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Effect of growth stages of chickpea on the genetic resistance of Ascochyta blight

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Abstract Ascochyta blight (AB, *Ascochyta rabiei* (Pass.) Lab.) is one of the most important foliar disease of chickpea (*Cicer arietinum* L.), globally. Chickpea is attacked by AB at any growth stage in cool and humid weather depending on the inoculum availability. However, the disease epidemics are most prominent during the flowering and podding growth stages. The main objective of this study was to determine the effect of growth stages of chickpea on the genetic resistance of AB and use this information in a resistance breeding program. Two susceptible and two moderately resistant chickpea cultivars were spray inoculated at seedling (GS1), post-seedling (GS2), vegetative (GS3), flowering (GS4) and podding (GS5) growth stages with *A. rabiei* conidial suspension under controlled environment conditions. Irrespective of crop cultivars the incubation period (IP) was shorter in GS1, GS4 and GS5 and was significantly extended in GS2 and GS3. Symptom development was delayed significantly in moderately resistant cultivars. The AB severity 10 days after inoculation ranged between 7 and 9 on susceptible cultivars and 3 and 5 on moderately resistant cultivars. Further the correlation coefficient of disease severity between GS1, GS4 and GS5 was highly significant (r=0.95) indicating that, evaluation for resistance to AB can be done at GS 1 (seedling stage), and or GS4 (flowering stage) to GS5 (podding stage) growth stages of chickpea. This supports the evaluation for AB resistance using 10-day-old-seedlings in controlled environment at ICRISAT and adult plant field screening at hot-spot locations in Dhaulakuan and Ludhiana in India.

Keywords *Ascochyta rabiei* · *Cicer arietinum* · Host plant resistance and Plant age

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Introduction

Chickpea (*Cicer arietinum* L.) is the world's third most important grain legume crop currently grown over about 11.12 million ha with 96% of the cultivation in the developing countries. India accounts for approximately 64% of the world chickpea production (FAO 2008). It is a major source of high quality protein in human diets and is a significant contributor to agricultural sustainability through atmospheric nitrogen fixation. Despite the large acreage under chickpea cultivation, the total production is quite low in most of the chickpea growing countries and a wide gap exists between the potential yield (5 tha^{-1}) and the actual yield (0.8 tha^{-1}) (FAO 2008). A primary cause for low yields of chickpea is the susceptibility of crop to a number of diseases such as Ascochyta blight, Botrytis gray mould, Fusarium wilt and root rots that affect the plant from seedling stage till harvesting.

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Lab. [teleomorph, *Didymella rabiei* (Kov.) v.

Arx], is one of the most important foliar diseases of chickpea and causes serious seed yield and quality losses, globally (Kaiser et al. 2000; Gaur and Singh 1996; Pande et al. 2005; Gan et al. 2006). It affects both desi and kabuli chickpeas equally. Over 80% of the world' chickpea area is under desi type and the countries where desi chickpea is widely grown and affected by AB include northern India, Pakistan, Australia, Canada and Ethiopia. Chickpea cultivation in Australia and Canada has been limited by outbreaks of AB because available commercial varieties became susceptible to the disease (Knights and Siddique 2002).

Symptoms of AB develop on all above-ground parts of the plant and are characterized by tan-coloured necrotic lesions with dark margins. Pycnidia develop within the lesions, often forming concentric rings around the infection site (Nene and Reddy 1987). Chickpea is attacked by AB at any growth stage in cool and humid weather (15–25°C and >150 mm rainfall during the crop season from October to April) depending on the inoculum availability. However, the disease epidemics are most prominent during the flowering and podding growth stages (Reddy and Singh 1984). Higher susceptibility of chickpea to AB in the reproductive growth stages may be due to senescence of the older tissues and or to environmental conditions becoming favourable for disease development. Plant age had been reported to have no impact on disease incidence in some cultivars (Trapero-Casas and Kaiser 1992). However, in others, it has been reported that resistance declines as the plant matures (Chongo and Gossen 2001; Gan et al. 2006; Nene and Reddy 1987). This change from resistance to susceptibility with maturity refutes the importance of resistance as the main strategy for managing this disease. Hence, information on resistance of chickpea to blight at different growth stages is essential for selecting the suitable stage for inoculating plants for screening for AB resistance. Therefore, the main objective of this study was to determine the effect of growth stages of chickpea on the genetic resistance to AB. This information will be used in a resistance breeding program.

Materials and methods

Cultivars

Four chickpea cultivars, two highly susceptible (ICC 4991 and ICCV 10) and two moderately resistant (ICCV 05562 and ICCV 04512) were used in the study. ICC 4991 (Pb 7), highly susceptible, is an old cultivar from Punjab (India) and ICCV 10 (ICC 15996) is a wilt resistant and widely adapted cultivar in India (Basandrai et al. 2007). ICCV 05562 (ICC 1069×ILC 3279) and ICCV 04512 [(C 235×NEC 138-2)×(FLIP P 87-4C×ILC 4421)] are AB moderately resistant ICRISAT breeding lines.

Pathogen

A single conidial isolate of *A. rabiei* from a hot spot location (where disease occur under natural environmental conditions) for AB in India, Hisar, Haryana (29º 10′ N latitude and 75º 46′ E longitudes) was used in the study. The isolate is highly virulent and was maintained on chickpea dextrose agar medium at 4°C.

Growth stages

Plants of the four test chickpea cultivars differing in their susceptibility to AB were raised in plastic pots (25 cm diameter) filled with a sterilized mixture of sand and vermiculite (3:1) and placed in the greenhouse at 25±3°C and 12 h photoperiod. Sowings were staggered to obtain plants at five growth stages; seedling (GS1-12 days old), post-seedling (GS2-24 days old), vegetative (GS3-36 days old), flowering (GS4-48 days old) and podding (GS5-72 days old) for inoculation at the same time.

Inoculation and incubation

Pots with plants of different growth stages were transferred to the controlled environment facility (specifically created for screening chickpea for AB resistance at ICRISAT) maintained at 20 ± 1°C and ~ 1500 lux light intensity for 12 h a day and allowed to acclimatize for 24 h. Components of controlled environment facility such as temperature (20±1°C), relative humidity (100%), photoperiod (12 h), inoculum concentration (5×10^4 conidia /ml) required for penetration, infection and colonization of *A. rabiei* were maintained. The *A. rabiei* inoculum for spray inoculation was mass multiplied on Kabuli chickpea seeds (Nene et al. 1981). For inoculum preparation, Kabuli chickpea seeds were soaked overnight in water and 50 g of these seeds were transferred in 250 ml flasks. Flasks were sterilized by autoclaving at 121°C

(15 psi) for 25 min. Highly sporulating inoculum of *A. rabiei* grown on chickpea dextrose agar was transferred aseptically on to the seeds in the flask. The inoculated flasks were incubated at 20±1°C with 12 h alternate light and dark period for eight days. The flasks were frequently shaken to avoid clumping of inoculum. After 8 days, conidia were harvested in sterilized distilled water. The plants were inoculated by spraying a conidial suspension (5×10^4 conidia/ml) of *A. rabiei* till run-off. The inoculated plants were allowed to partially dry for 30 min to avoid dislodging of spores and thereafter 100% relative humidity (RH) was maintained continuously for 4 days and then reduced to 6–8 h a day till the completion of experiment (Pande et al. 2005). An uninoculated control (sprayed with sterile distilled water) for each growth stage and cultivar was kept under the similar environmental conditions in the same controlled environment facility.

Design of experiment

Each treatment (four cultivars and five growth stages) was replicated four times and four pots (five seedlings /pot) constituted a replication. The pots were arranged in a randomized complete block design. The experiment was repeated twice following the same number of replication and experimental design.

Data recording and analysis

Data on incubation period (time from inoculation to the appearance of first symptoms) was recorded everyday and disease severity was recorded 3, 5, 7 and 9 days after inoculation (DAI) on a 1–9 rating scale where 1= no visible symptoms, 2=minute lesions (2 mm) prominent on the apical stem, 3=lesions up to 5 mm size and slight drooping of the apical stem, 4=lesions obvious on all plant parts, and clear drooping of apical stem, 5= lesions obvious on all plants/parts, defoliation initiated and breaking and drying of branches slight to moderate, 6=lesions as in 5, defoliation, broken, dry branches common, some plants killed, 7=lesions as in 5, defoliation, broken, dry branches very common, up to 25% of the plants killed, 8=symptoms as in 7 but up to 50% of the plants killed and 9=symptoms as in 7 but up to 100% of the plants killed.

The data of repeated measurements on disease severity was subjected to statistical analysis using SAS Proc Mixed first order ante-dependence model ANTE (1) (Littell et al. 2006). The ANTE (1) model is used where observations are taken repeatedly on a single unit over various intervals. The model assumes that the variance among observations changes over time and correlation between pairs of observations is the product of the correlations between adjacent times between observations, so that correlation may change over time. Analysis of variance and correlation coefficient between different growth stages was also calculated using GENSTAT 12th Edition.

Results

Incubation period

Irrespective of crop cultivars, the IP was shorter in GS1 (seedling), GS4 (flowering) and GS5 (podding) growth stages and was significantly ($P<0.05$) extended in GS2 (post-seedling) and GS3 (vegetative) stages. IP was shortest in susceptible cultivar ICC 4991 (2.8 days) and ICCV 10 (2.9 days) at GS5 and was statistically at par with the GS4 (3.0 and 3.1 days) and GS1 (3.1 and 3.4 days) growth stages, respectively. However, at post-seedling (GS2) and vegetative (GS3) stage IP was significantly prolonged and varied between 3.8 and 4.3 days. Symptom development was significantly prolonged in resistant cultivars by 2–3 days as compared to susceptible ones. ICCV 05562, moderately resistant cultivar showed the longest incubation period (5.0 days) at GS2 growth stage and was statistically at par with ICCV 04512 (4.9 days) (Table 1).

Disease severity and dynamics of disease development

The AB severity differed significantly ($P<0.001$) among susceptible and resistant cultivars; at 9 DAI, it was between 7 and 9 on susceptible cultivars and 3 and 5 on moderately resistant cultivars (Table 2). Irrespective of cultivars, disease severity was statistically at par at GS1, GS4 and GS5 growth stages and was significantly more than GS2 and GS3 stages (Fig. 1). On susceptible cultivar (ICC 4991), disease severity was very high (9 on 1–9 rating scale) at all the growth stages except at GS2 stage where it was 7 (Table 2). However, AB severity on resistant cultivars

Table 1 Effect of plant age and chickpea cultivar on the length of incubation period of Ascochyta blight

Growth stage	Incubation period (Days)					
	Susceptible			Resistant		
	ICC 4991	ICCV 10	Mean	ICCV 05562	ICCV 04512	Mean
GS1	3.1	3.4	3.2	4.3	4.5	4.4[a*]
GS2	3.9	4.3	4.1	5.0	4.9	4.9[c]
GS3	3.8	4.0	3.9	4.5	4.6	4.5[ab]
GS4	3.0	3.1	3.0	4.3	4.3	4.3[a]
GS5	2.8	2.9	2.9	4.2	4.2	4.2[a]

*Means with the same letter are not significantly different

LSD ($P<0.05$) Growth stage=0.36; Cultivar=0.26; Growth stage×cultivar=0.15

(ICCV 05562 and ICCV 04512) was significantly lower (3.0 on 1–9 scale) at GS2 and varied between 4 and 5 at other growth stages. In addition, on susceptible cultivars the number of lesions per stem increased as plants matured. However, in comparison to susceptible cultivars, lesions were smaller and evenly distributed on stems and leaves on resistant cultivars at all the growth stages.

AB severity of the test cultivars at different growth stages after 3, 5, 7 and 9 days of inoculation is given in Fig. 2. Repeated measurement analysis suggested that there was a clear difference between disease development on susceptible and resistant cultivars. ANOVA showed that all effects, i.e. treatment (cultivar×growth stage), days after inoculation and their interaction were highly significant ($P<0.0001$). Disease development was faster on susceptible cultivars and progressed more quickly at GS1, GS4 and GS5 as compared to GS2 and GS3. Irrespective of growth stage, there was a significant difference in the development of disease ($P<0.0001$) 3, 5 and 7 DAI on susceptible cultivars. However, the difference was non-significant (P values varied from 0.0011 to 0.50) 7 and 9 DAI. On the other hand, in resistant cultivars, the difference in disease severity was significant 3 and 5 DAI ($P<0.0001$), but was non-significant between 5, 7 and 9 DAI and P values varied from 0.0011 to 1.0.

The correlation coefficient of disease severity between GS1, GS4 and GS5 was highly significant ($r=0.95$) indicating that, evaluation for resistance to AB at GS1 (seedling stage), and or GS4 (flowering stage) to GS5 (podding stage) growth stages of chickpea would generate similar results.

Discussion

The development of AB in chickpea was affected by plant age, cultivar and their interaction. The effect of crop growth stages on development of AB was studied in cultivars varying in the level of resistance under controlled environment at ICRISAT, Patancheru. All the cultivars were bred in India, where the natural *A. rabiei* population is highly variable in virulence. High levels of resistance are not available against all pathotypes of *A. rabiei* in cultivated chickpea (Basandrai et al. 2005; Pande et al. 2005). Under such conditions, growing susceptible cultivars, namely ICC 4991 and ICCV 10 can result in a total

Table 2 Average disease severity of Ascochyta blight on chickpea cultivars inoculated at different growth stages under controlled environment

Growth stage	Disease reaction (1–9 scale)						Overall mean
	Susceptible			Resistant			
	ICC 4991	ICCV 10	Mean	ICCV 05562	ICCV 04512	Mean	
GS1	9	9	9	5	4	4.5	6.9[c]
GS2	7	7	7	3	3	3.0	5.0[a]
GS3	9	7	8	4	4	4.0	5.9[b]
GS4	9	9	9	5	5	5.0	6.8[c]
GS5	9	9	9	4	5	4.5	6.8[c]

*Means with the same letter are not significantly different

LSD ($P<0.05$) Growth stage=0.42; Growth stage×cultivar=1.4

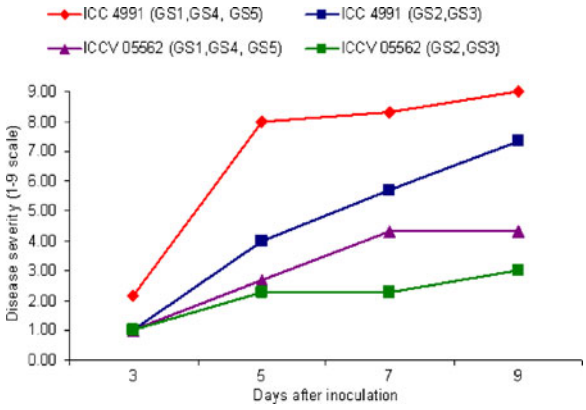

Fig. 1 Ascochyta blight development in susceptible and resistant cultivars at different growth stages

crop failure and even resistant cultivars can suffer heavy losses (Chongo and Gossen 2001; Chongo et al. 2000).

Plant age has been considered a factor affecting disease expression and susceptibility of chickpea to *A. rabiei* (Trapero-Casas and Kaiser 1992; Chongo and Gossen 2001; Basandrai et al. 2007). Irrespective of the growth stage, symptoms developed earlier on susceptible cultivars with an incubation period of 2.8 days (ICC 4991) and 2.9 days (ICCV 10). The IP was statistically longer in resistant cultivars. Irrespective of cultivar, IP was shorter at GS5 (podding stage) followed by flowering (GS4) and seedling stage (GS1). The extended IP at GS2 and GS3 was attributed to the development gene expression, as resistance genes were reported to be highly expressed during the post-seedling to vegetative growth stages than at maturity (Trapero-Casas and Kaiser 1992).

Disease severity was greater and disease development faster on susceptible cultivars at all the growth stages. These results are supported by earlier studies (Chongo and Gossen 2001; Trapero-Casas and Kaiser 1992) that showed that growth stage had no effect on disease development on susceptible cultivars. However, resistant cultivars showed less disease severity and slower disease development. Disease severity was greater at flowering, podding and seedling stage as compared to post-seedling and vegetative stage. This supports other studies that showed increased susceptibility as plants matured (Nene and Reddy 1987; Chongo and Gossen 2001). This shift in susceptibility is probably due to change in resistance response rather than the effect of crop canopy as this experiment was carried out under controlled conditions. Increased secretion of maleic acid (Singh and

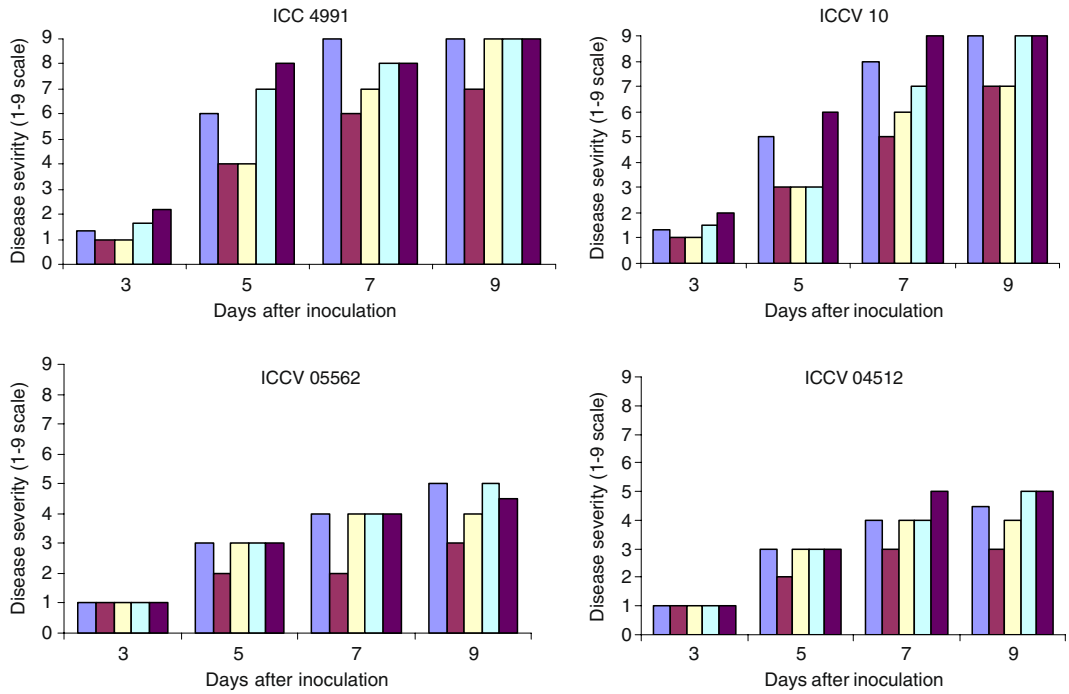

Fig. 2 Progress in Ascochyta blight development in chickpea cultivars at different growth stages

Sharma 1998), activity of chitinase and exochitinase (Nehra *et al*. 1997), phytoalexins, namely maackianan and their biosynthesic bioenzymes, lytic protein enzymes and other PR proteins (Hanselle and Barz 2001) may be responsible for delayed symptom appearance and lower severity in post seedling to vegetative growth stages. AB infection was also found to be less on leaves than on stems at each growth stage, suggesting different resistant genes may have operated in stems and leaves. This has also been reported for AB of lentil (*Lens culinaris* Medik.), where the expression of resistance in leaflets and stems differed among cultivars (Pedersen and Morrall 1994).

Assessment of resistance to AB at the seedling stage correlates well with the adult growth stages indicating that evaluation for resistance to AB can be done at GS 1 (seedling stage), and/or GS4 (flowering stage) to GS5 (podding stage) growth stages of chickpea. Sharma et al. (2006) also found a high correlation between greenhouse (seedling stage) and field screening (adult plant) for AB resistance. This supports the evaluation for AB resistance using 10-day-old-seedlings in a controlled environment at ICRISAT and adult plant field screening at hot-spot locations in Dhaulakuan and Ludhiana in India (Pande et al. 2009). Resistance to AB has been one of the major objectives in breeding programs. Present findings support the current screening method adopted at ICRISAT where a large number of germplasm and breeding lines has been screened using 10-day-old seedlings in controlled environment.

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