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Review Paper on Status, Distribution and the Management of Chickpea, Botrytis Grey Mould (Botrytis cinerea)

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Abstract
Botrytis grey mould (BGM), caused by *Botrytis cinerea* Pers. ex. Fr., is an economically important disease of chickpea (*Cicer arietinum* L.), especially in areas where cool, cloudy and humid weather persists. Several epidemics of BGM causing complete crop loss in the major chickpea producing countries have been reported. The pathogen *B. cinerea* mainly survives between seasons on infected crop debris and seeds. Despite extensive investigations on pathological, physiological and molecular characteristics of *B. cinerea* causing grey mould type diseases on chickpea and several other hosts, the nature of infection processes and genetic basis of pathogen variability have not been clearly established. Effective and repeatable controlled environment and field screening techniques have been developed for identification of HPR of the selected portion of chickpea germplasm evaluated for BGM resistance, only few accessions belonging to both cultivated and wild *Cicer* spp. were tolerant to BGM, and the search for higher levels of disease resistance continues. BGM management should not completely rely on the use of fungicides, as development of fungicide resistance in *B. cinerea* has been commonly observed. An adequate level of genetic resistance to BGM is not available in the cultivated genotypes and fungicides become ineffective during conditions of high disease pressure. Hence, integrated disease management (IDM) using the available management options is essential to successfully manage the disease and mitigate yield losses. Further information on the biology of *B. cinerea* and epidemiology of the disease is needed to strengthen the IDM programs. In this paper the biology of *B. cinerea* including its variability, epidemiology of BGM, identified sources of resistance and other management options and available information on biochemical and genetic basis of disease resistance have been reviewed with a mention of future research priorities.
Keywords: Chickpea, Botrytis grey mould disease management

1. Introduction

In Ethiopia, crops are grown annually on approximately 7.9 million hectares is appropriate to pulses. Pulse crops have diverse roles to play in the country and rank second as food after cereals and occupy about 17.7% of the total cultivated areas, and contribute about 12% of the total production. Chickpea *(Cicer arietinum L.*) is one of the important grain legume crop in Africa particularly in Ethiopia which widely grown in marginal soils and usually as rotational crops in highland and semi-highland regions of the country (Asfaw, 1993). As a result, the yield for most pulse crops in Ethiopia is low and ranges between 500-900 kg/ha, whereas the average yield potential is about 1760 kg/ ha. Because of its importance, the crop is widely produced by the Ethiopian farmers. According to FAO (2009), it is produced on 11 million ha with annual production of 9.7 million tones worldwide. However, among the pulse crops, chickpea has consistently maintained a much more significant status, ranking second in area (15.3% of total) and third in production (14.6%) after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Knights, 2007). Chickpea is widely used for food for its high protein content and also cash for the farmers and the country. Apart from this, because of its ability to fix nitrogen, it is used in crop rotation with the nationally important cereal crops like wheat, teff and barley. Chickpea is a good source of energy, protein, minerals (especially potassium, phosphorus, calcium, magnesium, copper, iron and zinc), vitamins (especially B vitamins), fiber and also contains potentially health-beneficial phytochemicals (Wood and Grusak, 2007). There are two types of chickpea: the 'Dessi' type (mostly brown seeded), traditionally grown in warmer climates of Asia and Africa, mostly in India, Pakistan, Bangladesh, Myanmar and Ethiopia; and the 'Kabuli' type (white-seeded), a large-seeded variety more suited to the temperate climates of Turkey, Mexico, the USA, Afghanistan and Iran. Some countries like Canada and Australia produce both 'Dessi' and 'Kabuli' types (Reddy *et al.,* 2007). 'Kabuli' type constitutes ~15% of global chickpea production and 'desi' type constitutes the remaining 85% (Singh and Malhotra, 1984). The national average yield of chickpea, 'Desi' type is 8.14 kg /ha; and Kabuli type is more than 15 kg /ha. The protein concentration of chickpea seed ranges from 16.7% to30.6% for 'Dessi' type and 12.6% to 29.0% for 'Kabuli' type, and is commonly 2–3 times higher than that of cereal grains (Wood and Grusak, 2007). Therefore, chickpea is an inexpensive, high-quality source of protein (Yadav *et al*., 2007). Chickpea exhibits higher lipid content than other pulses. The total lipid concentration of 'Dessi' and 'Kabuli' types ranges from 2.9% to 7.4% and 3.4% to 8.8%, respectively. Carbohydrates are the major nutritional component in chickpea, with 51– 65% in 'Dessi' type and 54 – 71% in 'Kabuli' type (Wood and Grusak, 2007). Since chickpea is high in fiber, low in sodium and fat, and also cholesterol free, it is a healthy food that is beneficial to the prevention of coronary and cardiovascular diseases

(Yadav *et al*., 2007). Chickpea is not only an important source of protein in human diets, but it also plays a significant role in maintaining soil fertility, through biological nitrogen fixation (Kantar et *al*., 2007).

Ethiopia is the largest producer of chickpea in Africa, accounting for about 46% of the continent's production during 1994-2006. It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (Menale Kassie *et al*., 2009). The total annual average during 1999 - 2008 chickpea production is estimated at about 173 thousand tones. During the same period, chickpea was third after faba bean and field pea in terms of area coverage (Menale Kassie.,2009). Despite of its importance, chickpea productivity is very low. The national average yield of chickpea in Ethiopia under farmers' production condition remains less than 1.5 t ha^{-1}(CSA, 2009). On the other hand, the potential of the crop under improved management condition is more than 3 t ha^{-1} (Legesse Dadi ., 2005). A number of limiting factors contribute to low productivity of chickpea. The large area under chickpea cultivation, total production and productivity is quite low in most chickpea growing countries and there is a wide gap between potential yield (5 tons ha^{-1}) and actual yield (0.8 tons ha^{-1}). The primary cause of low yields in chickpea is its susceptibility to a number of biotic and a biotic stresses. Among biotic constraints, drought is the most important factor limiting chickpea production (Singh *et al.,* 2008). Occurrence of drought is the common phenomenon in arid and semi-arid regions.

In Ethiopia, 16 diseases were reported in chickpea (Tadesse *et al.,* 1998). The crop suffers from serious diseases that affect it in all growth stages. About 50 and 38% of these diseases are caused by fungal and viral pathogens, respectively. The major threats to the production of the chickpea crops are the diseases of fungal origin particularly *Fusarium* wilt, *Botrytis* grey mould (BGM) and *Ascochyta* blight (Tadesse *et al.,* 1998). Among biotic stresses, Botrytis grey mold is caused by *Botrytis cinerea* Pers. ex. Fr., is a major disease of chickpea, especially in areas where cool, cloudy, and humid weather persists during the crop season and constrained chickpea production in Ethiopia and wide spread foliar disease that causes extensive crop losses up to 100% in most regions of the world. Susceptibility of chickpea to a number of fungal pathogens from seedling stage till harvest is the primary cause for low yields. Botrytis grey mould is the second most potentially important disease of chickpea after Ascochyta blight in Ethiopia (Mengistu and Negussie, 1994). Production of chickpea in the rainy season (main cropping) in Ethiopia could not be envisaged without fungicide application to control BGM. The BGM infestation is large in the cultivated fields of chickpea during this years in Ethiopia, due to this, the researchers are tried to use different management practices. However, there is inadequate information with regard to the status, distribution, plant growth promotion of chickpea, and its management of chickpea BGM in Ethiopia. Therefore, this review paper is aimed and carried out with the specific objective of to review on the status, distribution and the management of *Botrytis* grey mold in chickpea.

2. Materials and Methods

For the effectiveness of this paper, different sources such as proceedings, thesis works, journals, annual reports, fact sheets and publications regarding to irrigation water pollution and its minimization measures have been reviewed.

3. Discussion

3.1. Origin and domestication of chickpea

Van der Maesen (1987) recognized the present day southeastern part of Turkey adjoining Syria as the possible center of origin of chickpea (*Cicerarietinum* L.) based on the presence of the closely related annual species, *C. reticulatum* and *C. echinospermum*, in this region. Chickpea is also likely to have been domesticated for first time in south-east Turkey. This is supported by the distribution of early Neolithic chickpea which was confined to the Fertile Crescent, particularly in modern Anatolia and the eastern Mediterranean. In the late Neolithic era, chickpea spread westwards to modern Greece. By the Bronze Age, chickpea had been disseminated widely to Crete in the west, Upper Egypt in the south, eastwards through present-day Iraq to the Indian subcontinent. By the Iron Age, chickpea consolidated its distribution in South and West Asia, and appeared in Ethiopia for the first time (Redden and Berger, 2007). The primary center of diversity is in the Fertile Crescent where the crop was domesticated, and with the geographic spread of chickpea secondary centers of diversity developed, some older than 2000 years in Mediterranean, Europe, the Indian subcontinent and north-east Africa, and some more recently in Mexico and Chile with post-Columbus introduction (Redden and Berger, 2007). In line with the above idea, Muehlbauer and Rajesh (2008) added that after domestication, chickpea, along with other pulses and cereals, formed the basis of early agriculture in the Mediterranean and West Asian regions. Chickpea soonspread south to Ethiopia and east to South Asia where it became an important and popularlegume food crop and remains so to the present time. According to the same authors, chickpea, apparently taken to the Americas soon after the discovery of the New World, became an important food crop in the Pacific coastal regions of North, Central, and South America.

3.2. Agro-ecology and adaptation zones of chickpea in Ethiopia

Chickpea is widely grown in different agro-ecological zones falling between 1400 to 2300m above sea level where the mean annual rainfall ranges from 700 to 2000mm (Geletu Bejiga and Million Eshete, 1996). In line with thisidea, Menale Kassie*et* (2009) noted that the major chickpea producing areas are concentrated in the two regional states - Amhara and Oromia. These two regions cover more than 90% of the entire chickpea area and constitute about 92% of the total chickpea production. The top chickpea producing zones (North Gonder, South Gonder, North Shewa, East Gojam, South Wello, North Wello, West Gojam, and GonderZuria) belong to the Amhara region and account for about 80% of the country's chickpea production. In the Oromia region, the major producing zones are in West Shewa, East Shewa and North Shewa, which account for about 85% of the total area and production in this Regional State (MenaleKassie*et al*., 2009).

3.3. Chickpea production practices in Ethiopia

Chickpea is usually grown on black vertisol. Such soils are known for excess water and drainage problem during the main rainy period (June -August). Thus, to overcome this problem farmers plant chickpea late in the season (September- October) commonly on residual moisture (LegesseDadi*et al.,* 2005). According to Geletu Bejiga and AbebeTullu (1982), late sowing is important to avoid the unfavorable effects of water logging, diseases particularly Ascochyta leaf blight and insects. Chickpea is weeded at least once throughout the production season. Chickpea is mainly cultivated without application of fertilizers and herbicides. Pesticides are applied on chickpea fields to control disease or insects only when a specific disease or insect epidemic occurs in a specific location (Legesse Dadi *et al*., 2005). Chickpea harvesting is done by manual labor, either for green pod consumption or for dry seed. Harvesting time extends from October to January for green pods and February to March for harvesting dried seed (Legesse Dadi *et al*., 2005).

3.4. Major limiting factors of chickpea production

Chickpea production is exposed to different a biotic and biotic constrains which penalize seed yields. Some major limiting factors of chickpea production are discussed below. Considerable yield losses occur due to a biotic stresses like drought, salinity, cold and frost. Resistance or tolerance to these stresses is more complex (Salimath*et al*., 2007). The most common a biotic stresses affecting chickpea production are drought (particularly terminal drought), heat and cold. In addition, there are other a biotic stresses specific to some regions such as salinity, water logging, soil alkalinity and acidity, and nutrient deficiencies and toxicities. Drought may cause complete crop failure or a varying amount of reduction in biomass and seed yield (Serraj*et al*., 2003). The major biotic stresses which lead to yield reduction and instability are those caused by fungal, bacterial and viral diseases, insect pests and parasitic nematodes (Ranalli and Cubero, 1997). Ascochyta blight, caused by Ascochytarabiei, is a highly devastating foliar disease of chickpea in West and Central Asia, North Africa, North America and Australia.

It occurs mainly in areas where cool, cloudy and humid weather prevails during the crop season (Singh *et al.,* 2008). Fusarium wilt, caused by Fusariumoxysporum, is the most important root disease of chickpea, particularly in the semiarid tropics where the chickpea growing season is dry and warm (Gaur*et al*., 2007). Viral diseases have been reported to cause sporadic but significant yield loss in some areas. Major symptoms include discoloring (yellow, orange or brown) of foliage, browning of phloem and stunting of growth. Many viruses have been identified that can cause stunt disease (Gaur*et al*., 2007). Viruses isolated from chickpea include Alfalfa Mosaic, Bean Yellow Mosaic, Cucumber Mosaic, Pea Enation Mosaic, Pea Leaf Roll and Pea Streak (Winch, 2006).

Insects especially the gram caterpillar or gram podborer(*HelicoverpaarmigeraHubner*) can cause problems (Winch, 2006). The insect is highly polyphagous and sources with high levels of resistance are not available in chickpea germplasm (Gaur *et al*., 2007). Furthermore, seed beetle or bruchid (*Callosobruchusspp*.) is the most important storage pest of chickpea (Winch, 2006; Gaur *et al*., 2007). Leaf miner (*LiriomyzacicerinaRondani*) is an important insect pest of chickpea in West Asia and North Africa (WANA) and southern Europe. Efforts have been made to identify sources of resistance in the cultivated and wild species (Gaur *et al.,* 2007). Cyst nematode (*Heteroderaciceri*) is another major biotic stress to chickpea (Singh *et al*., 2008).

3.5. Geographical distribution and Ecological occurrence

The first occurrence of BGM on chickpea was reported from India by (Shaw.1915) and later by (Butler *et al* .1931). The first epidemic of BGM was reported by Carranza (1965) in Argentina, which have been reported from many chickpea-growing countries. This disease is very serious concern in India, Bangladesh, Nepal, Pakistan, Australia, and Argentina where yield losses of up to 100% were reported under conducive conditions (Davidson., 2004). BGM has also been reported from Canada, Chile, Colombia, Hungary, Mexico, Myanmar, Spain, Turkey, the USA, and Vietnam (Nene *et al*. 1984; Pande *et al*. 2002). The disease was reached epidemic

proportions in India during the 1978-1979 crop seasons, destroying about 20,000 ha of chickpeas (Grewal and Laha, *et al.*1983). In Nepal, this disease was occurs almost every year, with average yield losses of 15% (Joshi, *et al*. 1992). Also this disease was first documented in Bangladesh during 1981 and reached devastating proportions in 1988, destroying almost all the crop (Bakr and Ahmed, *et al.* 1992). Currently, it is considered the most damaging foliar disease of chickpeas in Bangladesh (Bakr *et al*. 2002).The effects of BGM on pod yield depend on the onset of the disease in relation to crop growth, and disease severity, both of which depend largely on weather conditions and inoculums levels of the pathogen.

Fig.1. Global occurrence of Botrytis grey mould in chickpea

Key Botrytis grey mould reported on chickpea

3.6. Causal organism

The genus of botrytis was first innovated by Micheli (1929), and since that it has become widely known as a group of fungi causing economically and potentially important plant diseases. This is particularly true of those forms which group together as the form of species tend to be concentrated in the temperature regions between 25 & 30 latitude were they occur on a variety of crop plants. The causal organism of BGM of chickpea is botrytis *cinirea* and its teleomorph is *Botryotinia* fuckeliana (de Bary) Whetzel (Grooves and Loveland 1953). The telemorphic state of this fungus has been produced from sclorotia of B.cenirea infecting chickpea in India (sigh 1997). *Botrytis cinerea* is a necrotrophic fungus well known for its extensive host range, wide distribution globally, extreme variability and adaptability to wide range of environmental conditions.

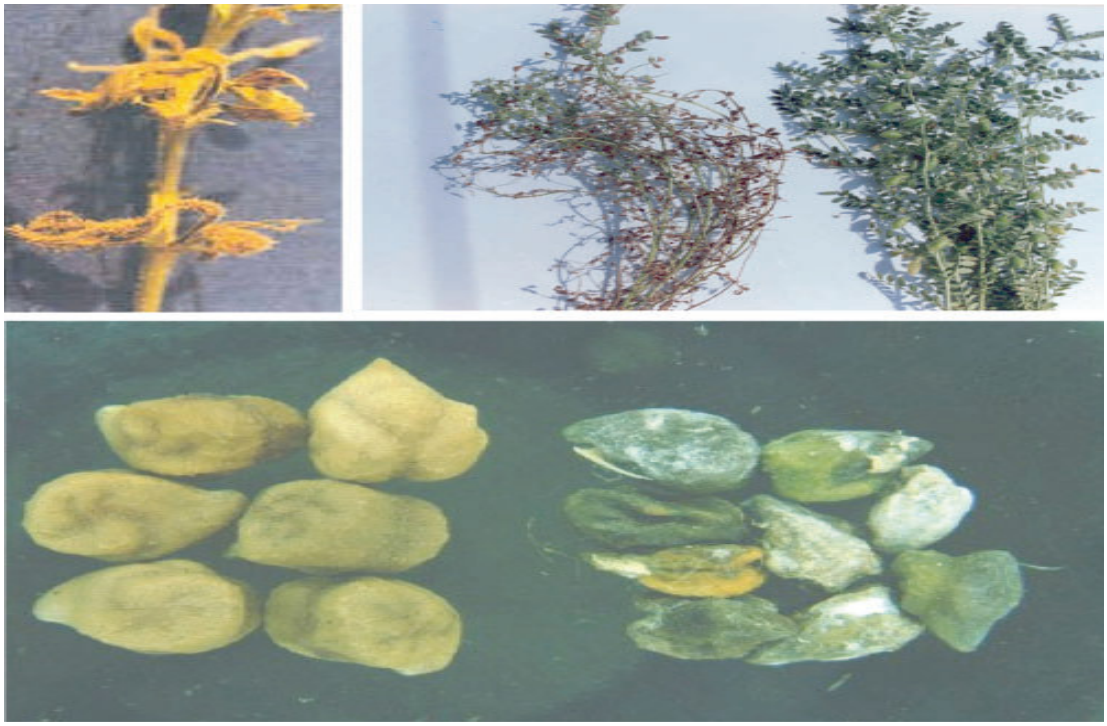

Figure 2**.** Symptoms of Botrytis grey mould (BGM) infection in chickpea on infected flowers, BGM infected and dead plant (left side) in comparison to healthy plant (right side) and seeds harvested from BGM infected pods (left side) and severely infect seed (right side). The asexual stage of the necrotrophic fungus *B. cinerea* (Moniliaceae, Hyphales) is dominant in chickpea crops. *B. cinerea* grown on potato dextrose agar (PDA) has a white, cottony appearance, which turns light grey with age. The young hyphae are thin, hyaline, and 8–16 µm wide, and they become brown and septate with age. Conidia from infected chickpea plants and on PDA measure 4-25×4-18 µm and 4-16×4-10 µm, respectively (Jarvis 1980; Nene and Reddy 1987; Pande *et al.* 2002). Sporodochia formed on the host surface measure 0.5–5.0 µm in diameter and may turn into hard sclerotial masses (Joshi and Singh, *et al*.1969). However, cultural characteristics and sporulation of *B. cinerea* largely depend on and vary with nutrient medium, temperature, and ecological factors. Sclerotia, which germinate asexually by producing conidiophores bearing conidia, can form on crop stubble. The teleomorphic stage of *B. cinerea*, *Botryotinia fuckeliana* (de Bary) Whetzel Family: Pezizales, Ascomycotina) is formed following fertilization of sclerotia with uninucleate micro conidia followed by their exposure to cold temperatures. There are no reports of the sexual state of *B. cinerea* occurring naturally on chickpea stubbles. However, it has been produced under laboratory conditions in India (Singh *et al*. 1997).

3.7. Disease diagnosis

3.7.1. Characteristic symptoms

All the aerial parts of chickpea are susceptible to the disease with growing tips and flowers being the most vulnerable (Bakr and Ahmed 1992; Grewal., 1992). Symptoms of BGM usually become apparent following crop canopy closure (Knights and Siddique., 2002). BGM often appears first as water-soaked lesions on the stem, near ground level, that extend along the stem, and lead to infection of other stems (Knights and Siddique., 2002). These lesions may be 10-30mm long and completely girdle the stem. Branches break off at the rotting point and the affected leaves and flowers turn into a rotting mass (Bakr., 2002; Pande., 2002). Initially, the disease is randomly distributed within a crop, with infected plants being scattered, with yellowing or dying branches, or if the lesions are at ground level, as scattered dead plants Drooping of the affected terminal branches is a common field symptom and branches may break off at the point of infection (Grewal *et al*. 1992). The fungus can form grey or brown to light brown lesions on leaflets, branches, and pods, covered with hairy sporophores and masses of single celled, hyaline spores (Haware and Mc Donald, 1992). Lesions on pods are water-soaked and irregular. Sometimes tiny black sclerotia are formed on dead tissue (Nene *et al*. 1991). Grey fungal growth and profuse sporulation will occur if conditions within the canopy are moist or humid and rapidly spread through the canopy resulting in patches of dead plants (Knights and Siddique., 2002). Flower drop is common leading to poor pod formation and low grain yields and often undetected unless the crop is closely monitored. Depending on the site of infection, mature seeds from diseased plants may be shrunken, dark coloured or, when the fungus has invaded the pod, the seeds are covered in a white/grey fungal mat (Tripathi, *et al* 1998).

Figure 3**.** Groups of plant infected by BGM appears yellow patches in the Crops

3.7.2. Seedling rot

The pathogen *Botrytis cinerea* is one of the many fungi associated with seedling disorders of chickpea creating a soft rot (Burgess *et al*. 1997). In most chickpea growing regions of the world foliar infection is considered most important such as in Australia, soft rot of young seedlings resulting from seed borne infection is also important and can result in total crop failure (Burgess *et al*. 1997). Symptoms include poor emergence, yellowing, wilting, and death of seedlings and pale yellow to light discoloration of the tap root. Most plants that develop soft rot become flaccid and then die within a few days. Plants seldom recover from the disease.

3.8. Epidemiology of Pathogen survival

3.8.1. Seed infection

Botrytis cinerea survives on chickpea seed without any visible symptoms for at least 5 years and may be internally or externally seed borne found it to be largely external (Grewal, 1983). The survival period on seed is affected by the storage temperature the longest being up to 5 years at 5◦C to 10◦C and relative humidity found that survival of the pathogen on chickpea seed was reduced from 95 to 2% after 12 months storage at 20◦C Heating moist seed to 50◦C for 5 min resulted in significant reduction in viable seed infection (Burgess, 1997: Pande., 2002). Seed from diseased plants may not show external symptoms and a laboratory seed-testing procedure is required for detection of the fungus (Haware *et al*. 1986). Seed infection levels up to 95% have been recorded from diseased crops (Burgess *et al*. 1997). Seed-borne inoculums appears to be most important under Australian conditions and seeds with infection level greater than 5% are considered unsuitable for planting (Wright *et al* 2000).

Figure 4a. Infected seeds are usually smaller than normal and are often covered with white to grey fungal growth.

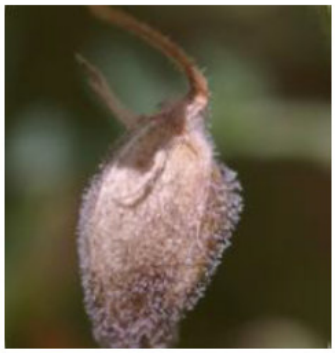

Figure 4b. Stems and on dead Leaves and petioles.

3.8.2. Plant debris and soil

Studies conducted at ICRISAT, Patancheru, showed that infected chickpea leaves decomposed within a few months but the stems took considerably longer (Haware and McDonald., 1992). In India, the pathogen survives on plant debris on the soil surface for up to eight months is considered the main source of primary inoculums (Metal 1986; Singh and Tripathi., 1993). The pathogen also survives in the soil as mycelia and sclerotia (Sinha *et al.* 1990). In Western Australia, *B. cinerea* remained viable for 9-11 months in the previous season's chickpea stubbles and survived over the hot greater than 35◦C, dry conditions of summer and through the following growing season (Galloway *et al*. 2004). Asexual sporulation of the fungus occurs on this stubble under warm

greater than 20◦C, moist conditions associated with prolonged periods of high relative humidity (Galloway *et al.* 2004). Spores can be blown several hundred meters from their source indicating that plant debris could also be a major source of primary inoculum for BGM in Australia, similar to India (MacLeod and Sweetingham *et al.* 2000). The fungus *B. cinerea* has been reported to survive in the soil in India even at a depth of 0.10-0.25m at 40◦C, from one crop season to the next, both in the form of mycelium and sclerotia (Singh and Tripathi 1992), whereas in Australia, *B. cinerea* did not survive on chickpea stubble buried at a depth of 0.5m (Galloway and MacLeod, 2003).The fungus *B. cinerea* is known to produce sclerotia on crop stubbles of many host species.

The sclerotia are thought to be the main means of the fungal long-termsurvival (Coley- Smith 1980). In Europe, apothecia emerge from the fertilized sclerotia and wind-dispersed ascospores are released mainly in the spring after chilling and periods of high rainfall on *Vicia* beans (Harrison *et al.* 1988). Sclerotia develop on the previous season's chickpea stubble in Australia after exposure to cold greater than 10◦C winter temperatures. As day-time temperatures increase in spring, sclerotia germinate asexually, forming conidia on conidiophores. The sclerotia remain viable for the rest of the growing season but do not survive the following hot and dry summer conditions hence, sclerotia are not considered to be a means of long-term survival in Australia (Galloway and MacLeod 200.). Chlamydospores of *B. cinerea* are formed in response to drought, nutrient and oxygen deficiency, attack by bacteria, and pH alterations. The chlamydospores germinate to produce mycelium, which either directly or after Production of macro conidia, serves as secondary inoculums (Urbasch., 1986).

3.8.3. Alternative hosts

Due to the wide host range of this pathogen, the role of alternative hosts is likely to play an important part in survival from one chickpea crop to another (Coley- Smith 1980; Knights and Pande., 2002). However, further studies are required to understand the host-specific pathogenicity of *Botrytis* isolates of chickpea.

3.9. Disease development

There is a wealth of literature available on the temperature and relative humidity requirements of *B. cinerea* on many crops of importance. It should, however, be noted that the temperature and relative humidity requirements for *B. cinerea* appear to be influenced by the host plant and even by the plant part being infected (Elad., 1992). On chickpeas, the optimum temperature for sporulation and conidial germination is 25◦C (Mahmood and Sinha 1990; Singh., 1997) and 20◦C respectively, with 5◦C and 30◦C being the minimum and maximum extremes for conidial germination. However, different isolates were found to require differential light intensities and relative humidity for conidial germination (Rewal and Grewal., 1989).BGM may develop rapidly over time and space, depending on the environmental conditions. Relative humidity, leaf wetness, and temperature are the most important factors (Pande *et al*. 2002). That disease increased at temperatures of 17–28◦C and 70–97% relative humidity (Bakr and Ahmed.,.1992). In Bangladesh, maximum disease severity was recorded at a temperature range of 20–28◦C and 25–30◦C, (Reddy., 1990; Tripathi and Rathi 1992). In the Indian sub-continent, BGM epidemics have occurred in years with high rainfall and a high number of rainy days (Tripathi & Rathi, 1992). The duration of leaf wetness appears to have some influence on the development of BGM on chickpeas. Disease severity increased with leaf wetness periods greater than 12 h/day (Singh and Kapoor.,1984). The epidemics can spread rapidly at 95% or above relative humidity and up to a maximum temperature of approximately 25◦C in a dense crop canopy.

3.9.1. Host range

Botrytis cinerea is a non-specialized pathogen well known for its global distribution and extensive host range of more than 100 plant species from different genera including ornamental Plants, vegetables, fruit, field and glasshouse crops, several weeds, and post-harvest produce. The host range includes species such as black gram, strawberry, grapevine, apple, cabbage, carrot, cucumber, eggplant, lettuce, lentil, mung bean, mustard, paddy, pea, pepper, pigeonpea, squash, tomato, chrysanthemum, dahlia, lily, rose, gladiolus, and tulip (Chand *et al.*1997). *B. cinerea* isolated from chickpea, infected 8 crops and 21 weed species under artificial inoculation conditions (Rathi and Tripathi, 1991.). Tested *B. cinerea* from chickpea on 20 plant species from 17 families under greenhouse conditions and found it infecting peas and 7 weeds, none of which was a previously reported host (Meeta, *et al*.1988).

3.9.2. Pathogen variability

The pathogen *B. cinerea* is reported to have extreme variability and adaptability to a wide range of environmental conditions. Joshi and Singh (1969) and Singh (1970) have been observed the formation of sclerotial and/or sporodochial bodies on *B. cinerea*-infected chickpea plants in the Tarai region of Nainital, India, which were not found later from the same area (Pandey. 1988). Singh and Bhan (1986) and Rewal and Grewal (1989) have been identified 4 and 5 patho types, respectively, among the *B. cinerea* isolates collected from northern India differentiated 8 chickpea isolates of *B. cinerea* collected from Nepal into distinct path types based on their morpho cultural characters and reaction on 39 differential lines (Kishore.,2005). Molecular markers such as micro satellites are powerful tools for accurate detection of genetic diversity because they are highly polymorphic across numerous loci and are reproducible. In chickpea, microsatellites have revealed genetic

variation among isolates of *Ascochyta rabiei* (Ph.an *et al*. 2003). A recent study that used microsatellite DNA markers developed specifically for the *B.cinerea genome* revealed genetic variation in *B. cinerea* isolates of chickpea from 4 regions in Bangladesh, India, and Nepal (Isenegger., 2005). Furthermore, hierarchical sampling of field sites in Bangladesh elucidated the level of genetic variation at various spatial scales. Consequently, high genetic diversity was determined within and among sub populations and was detected in the smallest spatial scale sampled within field sites (1-2 m). Multi locus microsatellite profiles showed considerable genotypic diversity and discriminated up to 50% of isolates examined within a field. Evidence for a mixed reproductive system and gene flow was revealed within and among sub- populations (Isenegger, *et al*. 2005). Isolates from all subpopulations from Bangladesh showed potential for a highly adapted pathogenic group to chickpea, which can threaten (or break down) long-term control with fungicides and durable host resistance. Ardley and Weichel (2005) have been differentiated *B. cinerea* isolates from different hosts based on the presence of transposable elements. Transposable element *Flipper* was found in lettuce and grapevine isolates, whereas, transposable element *Botrytis* was present in grapevine, chickpea, and lentil isolates. Genetic similarity of internal transcribed spacer (ITS) regions indicated that lentil and chickpea isolates were closely related. Previously, molecular evidence revealed the role of genetic recombination in *B. cinerea* from grapevine in France (Giraud*et al*. 1997). This is important, as genetic recombination can generate new genotypes, hence genetic diversity can spread quickly via asexual conidia. In other studies in *B. cinerea*, molecular markers have revealed high genetic diversity and high gene flow among populations from vegetable crops in Europe (Alfonso 2000 & Moyano., 2003).

Elucidating the genetic structure by measures of genetic diversity within and among fungal pathogen populations is of major importance as it infers adaptive potential that can hamper disease management based on fungicide and host resistance. Indeed, fungal populations with high genetic diversity, mixed reproductive systems, and gene flow are considered to be highly adaptable and therefore pose a risk of rapid breakdown of host resistance (McDonald and Linde 2002). Furthermore, the durability of resistance will depend on genetic control such as single gene or quantitative resistance, and deployment strategies that would require consideration on a regional to multi-regional scale (McDonald and Linde .2002).

4. Disease management

4.1. Cultural methods

Using pathogen-free seed can reduce seed transmission of the disease. Practices such as manipulating sowing dates, using erect cultivars, and lower plant densities are helpful in reducing the level of BGM in chickpeas (Haware *et al*. 1998). Late sowing reduces the vegetative growth and hence lowers disease incidence. However, this can also lead to reduced grain yield (Karki, 1993). Wider row spacing allows for more aeration of the crop canopy and reduced periods of leaf wetness and relative humidity, which in turn reduce the disease incidence (Pande *et al*. 2002). Increased plant spacing in paired rows and intercropping with linseed (Reddy *et al*. 1990) have been reported to reduce the disease and increase grain yield. Foliage de topping increases the duration and intensity of light to the lower canopy and makes the microclimate unfavorable for disease development (Rathi and Tripathi *et al*. 1995). Similarly plants with erect and compact growth habits show lower disease incidence than bushy spreading genotypes due to improved aeration (Sethi, 1993). Erect chickpea types may escape the disease as the open canopy allows air movement and an early drying of the foliage after rainfall (Haware, 1998). Crop lodging exacerbates disease through poor canopy ventilation and genotypes with different lodging susceptibilities suffer different levels of BGM (Knights and Siddique *et al*. 2002). In addition, crop rotations, burning infected debris, and deep ploughing reduce inoculum levels.

4.2. Chemical methods

4.2.1. Fungicides

Seed treatments with fungicides, *viz*.iprodione, mancozeb, thiabendazole, triadimefon, triadimenol, vinclozolin, thiram, benomyl, carbendazim, or captan are effective in reducing seed infection (Pande *et al*. 2002 and Davidson *et al*. 2004). Foliar sprays, used at regular intervals with the first appearance of the disease, can control an epidemic in the crop (Pande *et al*. 2002), particularly when used in combination with a seed-dressing fungicide (Grewal and Laha *et al*. 1983). Effective fungicides used as a foliar spray 50 days after sowing or with the first sign of the disease include captan, carbendazim, chlorothalonil, mancozeb, thiabendazole, thiophanate-methyl, thiram, triadimefon, triadimenol, or vinclozolin (Singh and Kaur 1990;Pande *et al*. 2002; Davidson *et al*. 2004). Sometimes multiple sprays are recommended, although generally one spray at flowering followed by another 10 days later on a moderately resistant chickpea cultivar provides the best protection against BGM on chickpea (Pande *et al*. 2002). Disease prediction models facilitate the timely application of fungicides for effective and economical disease control. An early appearance of disease preceding this period helped in initial inoculum build up and rapid disease take-off, if the subsequent conditions were favorable. A function of these 2 variables is used as a basis for a predictive scheme to schedule fungicide sprays for managing BGM (Pande *et al.*

2005). However, the use of fungicides has not been widely adopted by resource poor farmers in Asia and hence integrated management of BGM is encouraged using agronomic practices, erect cultivars, biological control agents, and targetted fungicidal sprays (Haware and McDonald 1992, 1993; Bakr *et al*. 2002; Pande *et al*.2002, 2005).

4.3. Biological control

Although repeated fungicide application can not alone achieve the effective management of BGM in chickpea. If the conditions is not are favorable for disease development biological control of B. *cinerea* using species of *Trichoderma* has been reported in some fruit and vegetable crops (Tronsmo 1986, Nels and Powels 1988, Elad 1994). Integrated a biocontrol agent with sub lethal dose of fungicide seems to be very promising in controlling plant pathogens without disturbing the biological equilibrium.

Table 1. Effect of *T.viride* and *Ranilan* on flower drop, area under disease progress curve (AUDPC), and grain yield of chickpea.

Treatments	Rampur ,Chitwan 1996			RARS, Tarahara 1997		
	Flower drop %	AUDPC	Grain yield (ton/ha)	Flower drop %	AUDPC	Grain yield (ton/ha)
control	17	631.0	0.39	43.6	2.0	1.3
Two sprays of *T.viride*(10^7-10^{10} spore ml^{-1})	18.3	370.5	0.42	31.0	1.1	1.2
Three sprays of T.viride	20.6	503.0	0.35	37.7	2.0	1.4
Two sprays of *Ranilan** (0.1%)	24.3	402.5	0.37	39.3	1.15	1.2
Three sprays of *Ranilan* *	16.6	303.6	0.38	35.9	2.2	1.2
Three sprays of *T.viride+Ronilan**	14.6	376.3	0.42	37.5	2.0	1.2
LSD (p=0.05)	NS	NS		NS	NS	

Source: Australian Journal of Agricultural Research 2006.

A set of fungicides was evaluated a long with biological control agents to be Include in the integrated management of BGM. A collaborative study involving ICRISAT was conducted at GLRP, Rampuper , during 1995/96 and at RARS, Tarahar a , during 1996 / 97 using chickpea cultivars Sita. The study had six treatments : (1) three sprays of *Trichoderma viride (107 - 1010 spor e s mL- 1) , (2) two* sprays of T. viride , (3) three sprays of Ronilari® or Bavistin®, (4) two sprays of Ronilan®, (5) three sprays of T. *viride + Ronilan®, and (6) control (water spray)* . No significant differences were observed among the different treatment combinations. However, three sprays of Ranilan® gave the best results in reducing disease severity (as measured by the are undre disease progress curve : AUDPC) followed by the three sprays of *T. viride and Ronilan® (Table 2).*

3.3.1. Host plant resistance

3.3.2. Screening for disease resistance

Different screening techniques have been used for screening the germplasm for BGM resistance under *in vitro*, greenhouse, and field conditions (Rewal and Grewal, 1989).The cut-twig technique developed by Singh *et al*. (1998) offers a non destructive sampling of the plants and is particularly useful in wide hybridization programs. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, a unique facility has been established for chickpea BGM screening under controlled-environment conditions (CEC) in a growth room. Ten-day-old seedlings of the test genotypes grown in plastic Trays (45 by 30 by 5 cm), filled with sterilised sand and vermiculite (4:1) and placed in a greenhouse at 25±2◦C, along with susceptible checks H 208/JG 62, are used for artificial inoculation. *B. cinerea* was multiplied on autoclaved flowers of *Tagetus erecta* (marigold) for 8 days at 25◦C and 12-h photoperiod. Conidia from the profusely sporulating culture are harvested into sterile distilled water and a conidial suspension at the concentration of 3×105 conidia/mL is Used as inoculum. Greenhouse-grown seedlings of the test genotypes and susceptible check are transferred to CEC in a growth room 24 h before inoculation. These are uniformly sprayed with the inoculum. The growth room is maintained at 15±2◦C and 95-100% RH with a 12 photoperiod of 2500-3000 lux intensity. The severity of the disease in all the test genotypes is recorded on a 1-9 rating scale (Table 1) after 14 days of inoculations or when the disease severity in the Susceptible check reaches 9. with high levels of resistance (Gurah *et al.* 2003; Davidson *et al*. 2004). Several wide and intraspecific hybridizations have been carried out to transfer the identified disease resistance in wild types and land races to commonly adopted and widely grown chickpea cultivars. Through these breeding programs a few interspecific hybrids with moderate levels of resistance to BGM and desirable agronomic traits have been identified (Singh *et al*.1998). Further details of other chickpea lines derived from the wide hybridization and their resistant parents were provided by Pande *et al*. 2002.

Table.2. Host-plant resistance against BGM infection in chickpea as determined by screening programs conducted in various countries

Resistance	Genotype	Reference
Wild species	ILWC35/S-1(C.echinospermum) and ILWC9/S-1 (C.pinnatifidum)	Singh *et al.* (1991)
	C.judaicum189,C.pinnatifidum 199, C.pinnatifidum , ILWC9/S-1,C.bijigum ILWC9/S-1 C.bijigum ILWC7/S-1, C.echinospermum ILWC35/S-1 and C.echinospermum ILWC9/39	Singh *et al.* (1998)
Land races		Singh and Kant (1999)
	GPC 14, HIMA, and P 6223	ICC 1069, 6250, 7574, and 10302 Rathi *et al.* (1984)
	ICC 1069, 6250, 7574, and 10302 ICC 466, ICC 478, ICC 662, ICC 755, ICC 756, ICC 799,	Tripathi and Rathi (2000)
	ICC 800, ICC 1069, ICC 1591, ICC 7574, ICC 10302,ICCL 87322	Singh and Kaur (1989)
	GL 84212 and ICC 1905	Pandey *et al.* (1982)
	GNG-3, C-235, and BG-249	Singh and Kapoor (1985
	P 919, CPI 56566, JM 995, and E 100 Y	

Source: Australian Journal of Agricultural Research 2006.

4.4. Integrated disease management

Effective management of BGM is very important or it will cause heavy damage to the chickpea crop. Since a high level of resistance to BGM in cultivated chickpea is not available, whatever resistance is available needs to be combined with management options that will minimize the disease. Although repeated application of fungicides can control BMG it may not be practicable for resource poor farmers in the main seasons of chickpea in BGM endemic areas of south Asia and could result in production of new/ resistant strains of fungus (Bhan and Chatrath, 1994).

Hence, integrated disease management (IDM) using the available management options is essential to successfully manage the disease and mitigate yield losses. Chemical control of BGM combined with wider row spacing (Reddy *et al*. 1993) or the use of *T. viride* (Agarwal *et al*. 1999) as a biocontrol agent has been attempted. Ahmed *et al*. (2002) reported that use of tolerant genotype ICCL 87322 in combination with wider row spacing and spraying with bavistin was the best combination followed by the use of the tolerant genotype ICCL 87322 in combination with wider row spacing and intercropping with linseed. Judicious use of fungicides as a seed treatment and/or foliar spray in an IDM system could be economical and affordable to the resource poor farmer. IDM program involving cultivation of a BGM tolerant genotype Avarodhi, soil application of diammonium phosphate, wider row spacing (0.60 m), seed treatment with carbendazim+thiram (2g/kg seed), and need-based Foliar application of carbendazim has been devised. Integrated disease management (IDM) of BGM in Bangladesh consisted of a BGM-tolerent cultivar such as Barichola 5 or ICCL 87322, lower seed rate (37.5 kg/ha), fungicide seed treatment, delayed sowing, and need-based foliar application of fungicides. Mean grain yield in IDM plots was 678–1610 kg/ha compared with 450-1373 kg/ha in non-IDM plots. Growing chickpea was found to be economically more viable than any other crop grown after rice, especially in rainfed rice fallows (Bakr *et al*. 2005; Pande *et al*. 2005).

4.4.1. Gene plant technology for BGM resistance

For gene technology to be effective in delivering new traits such as BGM resistance in chickpea, the development of reliable and efficient regeneration and transformation systems is essential. In addition, cloned and characterized genes that confer antifungal activity on *B. cinerea* are of particular importance. Thus, the expression of genes with antifungal metabolites is a feasible approach for BGM resistance in advanced breeders' lines or cultivars of chickpea. A range of antifungal proteins, such as the fungal cell-wall degrading hydrolytic enzyme chitinase, have been demonstrated to suppress fungal growth of *B. cinerea* within leaf tissue in transgenic plants such as tobacco (Kishimoto *et al*. 2002), cucumber (Tabei *et al*. 1998), and *Dendranthema morofolium* (Takatsu *et al*. 1999 *et al*. 1999). Interestingly, the expression of an iron-binding protein, ferritin, from alfalfa showed improved protection from the oxidative damage that was caused during necrosis by *B. cinerea* infection (Deak *et al*. 1999).

5. Conclusion and Recommendation

Botrytis grey mould (BGM), caused by *Botrytis cinerea* Pers. ex. Fr., is an economically important disease of chickpea (*Cicer arietinum* L.), especially in areas where cool, cloudy, and humid weather persists. The pathogen *B. cinerea* mainly survives between seasons on infected crop debris and seeds. Despite extensive investigations

on pathological, physiological and molecular characteristics of *B. cinerea* causing grey mould type diseases on chickpea and several other hosts, the nature of infection processes and genetic basis of pathogen variability have not been clearly established. For gene technology to be effective in delivering new traits such as BGM resistance in chickpea, the development of reliable and efficient regeneration and transformation systems is essential. Thus, the expression of genes with antifungal metabolites is a feasible approach for BGM resistance in advanced breeders' lines or cultivars of chickpea. Chemical control of BGM combined with wider row spacing or the use of *T. viride* (Agarwal As a bio control agent has been attempted. Disease prediction models facilitate the timely application of fungicides for effective and economical disease control. Biological control options for BGM management should be further exploited. In chickpea, BGM is a devastating disease and extensive studies on the biology of the pathogen and screening programs to identify host-plant resistance have failed. Despite the extensive investigations in other hosts, the infection process of *B. cinerea* on chickpea has not been Studied. Also, very little is known about the resistance mechanisms of chickpea against *B. cinerea*. Knowledge of the infection process and host defense mechanisms will help in devising management strategies for BGM. Resistance to BGM identified in wild *Cicer* spp. should be transferred to land races through wide hybridization programs. BGM management should not completely rely on the use of fungicides, as development of fungicide resistance in *B. cinerea* has been commonly observed. Hence, IDM programs suitable for adoption by resource poor farmers should be emphasised. It is advised that BGM management in chickpea should be based on the location specific disease predictive models. Farmers' participatory on-farm validation of the IDM programs, extension, and seed distribution systems should be the tools for promotion of IDM programs developed at research centres. Biological control options for BGM management should be further exploited. Transgenic plant technology using PGIPs and other antifungal proteins could be the possible approach for imparting disease resistance to commonly adapted cultivars in the future. Therefore, IDM programs suitable for adoption by resource poor farmers should be emphasized.

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