars; C 727, Punjab 91, Dasht, Parbat and ILC 263 were grown in the plastic pot (10.5 x 7.5 cm) filled with sterilized soil. There were three replicated pots, each containing five seedlings. These seedlings were inoculated 15 days after sowing with the spore suspension of *A.rabiei* in concentration of 5×10^4, 1×10^5, 2.5×10^5 and 5×10^5 spores per ml of were prepared. Seedlings were inoculated and incubated in a humid chamber for 72 h. Control plants were treated with SDW (simple distilled water) and incubated in a separate humid chamber at 20 ± 2^0C. Disease was assessed after 14 days of inoculation using 1-9 rating scale of Singh *et al.* (1981).

3.2.2 Effect of plant age on disease development

Two chickpea cultivars, a highly susceptible (C 727) and a moderately susceptible (Punjab 91) were tested at different physiological stages from 2 to 12 weeks after sowing, representing the seedling to the flowering stage in the green house conditions. Seeds were sown in earthen pots (30 x 20 cm) at 2-week intervals for 12 weeks to provide plants, at the time of inoculation at different growth stages to avoid any variation in the inoculum and greenhouse conditions. Plants were inoculated with a suspension of $5x10^5$ spores per ml) and incubated at 20 ± 2^0C. Non-inoculated plants served as control. Symptoms were assessed 14 days after inoculation using 1-9 rating scale of Singh *et al.* (1981).

3.2.3 Effect of leaf wetness periods on disease development

Four chickpea cultivars viz. C 727, Dasht, Parbat and Punjab 91 with varying range of disease susceptibility were grown in pots (10.5 x 7.5 cm). Five seedlings were raised and three pots were taken for each cultivar. Fifteen days after sowing, the seedlings were inoculated with the spore suspension @ $5x10^5$ spores pr ml and incubated at 20 ± 2^0C under different leaf wetness period to determine the latent period for disease development and disease was assessed according to 1-9 rating scale (Singh *et al.*, 1981) 14 days after inoculation.

- 4 days of incubation and no spray (4D/0S)
- 4 days of incubation and one spray (4D/1S)
- 4 days of incubation and two spray (4D/2S)
- 3 days of incubation and no spray (3D/0S)
- 3 days of incubation and one spray (3D/1S)
- 3 days of incubation and two spray (3D/2S)
- 2 days of incubation and no spray (2D/0S)
- 2 days of incubation and one spray (2D/1S)
- 2 days of incubation and two spray (2D/2S)
- 1 day of incubation and no spray (1D/0S)
- 1 day of incubation and one spray (1D/1S)
- 1 day of incubation and two spray (1D/2S)
- 0 day of incubation and no spray (0D/0S)
- 0 day of incubation and one spray (0D/1S)

- 0 day of incubation and two spray (0D/2S)

3.3 PATHOGENIC VARIABLITY IN *ASCOCHYTA.RABIEI*

3.3.1 Isolation of *A. rabiei*

Diseased samples were collected from the farmers' fields and research stations at various districts of Punjab and North Western Fronter Province (NWFP) for isolation of *A. rabiei.*

Blight infected samples of chickpea plants showing characteristic disease symptoms were cut into small pieces (4-6 cm) and were surface sterilized in 0.1% mercuric chloride solution for two minutes and then washed twice in sterilized water, dried on sterilized filter paper and were placed on potato dextrose agar (potato starch 20g, dextrose 20g, agar 20g and water 1lit) medium in petri-plates autoclaved at 15 pound per square inch (psi) for 15 minutes and were incubated at 20 $\pm$ 2 ^{0}C for two weeks under alternating 12 h (Philips TLD 18W/08) and 12 h darkness. Colonies of *A. rabiei* coming out of infected bits were later purified by spore streak method (Pathak, 1986). The purified cultures were identified according to the keys of Sutton (1980) and Punithalingam and Holliday (1972a, b & c). Pure cultures were grown on chickpea seed meal agar (CSMA) medium (chickpea seed meal 20 g, dextrose 20 g, agar 20g and water 1 lit) slants and maintained for further studies.

Forty-two isolates were recovered for the present study. Single spore cultures of these isolates were preserved on CSMA medium. Isolates were subjected to detailed morphological and cultural characteristics, viz., radial growth on medium (mm), colony colour, size of pycnidia (µm) and pycnidiospores (µm).

3.3.2 Pathogenicity tests of the isolates

Pathogenicity of isolates was determined using a set of seven chickpea cultivars viz; C 727, ILC 263, C 44, CM 72, Piadar, Noor-91 and Punjab 91. Prior to sowing, seeds were surface sterilized with Clorox (0.1% available Chlorine) and plastic pots (10.5 x 7.5 cm) were filled with sterilized sandy loam soil. Ten seeds of each cultivar were sown in

able-3.2: Isolates of *Ascochyta rabiei* collected from different chickpea growing areas of Pakistan

S. No	**Isolates**	**Locations**	**S. No.**	**Isolates**	**Locations**
1	KB-1	Khushab	22	AT-2	Attock
2	BR-4	Bhakhar	23	BN-1	Bannu
3	BR-3	Bhakkar	24	KB-2	Khushab
4	LY-4	Leiah	25	KN-1	Kagan
5	KT-2	Kohat	26	MN-3	Mianwali
6	MN-4	Mianwali	27	DI-1	D.I. Khan
7	KT-1	Kohat	28	BR-1	Bhakkar
8	MN-5	Mianwali	29	FD-2	Faisalabad
9	LY-2	Leiah	30	CL-2	Chakwal
10	AT-1	Attock	31	MN-1	Mianwali
11	BR-5	Bhakkar	32	ST-1	Sialkot
12	BR-2	Bhakhar	33	AT-3	Attock
13	KK-2	Karak	34	JN-1	Jhang
14	JN-2	Jhang	35	ST-2	Sialkot
15	KB-3	Khushab	36	AT-5	Attock
16	NC-3	Islamabad	37	AT-4	Attock
17	NC-1	Islamabad	38	MN-2	Mianwali
18	BN-2	Bannu	39	FD-1	Faisalabad
19	DI-2	D.I. Khan	40	CL-2	Chakwal
20	LY-3	Leiah	41	KK-1	Karak
21	NC-3	Islamabad	42	CL-3	Chakwal

these pots with three replications in complete randomized design. Fifteen days old plants were inoculated by spraying spore suspension (5 x 10^5 spores per ml) from 15 days old cultures of the isolates and incubated separately under humid chamber for 72 hours in the green house (Singh *et al.*, 1982). Relative humidity was maintained in the range of 85-95%. Disease observations were recorded after 14 days of inoculation by using 1-9 scale according to Singh *et al.*, (1981) and designation of pathotypes was followed by Habgood (1970).

The disease data were analyzed for 2 factor design statistics and numerical taxonomic techniques using the procedure of cluster and principal component analyses (Sneath and Sokal, 1973) with the help of computer software "Statistica" and "SPSS" for Windows 98. Based on results, similarity index was calculated and then converted to a dissimilarity matrix to construct dendrogram by the unweighted pair group method average (UPGMA) (Sneath & Sokal, 1973).

3.3.3 Combined effect of the least and the most aggressive isolates on blight

In order to determine the combining effect of isolates of *A. rabiei*, an aggressive (AT-2) and a least aggressive (BR-5) isolates were applied separately as well as in combination (1:1 ratio) on a s0et of sixteen chickpea cultivars viz; Dasht, Parbat, C 727, C 44, C 235, CM 72, NIFA 88, NIFA 95, Bittle, Noor-91, Punjab 91, Piadar 91, Bulksar, Wanhar, ILC 263 and DC-1. Pathogenicity study was determined according to the procedure described in Section 3.12.3.

3.4 SCREENING OF CHICKPEA FOR BLIGHT RESISTANCE

In order to identify the sources of resistance to blight, 248, 232 and 344 chickpea germplasm lines received during 1994, 1995 and 1996, respectively, from international and national sources (Table 3.3). These lines were tested under the conditions of artificial inoculation of chickpea blight.

3.4.1 Screening under greenhouse conditions

Seeds of germplasm accessions were surface-sterilized with Clorox solution (0.1% available chlorine) for 2 minutes and sown in disposable pots (7.5 x 10.5 cm) filled

Table 3.3: Sources of germplasm accessions screened against blight

Centre/ Institute	Number of Accessions		
	1994	1995	1996
International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria	20	47	48
Intentional Crop Research Institute for Semi-arid tropics (ICRISAT), India	23	19	14
Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan	127	-	122
Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan	78	166	121
Nuclear Institute for Food & Agriculture (NIFA), Peshawar, Pakistan	-		39
Total	248	232	344

with sterilized soil and sand mixture (2:1). Each pot contained five chickpea seedlings. A susceptible variety, C 727 was kept as control for comparison. Pots were kept in greenhouse at 20±2 ^{0}C in natural light for 15 days before inoculation. Pots were watered from the top prior to inoculation. Two week old seedlings were inoculated by spraying aqueous spore suspension having a concentration of 5 x 10^{5} spores/ml. The inoculum was prepared from 15 days old culture of *A. rabiei* multiplied on chickpea grains according to the procedure developed by Ilyas and Khan (1986). The inoculated seedlings were incubated in humid chamber for 72 hours in the greenhouse. Disease observations were taken when susceptible check lines were completely killed and recorded on 1-9 disease rating scale (Singh *et al.*, 1981).

3.4.2 Screening under field conditions

Same set of germplasm was screened under field conditions during simultaneous crop seasons of 1994, 1995 and 1996. One row of 4 m length was planted for each genotype in two replications. Susceptible check (C 727) was planted after every two rows of the germplasm for disease spread. When the entries were in early flowering stage, they were spray-inoculated with spore suspension of *A. rabiei* @ 5 x 10^{5} spores/ml.

At the time of inoculation, pycnidial inoculum on chickpea grains was thoroughly homogenized in distilled water (20 lit) to prepare spore suspension of the fungus. The spore suspension was sieved through a muslin cloth to remove seed debris and then sprayed on chickpea plants for inoculation. The inoculum was applied daily in the evening till the appearance of blight. Continuous spray of water supported to maintain RH for development of disease. The data for blight at vegetative stage was recorded according to Singh *et al.*, 1981, whereas at pod formation stage, disease was recorded in percentage of pod infection (Singh and Reddy, 1993).

3.5 EFFECT OF MORPHOLOGICAL TRAITS FOR BLIGHT RESISTANCE

Six chickpea cultivars (NIFA 88, Dasht, Parbat, C 44, C 727 and ILC 263) relating to each reaction group (resistant and susceptible) were sown in the field during 1998-99. The test lines were sown in single row subplots, 3 meter long with 30 cm and

10 cm row-to-row and plant to plant distance respectively. Green plant tops were collected randomly from all lines of the two reaction groups growing in the field. To ensure uniformity, fifth compound leaf from the top was selected for microscopic studies on different structural parameters (hair density, stomatal population, size of stomata and stomatal aperture).

3.5.1 Density of hair

Leaflets were removed from the leaf and placed under binocular stereoscopic microscope (WILD M3B Heerburg, Switzerland) for counting the density of hair on their dorsal and ventral side in an area of 5.5 mm^2. To get a more precise picture, three observations were recorded from the same leaflets.

3.5.2 Density and area of stomata, stomatal aperture and guard cells

For determining the frequency and size of stomata, the leaf cuticle was removed gently with the help of a scalpel and a pair of forceps (Randhawa, 1994). The cuticle layer was placed on a glass micro slide, 1"x3" size (in a small drop of safranin mixed well with two drops of Hoeyer's mounting medium). In this way permanent mounts were prepared for all the test lines of resistant and susceptible reaction groups.

The slides were examined under a compound microscope (Zeiss, Germany) at 80X to determine stomatal population in a specific area (1.52 mm^2). The size of stomata, guard cells and stomatal aperture was determined at 320X. Camera Lucida drawing of a typical stomata for each test lines was drawn in order to calculate the correction factor.

Observations on length and width of the stomata were recorded under research microscope (Zeiss, Germany) at 320X. The multiplication of length and width of a stomata (observed area) was multiplied by 0.7 on the calculative assumption that it would be nearest to the calculated area (corrected area). The area of stomatal aperture was also calculated using the formula. Area of guard cells was determined by the following formula;

Area of guard cells = Area of stomata - Area of stomatal aperture

3.6 RELATIONSHIP BETWEEN SDS-PAGE MARKERS AND CHICKPEA BLIGHT

3.6.1 Effect of chickpea plant extract on the growth of *A. rabiei*

Fifty seven commercial chickpea varieties/ cultivars and advanced lines were screened against blight according to the procedure as described in section 3.4.1 to confirm their disease reaction. In order to determine the role of plant extract of various chickpea cultivars on the growth of *A.rabiei*, 5 g shoot tips of each cultivar were weighed with an electronic balance and steeded in 100 ml distilled water. After two hours, the water extract was sieved to get a clear solution. This extract was kept under UV light for 24 hours for sterilization. It was used for further studies on the colony growth of *A. rabiei*.

About 10 ml water extract (as prepared above) was mixed with sterilized dextrose water agar medium (2%) and poured in sterilized petri dishes (90 mm dia). This was stirred with a glass rod while still hot under aseptic conditions. This was inoculated with the actively growing culture of the fungus with the help of a cork borer, and was kept at 20± 2^{0}C in an incubator for growth. Potato dextrose agar medium without plant extract served as control. The experiment was run in triplicate. Observations on colony growth were recorded 15 days after incubation.

3.6.2 Application of SDS-PAGE to determine the resistance

SDS-PAGE was applied to study its role to determine the resistant behavior of chickpea towards Ascochyta blight. For the extraction of proteins, single seed was ground to fine powder with pestle and mortar. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in eppendrof tube with a small glass rod. The extraction buffer contained the following final concentrations: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol Blue (BPB) was also added to the sample buffer as tracking dye. To purify extraction, the homogenate samples were mixed thoroughly by vortexing and centrifuging at 15,000 rpm for 5 minutes at RT. The extracted crude proteins were recovered as clear supernatant, transferred into new 1.5 ml eppendrof tubes and stored at -20 ^{o}C until electrophoresis.

3.6.2.1 Electrophoresis

Seed protein was analyzed through slab type SDS-PAGE using 11.25% Polyacrylamide gel. Electrophoresis was carried out at 100 V for half hour and then at 150 V until the Bromophenol blue marker reached the bottom of the gel (approximately two and half hour). In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. The molecular weights of the dissociated polypeptides were determined by using molecular weight protein standards "MW-SDS-70 kit" containing Albumin Bovine (66 kd), Egg Ovalbumin (45 kd), Pepsin (Porcine Stomach Mucosa) 34.7 kd, Trypsinogen (Bovine Pancreas) 24 kd, β-Lactoglobulin (Bovine Milk) 18.4 kd and Lysozyme (14.3 kd) from Sigma Chemical Company, USA.

SDS-PAGE of total seed protein was carried out in polyacrylamide slab gels in the discontinuous buffer system according to the method of Laemmli, (1970). Vertical gel slabs were prepared in a glass sandwich, which was tightened by a set of plastic clips lined with a band of foamed silicon rubber. The separating gels contained 11.25% of Acrylamide and 0.135% by weight of N.N-methylene-bis-acrylamide in 1 M Tris-HCl buffer (pH 8.8) with 0.27% SDS. The gels were polymerised chemically by the addition of 20 µl by volume of tetramethylethylene-diamine (TEMED) and 10% ammonium persulfate (APS). The stacking gels consisted of 30% acrylamide and 0.8% N.N-methylene-bis-acrylamide in 0.25 M Tris-HCl buffer (pH 6.8) containing 0.2% SDS. The stacking gels were polymerized chemically in the same way as for the separation gel. The electrode buffer contained Tris-glycine (9.0 g Tris HCl and 43.2 g glycine per 3 litres buffer solution at a pH 8.9) with 3.0 g (0.1%) SDS. Six µl of protein supernatant were applied into the wells in stacking gel sample wells with a microsyringe.

3.6.2.2 Staining and destaining

After electrophoresis, the gels were stained with 0.2% (w/v) coomassie brilliant blue R250 dissolved in a solution containing 10% (v/v) acetic acid, 40% (v/v) methanol and water in the ratio of 10:40:60 (v/v) for one hour. Gels were then destained by washing with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and water in the ratio of 5:20:75 (v/v) until the colour of background disappeared and electrophoresis

bands were clearly visible. After destaining, the gels were dried using gel drying processor for about 100 minutes.

3.6.2.3 Data analysis

Depending upon the presence or absence of polypeptide bands, similarity index was calculated for all possible pairs of protein types. To avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of the bands was taken as indicative. The scores were "1" for the presence and "0" for the absence of a band. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, Jaccard's similarity index (JSI) was calculated for all possible pairs of protein types electrophoregrams by the following formula (Sneath & Sokal, 1973):

$$S = W/(A + B - W)$$

where "W" is the number of bands of common mobility, "A" the number of bands in protein type A and B is the number of bands in protein type B.

The similarity matrix thus generated was converted to a dissimilarity matrix (Dissimilarity = 1- similarity) and used to construct dendrogram by the unweighed pair-group method with arithmetic means (Sneath & Sokal, 1973). All the analyses were carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993) and "STATISTIA" for Windows 95.

3.7 YIELD LOSSES IN CHICKPEA DUE TO BLIGHT

Since blight is a major problem of chickpea production, it is essential that the lines developed are likely to be released as varieties to be tested prior to their release against heavy induced blight attack to measure the extent of economic loss.

The experimental material comprised of seven chickpea genotypes (C 44, C 727, CM 72, Dasht, Parbat, NIFA 88 and Punjab 91) with varying level of tolerance against *Ascochyta* blight (Appendix-VIII). These genotypes were sown in the experimental field of Pulses programme, NARC, Islamabad during October, 1998 in a split plot randomized

complete block design with four replications. Two treatments T1 (disease created artificially), T2 (protected from disease) were placed in main plots and varieties were placed in subplots. The main plots were isolated from each other by growing a tall wheat variety (C 591) between them. Each subplot consisted of 6 rows of 4 meters length with inter row spacing of 30 cm and plant to plant distance of 10 cm. In T1, plants were inoculated with *A. rabiei* in the form of diseased debris of chickpea supplemented with spore suspension having concentration of 5×10^5 spore per ml. Plants in T2 were protected from disease with continuous sprays of daconil (Chlorthalonil WP 70) fungicide at an interval of 15 days starting one month after germination. In T2, humidity was created by continuous spray of water in the evening for developing the disease conducive conditions and initiation of infection.

The blight incidence on vegetative parts was scored on 1-9 scale (Singh *et al*, 1981) using 20 randomly selected plants from the middle rows of the sub-plot. Disease severity index (DSI) was calculated according to the following formula of Gemavat and Prasad (1969):

$$DSI = \frac{\text{Total of all disease ratings}}{\text{No. of plants observed}} \times \frac{100}{\text{Maximum disease rating}}$$

Percent infection on pods and seeds was observed on 200 randomly sampled pods. The data on number of pods/plant, number of seed/plant and grain weight/plant were recorded on 20 random plants of each plot. Yield per plot (6×1.8 m^2) was also recorded. Percent loss of yield and other components was calculated on the basis of difference between healthy and diseased plants.

CHAPTER-4

RESULTS

The survey to study the prevalence, incidence and severity of blight disease was carried out in major chickpea growing areas. During this survey 459 farms from 76 localities scattered over fourteen districts of Punjab and NWFP were inspected (appendix-I). Factors affecting blight disease development such as inoculum level, age of plant and leaf wetness period was determined. Forty two isolates of *A.rabiei* collected from various locations during the survey, were maintained for the study of morphological as well as pathogenic variability. Screening of chickpea germplasm from various national and international sources was obtained to screen them against blight under green house and field condition at the seedling to pod forming stages. Morphological as well as biochemical characteristics associated with blight were studied. Losses in yield and yield components in commercial and advanced chickpea lines were assessed.

4.1 SURVEYS FOR ASSESEEMENT OF CHICKPEA BLIGHT

4.1.1 Prevalence:

Ten districts of Punjab and four of North West Frontier Province (NWFP) where chickpea is the major winter crop were surveyed and samples of chickpea plants infected with blight were collected. Different numbers of samples were obtained from various locations depending on the disease incidence and severity. Three hundred and seventy three farms from 55 localities distributed across the 10 districts of Punjab and 86 farms in 21 localities of 4 districts in NWFP were surveyed. The disease was most prevalent in the districts of Attock, Rawalpindi, Sialkot and Norowal where 100% farms were affected with blight (Table 4.1). The disease prevalence in all the 14 districts surveyed varied from 9.35 % to 100%. The minimum disease prevalence was observed in Districts Jhang and Leiah. (Fig 4.1).

In the district of Chakwal 100% disease was prevalent in the areas of Baun, and

Balkasar with 88.7 % district mean of disease prevalence. Whereas in the areas of Thoa, Rupowal and Dhudial, the disease prevalence varied from 80% to 83.3%. The mean disease prevalence in the district of Mianwali was 76.2% with a range of 66.7-100%. Only in the Dab area, 100% farms were infected with the disease. Minimum prevalence of disease in this district was observed in Wanbhacran and Harnoli.

The disease prevalence data from district Khushab revealed that 33.4 to 47.1% disease prevailed on 70 farms of 8 localities. Maximum (47.1%) disease prevalence was observed at Hemoka where 9 farms were visited whereas minimum (33.4%) at Adhikot where 11 farms were inspected. Maximum farms were surveyed in district Bhakhar, which has maximum chickpea area. The disease prevalence in this district ranged from 16.7 to 38.1%. Maximum number of farms infested with disease in Bhakhar were located in Kalikow locality and minimum in Mankera. A total of 90 farms were visited and it was found that 27.4% farms were having blight disease in this district. In district of Leiah, 53 farms were visited. The disease prevalence ranged from 0 to 28.6.5%. No disease was observed in any farm at Nawankot and Fatepur. Disease prevalence was observed 14.3, 16.7 and 28.6%in the area of Kot sultan, Kharewala and Chaubara, respectively. Similarly, in the district of Jhang, the disease prevalence ranged from 0 to 25% and in most of the localities, blight was not observed at all. However, at the localities of Atharanhazari, Daultana and Dager, blight was 14.3, 16.7 and 25%, respectively.

In NWFP, maximum blight was recorded in the district of Karak with mean disease prevalence of 86.3% and in the other districts i.e., Lakki Marwat, Bannu and D.I. Khan, it was 43.0, 23.4 and 18.4%, respectively. In district Karak, 21 farms were surveyed and hundred percent disease prevailed in the areas of Bahadar Khel, Ahmadwala, and Banda Daud Shah whereas in the localities of Karak, Lachi and Shanwala, it ranged from 50-75%. In district Lakki Marwat, hundred percent disease was present in the area of Shehbaz Khel, where as no blight was observed in the area of Pezu. In other areas of this district, blight ranged from 25 to 50%. Mean disease prevalence in district Bannu was 23.4% with maximum disease prevalence (28.7%) at Sarae Norang and no disease was observed in the area of Domeli. Similarly, in District D.I. Khan, mean

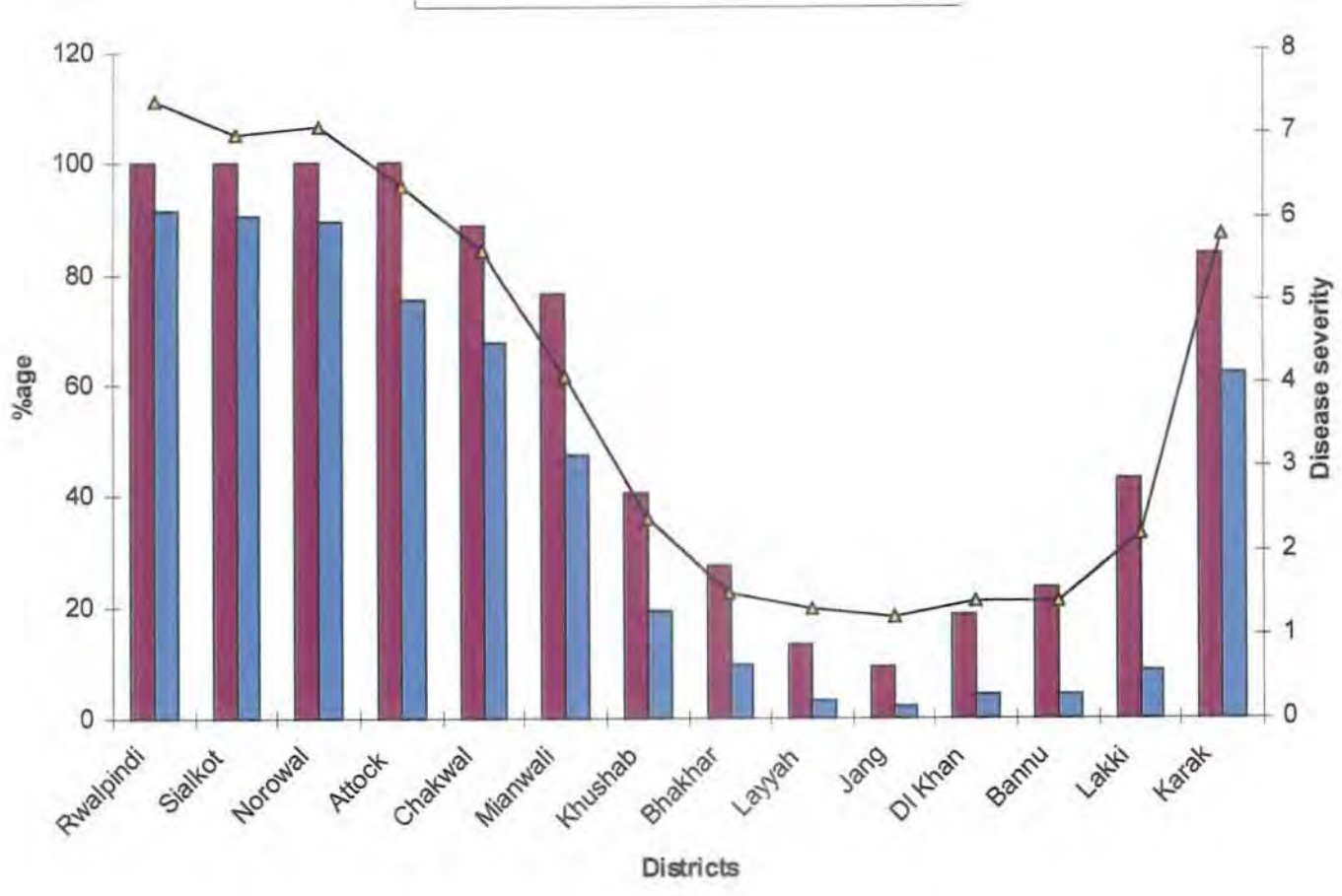


Fig 4.1: Mean disease prevalence, incidence and severity of chickpea blight in different districts where prevalence (%) is the disease observed in the area, incidence (%) is the disease in a field and severity is the infection level on the individual plant.

disease prevalence was 18.4% with a range of 0 to 28.6%.

4.1.2 INCIDENCE AND SEVERITY

The highest mean disease incidence (93.3%) was recorded at Jatli in district Rawalpindi, (Table 4.1). The maximum disease incidence range of 86-98% was recorded at Dolatala and lowest of 80-96% at Tarnol. Maximum mean disease severity of 8.3 was found at Dolatala whereas the minimum severity (6.3) with range 5-7 was found at Jatli. The highest mean disease incidence (92.5 %) in district Sialkot was recorded at Sahowali and minimum (88.8%) at Chowinda. The highest mean disease severity of 7.8 with the highest severity range (6-9) was found at Sahowali and the lowest mean disease severity of 6.2 with the severity range of 3-9 was found at Chowinda. District-wise mean disease incidence indicated that it was 91.5 and 90.7%, respectively for Rawalpindi and Sialkot.

The highest mean disease incidence of 93.0% in district Norowal was found at Mansoorwali that was followed by 92.7% incidence recorded at Zafarwal and Chak amru. The lowest mean disease incidence, which was 78.5%, was observed at Naina kot. Maximum disease incidence which ranged from 90 to 98% at Chak amru. The lowest disease incidence which ranged from 60 to 90% at Naina kot. Even a single farm in this district was not free from the disease. The highest mean disease severity (8) was found at Mansoorwali and followed by 7.7 each at Zafarwal and Chak amru. The lowest mean disease severity (5) was found at Naina kot. Similar sort of disease trend was observed in Dist. Attock, where maximum mean disease incidence of 85.6% was recorded at Nara followed by 82.3 at Jand. The minimum mean disease incidence 65.3% for this district was found at Pindi gheb. The maximum disease incidence with range of 70 to 94% was recorded at Jand followed by 80-90 and 72-90% at Nara and Attock, respectively. The lowest being 40-80% at Pindi gheb. The maximum and minimum for a farm was recorded at Jand and Pindi gheb, respectively. No farm in this district was free from the disease.

In district Chakwal, the highest mean disease incidence (89.2%) was found at Balkasar followed by 76% at Bhaun, whereas, the lowest mean disease incidence (56.4%) was found each at Thoa and Rupowal. The disease incidence range of 60-94% recorded

at Bhaun was followed by 80-90% at Balkasar. The lowest being 0-80% at Rupowal. Maximum disease incidence of 94% was found in a farm at Bhaun, whereas some farms at Thoa, Dhudial and Rupowal were free from the disease. In this district, highest mean disease severity of 6.7 was recorded at Bhaun, whereas the lowest mean disease severity of 4.8 was recorded at Thoa. The maximum disease severity range of 5-9 was found at Balkasar and the minimum severity range of 1-7 was at Thoa and Dhudail.

The highest mean disease incidence (65.3%) in district Mianwali was found at Dab followed by 44% and 42% at Harnoli and Wanbhachran, respectively. The lowest disease incidence (37.2%) was recorded at Chashma. The maximum disease incidence range (50-76%) was found at Dab. Some of the farms at Harnoli, Wanbhachran and Chashma were free from the disease.

In district Khushab mean disease incidence ranged from 12% to 29 %, which were respectively recorded at Ukhli mola and Adhikot. The maximum disease incidence 64% was found at Ukhli mola where disease free plots were also found. Minimum disease incidence ranging from 0-40% was observed at Adhikot. The highest mean disease severity (2.9) was found at Rangpur and the lowest (2.1) at Adhikot and Garot. The maximum disease severity where it ranged from 1-7 was recorded at Roda.

The mean disease incidence of various localities in Distt Bhakar varied from 3.0 (at Mankera) to 23.3% (at Kalurkot). The disease incidence data revealed that the maximum disease incidence that ranged from 0-70% was observed at Kallurkot whereas minimum disease incidence (0-20) at Mankera. In this district, the maximum mean disease severity (2.7) was found at Kallurkot and minimum 1.3 was found at Mankera, Dullewala and Goharwala.

In district Leiah, the highest mean disease incidence (8%) was recorded at Karor. The minimum mean disease incidence in this district was 2%, which was recorded at Kot sultan. Disease was not found even in a single farm at Nawankot and Fatehpur. The highest mean disease severity (1.6) was recorded at Karor followed by 1.4 at Chaubara. The lowest mean disease severity (1.3) was found at two locations, Rakh kharewala and Kot sultan. Similarly, in district Jhang, the highest mean disease incidence of 5.4% was

ble 4.1 Prevalence, incidence and severity of Ascochyta blight in major chickpea growing areas of Punjab and NWFP of Pakistan (1995)

strict	Locality	No. of Farms	Disease Prevalence (%)	Disease Incidence (%)		Disease severity (1-9 rating)	
				Range	Mean	Range	Mean
1	2	3	4	5		6	
walpindi	Jatli	3	100	90-96	93.3	5-7	6.3
	Doltala	4	100	86-98	92.5	7-9	8.3
	Tarnol	3	100	80-96	88.7	7-9	7.7
		10	**100**		**91.5**		**7.4**
lkot	Sahowali	4	100	84-98	92.5	6-9	7.8
	Chowinda	5	100	50-98	88.8	3-9	6.2
		9	**100**		**90.7**		**7.0**
rowal	Zafarwal	3	100	90-96	92.7	7-9	7.7
	Darman	5	100	80-98	90.8	5-9	7.2
	Chak amru	3	100	90-98	92.7	7-9	7.7
	Naina kot	4	100	60-90	78.5	3-7	5.0
	Mansoorwali	2	100	90-96	93.0	7-9	8.0
		17	**100**		**89.5**		**7.1**
ock	Attock	5	100	72-90	81.2	5-9	7.2
	Jand	7	100	70-94	82.3	7-9	6.3
	Fate jang	9	100	40-86	70.4	3-9	6.1
	Nara	5	100	80-90	85.6	7-9	7.8
	Bahtar	5	100	46-84	70.8	3-9	5.8
	Hassanabdal	4	100	52-80	71.0	3-7	5.5
	Pindigheb	6	100	40-80	65.3	3-7	5.8
		41	**100**		**75.3**		**6.4**
akwal	Thoa	5	80	0-86	56.4	1-7	4.8
	Dhudial	6	83.3	0-90	60.0	1-7	5.2
	Bhaun	6	100	60-94	76.0	3-9	6.7
	Balkasar	5	100	80-90	89.2	5-9	6.2
	Rupowal	5	80	0-80	56.4	1.8	5.2
		27	**88.7**		**67.6**		**5.6**
anwali	Harnoli	3	66.7	0-72	44.0	1-7	4.3
	Wanbachran	6	66.7	0-70	42.0	1-6	3.5
	Chashma	7	71.4	0-70	37.2	1-7	3.4
	Dab	3	100	50-76	65.3	3-7	5.0
		19	**76.2**		**47.2**		**4.1**
ushab	Rangpur	8	37.5	0-48	22.8	1-5	2.9
	Nurpur	9	38.9	0-46	15.6	1-5	2.3
	Adhikot	11	33.4	0-40	12.0	1-5	2.1
	Roda	10	45.0	0-58	16.4	1-7	2.4
	Girot	11	36.4	0-60	15.1	1-5	2.1
	Hemoka	9	47.1	0-44	21.6	1-5	2.5
	Mithantiwana	5	40	0-50	19.2	1-5	2.2
	Ukhli mola	7	46.6	0-64	29.0	1-5	2.4
		70	**40.6**		**19.0**		**2.4**

1	2	3	4	5		6	
khar	Bhakhar	8	26.7	0-48	11.0	1-3	1.5
	Kallurkot	12	38.1	0-70	23.3	1-5	2.1
	Basti sha alam	9	33.3	0-50	9.8	1-3	1.7
	Punj gran	10	35.0	0-44	14.4	1-3	1.6
	Darya khan	9	34.4	0-38	10.9	1-3	1.6
	Mankera	12	16.7	0-20	3.0	1-3	1.3
	Dulle wala	10	20.0	0-28	4.8	1-3	1.3
	Gohar wala	11	20.0	0-22	3.5	1-3	1.3
	Nawanjanan wala	9	22.2	0-24	4.7	1-3	1.4
		90	**27.4**		**9.5**		**1.5**
h	Kharewala	6	16.7	0-22	3.6	1-3	1.3
	Kaoror	7	28.6	0-30	8.0	1-3	1.6
	Kot sultan	7	14.3	0-14	2.0	1-3	1.3
	Chaubara	10	20.0	0-20	5.6	1-3	1.4
	Nawan kot	11	0.0	0	0	1	1.0
	Fatepur	12	0.0	0	0	1	1.0
		53	**13.3**		**3.2**		**1.3**
	Trimun	3	0.0	0	0	1	1.0
	Athran hazari	7	14.3	0-22	3.2	1-3	1.3
	Sanyasi wala	6	0.0	0	0	1	1.0
	Ueh	7	0.0	0	0	1	1.0
	Dager	8	25.0	0-28	5.0	1-3	1.5
	Daulatana	6	16.7	0-24	4.0	1-3	1.3
		37	**9.3**		**2.1**		**1.2**
Khan	DI Khan	5	20	0-32	6.4	1-3	1.4
	Akal ghar	4	25	0-20	5.0	1-3	1.5
	Kafir kot	6	16.7	0-24	4.0	1-3	1.3
	Kot jai	7	28.6	0-28	6.9	1-3	1.6
	Saggu	5	20	0-20	4.0	1-3	1.4
	Yarik	3	0	0	0	1	1.0
		30	**18.4**		**4.4**		**1.4**
nu	Sarae gambila	5	40	0-38	9.6	1-3	1.6
	Saraae nurang	7	28.7	0-16	4.0	1-3	1.6
	Ghori wala	4	25	0-12	3.0	1-3	1.5
	Domeli	2	0	0	0	1	1.0
		18	**23.4**		**4.2**		**1.4**
i marwat	Lakki	4	25	0-24	8.0	1-3	1.5
	Pezu	2	0	0	0	1	1.0
	Shehbaz khel	2	100	50-100	7.5	3-5	4.0
	Aba khel	4	50	0-40	15	1-5	2.5
	Isa khan	5	40	0-40	11.6	1-3	1.8
		17	**43.0**		**8.4**		**2.2**
k	Karak	4	75	0-90	57.5	1-9	5.5
	Bahadar khel	3	100	84-98	90.7	7-9	7.7
	Ahmad wala	6	100	70-100	71.7	5-9	6.7
	Banda daud shah	2	100	74-92	83.0	7-9	8.0
	Lachi	2	50	0-56	28.0	1-5	3.0
	Shan wala	4	75	0-70	40.0	1-7	3.8
		21	**83.3**		**61.8**		**5.8**

recorded at Dager followed by 4% at Daulatana. The minimum mean disease incidence 3.2% was found at Atharanhazari. In this district, most of the farms were quite free from the disease. The highest mean disease severity was 1.5, recorded at Dager followed by 1.3 each at Atharanhazari and Daulatana.

In district D.I. Khan, maximum mean disease incidence (6.9%) was recorded at Kot jai and minimum (4.02%) was found at Kafir kot and Saggu. No disease was found at Yarik. Mean for disease severity varied from 1.6 recorded at Kot jai to 1.3 recorded at Kafir kot Similarly, in district Lakki marwat, the highest mean disease incidence was 15.6% that was recorded at Aba khel followed by 11.6% and 8% at Isa khan and Lakki, respectively. The minimum mean disease incidence (7.5) was found at Shehbaz khel. In this district majority of the farm was free from the disease. The highest mean disease severity of 4 was recorded at Shehbaz khel followed by 2.5 at Aba khel whereas minimum 1.5 was found at Lakki.

In district Karak, the highest mean disease incidence that was 90.7% was recorded at Bahader khel followed by 83 and 71.7% at Banda Daud Sha and Ahmad wala, respectively. The lowest mean disease incidence of 28% was found at Lachi also with the lowest incidence range of 0-56%. The highest mean disease severity (8) was recorded at Banda daud shah followed by 7.7 and 6.7 at Bahader khel and Ahmad wala. The lowest mean disease severity of 3.0 was found at Lachi.

4.2 FACTORS AFFECTING DISEASE DEVELOPMENT

Before initiating the studies on pathogenic variability and screening of chickpea germplasm against blight, some factors (inoculum level, age of the plants at the time of inoculation and wetness/ incubation periods), which are prerequisite for blight, were determined.

4.2.1 Effect of inoculum concentration on disease development

There was a positive correlation between disease severity and inoculum concentration in used with the range of $5x10^4$, $1x10^5$, $1.5x10^5$ and $5x10^5$ spores per ml (Figure-4.2). The control plants remained healthy. Disease symptoms were generally

least severe on Dashat and Parbat varieties of chickpea, with mean disease severity of 3.4, 4.2, 4.8 and 5.0, respectively on Dashat while on Parbat, it was 3.2, 3.8, 5.0 and 5.0 at all inoculum concentration levels. Two varieties C-727, ILC-263 and Punjab-91showed mean disease severity of 4.6, 6.0 and 4.4 respectively, when inoculated with $5x10^4$ spores per ml. These cultivars showed mean disease rating in the range of 6.2 to 7.8 and 6.4 to 8.6, respectively at the inoculum of $1x10^5$ and $2.5x10^5$ spores per ml, respectively. ILC-263 and C-727 were completely killed at the inoculum concentration of $5x10^5$ (14 days after inoculation).

4.2.2 Effect of plant age on disease development

Punjab-91and C-727 were tested against blight at different physiological stages from 2 to 12 weeks after sowing, representing various stages from seedling to the pod forming stage. The results showed that disease was more severe on 2 week old seedlings of the genotypes than on older plants (Figure-4.3). The older plants showed pale yellowish discoloration of the entire foliage and leaf abscission was more severe compared to young plants. The control plants remained healthy and did not show any sort of symptoms. It was observed that the disease was more severe on C-727 as compared to Punjab-91.

4.2.3 Effect of spray and incubation periods on disease development

The results of this study revealed that in all the cultivars, incubation periods of 3 and 4 days when sprayed with water for 1 or 2 times a day had a significant effect. Longer incubation periods, i.e., 4 days was more effective when sprayed twice a day (Table-4.2). The maximum infection frequency occurred with 2-sprays/4 days of incubation period and also with 2spray/3days incubation period, whereas less infection and disease development occurred with no spray/1-day incubation period. No disease was developed on the plants that were not incubated and also water was not sprayed on them. Results showed that Dashat was resistant/tolerant for all leaf wetness periods, with or without water spray, whereas other cultivars were susceptible and increasing the period of leaf wetness as well as water spray increased disease severity. Only C-727 showed a susceptible reaction in all the treatments.

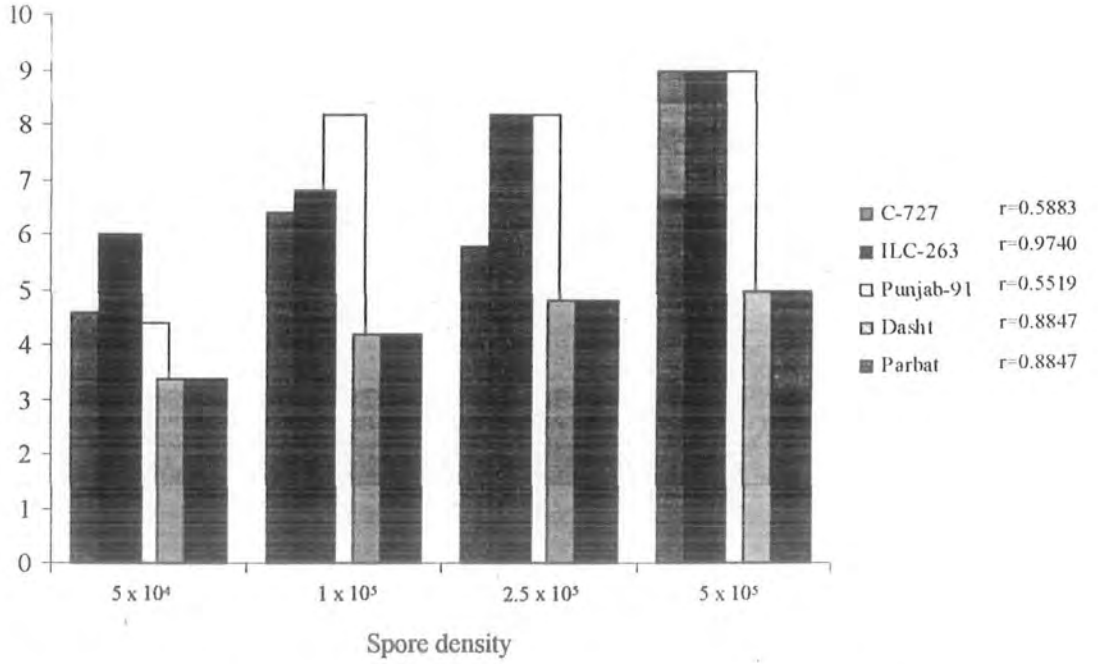


Fig. 4.2:- Effect of inoculum levels for the development of *Ascochyta* blight in chickpea

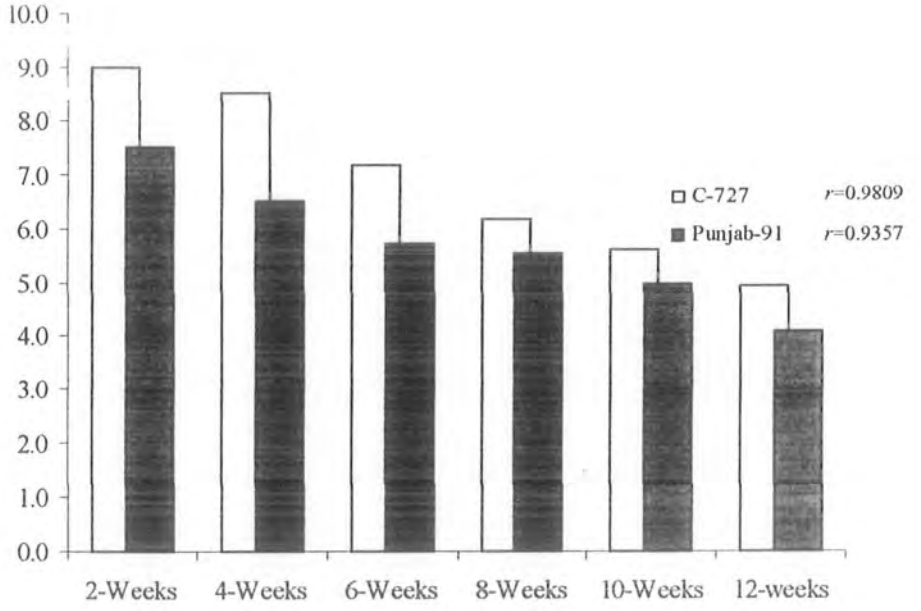


Fig. 4.3:- Response of *Ascochyta* blight at various growth stages of plants.

Table 4.2:- Effect of number of sprays and incubation period on blight infection in chickpea

Variety	Disease rating (1-9 scale)															
	0 S/0 D	0 S/1 D	0 S/2 D	0 S/3 D	0 S/4 D	1 S/0 D	1 S/1 D	1 S/2 D	1 S/3 D	1 S/4 D	2 S/0 D	2 S/1 D	2 S/2 D	2 S/3 D	2 S/4 D
C-727	1.0 p	7.0 f	7.6 e	8.1 bcd	9.0 a	1.0 p	7.0 f	7.9 cde	9.0 a	9.0 a	1.0 p	9.0 a	9.0 a	9.0 a	9.0 a
Parbat	1.0 p	3.5 mn	5.0 I	5.5 h	7.8 de	1.0 p	3.8 mn	6.8 f	7.0 f	7.0 f	1.0 p	7.0 f	7.0 f	9.0 a	9.0 a
Dasht	1.0 p	2.2 o	3.4 n	4.2 l	4.7 ij	1.0 p	2.3 o	3.4 n	3.7 mn	4.6 jk	1.0 p	3.9 lm	4.2 kl	4.6 jk	6.2 g
Pb-1	1.0 p	6.2 g	7.0 f	7.8 de	9.0 a	1.0 p	5.0 I	7.0 f	8.2 bc	8.5 b	1.0 p	7.0 f	9.0 a	9.0 a	9.0 a

Figures having same letters are insignificantly different at 5% probability.
S= No. of sprays D = days for incubation

4.3 VARIABILITY IN *ASCOCHYTA RABIEI*

Maorphological as well as pathogenic variability in *A. rabiei* was studied for the better understanding of the host-pathogen interaction. Resistance to blight in new generation of genotypes needs to be evaluated and variability in the pathogen used to guide resistance breeding.

4.3.1 Morphological variability in *A. rabiei*

Differences in morphological characters of *A.rabiei* such as radial growth on medium, colony colour, size of pycnidia and pycnidiospores were observed. In case of the radial growth of forty-two isolates of the fungus, a significant difference was recorded. It ranged from 2.6 to 6.7 cm when observed 15 days after incubation. The isolates BR-3 and KK-1 with a colony diameter 6.7 and 6.6 cm, respectively were considered fast-growing; isolates BN-1, MN-5, DI-1, and BR-1 (linear growth less than 3.0 cm) were rated as slow-growing and rest of the isolates were intermediate i.e., with linear growth between 3.0 to 5.1 cm. Amongst all the isolates, the BR-3 was found fast-growing (6.7 cm) while BR-1 (2.6 cm) was observed to be slowest growing (Table 4.3).

The isolates also showed some differences in colony colour that varied from light brown to black. There were five groups of the isolates on the basis of colony colour; light brown, brown, dark brown, grey and black. Sixteen isolates were light brown, eleven isolates were brown, six isolates were dark brown, two isolates were gray and seven isolates were black in colour (Table 4.3).

Data on cultural and morphological characteristics of the aforementioned 42 isolates, such as pycnidial formation revealed that abundant formation of pycnidia were obtained in case of 10 isolates while least pycnidial formation was observed in 14 isolates. Rest of the isolates showed the intermediate response for pycnidial formation (Table 4.3).

The data on size of pycnidia and pycnidisopres showed that maximum size of pycnidia was obtained from isolates of FD-2 (225 x 224μm) and AT-2 (204 x 205μm) respectively, with the pycnidiospores size of 10 x 4.5μm and 9.0 x 5.0μm. These isolates were followed by isolates KN-1 (194 x198μm) and FD-1 (194 x 196μm). The smallest size

Table-4.3: Morphological characteristics of various isolates of *Ascochyta rabiei*

S.No	Isolates	Colour	Pycnidial formation	Colony growth (Cm)	Pycnidial size (µm)	Pycnidio-spore size (µm)
1	KB-1	Light Brown	++	4.4 fg	132x127mno	11.0x5.0 c
2	BR-4	Light Brown	+	3.9 ijk	128x121 nop	11x5.0 c
3	BR-3	Brown	+	6.7 a	113x115 mn	9x5.0 h
4	LY-4	Light Brown	++	3.9 ijk	142x132 klm	10x5.0 ef
5	KT-2	Brown	+	3.7 klm	108x113 q	10x5.0 ef
6	MN-4	Light Brown	+	3.9 ijk	118x118 pq	10.0x6.0 b
7	KT-1	Brown	++	3.7 klm	110x110 q	13x5.0 a
8	MN-5	Black	+	2.8 op	124x122 op	8x4.0 k
9	LY-2	Brown	+	3.7 klm	89x94 r	9.0x5.0 h
10	AT-1	Light brown	++++	4.7 de	113x114 q	8.5x4.0 k
11	BR-5	Gray	+	3.0 o	138x131 lm	9.0x5.0 h
12	BR-2	Light brown	+	4.3 gh	113x123 pq	8.5x5.0 I
13	KK-2	Light brown	++	3.5 m	107x111 q	9.0x5.0 h
14	JN-2	Light brown	+	5.0 bc	113x113 q	8.5x5.5 h
15	KB-3	Gray	+	4.0 ij	91x85 r	7.5x5.0 j
16	NC-3	Dark brown	++++	4.0 ij	118x111 q	11x5.0 c
17	NC-1	Brown	++++	3.8 jkl	139x141 kl	10x5.0 ef
18	BN-2	Light brown	+	4.1 hi	101x95 r	8.4x5.0 I
19	DI-2	Light brown	+	3.6 lm	107x111 q	9.5x4.4 j
20	LY-3	Light brown	+	3.3 n	145x128 klm	9x5.0 h
21	NC-2	Light brown	++++	3.6 lm	115x119 pq	9.4x5.0 gh
22	AT-2	Black	++++	4.3 gh	204x205 b	9.0x5.0 h
23	BN-1	Black	++	2.9 o	152x150 j	11.0x5.0 c
24	KB-2	Brown	++	3.0 o	155x153 j	9.0x5.0 h
25	KN-1	Dark brown	+++	4.7 de	194x198 c	10x5.0 ef
26	MN-3	Light brown	++	5.1 b	181x183 f	11x5.0 c
27	DI-1	Black	++	2.7 pq	135x135 lm	10x5.0 ef
28	BR-1	Black	++	2.6 q	135x130 lm	10x5.4 cd
29	FD-2	Black	+	3.2 n	225x224 a	10x4.5 h
30	CL-2	Light brown	++++	4.9 cd	184x186 ef	11x5.1 c
31	MN-1	Dark brown	+++	4.4 fg	175x176 gh	11x5.5 b
32	ST-1	Dark brown	++++	4.3 gh	170x175 h	10x5.0 ef
33	AT-3	Brown	++++	4.6 ef	164x167 I	10x5.4 cd
34	JN-1	Dark brown	+	4.6 ef	186x196 cd	10x5.0 ef
35	ST-2	Brown	+++	4.9 cd	183x177 fg	10x5.5 c
36	AT-5	Black	+++	4.2 gh	162x167 I	9x5.5 fg
37	AT-4	Light brown	++++	4.0 ij	188x189 de	11x5.4 b
38	MN-2	Light brown	+++	4.9 cd	140x147 k	12x5.0 b
39	FD-1	Dark brown	++	3.8 jkl	194x196 c	9.5x5.5 de
40	CL-1	Brown	++++	4.7 de	191x186 de	10x5.0 ef
41	KK-1	Brown	+++	6.6 a	113x115 q	9x5.0 h
42	CL-3	Brown	+++	4.6 ef	164x167 I	10x5.4 cd

of pycnida was observed in isolates, KB-3 (91 x 85μ), LY-2 (89 x 94μ) and BN-2 (101 x 95μm), respectively (Table-4.3). On the basis of pycnidial size, the isolates were categorized into 23 groups whereas they were divided into 12 groups according to the size of pycnidiospores. The largest spore size was observed in isolate KT-1 (13 x 5.0μm) followed by isolates of MN-1 (11.0 x 5.5μm), MN-2 (12.0 x 5.0μm), MN-4 (10.0 x 6.0μm) and AT-4 (11.0 x 5.4μm), respectively, while the smallest spores were obtained in MN-5 (8.0 x 4.0μm) and AT-1 (8.5 x 4.0μm).

4.3.2 Pathogenic variability in *A. rabiei*

The reaction of 42 isolates of *A. rabiei* on seven differential cultivars of chickpea indicated that the cultivars C-727, C 44, ILC 263 and CM 72 were susceptible to all the isolates while remaining three varieties were tolerant and exhibited variation in disease reaction with different isolates. Isolates of *A. rabiei* greatly varied in their pathogenic reaction to 7 different genotypes (Table 4.4). The analysis of variance showed significant differences ($P < 0.001$) for both the factors. The interaction was also highly significant, this source of variation and that of the isolates represented high proportions of the total sum of squares (Table-4.5).

The virulence rating of each isolate on all the lines tested exhibited a large but continuous variability. All the cultivars showed symptoms involving both leaves and stems when treated with various isolates.

The factor analysis showed that three factors gave eigen values greater than unity, whereas others were < 1, hence first three principal components were considered important in contributing variation amongst 42 isolates. First three components contributed 75.9% of the total variability (Table-4.6). The first PC was more related to blight reaction with different varieties rather than morphological characters of isolates. Pycnidial formation was more related to first PC and second PC contributed more for isolate colony colour, whereas variability for other morphological traits was distributed among all the three components. All the variables except colony colour contributed positively to PC_1: thus this principal component is a weighted average of the characters. Figure-4.4 presents the virulence status of *A. rabiei* isolates collected from various

Table 4.4: Pathogenic variability of various isolates of *Ascochyta rabiei*.

S. No.	ISOLATE	DIFFERNTIAL OF CULTIVARS						
		C-727	ILC-263	C-44	CM-72	Piadar-91	Noor-91	Punjab-91
1	KB-1	S	S	S	S	S	S	S
2	BR-4	S	R	R	S	R	R	R
3	BR-3	S	S	S	R	S	S	R
4	LY-4	S	S	S	S	S	S	S
5	KT-2	S	S	S	R	R	R	R
6	MN-4	S	S	S	S	S	R	R
7	KT-1	S	S	S	S	S	S	S
8	MN-5	S	S	S	S	R	R	R
9	LY-2	S	S	S	S	S	S	S
10	AT-1	S	S	S	S	S	S	S
11	BR-5	R	R	R	R	R	R	R
12	BR-2	S	S	S	S	S	R	R
13	KK-2	S	S	S	S	S	S	S
14	JN-2	R	S	R	R	R	R	R
15	KB-3	S	S	S	S	S	S	R
16	NC-3	S	S	S	S	S	S	S
17	NC-1	S	S	S	S	S	S	S
18	BN-2	R	R	S	S	R	R	R
19	DI-2	S	S	S	S	R	S	R
20	LY-3	S	R	S	S	S	S	R
21	NC-2	S	S	S	S	S	S	S
22	AT-2	S	S	S	S	S	S	S
23	BN-1	S	S	S	S	R	S	S
24	KB-2	S	S	S	S	S	S	S
25	KN-1	S	S	S	S	S	S	S
26	MN-3	S	S	S	S	S	S	S
27	DI-1	S	S	S	S	R	R	S
28	BR-1	S	S	S	S	S	S	S
29	FD-2	S	S	S	S	S	S	S
30	CL-2	S	S	S	S	S	S	S
31	MN-1	S	S	S	S	S	S	S
32	ST-1	S	S	S	S	S	S	S
33	AT-3	S	S	S	S	S	S	S
34	JN-1	S	S	S	S	R	S	S
35	ST-2	S	S	S	S	S	S	S
36	AT-5	S	S	S	S	S	S	S
37	AT-4	S	S	S	S	S	S	S
38	MN-2	S	S	S	S	S	S	S
39	FD-1	S	S	S	S	S	S	S
40	CL-1	S	S	S	S	S	S	S
41	KK-1	S	S	S	S	S	S	S
42	CL-3	S	R	S	S	S	S	S

R = resistant, S = susceptible

able-4.5. Two-factor analysis of variance for the reaction of 7 chickpea varieties to 42 isolates of *Ascochyta rabiei* collected from Pakistan

ource of riation	Df	Sum of squares	Mean squares	*F* value	Probability	Standard error
eplication	2	1.75	0.87	4.00	0.02	0.02
arieties	6	264.09	44.01	201.65	0.00	0.04
olates	41	1379.97	33.66	154.20	0.00	0.10
teraction	246	431.51	1.75	8.04	0.00	0.27
rror	586	127.907	0.22			

oefficient of Variation: 6.22%

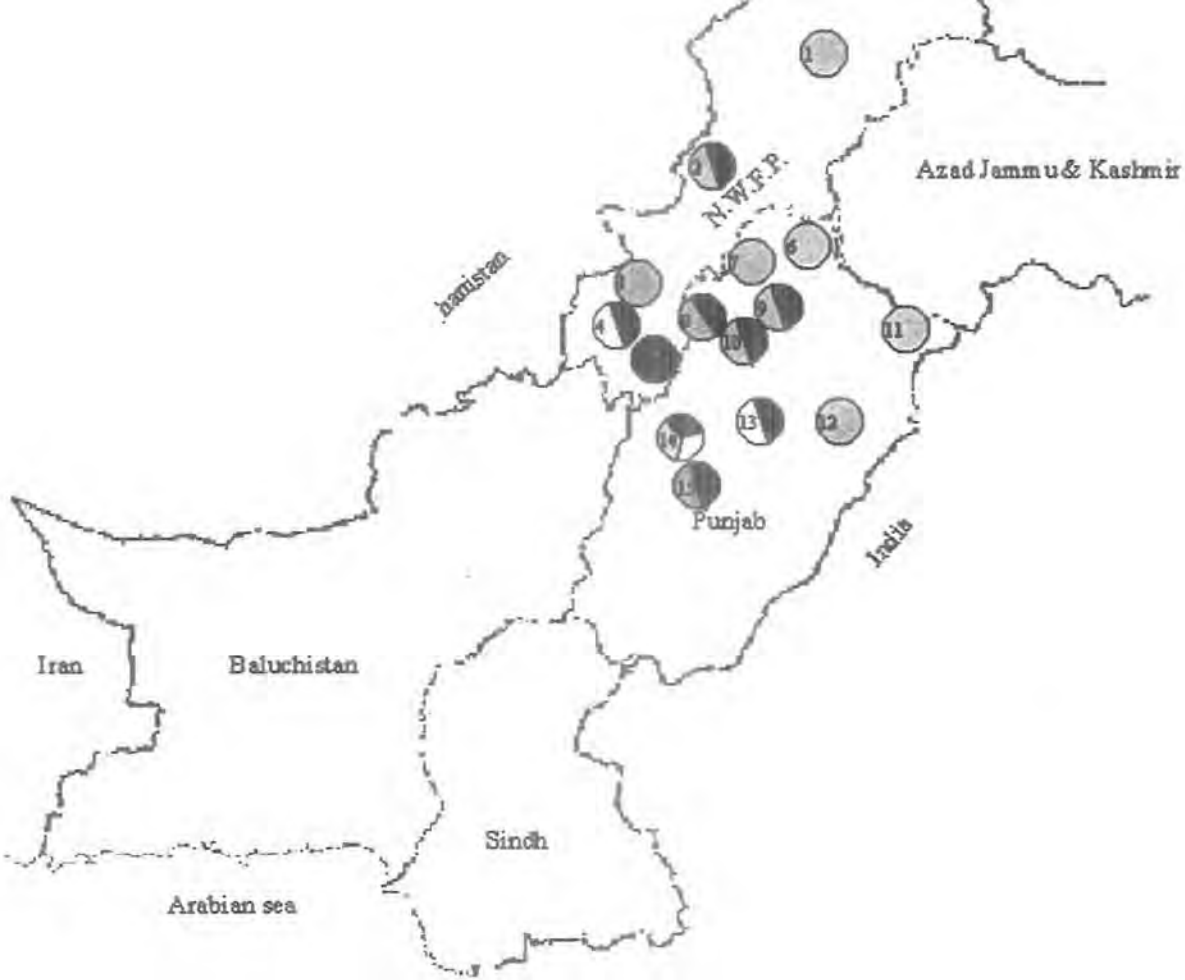


Fig.4-4- Collecting sites of *Ascochyta rabiei* in Pakistan. The symbol ◯ represents the collecting site of isolate. The serial numbers represent 1- Kaghan, 2- Kohat, 3- Karak, 4- Bannu, 5- DI Khan, 6- Islamabad, 7- Attock, 8- Mianwali, 9- Chakwal, 10- Khushab, 11- Sialkot, 12- Faisalabad, 13- Jhang, 14- Bhakkar and 15- Layyah

able 4.6:Principal Components (PCs) for cultural characterization and disease reaction of 42 isolates of *Ascochyta rabiei*

	PC_1	PC_2	PC_3
igen value	6.531	1.563	1.015
roportion of σ^2	54.426	13.025	8.458
umulative σ^2	54.426	67.452	75.91
	Eigen vectors		
olony colour	-0.14	0.86	-0.08
ycnidial formation	0.88	-0.06	-0.06
adial growth	0.28	-0.77	0.02
ycnidia	0.50	0.30	0.50
pores size	0.37	-0.13	0.80
isease reaction with variety C-727	0.85	0.27	-0.01
isease reaction with variety ILC-263	0.78	0.14	0.08
isease reaction with variety C-44	0.86	0.09	-0.02
isease reaction with variety CM-72	0.87	0.07	-0.19
isease reaction with variety Piadar	0.86	-0.14	-0.21
isease reaction with variety Noor-91	0.93	-0.05	-0.16
isease reaction with variety Punjab-91	0.92	-0.02	-0.05

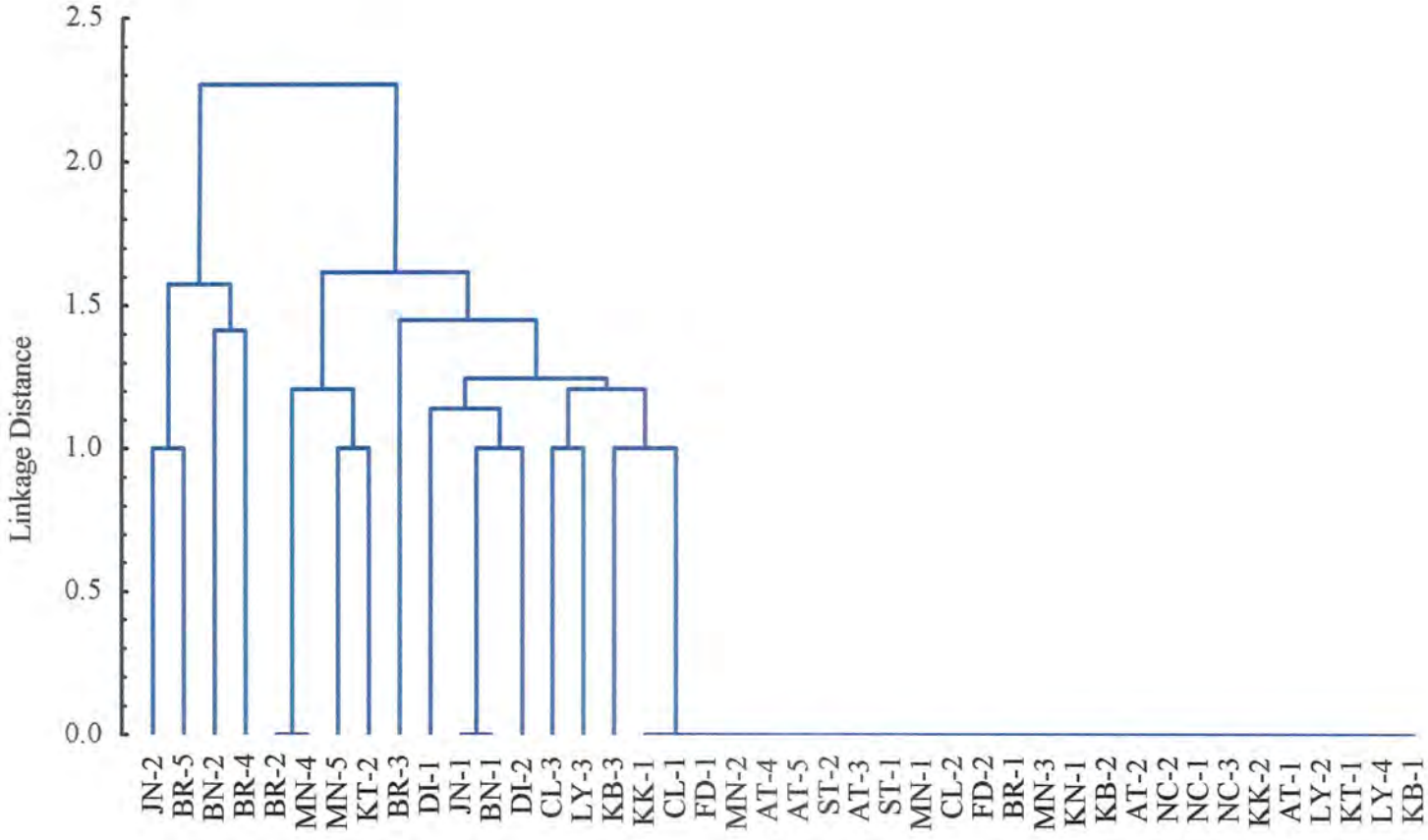


Fig.4.5. Differential response of 42 isolates of *Ascochyta rabiei*

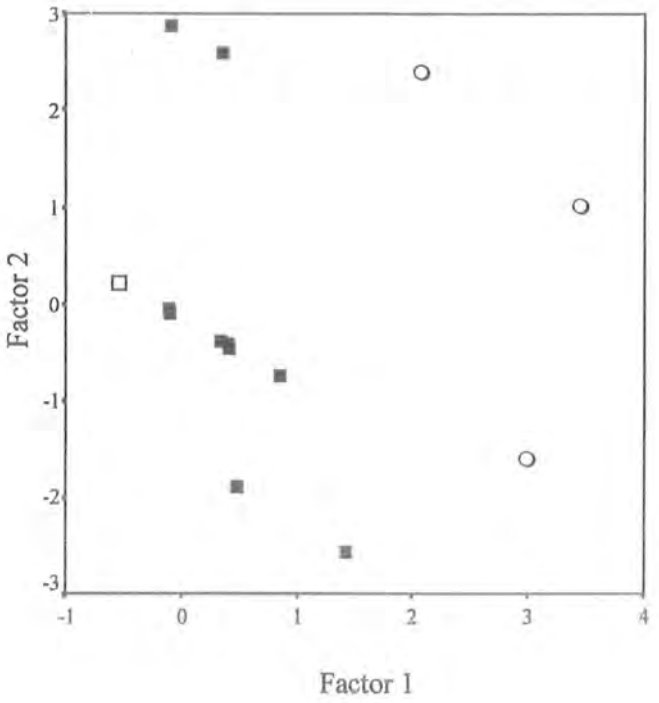


Fig. 4.6Scatter diagram for two factors in 42 isolates of *Ascochyta rabiei* collected from chickpea growing areas of Pakistan.
○ least virulent, ■ medium virulent and □ most virulent

localities/districts of two provinces; Punjab and NWFP that represents the major chickpea growing areas of Pakistan. The isolates collected from six sites (Kaghan, Kohat, Islamabad, Attock, Sialkot & Faisalabad) gave the most virulent reaction consistently (Table-4.6).

Two clusters were observed using UPGMA method for constructing dendrogram (Fig. 4.5). Cluster I consisted of four isolates and these all were less virulent originating from the districts of Jhang, Bhakar (Punjab) and Bannu (North West Frontier Province), whereas cluster II consisted 38 isolates of diverse origin. This cluster could be further separated in to two sub clusters, i.e., twelve isolates did not infect all the varieties, whereas other 26 isolates infected all the varieties at the same level and could be considered more virulent.

First two factors contributing more than 67 % of the variability were plotted for disease reaction and three distinct groups were observed (Fig. 4.6). The most virulent isolates gave the similar reaction and were observed one point in extreme left of the graph. Least virulent were grouped in the right half of the graph where 3 points were observed because of similar reaction by 2 isolates. The third category, i.e., intermediate were ranging from origin to 2 at x-axis, while scattered throughout in the y-axis. It was obvious that only virulent isolates could clearly be identified. Although other two groups were separated but exhibited little breeding value due to non-clear-cut identification. On the whole, multivariate analyses were able to distinguish isolates on the basis of virulence rather than origin or morphological/cultural characterization. Some of the isolates collected from the same area or source behaved differently.

4.3.3 COMBINED EEFECT OF THE LEAST AND THE MOST AGGRESSIVE ISOLATES ON BLIGHT

The reaction of chickpea 16 genotypes when tested against the least aggressive (BR-5) and the most aggressive (AT-2) individually as well as in their combination of 1:1 ratio was recorded on 1-9 scale (Single *et al.*, 1981). The results presented in Table 4.7, showed that there were significant differences both for isolates and genotypes for disease response. The disease score on genotypes inoculated with least aggressive isolate ranged

able 4.7: Effect of isolates of *Ascochyta rabiei* representing the most and the least aggressive nature when applied separately and in combination.

Cultivars	Most aggressive isolate	Least aggressive isolate	Mixed isolate	Means
Dasht	5.4	2.2	2.4	3.33
Parbat	7.0	3.2	3.6	4.60
C 727	9.0	4.4	7.0	6.80
C 44	9.0	5.0	6.6	6.87
C 235	9.0	7.0	6.0	7.33
CM 72	9.0	5.0	3.4	5.80
NIFA 88	7.0	2.6	3.8	4.47
NIFA 95	9.0	4.2	5.0	6.07
Bittle 98	7.0	5.4	5.8	6.07
Noor 91	9.0	3.4	5.4	5.93
Punjab 91	9.0	4.0	7.0	6.67
Piadar 91	9.0	3.4	5.8	6.07
Balkasar	5.0	2.2	2.4	3.20
Wanhar	6.0	3.0	3.2	4.07
ILC 263	9.0	4.6	7.0	6.87
DC 1	5.6	4.0	4.0	4.53
LSD (P < 0.05%)	0.9096	0.7431	0.7601	
EMS	0.517	0.345	0.361	
G. means	7.95	5.66	4.90	

Duncan's Multiple Range Test (DMRT) was performed at P< 0.05.

Table 4.8: Comparison of the most aggressive and the least aggressive isolates of *Ascochyta rabiei*

		Most aggressive isolate	Least aggressive isolate
Radial growth	$\bar{X}$±SD	3.00±1.00	4.20±1.00
	t-value	$1.4x10^{7}$ ***	
	Probability	0.000	
Pycnidial size	$\bar{X}$±SD	41826.7±165.10	1807.0±175.00
	t-value	170.97 **	
	Probability	0.000	
Spore size	$\bar{X}$±SD	45.0±12.0	45.0±12.0
	t-value	0.00 ns	
	Probability	1.00	

from 2.2 to 7 whereas in the case of aggressive isolate and mixture of two it ranged from 5.4 to 9 and 2.4 to 7, respectively. The isolate means of disease scores on 16 genotypes were 7.75, 3.98 and 4.90 respectively for aggressive, least aggressive and mixture of both (Table 4.7). The isolate means of disease score for least aggressive and most aggressive isolates were significantly different from each other. However, the mean disease score of the mixture of two isolates (4.90) was not significantly different from that of least aggressive isolate. The genotypic mean for disease of Balkasar and Dasht were respectively 3.20 and 3.33. The maximum mean disease with score 7.33 was exhibited by C-235, which was followed by C 44 and C 727 with respective disease scores of 7.33 and 6.87. The intensity of disease on each genotype was different under three different treatments. The disease score of Dasht increased from 2.2 (in the case of least aggressive isolate and mixture of two) to 5.4 (in the case of aggressive isolate). Similarly for Balkasar it increased from 2.2 to 5.

The cultural traits presented in Table 4.8 revealed significant differences between the two isolates (BR-5 and AT-2) tested for radial growth and pycnidial size. The spore size in both the isolates was however the same.

4.4 SCREENING OF CHICKPEA FOR BLIGHT RESISTANCE

From 1994 to 1996, more that eight hundered chickpea germplasm lines and breeding materials were evalauated for blight resistance from seedling to pod forming stages under greenhouse as well as in field conditions. The germplasm screening during 1993-94 revealed that none of the 248 genotypes was highly resistant at any stage, whereas one genotype (ICC 13555) was resistant at seedling stage and eight (86025, 83205, 86205K, 93164, 92003K, ICC 13555, 93069, 93027) at vegetative stage, whereas none of the lines was resistant at pod formation stage (Tables 4.9 and 4.10). During 1995, the results revealed that one genotype (NIFA 88) was resistant at seedling stage and four (FLIP 93-62C, ICC 3416, ICC 6373, NIFA 88) at advanced vegetative stage, whereas all the genotypes were in the range of susceptible or highly susceptible at pod formation stage except six which were moderately resistant. It was observed that most of the resistant genotypes originated from ICARDA except NIFA 88 that was developed by Nuclear Institute for Food and Agriculture, Peshawar. Similarly, the germplasm

Table 4.9: Screening of chickpea genotypes against blight during 1994 to 1996

		Seedling stage			Vegetative stage			Pod forming stage		
Disease Grade	Disease reaction	1994	1995	1996	1994	1995	1996	1994	1995	1996
1	Highly resistant	0*	0	0	0	0	0	0	0	0
2-3	Resistant	1	1	2	8	4	9	0	0	9
4-5	Moderately resistant	11	28	37	71	33	72	6	6	72
6-7	Susceptible	27	20	66	118	66	145	41	29	145
8-9	Highly susceptible	209	181	239	51	129	118	201	197	118

* Number of chickpea germplasm accessions

Table 4.10: Chickpea germplasm lines resistant/ moderately resistant to blight

	Disease Grade	Disease Reaction	1994		1995		1996	
lling e	1	Highly resistant	0	-	0	-	0	-
	2-3	Resistant	1	ICC 13555	1	NIFA 88	2	FLIP91-159C, ICC 3991
etative e	1	Highly resistant	0	-	0	-	0	-
	2-3	Resistant	8	86025, 93205, 86205K, 93164, 92003K, ICC 13555, 93069, 93027	4	FLIP93-62C, ICC 3416, ICC 6373, NIFA 88	9	FLIP92-159C, ICC 3991, FLIP94-508C, FLIP94-509C, FLIP94-510C, ICC 3919, ICC 12004 ICC 13279, ICC 1403
ation e	1	Highly resistant	0	-	0	-	0	-
	2-3	Resistant	0	-	0	-	0	-

screening during 1995-96 revealed that none of the 269 genotypes in the range was highly resistant at either stage. Two genotypes (FLIP 91-159C, ICC 3991) were resistant at seedling stage and nine (FLIP 92-159C, ICC 3991, FLIP 94-509C, FLIP 94-509C, FLIP 94-510C, ICC 3919, ICC 12004, ICC 13279, ICC 1403) at vegetative stages, whereas no genotype was resistant at pod formation stage. It was observed that all the resistant genotypes were of exotic origin. Overall the number of resistant genotypes were higher during 1995-96 that might be due to involvement of resistant material in the study from national and international sources. It is quite evident that none of the genotypes was resistant at pod formation stage during all the three years except thirty seven during 1993-94, seventy two during 1994-95 and twenty one during 1995-96, which were moderately resistant. The common genotypes which were resistant at seedling and vegetative stage are suggested to be utilized in breeding programme.

High relationship among three stages was observed for three years (Table 4.11) and seed sources (Table 4.12). This indicated that although screening could be conducted at any of these stages, to minimize labour and resources, screening could be done at seedling stage. Disease rating at three stages on the basis of seed source/origin and years are presented in the Fig. 4.7. It was observed that disease at vegetative stage was lower as compared to seedling and pod formation stage. Similar pattern of disease infection was exhibited by genotypes of all the sources. The lowest disease rating at vegetative stage was observed in the germplasm obtained from ICARDA that was followed by the germplasm originated from NIFA. The average disease rating of germplasm obtained from these two sources was in the range of moderate resistance whereas, the germplasm from other sources was in the range of susceptibility.

The disease severity was less at vegetative stage, whereas it was at equal level on vegetative and pod formation stages during all the three years. From the present investigation, it was concluded that Ascochyta blight at seedling stage and pod formation stage gave the similar infection level although a high relationship was observed among all these three stages (Fig. 4.8). Due to high relationship of disease between seedling and pod formation stage it was suggested to screen huge germplasm lines for blight resistance at seedling stage under greenhouse conditions. It has been estimated that for screening

Table 4.11:- Correlation among three stages of Ascochyta blight in chickpea during three years

Stages	Year	Vegetative	Pod formation
Seedling	1994	0.41	0.42
	1995	0.84	0.86
	1996	0.81	0.85
Vegetative	1994		0.75
	1995		0.77
	1996		0.76

Table 4.12:- Correlation among three stages of Ascochyta blight in chickpea based on origin of germplasm

Stages	Origin	Vegetative	Pod formation
Seedling	AARI	0.54	0.72
	ICARDA	0.77	0.67
	ICRISAT	0.78	0.75
	NIAB	0.46	0.52
	NIFA	0.77	0.65
Vegetative	AARI		0.75
	ICARDA		0.86
	ICRISAT		0.63
	NIAB		0.66
	NIFA		0.63

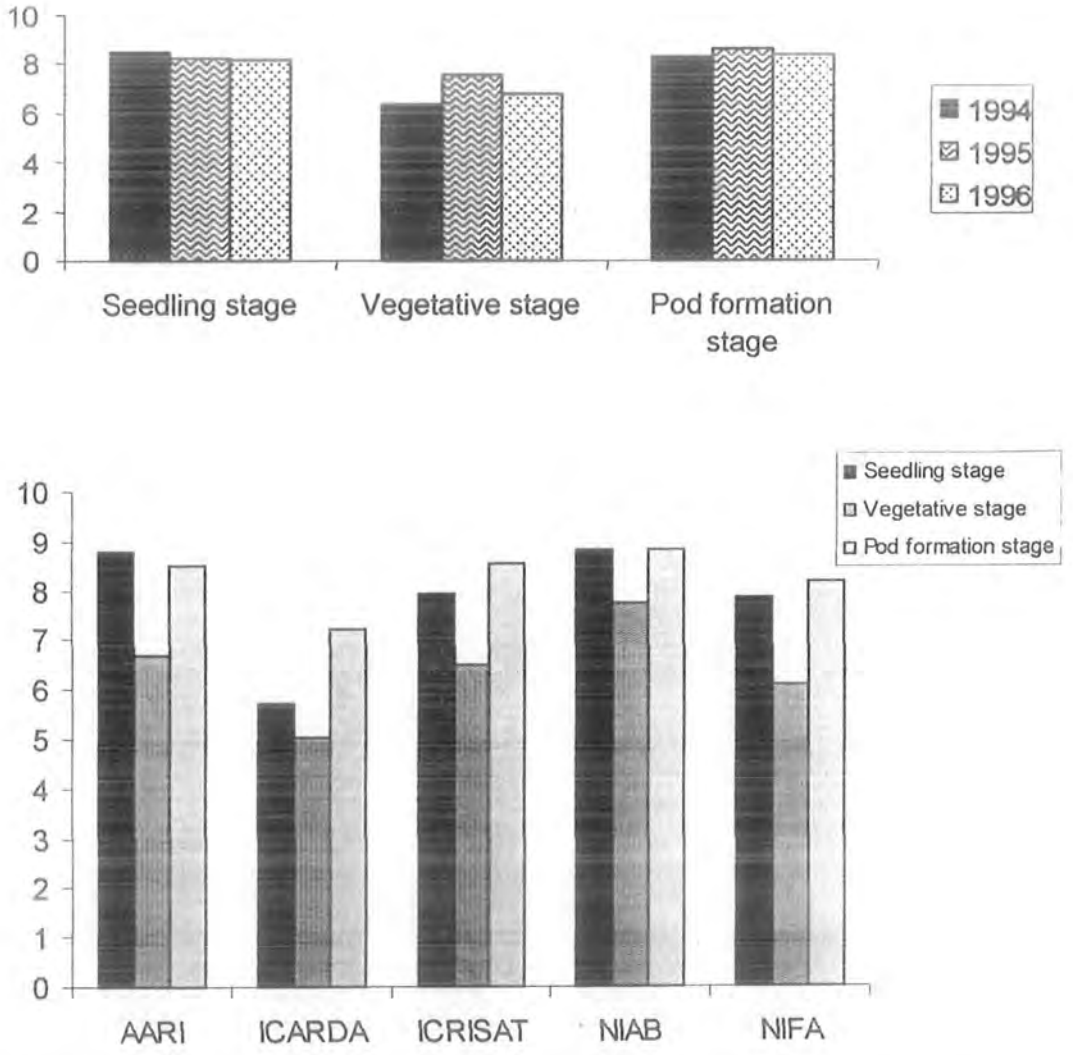


Fig. 4.7:- Disease rating at three plant stages of chickpea for three years (above) and five sources (below).

experiments under field conditions at least two weeks of continuous 90% RH are necessary for uniform spread of the disease.

Out of all the genotypes screened for disease, forty-eight genotypes representing various sources were repeatedly tested against Ascochyta blight for three years to investigate the year-genotype effect of the disease (Table 4.13). It was observed that the results were significant for genotypes at all the three stages of plant, whereas year effects were insignificant at pod formation stage and significant at other two stages. The amount of variation was higher at seedling and vegetative stage that indicated the influence of environmental change during early phase of experiment, from November to February when temperature range slightly favoured disease development but humidity was very low. Although water was sprayed to maintain RH at 90% but due to fluctuation in temperature and dryness of air RH was expected uneven. The variation in RH during this period exhibited high variation that made year effects significant. In-significant effects during pod formation period (March-April) were attributed to conducive environmental conditions for Ascochyta blight at Islamabad. The CV for disease rating at seedling stage and pod formation was in the range of acceptance limits, where CV for vegetative stage slightly exceeded.

4.5 EFFECT OF MORPHOLOGICAL TRAITS FOR BLIGHT RESISTANCE

Chickpea genotypes belonging to resistant and susceptible groups were selected to find out the relationship between disease resistance and morphological characters. Significant differences among genotypes for number of hairs on both sides were observed (Table 4.14). These two traits were significantly correlated (r=0.6421). The total numbers of hairs were also significantly correlated with both the components (Table 4.16). The range of hair density on dorsal surface of the leaf was 19.48 to 31.30 (highly variable). Within susceptible lines, values were 19.48 to 26.94 while within resistant lines, it was 20.23 to 31.30. In moderately resistant germplasm, values were 22.69 to 26.91. These traits did not exhibit any effect on infection as this character was randomly scattered without influencing disease pattern. All the genotypes, irrespective of their reaction to disease, were different from each other for this trait as revealed by Duncan's Multiple

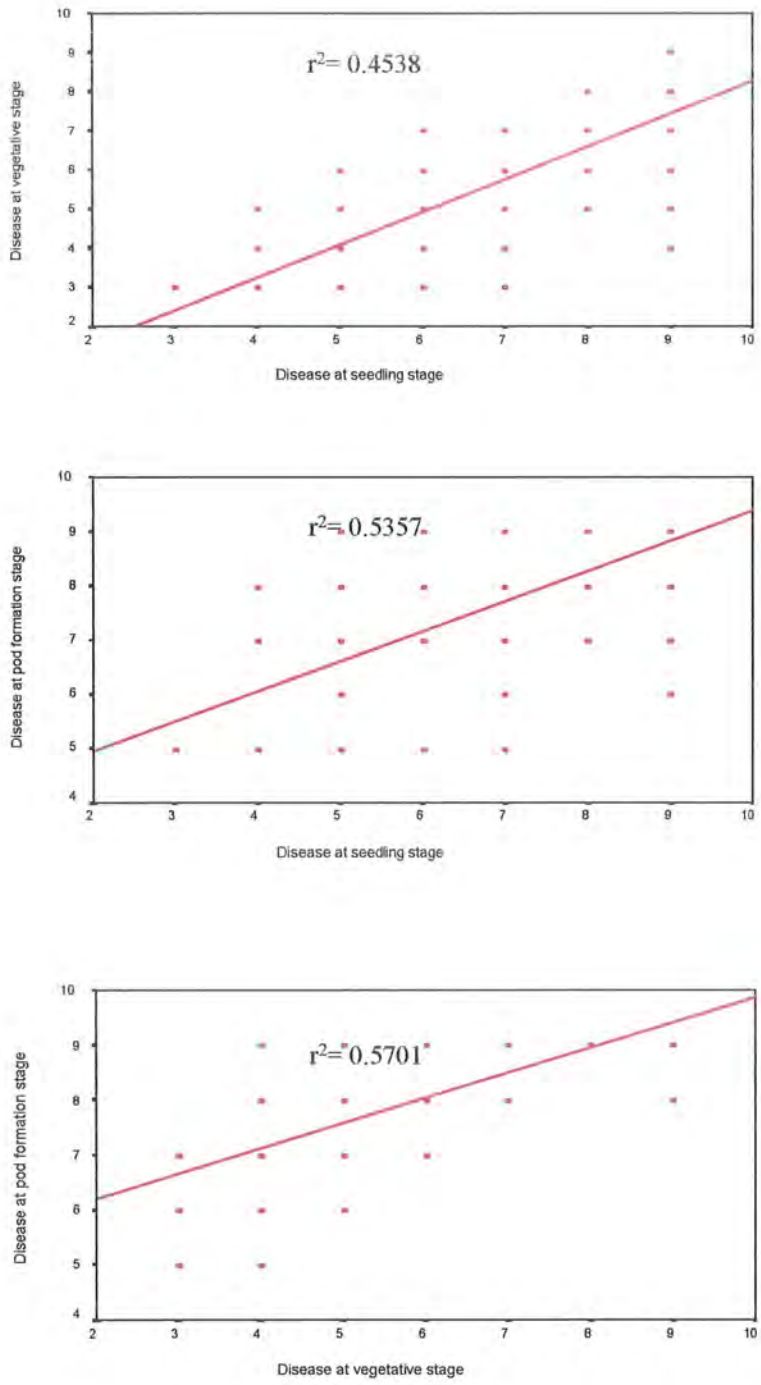


Fig. 4.8:- Relationship among Ascochyta blight at three plant stages of chickpea

Table 4.13:- Analysis of variance for disease reaction at three stages of chickpea

SOV	DF	Mean square		
		Seedling stage	Vegetative stage	Pod formation stage
Years	2	10.21	6.88	2.59
Genotypes	47	9.23	7.45	4.50
Error	94	1.07	1.39	0.88
Total	143			
F. ratio (y)		9.52**	4.94**	2.94ns
F. ratio (g)		8.60**	5.35**	5.12**
CV (%)		16.88	21.81	12.57
SE (y)		0.15	0.17	0.14
SE (g)		0.59	0.68	0.54

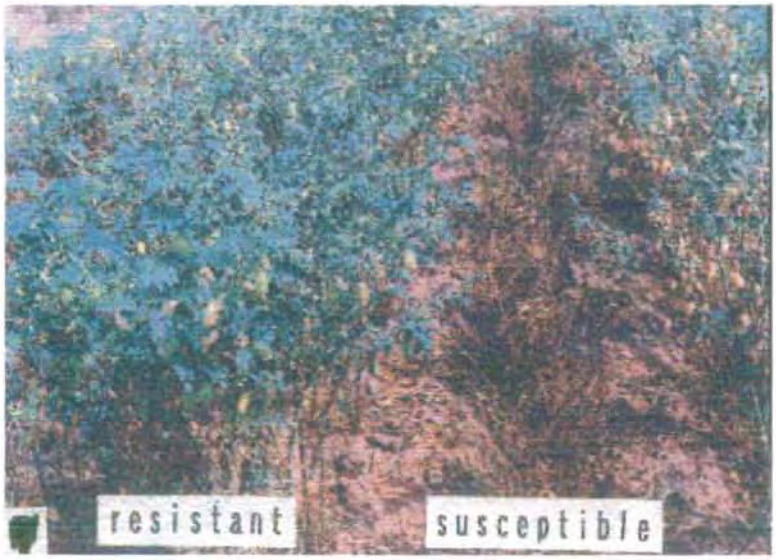


Fig 4.9: Resistant and susceptible cultivars of chickpea as a result of screening against blight at reproductive stage.

Range Test. On an average, by this character, no role towards resistance against blight was found.

A wide range of variation for number of hairs on ventral surface of the leaves was recorded (30.15 to 62.26). In case of susceptible and resistant cultivars, it ranged from 30.15 to 37.34 and 51.96 to 62.26, respectively. Data indicated highly significant difference between these genotypes. In moderately resistant cultivars, the range was 33.73 to 57.56 that indicated no association of this character with disease development, although susceptible cultivars had slightly less hairs. Total number of hairs showed highly significant difference and ranged from 72.05 to 93.51 and 49.64 to 64.29 in resistant and susceptible cultivars, respectively. Total number of hairs has significant association with number of stomata and area of guard cells (Table 4.16). The susceptible cultivars had slightly less number of hairs as compared to resistant ones, therefore, this trait needs to be investigated in a broader genetic stock to confirm association, if any.

For number of stomata per unit area, significant differences between genotypes were observed (Table 4.15), but no clear relationship was recorded for disease development. This trait was significantly correlated (r=0.3932) with cultivars (Table 4.16). Numbers of stomata were significantly associated with area of guard cells. Stomatal density varied from 29.37 to 52.12. In susceptible lines, the variation was 37.25 to 48.97 and in resistant lines 41.63 to 52.12, while in moderately resistant lines, 29.37 to 31.27. These results indicate possible role of stomatal density towards resistance to Ascochyta blight fungus. The number of stomata were, therefore, inversely proportional to degree of resistance.

Data regarding the area of stomata revealed significant differences among cultivars (Table 4.16). Area of stomata ranged from 374.7 to 474.5μm^2 for the genotypes. The values for area of stomata for resistant genotypes were 374.7 to 396.5μm^2 and for susceptible genotypes, 384.8 to 399.3μm^2. For moderately resistant genotypes, the values were 456.4 to 474.5μm^2. This indicated no response of stomatal area for disease development.

Significant differences were observed among the cultivars for area of guard cells.

Table 4.14: Mean number of hair on dorsal, ventral and dorso-ventral sides of the leaves of the reaction groups of chickpea cultivars.

		No. of hairs on dorsal side			No. of hairs on ventral side			No. of hairs on dorso-ventral side		
S.No.	Cultivars	R	MR	S	R	MR	S	R	MR	S
1	NIFA-88	31.30 a	-	-	62.26 a	-	-	93.57	-	-
2	Dashat	20.23 e	-	-	51.96 ab	-	-	72.19	-	-
3	C-44	-	22.69 d	-	-	37.73 bc	-	-	60.42	-
4	P-91	-	26.91 c	-	-	57.56 a	-	-	84.47	-
5	C-727	-	-	26.94 b	-	-	37.34 bc	-	-	64.28
6	ILC-263	-	-	19.48 f	-	-	30.15 c	-	-	49.63
LSD (0.05%) =			0.0182			18.33				
SE =			0.00577			5.816				
EMS =			0.00010			101.5				

Table 4.15: Mean area of guard cells, number of stomata, area of stomata and size of stomatal aperture of reaction groups of chickpea cultivars

		Area of guard cells (um^2)			Number of stomata (1.52 mm^2			Area of stomata (um^2)			Size of stomatal aperture (um^2)		
S.No	Cultivars	R	MR	S	R	MR	S	R	MR	S	R	MR	S
1	NIFA-88	268.5 a	-	-	41.6 b	-	-	396.5 d	-	-	73.2 ab	-	-
2	Dashat	238.4 b	-	-	52.1 a	-	-	374.7 f	-	-	67.9 b	-	-
3	C-44	-	239.7 b	-	-	29.4 d	-	-	456.4 b	-	-	65.3 b	-
4	P-91	-	273.2 a	-	-	31.3 d	-	-	474.5 a	-	-	79.6 ab	-
5	C-727	-	-	265.3 ab	-	-	48.9 a	-		384.5 e	-	-	79.7 ab
6	ILC-263	-	-	241.6 b	-	-	37.3 e	-		399.3 c	-	-	89.9 a
LSD (0.05%) =			25.35			3.414			0.08136			18.02	
SE =			0.050			1.083			0.02582			5.717	
EMS =			194.2			3.521			0.00200			98.06	

Table-4.16: Correlation between different morphological characters of chickpea cultivars susceptible and resistant to Ascochyta blight

Parameters	Number of hairs on dorsal side	Number of hairs on ventral side	Total number of hairs	Number of stomata	Area of stomata	Size of stomatal aperture	Area of guard cells
Number of hairs on dorsal side	1.0000						
Number of hairs on ventral side	0.6421	1.0000					
Total number of hairs	0.8126	0.9637	1.0000				
Number of stomata	0.4804	0.3932	0.4707	1.0000			
Area of stomata	0.6249	0.3844	0.4648	0.4466	1.0000		
Size of stomatal aperture	0.2130	0.1009	0.0193	0.2593	0.3267	1.0000	
Area of guard cells	0.7981	0.5848	0.6874	0.7378	0.8773	0.8773	1.0000

The range of size of guard cells was 238.4 to 268.5 μm^2. In case of susceptible lines, the values were 241.6 to 265.3 μm^2, while in the resistant lines, range was 238.4 to 268.5 μm^2). In case of moderately resistant group, it was 239.7 to 273.2 μm^2. Similarly, significant differences for size of stomatal aperture were observed. The size of stomatal aperture ranged from 65.25 to 88.97 μm^2 and there was no relationship for various categories on the basis of disease development.

4.6 RELATIONSHIP BETWEEN PLANT EXTRACT AND SDS-PAGE ON BLIGHT RESISTNCE IN CHICKPEA

The relationship of chickpea plant extract on the growth of fungus and using under SDS-PAGE on blight was investigated.

4.6.1 Disease response of chickpea genotypes

Fifty-seven chickpea genotypes were evaluated for the sources of resistance against *A.rabiei* and none of the line was found completely free from disease (Table 4.17). Among the Kabuli types, six genotypes (ILC 482, ILC 72, ILC 195, ILC 200, FLIP 97-192C, FLIP 97-179C) were tolerant to blight and all these obtained from ICARDA. None of local Kabuli type was tolerant. In case of desi types, eleven genotypes (Dasht, Parbat, Balkasar, Wanhar, NIFA 88, NCS 5010, NCS 950212, 97047, 92A043, CM 72/ILC 3279, DC 1) were tolerant and all of these have been developed by the local breeders (Table 4.18).

4.6.2 Effect of chickpea plant extract on the growth of Ascochyta rabiei

No relationship was observed between disease reaction of 57 genotypes and in vitro growth of *A. rabiei* grown on sap extract from the same genotypes. Fungus growth was fast in sap from Kabuli types, but no clear indication was observed that might help in determining the resistance mechanism during *in-vitro* investigation (Table 4.16). Similar response was observed in case of susceptible genotypes. However, in case of some susceptible genotypes, fungus growth was increased. But on the other hand, in case of some susceptible genotypes, the growth was minimum as compared with others. It is interesting to note that the genotypes CM 72/ILC 3279 (F_9 generation) was tolerant

Table 4.17: Chickpea genotypes included for their biochemical aspects associated with blight resistance

S.No	Cultivars	Source	Seed type	Disease scale (1-9)	Disease reaction	Radial growth (cm)
1	Dasht	NARC	D	3.3	R	4.6
2	Parbat	NARC	D	4.0	MR	5.1
3	C-727	AARI	D	9.0	S	4.9
4	C44	AARI	D	8.0	S	4.7
5	Punjab-91	AARI	D	8.0	S	4.7
6	Pindar	AARI	D	9.0	S	4.8
7	Noor-91	AARI	K	7.6	S	4.4
8	Bittle-98	AARI	D	7.0	S	4.6
9	Balkasar	BARI	D	3.0	R	4.7
10	Wanhar	BARI	D	4.7	MR	4.8
11	CM-2000	NIAB	K	7.0	S	5.0
12	CM-98	NIAB	D	7.0	S	5.3
13	CM-88	NIAB	D	7.3	S	5.5
14	CM-72	NIAB	D	7.7	S	5.0
15	NIFA-88	NIFA	D	4.7	MR	4.8
16	DG-92	RRI	K	8.7	S	5.3
17	DG-89	RRI	D	8.3	S	5.3
18	ILC 202	ICARDA	K	7.4	S	5.2
19	Pb-1	AARI	K	9.0	S	4.7
20	ILC-482	ICARDA	K	5.0	MR	5.5
21	ILC-1929	ICARDA	K	5.3	MR	5.3
22	ILC-3279	ICARDA	K	6.0	S	4.5
23	ILC-72	ICARDA	K	4.7	MR	3.9
24	ILC-194	ICARDA	K	6.3	S	4.5
25	ILC195	ICARDA	K	4.3	MR	4.6
26	ILC-200	ICARDA	K	3.7	R	4.6
27	ILC-201	ICARDA	K	6.3	S	4.4
28	AUG-424	UAF	D	9.0	S	4.6
29	NIFA-95	NIFA	D	6.7	S	4.4
30	C-235	AARI	D	9.0	S	4.6
31	Karak-1	ARS	D	7.0	S	4.5
32	V88194K	AARI	K	8.7	S	4.4
33	AAR-1	AARI	K	7.7	S	4.3
34	FLIP96-60C	ICARDA	K	7.0	S	4.3
35	FLIP97-17C	ICARDA	K	6.0	S	3.5
36	FLIP97-192C	ICARDA	K	5.0	MR	4.1
37	FLIP97-179C	ICARDA	K	5.0	MR	4.3
38	CH41/91	NIAB	K	7.7	S	4.0
39	NCS-2001	NARC	K	8.3	S	4.5
40	FLIP95-68C	ICARDA	K	5.7	MR	5.1
41	NCS-950183	NARC	D	5.3	MR	4.3
42	NCS-95004	NARC	D	5.3	MR	4.6
43	NCS-95010	NARC	D	4.7	MR	4.9
44	NCS-950212	NARC	D	4.3	MR	4.5
45	92080	AARI	D	6.0	S	4.9
46	97047	AARI	D	4.7	MR	4.6
47	90280	AARI	D	7.0	S	4.5
48	96052	AARI	D	7.7	S	4.5
49	96051	AARI	D	8.3	S	4.1
50	PBC-2000	AARI	D	6.7	S	5.2
51	93A082	AZRI	D	6.0	S	4.1
52	92A043	AZRI	D	5.0	MR	4.2
53	CM72XILC3279	NARC	D	5.0	MR	4.4
54	DC-1	RRI	D	4.0	MR	3.5
55	CH40/89	NIAB	D	5.3	MR	4.3
56	CM738/92	NIAB	D	8.0	S	3.5
57	CM2325/96	NIAB	D	8.0	S	3.4
	EMS			0.623		0.150
	LSD			1.276		0.482
	COV			12.15		8.56

NARC- National Agricultural Research Centre, Islamabad, AARI- Ayub Agricultural Research Institute, Faisalabad, BARI- Barani Agricultural Research Institute, Chakwal, NIAB- Nuclear Institute for Agriculture and Biology, Faisalabad, NIFA- Nuclear Institute for Food and Agriculture, Peshawar, RRI- Rice Research Institute, Dokri, Sindh, ICARDA- International Centre for Agricultural Research in Dry Areas, Allepo, Syria, UAF- University of Agriculture, Faisalabad, ARS- Agricultural Research Station, Karak, AZRI- Arid Zone Research Institute, Bhakhar- Kabuli (white seeded), D- Desi (brown seeded)

Table 4.18: Grouping of chickpea genotypes according to the reaction against blight

Tolerant	
Desi type	Dasht, Parbat, Balkasar, Wanhar, NIFA-88, NCS-95010, NCS-950212, 97047, 92A043, CM72/ILC3279, DC-1
Kabuli type	ILC-482, ILC-72, ILC-195, ILC-200, FLIP97-192C, FLIP97-179C
Susceptible	
Desi type	C-44, Punjab-91, Piadar, Bittle-98, CM-98, CM-88, CM-72, DG-89, AUG-424, NIFA-95, C-235, Karah-1, NCS-950183, NCS-95004, 92080, 90280, 96052, 96051, PBC-2000, 93A082, CH40/89, CM738/92, CM2325/96
Kabuli type	Noor-91, CM-2000, DG-92, ILC-202, Pb-1, ILC-1929, ILC-3279, ILC-194, ICC-201, V88194K, AAR-1, FLIP96-60C, FLIP97-17C, CH41/91, NCS-2001, FLIP95-68C, ILC-263

although both of the parents were susceptible to disease. This might be due to additive genes controlling resistance mechanism present at various loci.

4.6.3 Seed Proteins

On SDS-PAGE, 12 protein bands were observed with the molecular weight (MW) of 34 to 66 kd. Many protein subunits of lower MW were also observed but due to inconsistency in reproducibility they were not recorded. Occasionally, variation was also observed in the density or sharpness of a few bands but this variation was not taken in consideration. Out of 12 protein subunits, 6 were polymorphic and 6 were monomorphic. On the basis of banding pattern, gel was divided into three regions (Fig. 4.10).

Region I had bands of more than 66 kd MW of which 2 were polymorphic. Region II ranged from 34 to 66 kd having eight protein peptides, out of which 4 were polymorphic. In this region, the protein bands were observed with high degree of variation in quantitative term. The quantitative intensity of bands was not recorded at present although these may provide some information specific to chickpea. Weak protein bands were observed in the region III of lower molecular weight, hence not recorded due to inconsistency in presence. On the basis of disease rating and radial growth, three clusters were observed (Fig. 4.11).

Cluster I consisted 14 genotypes, cluster II comprised 21 and cluster III of twenty-two genotypes. Out of 17 tolerant genotypes, 10 were grouped in the cluster III, six in cluster II and one in cluster I. The genotypes were plotted on the basis of SDS-PAGE and if cut at 1.5 linkages distance four clusters were observed (Fig. 4.12). Many genotypes overlap each other due to similarity on the basis of SDS-PAGE markers. Cluster I consisted three genotypes (AUG 424, C 235, NCS 2001), whereas cluster II consisted two genotypes (CM 2000, CM72/ILC 3279) and both of these were tolerant genotypes. One genotype (CH 41/91) was in cluster III and all the other fifty-one genotypes were in cluster IV. Within this cluster, this cluster comprised of mixed genotypes of susceptible and tolerant nature both Kabuli and desi types that indicated no relationship between disease reaction and SDS-PAGE. A low level of genetic diversity was observed among 57 genotypes although these originated from diverse sources that might indicate

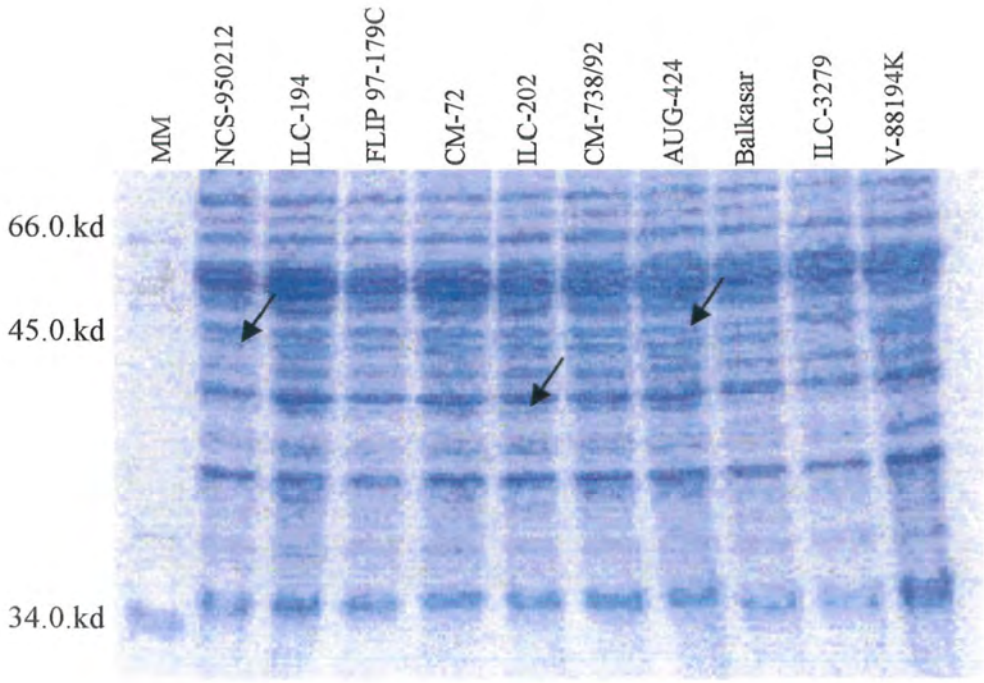


Fig.4.10: Variation in seed proteins of chickpea genotypes. The molecular marker used in the gel was SDS-70 Kit. The arrows indicate variation in different regions

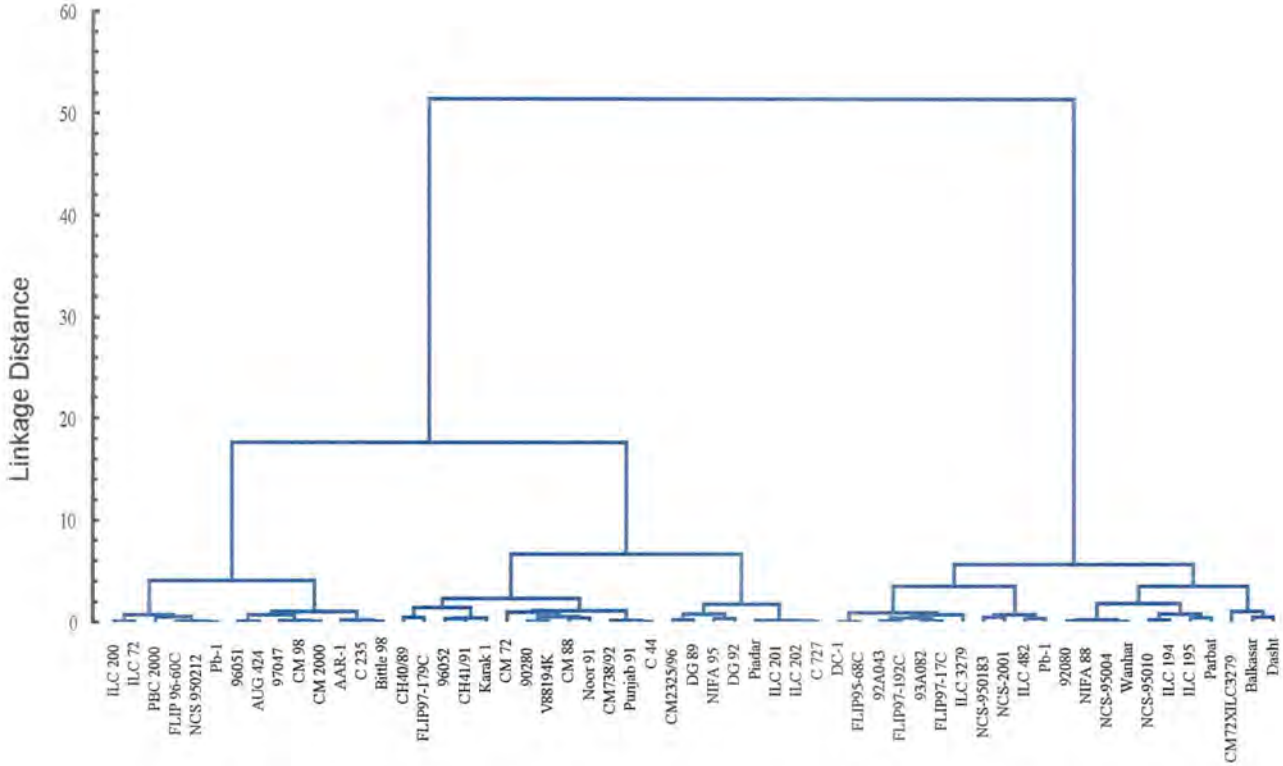


Fig.4.11:- Pheneogram based on disease rating and radial growth in 57 genotypes of chickpea

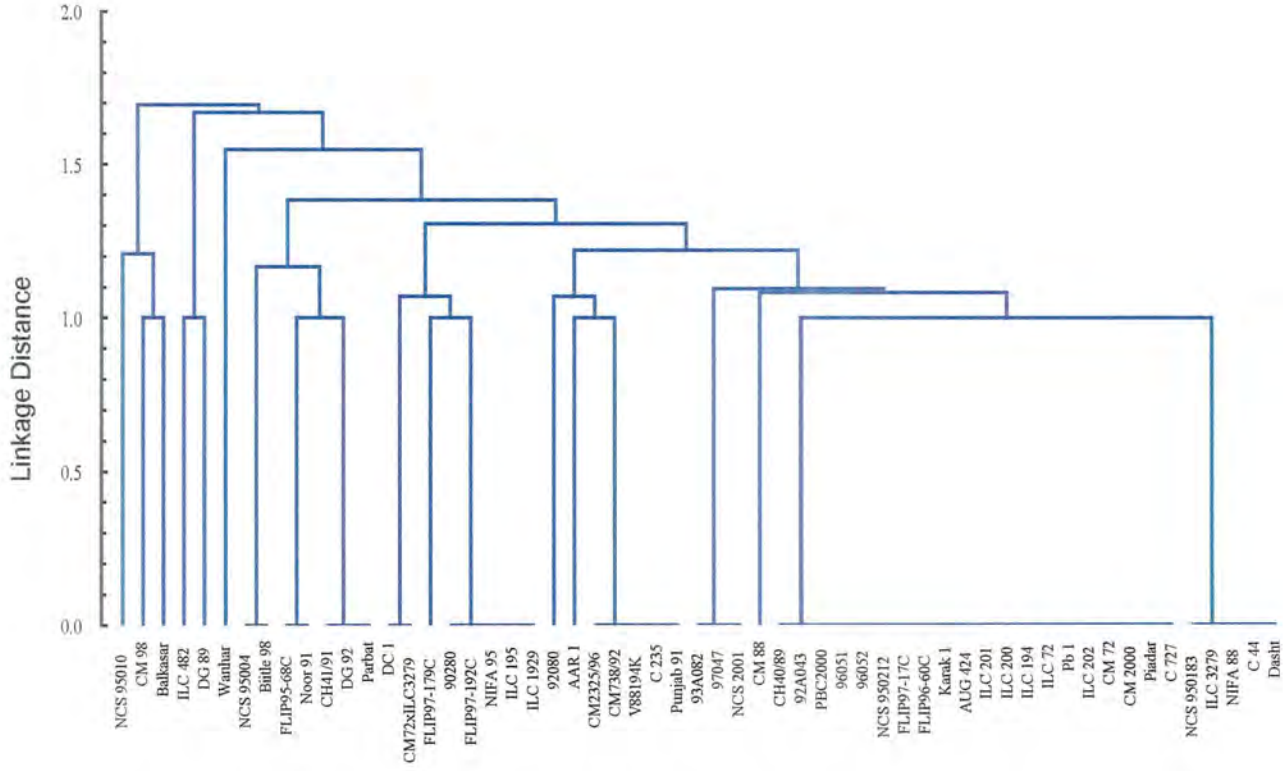


Fig.4.12:- Dendrogram based on six protein markers in 57 genotypes of chickpea

exploitation of a portion of genetic diversity for chickpea improvement.

4.7 LOSSES IN YIELD COMPONENTS OF CHICKPEA CAUSED BY BLIGHT

Resistant and susceptible chickpea genotypes were included in this study to determine the losses in yield and yield components as affected by blight. The results of the analysis of variance revealed that there were significant differences among genotypes for all the characters, both under diseased and disease free conditions (Table-4.19). High portion of genetic variance was attributed by disease that was strengthened by varieties and interaction. Data on plant height, number of pods, seeds per plant, 100-seed weight, yield per plant and yield per plot recorded under diseased and healthy conditions are given in Table-4.20.

Blight severity on various genotypes expressed as disease severity index (DSI) and percent seed and pod infection is given in Fig-4.13. The healthy plots were absolutely free of disease, while the disease score on infected plots varied from 4-8 on 1-9 scale, depending upon the genotypes. Maximum disease score (8) was recorded on C727 and minimum disease score (4) was observed on Dasht. Similarly, the disease severity index ranged from 44% to 82%. Maximum DSI at vegetative stage was observed on C-727 and it was followed by C-44, Punjab-91 and CM-72 whereas minimum DSI was recorded on Dasht i.e., 44% (Fig-4.13). The response of Parbat and NIFA-88 to disease in terms of DSI at vegetative stage was almost similar with respective DSI score 55% and 49%. Pod infection varied from 17 to 90%. It was the highest (90%) in case of C-727, followed by C-44 (68%) and Punjab-91 (61%), while it was lowest in Dasht (17%). The pod infection in Parbat and NIFA-88 was 23% and 27%, respectively. Maximum seed infection was recorded in C-727 (42%) and minimum seed infection in NIFA-88 (2%).

Under disease free conditions, maximum grain yield (4722.3 kg/ha.) was expressed by C-727 which was followed by Punjab-91 and CM-72 (Table-4.21). Grain yield losses due to blight ranged from 13.6 to 17.6%. Maximum reduction in grain yield

Table 4.19: Effect of Ascochyta blight on yield and yield components of chickpea cultivars.

	Plant height (Cm)		Pods/plant		Seeds/plant		Yield/plant (g)		100-g weight (g)		Yield/plot (Kg)	
	H	D	H	D	H	D	H	D	H	D	H	D
CM-72	61.2	60.05	51.25	28.25	89.5	13.25	17.08	8.63	23.75	19.17	0.6	0.17
C-44	70.58	64.43	61.5	29.25	60.0	25.75	13.97	5.7	26.15	21.7	0.85	0.43
Dasht	69.65	63.7	50.5	31.25	62.25	33.0	11.77	9.52	25.53	25.33	0.44	0.38
Punjab-91	72.22	61.53	76.75	14.0	75.0	5.25	17.25	0.5	24.65	20.17	0.73	0.22
Parbat	76.7	63.47	53.0	33.0	77.5	29.75	13.85	8.33	24.55	21.45	0.41	0.27
NIFA-88	72.88	69.45	49.0	30.25	67.5	30.75	14.27	7.6	22.4	20.75	0.62	0.45
C-727	72.13	35.75	59.5	6.75	53.25	37.75	18.6	1.55	23.48	16.0	0.68	0.16
St. Error	2.36	2.04	3.29	1.29	2.37	1.69	1.12	0.42	1.17	0.91	0.03	0.02
CD-1	7.02	6.07	9.80	3.85	7.03	5.03	3.33	1.26	3.46	2.72	0.08	0.06
CD-2	9.62	8.32	13.43	5.28	9.63	6.89	4.56	1.72	4.75	3.72	0.11	0.08

H- healthy and D- diseased plots.

Table 4.20: Two-factors analysis of variance for six yield components in chickpea

		Mean Square					
SOV	df	Plant height	Pods/plant	Seeds/plant	Yield/plant	100-seed weight	Yield/plot
Replications	3	33.34	30.64	13.07	1.24	0.953	0.008
Varieties	6	289.94**	143.68**	169.87**	12.43**	27.21**	0.082**
Disease	1	1692.90**	14950.45**	27192.07**	1198.80**	192.03**	1.472**
Varieties x disease	6	284.12**	645.36**	948.03**	63.09**	10.99**	0.072*
Error	39	19.59	23.90	17.79	2.75	4.09	0.002
SE (Varieties)		1.57	1.73	1.49	0.5863	0.72	0.017
SE (Disease)		0.84	0.92	0.79	0.31	0.38	0.009
SE (Interaction)		2.21	2.45	2.11	0.83	1.01	0.042
CV		6.78%	11.92%	8.93%	15.60%	8.99%	10.57

occurred in C-727 followed by Punjab-91which showed 76.5% and 71.7% loss respectively. The minimum loss in yield (13.6%) was recorded in Dasht. A variable response of genotypes with respect to effect of blight on plant height was observed. Under diseased condition it varied from 35.75 to 69.45 cm. (Table-4.19). A comparison of data from healthy and diseased conditions revealed that reduction in plant height due to disease ranged from 1.9 to 50.4%. Maximum decrease in height was observed in C-727.

The effect of disease on number of pods per plant given in Fig-4.14 shows that reduction in pod number due to blight ranged from 37.7 to 88.7%. Whereas the actual number of pods in healthy treatment ranged from 49.0 to76.8 and in diseased treatment it ranged from 6.8 to 33.0 per plant (Table-4.20). Due to the infection of Ascochyta blight, the genotypes showed different response with respect to seeds per plant. Maximum reduction in seeds per plant was observed in cultivars Punjab-91 (93%) and CM-72 (85.2%). There was a significant reduction in all the yield components in case of diseased treatment when compared with healthy plant. Loss in yield/plant was observed as minimum in case of Dasht (19.1%) followed by Parbat (39.9%) and NIFA-88 (46.7%). Maximum loss was observed in Punjab-91 (97.1%) and C-727 (91.7%). Maximum loss in 100-grain weight was recorded in C-727 (31.9%) and it was followed by CM-72 with 19.3% loss. Pods per plant, seeds per plant and grains per plant were negatively correlated in diseased and healthy plants whereas 100 seed weight exhibited positive association (Fig-4.15).

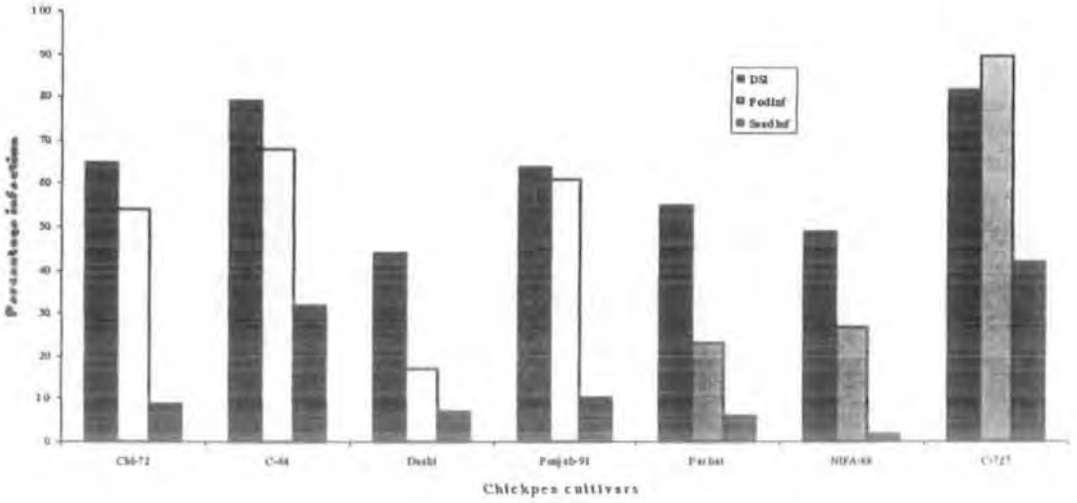


Fig. 4.13:- Disease reaction of chickpea cultivars against *Ascochyta* blight

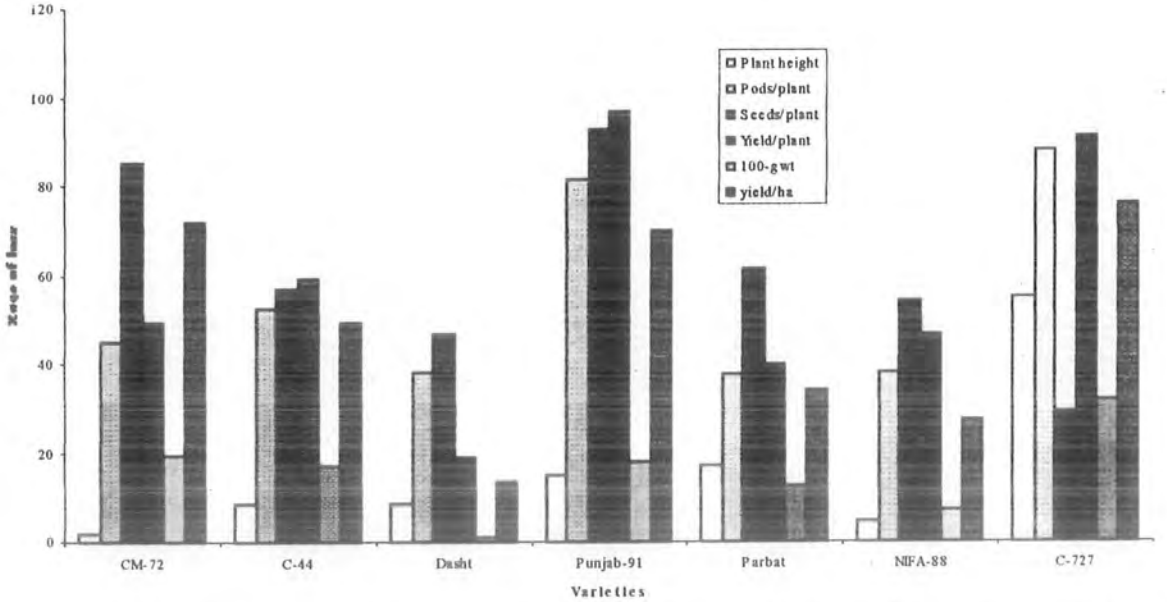
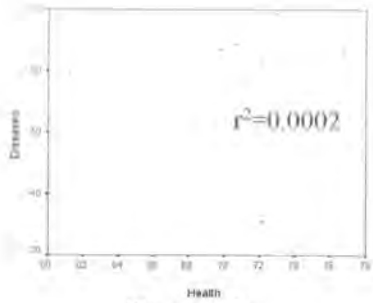
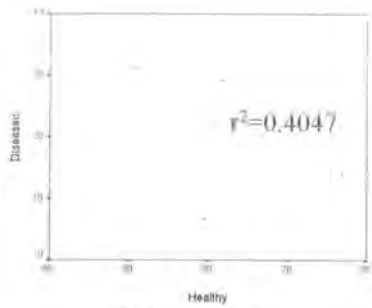


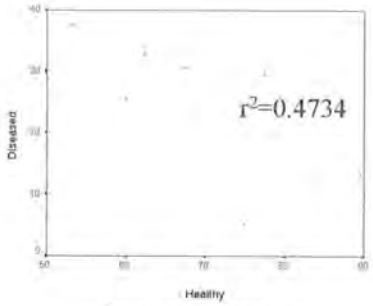
Fig. 4.14:- Percentage of losses in yield parameters of chickpea due to *Ascochyta* blight



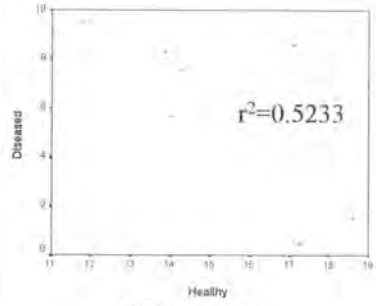
Plant height



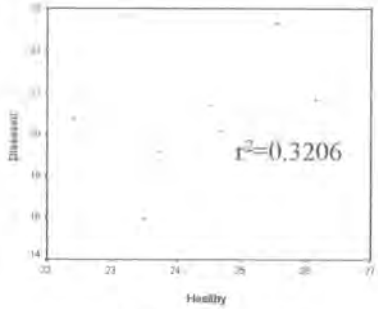
Pods per plant



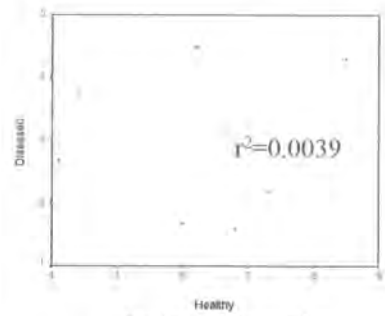
Seeds per plant



Yield per plant



100-seed weight



Grain yield per plot

Fig. 4.15: Relationship between healthy and diseased samples for six characters

DISCUSSION

Ascochyta blight of chickpea is a serious disease, which is mostly prevalent in the Northern areas of Pakistan where annual rainfall is higher than the other parts of the country. This disease was reported for the first time in these areas (Butler, 1918). A. Blight causes significant yield losses in different chickpea areas depending upon its prevalence and intensity. Present survey was systematically conducted to assess its prevalence and losses caused by it. Isolates of the pathogen were also collected during the survey. Morphological as well as pathogenic variability was determined. Screening of chickpea germplasm was carried out to identify the resistant sources. Possibility of morphological traits to the association of blight resistance was also worked out. Losses in yiled and yield component of some promising cultivars estimated.

5.1 SURVEY OF CHICKPEA AREAS FOR BLIGHT ASSESSMENT

Ascochyta blight is the most devastating disease of chickpea that is found throughout the chickpea growing areas of Punjab and NWFP. It is a major limiting factor to chickpea production. Since its first report in district of Attock in Pakistan (Butler, 1918), it has caused losses in chickpea worth million of rupees (Malik *et al*, 1984). Chickpea is generally planted in rainfed areas of Punjab and NWFP and its area is divided into three ecological zones on the basis of rainfall and crop duration.

1. Low temperature and high rainfall area
2. Medium temperature and medium rainfall area
3. High temperature and low rainfall area

These three ecological zones have different characteristics that have different impacts on chickpea production. In the present survey, it was observed that maximum prevalence, incidence and severity of blight occurred in the districts of Rawalpindi, Sialkot, Norowal, Attock and Chakwal that fall in long duration environment. Presently these are not the major chickpea growing areas due to blight hazards. There were not significant

differences among these districts for disease prevalence, incidence and severity. These districts fall in the category of long duration environment, where conditions for disease development are very conducive. Consequently, the chances of disease incidences and prevalence are quite high in these districts. The high rainfall (mean annual 400 to 1000 mm) and fertile soils in these areas result into luxurious growth of the crop. The plant canopy of the crop helps to increase the crop humidity, which also remains high in the atmosphere for longer period due to high and prolonged rains. High relative humidity generally more than 60% and low temperature in the range of 9-24^{0}C are the prerequisites for chickpea blight (Haware, 1998). Moreover, on account of being a neglected crop in the area, proper attention by researchers and extension workers was not given therefore, the farmers intended to grow local land races, which are susceptible to blight. Moreover, the blight isolates prevalent in this area, are the most virulent (Qureshi and Alam, 1984; Jamil *et al.*, 1995; Hussain and Malik, 1991). All these factors contributed to high disease development, resulting in elimination of chickpea from this area. Recently new blight resistant varieties like Dasht, Parbat and Bulksar and Wanhar have been released specially for this area. Due to the introduction of these varieties in the high rainfall area, the farmers are gradually increasing intended to increase the acreage of chickpea crop in this zone.

Other two zones comprise of Thal, which is a major chickpea growing area in Pakistan. Thal occupies 23310 Km^2 of Mianwali, Khushab, Bhakahr, Leiah, and Jang (Directorate of Soil Survey, 1968). The area is roughly triangular in shape with the base to the North and apex to the South, and is located between latitude 29^0 58 N and 32^0 35N, longitude 70^0 43 E and 72^0 18 E. The area is surrounded by the piedmont of the Salt Range in the North, the river Indus floodplains to the East and the 30^0 parallel latitude to the South. The climate is hot and windy in summer and mild in winter. The average annual rainfall varies between 261 mm to 385 mm in the northwest and around 169 mm in the south. The annual rainfall follows the bimodal pattern, with more than 69% occurring in Summer (June to September).

Thall is divided into two major parts (Zone-2 and Zone-3) according to its climate (rainfall), topography and crop duration. The upper Thall falls in the Zone-2 with medium duration environment and medium rainfall area. This zone comprises the districts of

Mianwali, Khushab (Punajb), Bannu, Lakki marwat and Karak (NWFP). The annual rainfall in these districts varies from 261 to 385 mm. It was observed during the present survey that there was more disease in the districts of Mianwali and Karak as compared to other districts falling under the same zone. The development of more disease in these districts may be due to cultivation of blight susceptible varieties on maximum area. Moreover, the chickpea area of these districts lies close to River Indus that may be responsible for high air humidity in this area and may have encouraged the disease development. Whereas the chickpea area in the rest of the districts of this zone is far from the rivers and new blight tolerant varieties had occupied a lot of acreage in these districts. The spread of new blight resistant varieties in the districts of Leiah, Bhakhar, Khushab, Mianwali and Jang appears to be the major factor for low disease development in these districts.

The second part of the Thal or Zone-3, comprise of districts Bhakhar, Leiah and Jang (Punajb) and DI Khan (NWFP). The disease prevalence, incidence and severity were minimum in these districts. The rainfall in these districts is very low during cropping period that varies from 100 to 150 mm. The temperature in these districts rises sharply after January, therefore limited moisture is available for a limited time in rainfed areas. The chances of blight occurrence in these areas are generally very rare. The risk of disease development in this zone has been further minimized due to spread of new resistant varieties. For these reasons, low disease was recorded in these districts.

It appeared from the present survey that there was strong correlation between disease prevalence, incidence and severity in all the districts included in the survey. This relationship prevailed everywhere irrespective of the magnitude of disease problem.

5.2 FACTORS AFFECTING BLIGHT DEVELOPMENT

Effect of spore concentration on the development of disease revealed that disease severity could vary according to different inoculum concentrations. High inoculum concentration will lead to increased disease severity, although this depends on the susceptibility of chickpea cultivars and other environmental conditions. However, the concentration of $1.5x10^5$ and $5x10^5$ are statitistically non-sginificant. Thus the spore load above this concentration is not recommended for screening of chickpea against Ascochyta blight. It has been already reported that inoculum concentration has an

important impact on the screening of chickpea germplasm for disease resistance (Nene, 1984; Nene and Reddy, 1987; Del-Serrone *et al.*, 1987). In the present study, the inoculum concentration of $5x10^5$ spores per ml was optimal for inoculation experiments because the response of the highly susceptible cultivars; C-727 and ILC-263 was insignificantly different at concentrations between 2.5 x 10^5 and 5 x 10^5 spores per ml. This concentration has been reported previously as appropriate for artificial inoculation under glasshouse and field conditions (Del-Serrone *et al.*, 1987; Trapero-Casas and Kaiser, 1992).

Plant age is another important factor that affects disease development and host susceptibility to Ascochyta blight on different crops (Sattar, 1933; Hafiz, 1952; Puerto-Romero, 1964). Some workers reported that older plants were more susceptible than seedlings (Sattar, 1933; Reddy and Singh, 1984; Singh and Reddy, 1993b). In the present study, 2-week old seedlings were found to be more susceptible than older plants. Susceptibility of young chickpea plants had been indicated by Hafiz (1952), Trapero-Casas and Kaiser (1992). Similarly, it has been reported that the disease severity in lentil plants inoculated with *A. lentis* was more at seedling stage than that at pods formation stage (Pedersen and Morrall (1994). These authors suggested that the resistance was related with age of tissue and that newly developed leaves were more susceptible to the pathogen than older leaves. A similar phenomenon has been reported in rice blast disease (Roumen *et al.*, 1992). Similarly in chickpea, the younger and tender leaves may make the seedling stage more susceptible to *A. rabiei* than the older leaves of mature plants.

In Pakistan, blight conditions are generally conducive during the month of March-April when the chickpea is at flowering to pod forming stage. Therefore, the blight epidemics reported in Pakistan supported the idea of susceptibility at reproductive stage. In fact, during November to January, chickpea is at seedling satge and the temperature remains low (0-15^{0}C) and sometimes beyond ^{0}c, that is why there are no chances of blight epidemic.

Relative humidity (RH) is an other important factor, which is directly related to disease severity. In this study, the optimum conditions of Ascochyta blight on C-727, ILC-263, Punjab-91, Dasht and Parbat at 20-25^{0}C and 48-72 h wetness period, and

disease severity was less at wetness period below 24 h. The results are in agreement with those reported by Luthra *et al.* (1935), Weltzein and Kaack (1984), Nene and Reddy (1987), and Trapero-Casas and Kaiser (1992), and contradict those of Chauhan and Sinha (1973), who reported that a minimum 60-h wetness period was required for blight disease development at the optimum temperature of 20^0C. These authors found that wetness period of at least 144 h were conducive for disease development. In addition to temperature and wetness period, the chickpea cultivar, microclimate and the isolates of *A. rabiei* affect disease development. It has been reported that 24 h was the minimum wetness period required to produce 100% disease in a susceptible cultivar whereas the same level of disease in the resistant cultivar needed a 96 h wetness period (Hafiz, 1952; Anonymous, 1989).

The effect of wetness period on disease development as observed in the present study are in partial agreement with those of Trapero-Casas and Kaiser (1992), who found that, for 2-week old seedlings, approximately 20^0C and inoculum of $5x10^5$ spores per ml were optimal for disease development under controlled environmental conditions, and that increasing the wetness period above 6-h humidity after inoculation or after 6-h of wetness had no effect on disease severity, as disease following 48 h of wetness was the same if no dry period was imposed. However, dry periods of 22 h did reduce disease severity when imposed either immediately after inoculation or after a 6-h wetness period. Disease severity decreased as the length of the dry period increased. However, disease severity of 20 -25% after 96 h dry periods demonstrated that Ascochyta blight can develop in alternating wet and dry conditions, as likely to occur in the field. On the basis of experimental results, it is concluded that under controlled conditions, disease severity could vary according to the inoculum concentration, plants age and leaf wetness duration following inoculation.

5.3 VARIABLITY IN *ASCOCHYTA RABIEI*

There was a considerable variation among the isolates for morphological characters such as radial growth, colony colour, Pycnidial size and spore size. Similar variability among various isolates of *A. rabiei* has already been reported by Singh and Reddy (1990); Grewal (1984); Gowen (1986); Gowen *et al.*, (1989) and Qureshi and

Alam (1984) using different chickpea cultivars. Grewal (1984) found the fast growing and less sporulation isolates to be less virulent and the slow growing and abundantly sporulating isolates to be more virulent. Pathogenic variability in *A. rabiei* has also been demonstrated by Aujla (1964); Kaiser (1973); Vir and Grewal (1974); Reddy and Kabbabeh (1985); Nene and Reddy (1987); Porta Pulgia *et al.*, (1986 and 1996) and Porta Pulgia (1992). Some of these authors designated the pathogenic groups as races of different cultivars. Available information, however, does not allow the term "race" but to distinguish in pathogenicity the word "isolate" may be used (Haware, 1987).

Although genetic diversity in isolates was observed and they could broadly be classified in three groups, but a clear-cut host-pathogen reaction was not observed. This situation did not favour the nomenclature of race rather variation in pathogenicity. Recommendations to standardize race characterization have been made since 1989, but to date standard methodology has yet not been agreed upon and no clear differential has been identified that should be acceptable universally. This problem might be associated with complex nature of gene-action involved in *A. rabiei* resistance (Malik, 1990). The need exists to use multiple crosses due to quantitative nature of gene-action involved for disease reaction. This will help to build resistance pyramids that could be obtained by involving parents of diverse origin and known tolerant to disease.

Chickpea cultivars included in the study as shown by multivariate analyses, susceptible genotypes and virulent isolates were identified but clear-cut standard for resistance was not observed. Inconsistent clustering pattern for various isolates collected from the same origin may be attributed towards frequent exchange of breeding material and disease cultures among the researchers. Several reasons have been reported, such as the increase of chickpea-growing area and the introduction of resistant cultivars that contribute to extending the variability of *Ascochyta* population (Crino *et al.*, 1985; Hussain and Barz, 1997). More variation could be expected, taking into account the heterothallic nature of the fungus (Trapero-Casas and Kaiser, 1992) and the recent development of new isolates that makes possible the appearance of the teleomorph of the fungus. Variation in isolates originated from same area need to be investigated using biochemical analyses, although isolates collected from the single field could vary for

disease infection (Morjane *et al.*, 1994).

Occurrence of isolates belonging to one cluster that are able to infect all the genotypes suggests the need for more suitable sources of resistance. Promising levels of resistance have been reported in wild species of *Cicer* (Singh *et al.*, 1992; Singh and Reddy, 1993a) and cultivated chickpea (Iqbal *et al.*, 1989 and 1994; Singh and Reddy, 1989). Even after 90 years of research on chickpea blight, the problem is yet unsolved and further studies on the host pathogen relationship of Ascochyta blight is still needed although effects of environments are well known (Hafiz, 1986). Further study involving biochemical analysis using known material (host and pathogen) should be streamlined for a comprehensive understanding of this complex disease.

A complex pathogenic variability is not surprising since the pathogen has a sexual stage that can generate new recombinants with varying virulence spectrum (Kaiser, 1992). The role of weak pathotypes in generating aggressive pathotypes either through accumulation of virulence and genetic recombination is not yet understood and needs to be explored. The use of field isolates in resistant screening representing populations of the pathogen, rather than individual or mixed races, has been suggested (Mmbaga *et al.*, 1994). The relatedness of the isolates on the basis of host parasite interaction can be determined through multivariate analyses (Shane, 1987). Such results are useful for choosing representative pathotypes that may be used to identify specific resistant groups for utilization in breeding programme. This study indicated that *A. rabiei* isolates collected from Pakistan were composed of various pathotypes and these cannot be stated as races according to standard definition. A continuous breakdown of resistance in host emphasizes the need for up to date knowledge of physiologic pathotypes prevalent in different regions to develop chickpea cultivars having stable resistance against Ascochyta blight. The variety CM 72 was released as blight tolerant but with the passage of time and by mixing or development of new strains of *A. rabiei*, this variety is no longer tolerant, therefore a need exists to evolve varieties with durable resistance. Similarly, ILC 263 is being used as susceptible check in most of the ICARDA experiments, but two varieties (C 727 and C 44) exhibited higher degree of susceptibility than ILC 263, and these are suggested to use as susceptible check in screening experiments.

5.3.1 Combined effect of the least and most aggressive isolates on blight development

All the cultivars subjected to disease infection (single or combined) showed blight symptoms. Isolates of *A. rabiei* greatly varied in their pathogenic reaction in 16 genotypes. The analysis of variance showed significant differences ($P < 0.001$) between genotypes as well as between treatments. The aggressiveness rating of each *A. rabiei* isolate toward all the lines tested exhibited a large but continuous variability. The results showed that there was remarkable variation in pathogenicity between two isolates for disease development. This was obvious from the genotypic means of disease scores for individual isolates. The disease development on individual genotypes (irrespective of their resistance level) under each isolate also showed variation between the two. A consistent trend of increased disease rating under aggressive isolate as compared to that of least aggressive isolate was observed in all the genotypes. A similar grouping of *A. rabiei* isolates on the basis of aggressiveness using different isolates and chickpea cultivars, have been reported by Singh (1985), Singh (1987), Vir and Grewal (1974), Singh (1990), Grewal (1984), Qureshi and Alam (1984).

The most aggressive pathotypes tend to be associated with areas where selection pressure is higher (Gowen, 1986). High adaptability of *A. rabiei* to its host has also been indicated in host-pathogen interaction studies that may partly explain resistance instability (Gowen, 1986). The present results and previous studies provided evidence that isolates of *A. rabiei* differ in both aggressiveness and in their specific virulence patterns. The occurrence of a complex pathogenic variability is not surprising since the pathogen has a sexual stage that can generate new recombinants with varying virulence spectrum (Kaiser, 1992). When the most aggressive and the least aggressive isolates were applied as 1:1 mixture, the aggressiveness of this mixture was similar to that observed in least aggressiveness isolate. This indicated dominance of less aggressive isolate over the aggressive isolate. The chickpea cultivars Parbat, C-235, CM-72, NIFA-88 and NIFA-95, which were susceptible to the aggressive isolate appeared to be resistant/ tolerant to the mixed population of isolates as observed for least aggressive isolate. Similarly, the resistance behavior of other cultivars became similar to that observed for least aggressive isolate when subjected to the mixture of isolates. In other words, the aggressive isolate

lost its aggressiveness when applied in combination with the least aggressive isolate. This may be due to weak isolate having occupied the site of infection that did not allow the aggressive isolate to cause severe infection (Ali *et al.*, 1993), It is also expected due to rapid multiplication of less aggressive isolate that suppressed the growth of aggressive isolate. Since disease is caused through the production of toxins (Alam *et al.*, 1989; Hohl *et al.*, 1991; Kaur, 1995).

It would be appropriated to conduct more studies on different mixtures of aggressive and less aggressive isolates to confirm these results. If it is confirmed that the least aggressive isolate reduces the disease developing capability of more aggressive isolate (as observed in the present study), the introduction of less aggressive isolate in the areas of more aggressive isolates would reduce the risk of disease development in that area and chickpea lines with moderate resistance level would be appropriate for that area. This will give an advantage of introducing genotypes with relatively high yield potential as blight resistance and yield potential are negatively correlated. Pizano (1997) also proposed the introduction of less aggressive isolate of Fusarium wilt of carnation in the areas where more aggressive isolates exist to reduce the severity of wilt disease.

The role of weak pathotypes in suppressing the aggressiveness of virulent pathotypes either through inactivation of virulence or genetic recombination is not yet understood and needs to be explored. Previously, the use of field isolates in resistant screening representing populations of the pathogen, rather than individual or mixed races, has been suggested (Mmbaga *et al.*, 1994). However, broad resistance that is effective against entire population is not always available and must be developed through breeding (Singh *et al.*, 1992). The relatedness of the isolates on the basis of host parasite interaction can be determined through multivariate analyses (Shane, 1987). Such results could be useful for choosing representative pathotypes that may be used to identify specific resistant groups for utilization in breeding programme.

5.4 SCREENING OF CHICKPEA GERMPLASM AGAINST BLIGHT

The number of resistant genotypes was higher during 1995-96 and that indicated that all of material included in the experiments. None of the genotype was resistant at pod

formation stage that indicated the conducive environmental conditions at terminal stage of the crop. Some of the lines, viz., ICC 12004, ICC 13269, ICC 13416, ICC 13508 and ICC 13555 observed resistant in the present study have already been reported resistant to blight with the similar type of study (Iqbal *et al.*, 1994). The chickpea line ILC-72 was reported as resistant (Singh *et al.*, 1981; Singh *et al.*, 1984; Reddy and Singh, 1990) while ILC-3279 has also been reported to be resistant (Singh *et al.*, 1981; Singh *et al.*, 1984; Crino *et al.*, 1985: Reddy and Singh, 1990, 1993). Bashir and Haware *et al.* (1986) reported moderate resistance of ICC-3996 and ICC- 4475 and similarly a line FLIP 87-507C has been reported resistant by Ilyas *et al.* (1991) At pod forming stage none of the test lines was highly resistant against the pathogen. However, five lines viz ILC-72, ILC-3279, FLIP 84-182C, FLIP 91-150C and ICC-13555 were found to be moderately resistant.

The chickpea germplasm line ICC 13555, NIFA 88, FLIP 91-159C and ICC 3991 were resistant at seedling stage under greenhouse conditions, whereas at vegetative stage, 21 genotypes (86025, 93205, 86205K, 93164, 92003K, ICC 13555, 93069, 93027, FLIP 93-62C, ICC 3416, ICC 6373, NIFA 88, FLIP 92-159C, ICC 3991, FLIP 94-508C, FLIP 94-509C, FLIP 94-510C, ICC 3919, ICC 12004, ICC 13279, ICC 1403) were resistant. These genotypes could not prove their worth at pod formation stage, and this type of infection might be due to different genes involved for resistance mechanism at various plant stages or may be because of variation in mode of infection at various stages (Ilyas *et al.*, 1991, Reddy and Singh, 1984, 1990). Anyhow this situation is yet to be resolved by conducting more experiments on mode of inheritance and infection of Ascochyta blight.

At ICARDA several sources of resistance to Ascochyta blight have been reported (Reddy and Singh, 1984; Singh *et al.*, 1984). Some of these lines i.e., ILC-72 and ILC-3279 have resistance in several other countries. However, none was highly resistant in India and Pakistan, the two major chickpea growing countries. Therefore resistant genotypes those originated from ICARDA need to be re-tested for their resistance using aggressive pathotypes. It is now well established that the fungus *A. rabiei* is highly variable and the pathotypes present in Pakistan and India are more aggressive than those prevalent in the Mediterranean region (Singh *et al.*, 1984). Lines with resistance at

vegetative stage to isolates of *A. rabiei* are reported in India (Singh *et al.*, 1988) and in Pakistan (Iqbal *et al.*, 1989).

The knowledge generated on the resistance to *A. rabiei* indicated that there is sufficient genetic variation in resistance to Ascochyta blight in the present chickpea germplasm that can be exploited for disease control by building disease resistance pyramids due to complex nature of disease. Immunity or high level of resistance was reported to be absent in chickpea germplasm (Nene, 1980). However, the frequency of highly resistant lines is very low. Only four lines were resistant at seedling and 21 at vegetative stage during the screening period from 1994 to 1996. Whereas none of the lines was found resistant at pod forming stage.

Bashir *et al.*, (1985) evaluated 3360 chickpea germplasm accessions obtained from ICRISAT for disease reaction to blight at NARC, Islamabad during 1983-84, and reported that only 55 accessions were resistant. Iqbal *et al.* (1989) screened 759 chickpea lines and found that only one breeding line (PK51863 x NEC 138-2) was resistant to blight. This indicates that there is either high aggressiveness or narrow diversification of genetic materials studied. Many workers have reported the occurrence of moderate resistance to blight. (Eliades, 1983; Shukla *et al*, 1984; Kalia, 1984, Katiyar and Sood, 1985; Bashir *et al.*, 1985; Gaur and Singh, 1987; Del-Serrone *et al.*, 1987; Reddy and Singh, 1990; Ilyas *et al.*, 1991; Reddy and Singh, 1993). The significant differences among breeding lines of chickpea with regards to disease reaction have been reported (Singh *et al.*, 1981, Reddy and Singh, 1984). Although sources of resistance to blight caused by *A. rabiei* have been reported at national and international levels but in this study, no genotype was resistant at pod formation stage. Therefore, development of disease tolerant cultivars those could survive under high incidence of pathogen could be one option to resolve this problem.

5.5 EFFECT OF MORPHOLOGICAL TRAITS FOR BLIGHT RESISTANCE

In an attempt to find out morphological basis of blight resistance in chickpea various morphological traits were studied in resistant and susceptible cultivars. These traits included leaf hair, number of stomata and stomata size, etc. For counting the

number of hairs on both the sides of leaf, 5th compound leaves from the top were selected from all the chickpea cultivars because it was reported that older leaves below 4-5th nodes are resistant to Ascochyta blight fungus (Pedersen and Morrall, 1994).

On the basis of this study, it was found that the hair density on the dorsal surface of leaves was insignificantly different in lines of all the reaction groups. The hair density on ventral surface of the lines was significantly higher in case of resistant lines compared to susceptible ones. This was further supported by significantly higher hair density in case of resistant lines. Genotypes with higher hair density may have some role in the resistance to blight. It is assumed that the hair would help keep the spores away from the leaf-surface and the spore clinging to the hair might fail to establish a direct contact with the leaf. Hence, even if they germinate while clinging to the hair, the germ tube may not be long enough to reach the cuticle. Earlier studies had indicated that resistant cultivars possessed larger number of hair on stem and leaves than susceptible types (Hafiz, 1952; Ahamd *et al.*, 1952). Similarly resistant cv. E100Y (M) and pods of E100Y bear more hair than susceptible types (Hari Chand *et al*, 1988). On this basis, however, difference in hair number could only be related to disease reaction but it could not fully explain the phenomenon of resistance. The present studies do not give indications that the hair number could be a sound basis to differentiate between resistant and susceptible cultivars of chickpea and therefore, cannot be effectively utilized as a screening parameter for disease resistance. The data obtained in the present studies were at variance with that of Koundal and Sinha (1983) who showed direct relationship between the number of glandular hairs and amount of malic acid secreted. These enzymic activities and concentration of malic acid was not taken into account in this study.

Reddy and Khare (1984) observed higher stomatal density in the lentil cultivars susceptible to rust as compared to resistant ones. Presence of higher population of stomata in the susceptible cultivars increased the rate of transpiration upon infection by the pathogen. In the present study, maximum number of stomata was observed in Dasht (resistant cultivar) and C-727 (susceptible cultivar), which indicated that the number of stomata has no role for the initiation of blight. Similarly, other parameters concerning with stomata such as area of stomata, area of guard cells and size of stomatal aperture did

not clearly exhibited any relationship with blight. Our results were supported by the earlier findings of Roundhill *et al.* (1995) who have reported that infection due to Ascochyta took place through direct invasion of the epidermal layer.

5.6 RELATIONSHIP BETWEEN PLANT EXTRACT AND SDS-PAGE ON BLIGHT RESISTANCE IN CHICKPEA

Fifty-seven chickpea genotypes were evaluated for *A.rabiei* reaction and seed protein through SDS-PAGE using vertical slab type apparatus and 11 genotypes were tolerant. Out of eleven tolerant genotypes five were approved varieties and others were advanced lines that indicated the visualization of breeding against chickpea blight by the national researchers. The tolerant genotypes are supposed to be the best sources for developing resistant cultivars by gene pyramiding as suggested by Horn (2001). There was no relationship in clustering on the basis of seed type, desi or kabuli both for disease rating and seed protein analysed for seed protein. Low level of variation was observed for seed protein among chickpea genotypes included in the present study and similar results had already been reported by Thakare *et al.* (1987), Iqbal, (2001), Mehrani, (2002) and Ghafoor *et al.*, (2002) in legumes who observed low intra-specific variation within one species in their studies. SDS-PAGE showed that the method provided a tool for reliable germplasm discrimination based on genetic differences in seed storage protein comparison in chickpea, but no relationship among disease, seed type and protein peptides was observed. The genotypes with similar banding patterns may be duplicated, but these are suggested to be confirmed by the use of other biochemical markers including 2-D electrophoresis and DNA markers (Beckstrom-Sternberg, 1989 and Higginbotham *et al.* 1991).

In the present study intra-specific variation was limited and it was observed that seed protein alone did not exhibit high level of intra-specific variation, therefore, diverse germplasm based on seed protein is suggested to be acquired from various sources, preferably from centre of diversity to build a broad based gene pool with maximum variability. Further, there was no relationship observed among three parameters, i.e., *in-vitro* growth of fungus, disease rating and SDS-PAGE for seed proteins, therefore for comprehensive knowledge of agricultural, biochemical data and Ascochyta blight

reaction, there is a need to enhance the level of biochemical markers (protein and DNA).

Seed protein did not yiel any clue either for fungal growth, disease reaction, seed type (desi or kabuli) or origin. For most genotypes and protein subunits, no clear observation was recorded which could facilitate selection on the basis of seed protein for improving disease resistance in chickpea from the material under investigation. Analysis based on disease rating and radial growth was more reliable than on the basis of protein peptides that indicated the use of enhanced biochemical markers. This situation indicated the independence of seed protein from disease reaction or complexity of genetics of this disease although DNA markers have been reported for Ascochyta blight in chickpea (Horn, 2001). Seed protein was not very effective for studying intra-specific genetic diversity in cultivated chickpea and disease status alone rather wild *Cicer* could be included. Further, biochemical markers are suggested to enhance by adding DNA markers in relation to Ascochyta blight should be included for further evaluation and screening that will help in marker assistant breeding.

5.7.1 YIELD LOSSES IN CHICKPEA CAUSED BY BLIGHT

The objective of this study was to estimate the yield losses caused by blight in chickpea. The genotypes used for this purpose were the commercial varieties that are generally cultivated in the country. The DSI was used as an indicator of resistance /susceptibility at vegetative stage (Gemavat and Prasad, 1969). On the basis of DSI and percent pod infection records, Dasht was the most resistant and C-727 was the most susceptible genotype among the genotypes used in the study. There was low seed infection in NIFA-88 despite its high pod infection. This may be due to the presence of some mechanism that restricted disease infection to pod level and resisted its penetration down to the seed. This was due to cell necrosis around the infected spots. Rapid cell necrosis and accumulation of phenolic compounds around the infected spot limits penetration and colonization of the fungus in resistant chickpea cultivars (Hohl *et al*, 1990).

The variety NIFA-88 was tolerant at reproductive stage, which produced quite a high number of disease free seeds despite infection at pods and vegetative parts. This

implies that genotypic response to blight varies at different developmental stages of plants (Reddy and Singh, 1984; Singh and Reddy, 1993; Tripathi, 1985). Loss in grain yield of C-727, CM-72, and Punjab-91was mainly due to reduction in pods and seed number and seed weight. Although, Punjab-91 and C-727 were among the top producers with respect to pod and seed numbers under healthy condition but they could not be selected for wider cultivation due to susceptibility to blight.

Three components (pods per plant, seed weight and number of seeds per plant) were less affected in resistant genotypes, although there was a negative association between diseased and healthy treatments. It also appeared that resistant genotypes could survive for a longer period under high disease pressure as compared to the susceptible. As soon as the disease pressure is reduced they produce new shoots and pods that compensate the loss caused by the disease to a reasonable extent. On the contrary, susceptible genotypes got most of their vegetative parts killed and were unable to compensate the damage either due to complete killing of plants or due to killing of most vegetative parts. Moreover the pod and seed infection in resistant genotypes were superficial causing less damage to the seed. Similar sort of results had been reported by Malik *et al.* (1991)

The results indicated that blight affected pod number without influencing seeds per plant and seed weight. These findings have practical implications for the chickpea breeders who breed for Ascochyta blight resistance (Reddy and Singh, 1990). Generally, segregating populations showing pod infection are almost certainly discarded without looking at seeds, hence the breeding methodologies need to be revised for developing blight resistant chickpea cultivars. The chickpea growing areas of Pakistan fall into three different categories with respect to rainfall (Haqqani *et al.*, 2000). The high rainfall area (Potowar) is a hot spot for blight incidence due to the conducive conditions for blight epidemics (Butler, 1918). The varieties, C-727, Punjab 91 and CM72 that showed up-to 50% yield losses due to blight infection may not be recommended for cultivation in such areas. The yield losses exhibited by these genotypes agree with the reported national yield loss of about 50% recorded in the epidemic years. Due to a continuous break-down in tolerance level, resistant chickpea cultivars are needed to be developed after strict

screening under high rainfall areas. Resistant varieties like NIFA-88 and Dasht may be recommended for high rainfall areas. However, in low rainfall areas, the genotypes with relatively less resistance and high yield potential may be recommended for cultivation.

RECOMMENDATIONS

- Research should have to be persued to raise yield potential and stability of chickpea crop along with increased level of tolerance/resistance to blight.

- Continuous survey for the assessment of blight in chickpea production areas is suggested to be carried out each year, particularly in the months of February and March. Meteorological data should also be recorded to determine its relationship to the epidemic occurrence of blight and disease forecasting.

- Even after 90 years of research on chickpea blight, the problem is still unsolved and further studies regarding host pathogen relationship of Ascochyta blight is still needed, although effects of environments are well known. Further study involving biochemical analysis including proteins and DNA markers should be streamlined for a comprehensive understanding of this complex disease.

- Pathogenic variability in *A. rabiei* has been confirmed but more studies are needed to solve the issues of races. Pathogenic variability indicated that the teleomorphic stage of *A. rabiei* existes in Pakistan. Therefore, chickpea breeders should be aware of potential problems that may arise when the sexual stage is a component of the disease cycle.

- In cooperation with plant pathologists, chickpea breeders may be able to utilize new molecular techniques to identify the presence of new pathotypes of *A. rabiei* that may adversely affect their resistance breeding.

- It would be appropriate to conduct more studies on different mixtures of aggressive and less aggressive isolates to confirm the aggregated effects of the isolates. If it is confirmed that the least aggressive isolate reduces the disease

developing capability of more aggressive isolate (as observed in the present study), the introduction of less aggressive isolate in the areas of more aggressive isolates would reduce the risk of disease development.

- Several sources of resistance to Ascochyta blight have been reported in various countries. The resistant genotypes so far identified need to be tested for their resistance using aggressive pathotypes of Indo-Pak, the major chickpea region. Furthermore, disease escape strategies wherever possible and meaningful, need to be adopted.

- The genotypes, which were resistant at seedling and vegetative stage, should be utilized in breeding programmes to build disease resistance pyramids due to complex nature of Ascochyta blight.

- Seed protein observed by SDS-PAGE was not very effective for studying intra-specific genetic diversity in cultivated chickpea and disease status. Further, biochemical markers are suggested to be developed and used for variability studies in isolates and host resistance breeding.

- At present, susceptible segregating populations at pod stage are being discarded without looking at seeds. The present investigation suggests a modification in breeding methodology for blight resistance, i.e., to take in consideration all the parameters of disease and grain yield till harvest and then to decide for selection or rejection of the genotypes. Advanced chickpea breeding lines and candidate cultivars should continuously be tested for the assessment of yield losses before their release.

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APPENDICES

Appendix I: Prevalence, incidence and severity of Ascochyta blight of chickpea in major chickpea growing areas of Punjab and NWFP of Pakistan

District	Locality/ Area	Number of farms surveyed	Disease prevalence (%)	Disease incidence (%)	Disease severity (1-9) rating
1	2	3	4	5	6
Rawalpindi	Jatli	3	100		
	1			96	7
	2			94	7
	3			90	5
	Doltala	4	100		
	1			90	9
	2			96	7
	3			86	7
	4			98	9
	Tarnol	3	100		
	1			96	9
	2			90	7
	3			80	7
Sialkot	Sahowali	4	100		
	1			90	7
	2			96	9
	3			84	6
	4			98	9
	Chowinda	5	100		
	1			80	7
	2			86	7
	3			50	5
	4			50	3
	5			98	9
Norowal	Zafarwal	3	100		
	1			96	9
	2			92	7
	3			90	7
	Darman	5	100		
	1			94	8
	2			92	7
	3			98	9
	4			90	7
	5			80	5
	Chak amru	3	100		
	1			98	9
	2			90	7
	3			90	7
	Nainakot	4	100		

1	2	3	4	5	6
	1			84	5
	2			80	5
	3			90	7
	4			60	3
	Mansoorwali	2	100		
	1			90	7
	2			96	9
Attock	GRS, Attock	5	100		
	1			76	5
	2			90	9
	3			72	6
	4			80	7
	5			88	9
	Jand	7	100		
	1			82	7
	2			94	9
	3			70	6
	4			84	7
	5			78	7
	6			88	8
	7			80	
	Fatehjang	9	100		
	1			52	3
	2			80	8
	3			86	9
	4			72	7
	5			40	3
	6			74	6
	7			84	8
	8			76	6
	9			70	5
	Nara	5	100		
	1			80	7
	2			86	7
	3			84	7
	4			88	9
	5			90	9
	Bahtar	5	100		
	1			80	7
	2			74	6
	3			82	8
	4			70	5
	5			46	3
	Hasanabdal	4	100		
	1			52	3

1	2	3	4	5	6
	2			80	7
	3			78	7
	4			74	5
	Pindigheb	6	100		
	1			80	7
	2			70	6
	3			64	5
	4			40	3
	5			60	7
	6			78	7
Chakwal	Thoa	5	80		
	1			86	7
	2			60	5
	3			72	6
	4			64	5
	5			0	1
	Dhudial	6	83.3		
	1			90	7
	2			66	5
	3			70	7
	4			70	5
	5			64	6
	6			0	1
	Bhaun	6	100		
	1			94	9
	2			90	9
	3			80	7
	4			60	3
	5			70	7
	6			62	5
	Balkasar	5	100		
	1			90	7
	2			98	7
	3			92	7
	4			86	5
	5			80	5
	Rupowal	5	80		
	1			0	1
	2			68	7
	3			60	5
	4			74	6
	5			80	8
Mianwali	Harnoli	3	66.7		
	1			0	1
	2			60	5

1	2	3	4	5	6
	3			72	7
	Wanbhachran	6	66.7		
	1			70	6
	2			60	5
	3			68	5
	4			56	3
	5			0	1
	6			0	1
	Chashma	7	71.4		
	1			60	5
	2			0	1
	3			68	7
	4			70	6
	5			62	5
	Dab	3	100		
	1			70	7
	2			76	5
	3			50	3
Khushab	Rangpur	8	37.5		
	1			40	5
	2			28	3
	3			0	1
	4			30	3
	5			36	4
	6			48	5
	7			0	1
	8			0	1
	Nurpur	9	38.9		
	1			0	1
	2			46	5
	3			38	5
	4			0	1
	5			24	3
	6			0	1
	7			0	1
	8			32	3
	9			0	1
	Adhikot	11	33.4		
	1			0	1
	2			0	1
	3			32	5
	4			0	1
	5			36	3
	6			24	3
	7			0	1

1	2	3	4	5	6
	8			0	1
	9			40	5
	10			0	1
	11			0	1
	Roda	10	45.0		
	1			58	5
	2			42	7
	3			0	1
	4			0	1
	5			30	3
	6			0	1
	7			0	1
	8			0	1
	9			34	3
	10			0	1
	Girot	11	36.4		
	1			24	3
	2			38	5
	3			0	1
	4			0	1
	5			0	1
	6			60	3
	7			0	1
	8			0	1
	9			0	1
	10			44	5
	11			0	1
	Hemoka	9	47.1		
	1			0	1
	2			30	5
	3			38	3
	4			0	1
	5			40	3
	6			44	4
	7			42	3
	8			0	1
	9			0	1
	Mithatiwana	5	40		
	1			0	1
	2			0	1
	3			50	3
	4			46	5
	5			0	1
	Ukhlimola	7	46.2		
	1			0	1

1	2	3	4	5	6
	2			50	3
	3			58	5
	4			65	3
	5			0	1
	6			0	1
	7			30	3
Bhakhar	AZRI, Bhakkar	8	26.7		
	1			40	3
	2			48	3
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	Kallurkot	12	38.1		
	1			52	3
	2			48	3
	3			70	3
	4			50	5
	5			60	4
	6			0	1
	7			0	1
	8			0	1
	9			0	1
	10			0	1
	11			0	1
	12			0	1
	Basti sha alam	9			
	1			0	1
	2			30	3
	3			0	1
	4			0	1
	5			20	3
	6			38	3
	7			0	1
	8			0	1
	9			0	1
	Punaj garan	10	35.0		
	1			40	3
	2			44	3
	3			0	1
	4			0	1
	5			26	3
	6			0	1

1	2	3	4	5	6
	7			0	1
	8			0	1
	9			34	1
	10			0	1
	Darya khan	9	33.4		
	1			38	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			28	2
	7			32	3
	8			0	1
	9			0	1
	Mankera	12	16.7		
	1			20	3
	2			16	2
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	9			0	1
	10			0	1
	11			0	1
	12			0	1
	Dullewala	10	20.0		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			28	3
	7			18	2
	8			0	1
	9			0	1
	10			0	1
	Goharwala	11	20.0		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1

1	2	3	4	5	6
	7			0	1
	8			0	1
	9			0	1
	10			22	3
	11			16	2
	Nawan Jandawala	9	22.2		
	1			18	3
	2			0	1
	3			24	3
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	9			0	1
Leiah	Kharewala	6	16.7		
	1			0	1
	2			22	3
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	Karor	7	28.6		
	1			0	1
	2			0	1
	3			0	1
	4			30	3
	5			26	3
	6			0	1
	7			0	1
	Kot Sultan	7	14.3		
	1			14	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	Chaubara	10	20		
	1			18	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			20	3

1	2	3	4	5	6
	7			0	1
	8			0	1
	9			0	1
	10			0	1
	Nawan Kot	11	0.00		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	9			0	1
	10			0	1
	11			0	1
	Fatepur	12	0.00		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	9			0	1
	10			0	1
	11			0	1
	12				
Jang	Trimun	3	0.00		
	1			0	1
	2			0	1
	3			0	1
	Atharan Hazari	7	14.3		
	1			22	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	Sanayasi wala	6	0.00		
	1			0	1
	2			0	1
	3			0	1

1	2	3	4	5	6
	4			0	1
	5			0	1
	6			0	1
	Ueh	7	0.00		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	Dager	8	25.0		
	1			12	3
	2			28	3
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	8			0	1
	Daultana	6	16.7		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			24	3
	6			0	1
D.I. Khan	D.I. Khan	5	20.0		
	1			32	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	Akal Ghar	4	25.0		
	1			0	1
	2			20	3
	3			0	1
	4			0	1
	Kafir kot	6	16.7		
	1			24	3
	2			0	1
	3			0	1
	4			0	1
	5			0	1
	6			0	1

1	2	3	4	5	6
	Kot jai	7	28.6		
	1			20	3
	2			28	3
	3			0	1
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	Saggu	5	20.0		
	1			0	1
	2			0	1
	3			0	1
	4			0	1
	5			20	3
	Yarik	3	0.0		
	1			0	1
	2			0	1
	3			0	1
Bannu	Sara-e-Ghambila	5	40.0		
	1			38	3
	2			0	1
	3			0	1
	4			10	2
	5			0	1
	Sara-e-Norang	7	28.75		
	1			0	1
	2			12	3
	3			16	3
	4			0	1
	5			0	1
	6			0	1
	7			0	1
	Ghori wala	4	25.0		
	1			0	1
	2			12	3
	3			0	1
	4			0	1
	Domeli	2	0.0		
	1			0	1
	2			0	1
Lakki Marwat	Lakki	4	25.0		
	1			0	1
	2			24	3
	3			0	1
	4			0	1

1	2	3	4	5	6
	Pezu	2	0.0		
	1			0	1
	2			0	1
	Shehbaz khel	2	100		
	1			100	5
	2			50	3
	Aba khel	4	50.0		
	1			0	1
	2			0	1
	3			40	5
	4			20	3
	Isa khan	5	40.0		
	1			40	3
	2			0	1
	3			18	3
	4			0	1
	5			0	1
Karak	Karak	4	75.0		
	1			0	1
	2			90	9
	3			62	5
	4			78	7
	Bahadar khel	3	100		
	1			90	9
	2			98	7
	3			84	7
	Ahmad wala	6	100		
	1			100	9
	2			56	5
	3			80	7
	4			60	6
	5			64	6
	6			70	7
	Banda daud shah	2	100		
	1			74	7
	2			92	9
	Lachi	2	50.0		
	1			0	1
	2			56	5
	Shanwala	4	75.0		
	1			70	7
	2			60	4
	3			30	3
	4			0	1

Appendix II: Effect of inoculum level (concentration of spores per ml) on disease development.

Cultivars	Disease rating			
	50,000 spores/ml	100,000 spores/ml	150,000 spores/ml	200,000 spores/ml
C-727	4.6 de	6.2 c	6.4 c	9.0 a
ILC-263	6.0 c	6.8 c	7.8 b	9.0 a
Punjab-91	4.4 de	7.8 b	8.6 ab	8.6 ab
Dash	3.4 fg	4.2 def	4.8 de	5.0 d
Parbat	3.2 g	3.8 efg	5.0 d	5.0 d

* Figures having the same letters are non-significant at 5% level of probability.

Appendix III: Response of chickpea genotypes to blight at different growth stages

Growth stages	Variety "C-727"	Variety "Punjab-91"
2-Weeks	9.0 a	7.5 b
4-Weeks	8.5 a	6.5 c
6-Weeks	7.2 b	5.7 de
8-Weeks	6.2 cd	5.5 ef
10-Weeks	5.6 de	5.0 fg
4.1 12-Weeks	4.9 g	4.1 h

* Figures having the same letters are non-significant at 5% level of probability.

Appendix IV: Pathogenic variability of various isolates of *Ascochyta rabiei*.

S.No	ISOLATE	DIFFERNTIAL OF CULTIVARS						
		C-727	ILC-263	C-44	CM-72	Piadar-91	Noor-91	Punjab-91
1	KB-1	7.8	6.8	8.4	9.0	8.0	7.3	7.4
2	BR-4	6.5	5.4	5.6	6.5	5.0	5.3	4.6
3	BR-3	7.5	6.1	6.2	5.8	6.2	6.6	5.7
4	LY-4	8.0	7.5	8.3	8.1	7.1	6.1	7.0
5	KT-2	4.9	6.2	7.6	4.0	3.5	3.1	3.0
6	MN-4	8.0	7.5	6.9	7.1	6.9	5.8	5.9
7	KT-1	8.5	6.9	8.7	8.6	7.4	6.7	7.4
8	MN-5	7.2	6.4	6.6	6.3	5.9	4.6	4.8
9	LY-2	7.7	7.0	8.4	7.6	6.9	6.0	6.1
10	AT-1	9.0	9.0	9.0	9.0	9.0	9.0	9.0
11	BR-5	4.5	4.5	5.1	5.0	3.5	3.5	4.1
12	BR-2	7.5	7.4	8.2	8.7	7.6	5.0	5.1
13	KK-2	9.0	7.0	8.5	7.8	6.5	7.5	8.2
14	JN-2	3.8	7.0	5.0	3.6	3.1	3.8	4.8
15	KB-3	6.3	7.2	7.0	6.8	5.9	6.7	5.4
16	NC-3	9.0	8.0	9.0	9.0	8.0	8.0	9.0
17	NC-1	9.0	9.0	9.0	9.0	9.0	9.0	9.0
18	BN-2	5.4	5.4	6.7	7.1	4.2	4.1	5.4
19	DI-2	9.0	7.5	8.0	7.3	5.1	6.1	5.6
20	LY-3	8.2	5.6	9.0	8.0	6.0	6.4	5.7
21	NC-2	9.0	9.0	9.0	9.0	7.0	9.0	9.0
22	AT-2	9.0	9.0	9.0	9.0	9.0	9.0	9.0
23	BN-1	9.0	7.3	8.8	8.7	4.5	6.3	7.0
24	KB-2	8.7	6.9	9.0	9.0	6.5	6.8	6.8
25	KN-1	8.2	8.6	9.0	9.0	9.0	7.9	6.3
26	MN-3	8.4	7.5	9.0	8,9	7.3	7.3	6.3
27	DI-1	9.0	9.0	8.6	7.7	5.6	5.3	6.4
28	BR-1	7.8	7.9	7.7	7.3	6.9	6.6	7.5
29	FD-2	8.6	7.5	7.3	7.2	6.5	6.5	6.9
30	CL-2	8.5	9.0	9.0	9.0	9.0	7.8	9.0
31	MN-1	9.0	9.0	9.0	9.0	7.0	7.0	9.0
32	ST-1	9.0	9.0	9.0	9.0	7.0	8.0	9.0
33	AT-3	9.0	9.0	9.0	9.0	9.0	9.0	9.0
34	JN-1	9.0	9.0	9.0	7.0	5.3	6.0	6.6
35	ST-2	9.0	9.0	9.0	7.0	7.0	8.0	8.0
36	AT-5	9.0	8.8	9.0	8.6	7.0	8.3	8.0
37	AT-4	9.0	9.0	9.0	9.0	7.4	9.0	9.0
38	MN-2	9.0	9.0	9.0	8.0	7.0	7.0	9.0
39	FD-1	9.0	8.4	8.9	7.3	6.3	6.7	6.7
40	CL-1	9.0	9.0	9.0	9.0	9.0	9.0	9.0
41	KK-1	7.8	8.0	9.0	9.0	8.5	8.0	8.8
42	CL-3	8.3	5.7	9.0	9.0	9.0	8.5	9.0

Appendix-V: Screening of chickpea germplasm during 1994

S. No	Variety	Source	Seedlings	Vegetable	Podding
1	2	3	4	5	6
1	93001	NIAB	9	9	9
2	93002	NIAB	9	7	9
3	93003	NIAB	9	7	9
4	93004	NIAB	9	7	9
5	93005	NIAB	9	9	9
6	93006	NIAB	9	5	7
7	93007	NIAB	9	6	9
8	93008	NIAB	9	7	9
9	93009	NIAB	9	9	9
10	93010	NIAB	9	7	9
11	93011	NIAB	9	5	9
12	93012	NIAB	9	4	8
13	93013	NIAB	9	5	7
14	93014	NIAB	9	5	8
15	93015	NIAB	9	9	9
16	93016	NIAB	9	7	9
17	93017	NIAB	9	7	9
18	93018	NIAB	9	6	8
19	93019	NIAB	9	5	8
20	93020	NIAB	9	6	8
21	93021	NIAB	9	7	9
22	93022	NIAB	9	5	8
23	93023	NIAB	9	4	8
24	93024	NIAB	9	5	7
25	93025	NIAB	9	6	8
26	93026	NIAB	9	5	8
27	93027	NIAB	5	3	6
28	93028	NIAB	9	5	7
29	93029	NIAB	9	5	7
30	93030	NIAB	9	7	9
31	93031	NIAB	9	8	9
32	93032	NIAB	9	7	9
33	93033	NIAB	9	9	9
34	93034	NIAB	9	9	9
35	93035	NIAB	9	5	8
36	93036	NIAB	9	5	9
37	93037	NIAB	9	6	9

1	2	3	4	5	6
38	93038	NIAB	9	6	9
39	93039	NIAB	9	7	9
40	93040	NIAB	9	7	9
41	93041	NIAB	9	6	9
42	93042	NIAB	9	5	7
43	93043	NIAB	9	6	8
44	93044	NIAB	9	6	8
45	93045	NIAB	9	6	8
46	93046	NIAB	9	7	9
47	93047	NIAB	9	7	8
48	93048	NIAB	9	6	8
49	93049	NIAB	9	6	9
50	93050	NIAB	9	6	9
51	93051	NIAB	9	6	8
52	93052	NIAB	9	5	7
53	93053	NIAB	9	4	7
54	93054	NIAB	9	4	7
55	93055	NIAB	9	6	8
56	93056	NIAB	9	9	9
57	93057	NIAB	9	9	9
58	93058	NIAB	9	7	9
59	93059	NIAB	9	6	9
60	93060	NIAB	9	9	9
61	93061	NIAB	9	9	9
62	93062	NIAB	9	6	9
63	93063	NIAB	9	6	9
64	93064	NIAB	9	6	9
65	93065	NIAB	9	6	9
66	93066	NIAB	9	7	9
67	93067	NIAB	9	5	7
68	93068	NIAB	9	9	9
69	93069	NIAB	5	3	5
70	93070	NIAB	9	5	7
71	93071	NIAB	9	7	9
72	93072	NIAB	9	7	9
73	93073	NIAB	9	6	9
74	93074	NIAB	9	5	9
75	93075	NIAB	9	7	9
76	93076	NIAB	7	5	8

1	2	3	4	5	6
77	93077	NIAB	9	5	8
78	93078	NIAB	9	7	9
79	93005A	AARI	9	8	9
80	93011A	AARI	9	7	9
81	93012A	AARI	9	7	9
82	93016A	AARI	9	8	9
83	93020A	AARI	9	8	9
84	93045A	AARI	9	7	9
85	93050A	AARI	9	7	9
86	93051A	AARI	9	7	9
87	93058A	AARI	9	8	9
88	93066A	AARI	9	7	9
89	93069A	AARI	9	6	8
90	93072A	AARI	9	7	8
91	93081	AARI	9	7	8
92	93098	AARI	9	7	8
93	93105	AARI	9	9	9
94	93110	AARI	9	9	9
95	93115	AARI	9	5	8
96	93117	AARI	9	7	9
97	93118	AARI	9	9	9
98	93120	AARI	9	8	9
99	93127	AARI	9	7	9
100	93137	AARI	9	5	9
101	93139	AARI	9	8	9
102	93140	AARI	9	6	9
103	93149	AARI	9	6	9
104	93152	AARI	9	7	9
105	93153	AARI	9	6	9
106	93158	AARI	9	7	9
107	93159	AARI	9	8	9
108	86160	AARI	9	7	9
109	86205	AARI	6	3	5
110	93164	AARI	7	3	5
111	93165	AARI	9	7	9
112	93166	AARI	9	8	9
113	93168	AARI	9	8	9
114	93183	AARI	9	5	9
115	93184	AARI	9	6	9

1	2	3	4	5	6
116	93188	AARI	9	6	9
117	93190	AARI	9	7	9
118	93197	AARI	9	7	9
119	93200	AARI	9	8	9
120	93280	AARI	9	6	9
121	93291	AARI	9	5	8
122	93293	AARI	9	9	9
123	93297	AARI	9	9	9
124	93300	AARI	9	8	9
125	93303	AARI	9	7	9
126	93306	AARI	9	7	9
127	93314	AARI	9	5	7
128	93321	AARI	9	5	8
129	93325	AARI	9	7	9
130	86135	AARI	9	7	8
131	93334	AARI	9	9	9
132	93339	AARI	9	9	8
133	93345	AARI	9	7	8
134	93346	AARI	9	6	8
135	93348	AARI	9	7	9
136	93362	AARI	9	7	9
137	93363	AARI	9	7	9
138	93365	AARI	9	7	9
139	93366	AARI	9	7	9
140	93368	AARI	9	8	9
141	93205	AARI	6	3	5
142	93369	AARI	9	9	9
143	93372	AARI	9	5	7
144	93380	AARI	9	5	8
145	93386	AARI	9	6	9
146	93390	AARI	9	9	9
147	93401	AARI	9	5	7
148	93402	AARI	9	7	9
149	93403	AARI	9	6	7
150	93405	AARI	9	7	8
151	93409	AARI	9	9	9
152	93410	AARI	9	8	9
153	93411	AARI	9	8	9
154	88173K	AARI	9	7	9

1	2	3	4	5	6
155	88193K	AARI	9	8	9
156	88194K	AARI	9	7	9
157	89169K	AARI	9	6	8
158	89171K	AARI	9	4	6
159	89174K	AARI	9	6	8
160	89178K	AARI	9	5	7
161	89181K	AARI	9	6	8
162	86135K	AARI	9	8	9
163	89182K	AARI	9	7	9
164	90368K	AARI	9	6	8
165	90369K	AARI	9	6	8
166	90372K	AARI	9	7	9
167	90373K	AARI	9	6	9
168	90374K	AARI	9	5	7
169	90377K	AARI	9	5	7
170	90378K	AARI	9	5	7
171	90385K	AARI	9	5	7
172	90386K	AARI	9	5	8
173	86205K	AARI	6	3	5
174	90388K	AARI	9	6	8
175	90391K	AARI	9	9	9
176	90392K	AARI	9	6	8
177	90394K	AARI	9	7	9
178	90395K	AARI	9	7	9
179	90398K	AARI	9	5	7
180	90399K	AARI	9	4	7
181	90402K	AARI	9	5	7
182	91301K	AARI	9	7	9
183	91302K	AARI	9	6	9
184	89113	AARI	9	5	7
185	91304	AARI	9	5	8
186	91305K	AARI	9	5	9
187	91306K	AARI	9	5	8
188	91307K	AARI	9	6	9
189	91309K	AARI	9	5	8
190	91310K	AARI	9	7	9
191	91311K	AARI	9	7	9
192	91315K	AARI	9	6	9
193	92001K	AARI	9	6	9

1	2	3	4	5	6
194	92003K	AARI	7	3	7
195	86235	AARI	9	7	9
196	92004K	AARI	9	5	8
197	92005K	AARI	9	4	7
198	92006K	AARI	9	5	7
199	92007K	AARI	9	4	7
200	92009K	AARI	9	5	8
201	92010K	AARI	9	7	9
202	92011K	AARI	9	9	9
203	92012K	AARI	9	4	7
204	92016K	AARI	9	9	9
205	92027K	AARI	9	9	9
206	FLIP 84-87C	ICARDA	7	7	9
207	FLIP 84-92C	ICARDA	8	5	7
208	FLIP 88-85C	ICARDA	5	5	7
209	FLIP 90-56C	ICARDA	7	5	7
210	FLIP 90-112C	ICARDA	5	4	7
211	FLIP 91-14C	ICARDA	7	5	8
212	FLIP 91-62C	ICARDA	7	4	6
213	FLIP 92-13C	ICARDA	6	7	8
214	FLIP 92-16C	ICARDA	6	6	8
215	FLIP 92-18C	ICARDA	6	7	9
216	FLIP 92-34C	ICARDA	5	5	8
217	FLIP 92-52C	ICARDA	5	6	9
218	FLIP 92-64C	ICARDA	4	5	8
219	FLIP 92-72C	ICARDA	5	6	9
220	FLIP 92-113C	ICARDA	5	5	8
221	FLIP 92-133C	ICARDA	6	5	9
222	FLIP 92-151C	ICARDA	5	6	8
223	FLIP 92-181C	ICARDA	7	6	7
224	FLIP 92-187C	ICARDA	5	5	8
225	ICC 13555	ICARDA	3	3	5
226	NARC 9002	ICRISAT	6	5	8
227	NARC 9005	ICRISAT	7	6	8
228	NARC 9006	ICRISAT	7	6	9
229	NARC 9008	ICRISAT	7	5	8
230	NARC 9009	ICRISAT	7	5	7
231	NARC 9010	ICRISAT	7	6	8
232	GL 85086	ICRISAT	9	8	9

1	2	3	4	5	6
233	ICCL 87322	ICRISAT	9	8	9
234	ICCV 88510	ICRISAT	8	8	9
235	ICCX 860023-BP-3P	ICRISAT	9	9	9
236	ICCX 860027-BP-9P	ICRISAT	7	5	9
237	ICCX 850622-BH-25H	ICRISAT	6	6	8
238	FLIP 82-52CK	ICRISAT	7	6	9
239	FLIP 83-13CK	ICRISAT	7	4	7
240	ICC 10302K	ICRISAT	9	9	9
241	ICC 1136	ICRISAT	9	9	9
242	ICC 16331	ICRISAT	8	6	9
243	ICC 16332	ICRISAT	7	5	7
244	ICC 16334	ICRISAT	8	6	8
245	ICC 16343	ICRISAT	9	8	9
246	ICC 16344	ICRISAT	9	8	9
247	ICCX 860047-BP-20H	ICRISAT	6	4	7
248	ICCX 830697-10H-BH	ICRISAT	9	8	9

Appendix VI: Screening of chickpea germplasm during 1995

Sr. No	Variety	Source	Seedlings	Vegetable	Podding
1	2	3	4	5	6
1	94001	NIAB	9	7	9
2	94002	NIAB	9	9	9
3	94003	NIAB	9	9	9
4	94004	NIAB	9	9	9
5	94005	NIAB	9	7	9
6	94006	NIAB	9	9	9
7	94007	NIAB	9	9	9
8	94008	NIAB	9	9	9
9	94009	NIAB	9	7	9
10	94010	NIAB	9	7	9
11	94011	NIAB	9	9	9
12	94012	NIAB	9	7	9
13	94013	NIAB	9	9	9
14	94014	NIAB	9	9	9
15	94015	NIAB	9	9	9
16	94016	NIAB	9	7	9
17	94017	NIAB	9	9	9
18	94018	NIAB	9	9	9
19	94019	NIAB	9	9	9
20	94020	NIAB	9	9	9
21	94021	NIAB	9	9	9
22	94022	NIAB	9	9	9
23	94023	NIAB	9	9	9
24	94024	NIAB	9	9	9
25	94025	NIAB	9	9	9
26	94026	NIAB	9	9	9
27	94027	NIAB	9	9	9
28	94028	NIAB	9	9	9
29	94029	NIAB	9	9	9
30	94030	NIAB	9	9	9
31	94031	NIAB	9	9	9
32	94032	NIAB	9	9	9
33	94033	NIAB	9	9	9
34	94034	NIAB	9	9	9
35	94035	NIAB	9	9	9
36	94036	NIAB	9	9	9
37	94037	NIAB	9	9	9

1	2	3	4	5	6
38	94038	NIAB	9	9	9
39	94039	NIAB	9	7	9
40	94040	NIAB	9	9	9
41	94041	NIAB	9	9	9
42	94042	NIAB	9	9	9
43	94043	NIAB	9	9	9
44	94044	NIAB	9	9	9
45	94045	NIAB	9	9	9
46	94046	NIAB	9	9	9
47	94047	NIAB	9	6	7
48	94048	NIAB	9	8	9
49	94049	NIAB	9	8	9
50	94050	NIAB	9	9	9
51	94051	NIAB	9	7	9
52	94052	NIAB	9	8	9
53	94053	NIAB	9	9	9
54	94054	NIAB	9	9	9
55	94055	NIAB	9	9	9
56	94056	NIAB	9	9	9
57	94057	NIAB	9	9	9
58	94058	NIAB	9	9	9
59	94059	NIAB	9	9	9
60	94060	NIAB	9	9	9
61	94061	NIAB	9	9	9
62	94062	NIAB	9	9	9
63	94063	NIAB	9	9	9
64	94064	NIAB	9	9	9
65	94065	NIAB	9	9	9
66	94066	NIAB	9	9	9
67	94067	NIAB	9	9	9
68	94068	NIAB	9	9	9
69	94069	NIAB	9	9	9
70	94070	NIAB	9	9	9
71	94071	NIAB	9	9	9
72	94072	NIAB	9	9	9
73	94073	NIAB	9	7	9
74	94074	NIAB	9	9	9
75	94075	NIAB	9	9	9
76	94076	NIAB	9	9	9

1	2	3	4	5	6
77	94077	NIAB	9	9	9
78	94078	NIAB	9	9	9
79	94079	NIAB	9	9	9
80	94080	NIAB	9	9	9
81	94081	NIAB	9	9	9
82	94082	NIAB	9	9	9
83	94083	NIAB	9	7	9
84	94084	NIAB	9	8	9
85	94085	NIAB	9	7	9
86	94086	NIAB	9	8	9
87	94087	NIAB	9	7	9
88	94088	NIAB	9	7	9
89	94089	NIAB	9	9	9
90	94090	NIAB	9	9	9
91	94091	NIAB	9	9	9
92	94092	NIAB	9	8	9
93	94093	NIAB	9	9	9
94	94094	NIAB	9	8	9
95	94095	NIAB	9	7	9
96	94096	NIAB	9	9	9
97	94097	NIAB	9	9	9
98	94098	NIAB	9	9	9
99	94099	NIAB	9	9	9
100	94100	NIAB	9	9	9
101	94101	NIAB	9	9	9
102	94102	NIAB	9	9	9
103	94103	NIAB	9	7	9
104	94104	NIAB	9	9	9
105	94105	NIAB	9	7	9
106	94106	NIAB	9	8	9
107	94107	NIAB	9	9	9
108	94108	NIAB	9	8	9
109	94109	NIAB	9	8	9
110	94110	NIAB	9	7	9
111	94111	NIAB	9	9	9
112	94112	NIAB	9	9	9
113	94113	NIAB	9	9	9
114	94114	NIAB	9	6	9
115	94115	NIAB	9	6	7

1	2	3	4	5	6
116	94116	NIAB	5	4	7
117	94117	NIAB	9	7	9
118	94118	NIAB	9	7	9
119	94119	NIAB	9	7	9
120	94120	NIAB	9	8	9
121	94121	NIAB	9	8	9
122	94122	NIAB	9	7	9
123	94123	NIAB	9	7	9
124	94124	NIAB	9	9	9
125	94125	NIAB	9	9	9
126	94126	NIAB	9	8	9
127	94127	NIAB	9	8	9
128	94128	NIAB	9	8	9
129	94129	NIAB	9	6	8
130	94130	NIAB	5	5	7
131	CMN 1-4	NIAB	9	7	9
132	CMN 1-7	NIAB	9	7	9
133	CMN 5	NIAB	9	7	9
134	CMN 7	NIAB	9	7	9
135	CMN 37	NIAB	9	7	9
136	CMN 64	NIAB	9	7	9
137	CMN 122	NIAB	9	9	9
138	CMN 2-8	NIAB	9	7	9
139	CMN 3-23	NIAB	9	7	9
140	CMN 15	NIAB	9	7	9
141	CMN 115	NIAB	9	7	9
142	CMN 426-1	NIAB	9	7	9
143	CMN 446-4	NIAB	9	7	9
144	CMN 447-4	NIAB	9	9	9
145	CMN 158	NIAB	9	8	9
146	CMN 72	NIAB	9	6	9
147	CMN 35	NIAB	9	7	9
148	CMN 114	NIAB	9	9	9
149	CMN 121	NIAB	9	7	9
150	CMN 144	NIAB	9	7	9
151	CMN 187	NIAB	9	9	9
152	CMN 194	NIAB	9	8	9
153	CMN 662-3	NIAB	9	9	9
154	CMN 678-6	NIAB	9	9	9

1	2	3	4	5	6
155	CMN 730-10	NIAB	9	9	9
156	CMN 391-9	NIAB	9	9	9
157	CMN 539-3	NIAB	9	9	9
158	CMN 560-13-4	NIAB	9	9	9
159	CMN 692-13-5	NIAB	9	9	9
160	CMN 884-3-7	NIAB	9	9	9
161	CMN 998-10	NIAB	9	9	9
162	CMN 1098-113-13	NIAB	9	9	9
163	CMN 1105-13-14	NIAB	9	9	9
164	CMN 1224-15	NIAB	9	9	9
165	CMN 1248-13-15	NIAB	9	9	9
166	NIFA 88	NIAB	3	3	5
167	ILC 72	ICARDA	5	5	7
168	ILC 200	ICARDA	5	5	7
169	ILC 3279	ICARDA	5	5	7
170	FLIP 84-182C	ICARDA	5	4	7
171	FLIP 88-83C	ICARDA	5	5	7
172	FLIP 89-78C	ICARDA	5	5	7
173	FLIP 90-58C	ICARDA	5	5	7
174	FLIP 90-76C	ICARDA	9	7	9
175	FLIP 90-85C	ICARDA	5	4	5
176	FLIP 91-8C	ICARDA	9	7	9
177	FLIP 91-23C	ICARDA	9	7	9
178	FLIP 91-52C	ICARDA	9	6	9
179	FLIP 91-150C	ICARDA	7	7	9
180	FLIP 91-196C	ICARDA	5	5	7
181	FLIP 92-116C	ICARDA	7	7	9
182	FLIP 92-45C	ICARDA	7	6	9
183	FLIP 92-70C	ICARDA	5	5	6
184	FLIP 92-78C	ICARDA	5	5	7
185	FLIP 92-96C	ICARDA	5	5	7
186	FLIP 92-132C	ICARDA	8	7	9
187	FLIP 92-139C	ICARDA	9	7	9
188	FLIP 92-152C	ICARDA	5	5	7
189	FLIP 92-155C	ICARDA	7	6	9
190	FLIP 92-159C	ICARDA	5	4	7
191	FLIP 92-172C	ICARDA	5	5	7
192	FLIP 92-174C	ICARDA	7	6	9
193	FLIP 92-175C	ICARDA	7	6	9

1	2	3	4	5	6
194	FLIP 92-179C	ICARDA	5	4	7
195	FLIP 92-189C	ICARDA	5	5	7
196	FLIP 92-190C	ICARDA	6	5	9
197	FLIP 92-194C	ICARDA	6	5	9
198	FLIP 93-62C	ICARDA	4	3	5
199	FLIP 93-63C	ICARDA	7	7	9
200	FLIP 93-131C	ICARDA	7	7	9
201	FLIP 93-141C	ICARDA	7	7	9
202	FLIP 93-146C	ICARDA	6	5	7
203	FLIP 93-158C	ICARDA	6	5	7
204	FLIP 93-160C	ICARDA	7	6	9
205	FLIP 93-176C	ICARDA	7	6	9
206	ICC 4475	ICARDA	6	5	7
207	ICC 13269	ICARDA	5	4	6
208	ICC 13416	ICARDA	4	3	5
209	ICC 13508	ICARDA	5	4	7
210	FLIP 87-505C	ICARDA	5	4	5
211	FLIP 87-506C	ICARDA	7	7	9
212	FLIP 87-507C	ICARDA	7	7	9
213	FLIP 87-508C	ICARDA	7	7	9
214	ICC 652	ICRISAT	9	9	9
215	ICC1136	ICRISAT	9	9	9
216	ICC1416	ICRISAT	9	8	9
217	ICC1468	ICRISAT	5	4	7
218	ICC 3996	ICRISAT	5	4	7
219	ICC 4018	ICRISAT	9	8	9
220	ICC 6373	ICRISAT	5	3	5
221	ICC 8489	ICRISAT	9	9	9
222	ICC 9800	ICRISAT	7	5	7
223	ICC 10302	ICRISAT	9	9	9
224	ICC 15987	ICRISAT	7	5	9
225	ICC 15988	ICRISAT	9	6	9
226	ICC 16337	ICRISAT	9	6	9
227	ICC 16953	ICRISAT	7	5	9
228	ICC 16954	ICRISAT	9	7	9
229	ICC 16955	ICRISAT	5	4	7
230	ICC 15989	ICRISAT	9	7	9
231	ICC 16956	ICRISAT	9	9	9
232	Pb-7	ICRISAT	9	9	9

Appendix VII: Screening of chickpea germplasm during 1996

S. No.	Variety	Source	Seedlings	Vegetable	Podding
1	2	3	4	5	6
1	95001	NIAB	9	7	9
2	95002	NIAB	9	7	9
3	95003	NIAB	9	8	9
4	95004	NIAB	9	9	9
5	95005	NIAB	9	9	9
6	95006	NIAB	7	6	9
7	95007	NIAB	7	6	9
8	95008	NIAB	7	6	9
9	95009	NIAB	7	6	9
10	95010	NIAB	9	8	9
11	95012	NIAB	9	9	9
12	95013	NIAB	9	9	9
13	95014	NIAB	9	9	9
14	95015	NIAB	9	9	9
15	95016	NIAB	7	5	8
16	95017	NIAB	7	6	9
17	95018	NIAB	9	7	9
18	95019	NIAB	9	7	9
19	95020	NIAB	9	8	9
20	95021	NIAB	9	8	9
21	95022	NIAB	7	6	9
22	95023	NIAB	7	6	9
23	95024	NIAB	9	7	9
24	95025	NIAB	9	7	9
25	95026	NIAB	9	8	9
26	95027	NIAB	9	8	9
27	95028	NIAB	9	8	9
28	95029	NIAB	7	5	7
29	95030	NIAB	9	9	9
30	95031	NIAB	9	9	9
31	95032	NIAB	9	9	9
32	95033	NIAB	9	8	9
33	95034	NIAB	9	8	9
34	95035	NIAB	9	8	9
35	95036	NIAB	9	8	9
36	95037	NIAB	9	9	9
37	95038	NIAB	9	8	9

1	2	3	4	5	6
38	95039	NIAB	9	8	9
39	95040	NIAB	9	8	9
40	95041	NIAB	9	8	9
41	95042	NIAB	9	9	9
42	95043	NIAB	9	8	9
43	95044	NIAB	9	9	9
44	95045	NIAB	9	9	9
45	95046	NIAB	9	9	9
46	95047	NIAB	9	9	9
47	95048	NIAB	9	7	9
48	95049	NIAB	9	7	9
49	95050	NIAB	9	8	9
50	95051	NIAB	9	8	9
51	95052	NIAB	9	9	9
52	95053	NIAB	9	9	9
53	95054	NIAB	9	9	9
54	95055	NIAB	9	9	9
55	95056	NIAB	9	9	9
56	95057	NIAB	9	9	9
57	95058	NIAB	9	8	9
58	95059	NIAB	9	8	9
59	95060	NIAB	9	8	9
60	95061	NIAB	9	8	9
61	95062	NIAB	9	9	9
62	95063	NIAB	9	8	9
63	95064	NIAB	9	7	9
64	95065	NIAB	9	7	9
65	95066	NIAB	9	7	9
66	95067	NIAB	9	9	9
67	95068	NIAB	9	8	9
68	95069	NIAB	9	7	9
69	95070	NIAB	9	7	9
70	95071	NIAB	9	7	9
71	95072	NIAB	9	9	9
72	95073	NIAB	9	7	9
73	95074	NIAB	7	6	9
74	95075	NIAB	7	6	9
75	95076	NIAB	9	9	9
76	95077	NIAB	9	9	9

1	2	3	4	5	6
77	95078	NIAB	9	9	9
78	95079	NIAB	9	9	9
79	95080	NIAB	9	9	9
80	95081	NIAB	9	9	9
81	95082	NIAB	9	9	9
82	95083	NIAB	9	9	9
83	95084	NIAB	9	7	9
84	95085	NIAB	9	9	9
85	95086	NIAB	9	9	9
86	95087	NIAB	9	9	9
87	95088	NIAB	9	9	9
88	95089	NIAB	9	9	9
89	95090	NIAB	9	9	9
90	95091	NIAB	9	8	9
91	95092	NIAB	9	9	9
92	95093	NIAB	9	8	9
93	95094	NIAB	9	8	9
94	95095	NIAB	9	8	9
95	95096	NIAB	9	8	9
96	95097	NIAB	9	9	9
97	95098	NIAB	9	8	9
98	95099	NIAB	9	9	9
99	95100	NIAB	9	8	9
100	95101	NIAB	9	8	9
101	95102	NIAB	9	9	9
102	95103	NIAB	9	9	9
103	95104	NIAB	9	9	9
104	95105	NIAB	7	5	7
105	95106	NIAB	9	7	9
106	95107	NIAB	9	9	9
107	95108	NIAB	9	8	9
108	95109	NIAB	9	7	9
109	95110	NIAB	9	7	9
110	95111	NIAB	9	7	9
111	95112	NIAB	9	9	9
112	95113	NIAB	9	7	9
113	95114	NIAB	9	7	9
114	95115	NIAB	9	7	9
115	95116	NIAB	9	7	9

1	2	3	4	5	6
116	95117	NIAB	9	9	9
117	95118	NIAB	9	7	9
118	95119	NIAB	7	6	9
119	95120	NIAB	7	6	9
120	95121	NIAB	7	6	9
121	95122	NIAB	9	9	9
122	CMN-5	NIFA	7	6	9
123	CMN-7	NIFA	7	5	9
124	CMN-15	NIFA	7	6	9
125	CMN-37	NIFA	9	7	9
126	CMN-64	NIFA	9	9	9
127	CMN-114	NIFA	9	6	9
128	CMN-115	NIFA	7	5	9
129	CMN-121	NIFA	7	5	9
130	CMN-122	NIFA	7	6	9
131	CMN-158	NIFA	7	6	7
132	CMN-124	NIFA	7	5	7
133	CMN-2-8	NIFA	7	5	7
134	CMN-3-23	NIFA	7	5	7
135	CMN-4-26	NIFA	7	5	7
136	CMN-11-61	NIFA	7	5	7
137	CMN-1-1/86	NIFA	7	5	7
138	CMN-1-3/86	NIFA	9	9	9
139	CMN-220-4	NIFA	9	7	9
140	CMN-227-3	NIFA	9	9	9
141	CMN-316-54	NIFA	9	9	9
142	CMN-336-61	NIFA	9	9	9
143	CMN-342-56	NIFA	9	9	9
144	CMN-426-1	NIFA	9	7	9
145	CMN-446-4	NIFA	9	7	9
146	CMN-539-3	NIFA	9	6	9
147	CMN-560-3-4	NIFA	9	6	9
148	CMN-662-3	NIFA	9	6	9
149	CMN-667-4	NIFA	9	7	9
150	CMN-668-6	NIFA	7	5	7
151	CMN-668-7	NIFA	7	5	7
152	CMN-728-5	NIFA	7	5	7
153	CMN-729-2	NIFA	7	5	7
154	CMN-730-2	NIFA	7	5	7

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155	CMN-730-10	NIFA	9	6	7
156	CMN-731-4	NIFA	7	4	7
157	CMN-854	NIFA	7	4	7
158	CMN-998-10	NIFA	9	6	9
159	CMN-1098-13-13	NIFA	7	5	8
160	CMN-1248-13-15	NIFA	7	5	8
161	95002K	AARI	9	9	9
162	95010A	AARI	9	9	9
163	95011	AARI	9	7	9
164	95012A	AARI	9	9	9
165	95013A	AARI	9	7	9
166	95014A	AARI	9	9	9
167	95018A	AARI	9	7	9
168	95021A	AARI	9	9	9
169	95024A	AARI	9	9	9
170	95025A	AARI	9	9	9
171	95032A	AARI	9	7	9
172	95034A	AARI	9	9	9
173	95038A	AARI	9	7	9
174	95040A	AARI	9	9	9
175	95041A	AARI	9	7	9
176	95049A	AARI	9	9	9
177	95051A	AARI	9	9	9
178	95054A	AARI	9	9	9
179	95055A	AARI	9	8	9
180	95057A	AARI	9	8	9
181	Local	AARI	9	9	9
182	95059A	AARI	9	7	9
183	95002A	AARI	9	7	9
184	95003A	AARI	9	9	9
185	95004A	AARI	7	5	7
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187	95006A	AARI	9	6	9
188	95007A	AARI	9	6	9
189	95008A	AARI	9	8	9
190	95009A	AARI	9	8	9
191	95001A	AARI	9	7	9
192	95015A	AARI	9	7	9
193	95016A	AARI	9	7	9

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194	95017A	AARI	9	9	9
195	95019A	AARI	9	7	9
196	95020A	AARI	9	7	9
197	95022A	AARI	9	7	9
198	95023A	AARI	9	7	9
199	95024	AARI	9	9	9
200	95025	AARI	9	8	9
201	95026A	AARI	9	9	9
202	95027A	AARI	9	9	9
203	95028A	AARI	9	8	9
204	95029A	AARI	9	7	9
205	95030A	AARI	9	7	9
206	95031A	AARI	9	7	9
207	95032	AARI	9	7	9
208	95033A	AARI	7	4	7
209	95034	AARI	9	7	9
210	95035A	AARI	7	4	7
211	95036A	AARI	9	7	9
212	95037A	AARI	7	5	7
213	95038	AARI	7	4	7
214	95039A	AARI	7	5	7
215	95040	AARI	7	5	7
216	95041	AARI	7	4	7
217	95042A	AARI	7	4	7
218	95043A	AARI	9	7	9
219	95044A	AARI	9	7	9
220	95045A	AARI	9	7	9
221	95046A	AARI	9	7	9
222	95047A	AARI	9	7	9
223	95048A	AARI	7	4	7
224	95049	AARI	9	7	9
225	95050A	AARI	9	7	9
226	95051A	AARI	7	5	7
227	95052A	AARI	9	5	7
228	95053A	AARI	9	6	9
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232	95057A	AARI	9	6	9

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234	95059A	AARI	9	7	9
235	95060A	AARI	9	7	9
236	95061A	AARI	9	6	9
237	95062A	AARI	9	6	9
238	95063A	AARI	9	6	9
239	95064A	AARI	9	7	9
240	95065A	AARI	7	5	7
241	95066A	AARI	7	5	7
242	95067A	AARI	9	7	9
243	95068A	AARI	9	7	9
244	95069A	AARI	9	7	9
245	95070A	AARI	9	7	9
246	95071A	AARI	9	7	9
247	95072A	AARI	9	7	9
248	95073A	AARI	9	7	9
249	95074A	AARI	9	7	9
250	95075A	AARI	9	7	9
251	95076A	AARI	9	7	9
252	95077A	AARI	9	7	9
253	95078A	AARI	9	6	9
254	95079A	AARI	9	7	9
255	95080A	AARI	9	7	9
256	95081A	AARI	9	6	9
257	95082A	AARI	9	6	9
258	95083A	AARI	9	7	9
259	95084A	AARI	9	7	9
260	95085A	AARI	9	6	9
261	95086A	AARI	9	6	9
262	95087A	AARI	9	6	9
263	95088A	AARI	9	7	9
264	95089A	AARI	9	7	9
265	95090A	AARI	9	7	9
266	95091A	AARI	9	7	9
267	95092A	AARI	9	7	9
268	95093A	AARI	5	4	5
269	95094A	AARI	9	7	9
270	95095A	AARI	9	7	9
271	95096A	AARI	9	7	9

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272	95097A	AARI	9	7	9
273	95098A	AARI	9	7	9
274	95099A	AARI	9	7	9
275	95100A	AARI	9	7	9
276	95101A	AARI	9	7	9
277	95102A	AARI	9	9	9
278	95103A	AARI	9	7	9
279	95104A	AARI	9	6	9
280	95105A	AARI	7	5	7
281	95106A	AARI	7	5	7
282	95107A	AARI	7	5	7
283	FLIP84-182C	ICARDA	5	4	5
284	FLIP88-83C	ICARDA	7	7	9
285	FLIP89-78C	ICARDA	7	6	9
286	FLIP90-58C	ICARDA	7	6	9
287	FLIP90-76C	ICARDA	5	4	5
288	FLIP90-85C	ICARDA	7	6	8
289	FLIP91-8C	ICARDA	7	6	8
290	FLIP91-23C	ICARDA	5	4	7
291	FLIP91-149C	ICARDA	4	4	7
292	FLIP91-196C	ICARDA	5	5	7
293	FLIP91-219C	ICARDA	7	6	7
294	FLIP92-45C	ICARDA	5	4	5
295	FLIP92-105C	ICARDA	5	4	5
296	FLIP92-142C	ICARDA	5	4	5
297	FLIP92-155C	ICARDA	5	4	5
298	FLIP92-159C	ICARDA	3	3	5
299	FLIP92-162C	ICARDA	5	5	7
300	FLIP92-164C	ICARDA	5	4	7
301	FLIP92-172C	ICARDA	5	4	7
302	FLIP92-174C	ICARDA	7	6	7
303	FLIP92-175C	ICARDA	5	4	7
304	FLIP92-179C	ICARDA	5	4	7
305	FLIP92-189C	ICARDA	5	5	7
306	FLIP92-190C	ICARDA	5	4	7
307	FLIP92-194C	ICARDA	7	5	7
308	FLIP93-106C	ICARDA	5	6	9
309	FLIP93-114C	ICARDA	5	4	7
310	FLIP93-128C	ICARDA	5	4	7

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311	FLIP93-130C	ICARDA	7	6	9
312	FLIP93-131C	ICARDA	5	5	7
313	FLIP93-146C	ICARDA	5	5	7
314	FLIP93-160C	ICARDA	5	4	5
315	FLIP93-174C	ICARDA	5	4	5
316	FLIP93-175C	ICARDA	5	4	5
317	FLIP93-176C	ICARDA	5	4	5
318	FLIP93-177C	ICARDA	5	4	5
319	FLIP93-181C	ICARDA	5	5	7
320	FLIP93-186C	ICARDA	5	4	7
321	FLIP94-508C	ICARDA	5	3	5
322	FLIP94-509C	ICARDA	5	3	5
323	FLIP94-510C	ICARDA	5	3	5
324	ICC 3912	ICARDA	5	5	7
325	ICC 3919	ICARDA	5	3	5
326	ICC 3991	ICARDA	3	3	5
327	ICC 12004	ICARDA	5	3	5
328	ICC 13729	ICARDA	5	3	5
329	ICC 14903	ICARDA	5	3	5
330	ILC 263	ICARDA	9	9	9
331	ICC 801	ICRISAT	7	6	9
332	ICC 1416	ICRISAT	9	8	9
333	ICC 7567K	ICRISAT	7	4	9
334	ICC 15976K	ICRISAT	9	8	9
335	ICC 15989K	ICRISAT	9	6	9
336	ICC 15990K	ICRISAT	9	7	9
337	ICC 16954K	ICRISAT	7	5	9
338	ICC 850622-BH-BH-25H-BH-BH	ICRISAT	9	6	9
339	ICC 860047BP-BH-25-BP-BG	ICRISAT	7	5	9
340	ICC 860678-BP-BH-17H-BH-BH	ICRISAT	9	7	9
341	ICC 860692-BP-BH-6H-BH-BH	ICRISAT	9	6	9
342	ICC 860693BP-BH-10H-BH-BH	ICRISAT	9	4	9
343	ICC 17000	ICRISAT	9	6	9
344	ICC 12966K	ICRISAT	9	9	9

Appendix VIII: Some information about the chickpea varieties included in this study

Variety	Description
CM-72	A gamma irradiated mutant of the genotype 6153. It was released in 1983 and a commercial variety by the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. At present, it is being widely grown in the NWFP and some parts of the Punjab Province of Pakistan. It is a desi type having some tolerance to blight.
C-44	Released as a commercial variety by the Ayub Agricultural research Institute (AARI), Faisalabad in 1983. It is being widely grown in the Thal (desert) area of the Punjab province where most of the chickpea is grown. It is susceptible to iron-chlorosis, has some tolerance to blight, and good yield potential. It is a desi type and bold seeded having a good seed characteristics.
C-727	A desi type, released in 1963 by AARI as a blight resistant variety but later on it lost its resistance most probably due to the prevalence of most virulent races. It is still grown by some farmers since it yields well in blight free years.
Punjab-91	A desi type variety and released by AARI in 1991. Resistant to blight and iron chlorosis, good cooking quality. Wide adoptability under irrigated and rainfed conditions.
NIFA-88	Released by Nuclear Institute of Food and Agriculture (NIFA), Peshawar. Growth semi erect, seed colour yellowish brown. Early maturity, seed size medium. Tolerant to blight and resistant to pod borer.
Dasht	Released by National Agricultural Research Centre (NARC), Islamabad mainly for potwar area because of good tolerance to blight. Seed size bold, broad leaves, seed colour brown.
Parbat	Released by NARC also for potwar area because of tolerance to blight. Yellowish brown seed coat, seed size bold. Leaves small as compared to Dasht.
ILC-263	Bold seeded, kabuli genotype, highly susceptible to blight. Used as spreader/ check in the ICARDA's blight screening nurseries.