Research Article

Screening of Elite Chickpea Germplasm against *Ascochyta* Blight under Controlled Conditions

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Abstract | *Ascochyta* blight (*AB*) caused by *Ascohyta rabiei* is considered as most disastrous and widespread fungal disease of chickpea across the world. Under favorable environmental conditions *AB* epidemics may cause partial to complete yield loss. Chickpea faces several biotic and abiotic stresses. Among biotic factors chickpea blight is most important factor responsible for drastic decline in productivity. Other disease management approaches are not more efficient and economical except to exploit the host plant resistance mechanism. The present study for screening of 60 elite chickpea lines was carried out for two consecutive years during 2017-18 and 2018-19 under controlled environment at pulses research Institute Faisalabad, Pakistan. Favorable conditions for disease incidence were developed by maintaining the temperature between15-20 °C and humidity >70%. Test entries were inoculated equally by spraying fungal suspension during initial flowering and pod filling stages. Observations on disease incidence were recorded by employing 1-9 Disease Rating Scale. Result showed that no line was resistant whereas, only eight chickpea desi lines were found moderately resistant, three lines were tolerant, seventeen were moderately susceptible and the rest thirty-two lines were found susceptible and highly susceptible. Identified moderately resistant lines (D-17001, D-17005, D-17008, D-17009, D-17011, D-17023, D-17024 and D-17032) may be included in chickpea breeding program for development of *AB* tolerant chickpea cultivars.

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Introduction

Chickpea (*Cicer arietinum* L.) is an important rabi pulse crop being grown in more than forty countries across the world (Hirich *et al*., 2014; Mahmood *et al*., 2019). It is the mostly grown pulse legume crop in the world after bean and peas. Chickpea (*Cicer arietinum* L.) is an important food legume crop with an annual global production of about 14.78 million tons (FAOSTAT, 2021). Chickpea is major source of high-quality protein for animals and human beings (Malik *et al*., 2011). It also helps in the management of soil fertility through biological nitrogen fixation (Islam *et al*., 2011). Among the chick pea diseases, *Ascochyta blight* (*AB*) results into significant yield loss. Occurrence of this disease has been reported in most of chickpea growing countries of world however more significant yield losses have been reported in India, Pakistan, Australia, Morocco, Spain, Syria, USA, Iran and Canada (Gayacharan *et al*., 2020). Humid, cool and cloudy climatic conditions are most favorable condition for disease spread (Pande *et al*., 2005). *AB*

epidemics under favorable environmental conditions may cause partial to complete yield loss (Mahmood *et al*., 2019). Yield losses up to 100% have been reported in the areas where temperature ranges between 10-25 ^{0}C and the relative humidity is more than 60% during the crop season (Jamil *et al*., 2010).

Breeding efforts for development of AB resistant cultivars were initiated on large scale by different research centers during last two decades of twentieth century (Pande *et al*., 2005). ICRISAT (International Crops Research Institute for Semi-arid Tropics) initiated systematic breeding efforts for exploration of *AB* resistant germplasm and thousands of chickpea genotypes were screened out for identification of resistant genetic resources (Chongo *et al*., 2003; Baite *et al*., 2016). Hybridization attempts for development of *AB* tolerant genotypes were initiated at ICARDA in 1978 (Islam *et al*., 2011). ICARDA released and freely shared more than 3000 AB resistant lines between 1981-2002 (Malhotra *et al*., 2003). In USA, two AB resistant cultivars, Sanford and Dwelly were released in 1990 (Khan *et al*., 2018).

The fungus can infect all above ground parts of the plant and survives on infested crop residue and seed. The fungus is extremely destructive and significantly affects the yield and quality of chickpea. The yield losses can reach up to 100% under favorable conditions (Pande *et al*., 2005). The presence of a sexual phase (Didymella rabiei) in the life cycle of the pathogen leads to high level of variability in aggressiveness within the pathogen populations (Pande *et al*., 2005). The most efficient strategy to manage this disease is to identify the genetic sources for resistance and to exploit such host plant resistance for development of *AB* resistant cultivars (Gan *et al*., 2006; Duzdemir *et al*., 2014; Gayacharan *et al*., 2020). Shah *et al*. (2015) screened 54 elite chickpea advance lines against blight under controlled environmental at NIAB, Faisalabad and concluded that 23 lines were found resistant and 16 as moderately resistant. The entry of new pathotype unable resistant mechanism of chickpea, so there is need of new improved genetic source for AB resistance in Chick pea (Jamil *et al*., 2010).

Several researchers (Megersa *et al*., 2017; Rubiales *et al*., 2018; Mahmood *et al*., 2019) have emphasized the use of 1-9 disease scale for screening of chickpea germplasm already suggested and proposed by Reddy and Singh (1984) and further elaborated by (Toker *et al*.,1999). For this purpose, artificial spore suspensions are prepared, and the screening nurseries are inoculated. Entries are screened out by keeping controlled environmental conditions and the resistant sources are identified for their further exploitation in development of AB resistant cultivars (Shah *et al*., 2015).

Screening of germplasm for identification of resistant strains and exploitation of these resistant sources are the key components for development of resistant cultivars (Sarwar *et al*., 2012). The present study was planned to identify the resistant sources against *Ascochyta* blight among the elite chickpea advance lines for their further exploitation in development of AB resistant chickpea cultivars.

Materials and Methods

Sixty advance chickpea lines were screened out against chickpea blight under controlled environmental conditions at experimental area of Pulses Research Institute, AARI, Faisalabad, Pakistan for two consecutive years during the rabi season of 2017-18 and 2018-19. The experimental material was laid down under augmented design.

Isolation of the pathogen

Plant samples of *Ascochyta* blight infected collected from chickpea fields. Infected stems, pods and leaflets presenting clear blight lesions were treated with solution of 5% sodium hypochloride and dehydrated on uncontaminated filter paper. For fungal growth, this material was plated on 2% water agar and incubated for 5-7 days at 20°C±2 with 12 hour light/dark cycle. Fungus colonies were developed after incubation on this media for 1-2 weeks. Similar procedure was adopted by Alam and Strange (1987) who reported that incubation of this material for 1-2 weeks results in fungal colonies with pycnidia.

Multiplication

For multiplication of fungus, chickpea grains were boiled for 15-30 minutes in water drained to soften the seeds and autoclaved at 121°C in a conical flask for 30 minutes. Sterilized distilled water was added for preparation of spore suspension from the slant of the fungus growing on chickpea seed agar. Haemocytometer was used for the determination of concentration of the spore suspension. It was diluted with distilled water and adjusted to10^6 spores/ml.

Adjusted volume of spore suspension was added to wet the seeds and distribution of inoculm was done by shaking the flask. This material was incubated for 7-10 days at 20°C. Developing pycnidia were observed on seeds and its agitation with sterilized distilled water resulted in spore suspension. This suspension was filtered through muslin cloth (Shah *et al*., 2015).

Sowing and inoculation

Sowing of test entries was done in plastic tunnel during last week of October in blight screening nursery at Pulses Research Institute, Faisalabad, Pakistan during 2017-2019. Each genotype was planted in single row measuring by dibbler. Highly susceptible chickpea genotype AUG-424 was planted as check after each two test entries. Artificial humidity was created by a sprinkler system for disease development. Inoculm of *Ascochyta* fungal suspension was equally sprayed on all genotypes during initial flowering and pod filling stages (Figure 1 and 2) as described by Singh and Reddy (1993) and practiced by Shah *et al*. (2015) and several others to ensure good disease development.

Data recorded

Disease rating scale 1-9 was utilized for screening of genotypes against *Ascochyta* blight proposed by Reddy and Singh (1984) (Table 1). Severity of blight disease was recorded on the vegetative stage by using the 1-9 rating scale as described by Toker *et al*. (1999). The genotypes rated 1-3 were considered to be resistant, 4 moderately resistant 5, 6 Moderately susceptible, 7 were observed susceptible and 8-9 were observed highly susceptible as given in Table 1. Means of data were compared by *t*-test (Shah *et al*., 2015). Disease rating scores of each line were recorded during both years and averaged.

Figure 1: *Spray of inoculum in Ascochyta blight screening nursery and symptoms of AB during pod bearing stage.*

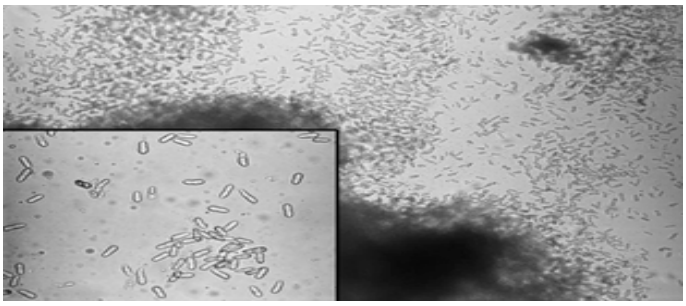

Figure 2: *Spores of Ascochyta Blight under microscope.*

Results and Discussion

The experimental lines were categorized according to disease rating scale (1-9). Rating of genotypes showed different host plant responses indicating a wide range of resistance among the studied elite germplasm. Data regarding the disease severity rating is evident that the genetic material was diverse in nature and the genotypes behaved differentially (Table 2). Reddy and Singh (1993) also found differential performance of genotypes regarding AB resistance and narrated that resistance mechanism could be controlled by single recessive or dominant gene.

Table 1: *Disease rating scale for Ascochyta blight (1-9).*

S. No	Symptoms	Infected area %	Scale/ Rating	Resistance class
1	Immune, with no symptoms on plants.	0	1	With no infections
2	Minute spots/lesions on the apical stem	1-5	2	Highly Resistant (HR)
3	Apical stem - slight drooping with elongating lesions	6-10	3	Resistant (R)
4	Apical stem- clear drooping with obvious lesions	11-15	4	Moderately Resistant (MR)
5	All plant parts-Obvious lesions, slightly to moderate drying with breaking branches	16-40	5	Tolerant (T)
6	Some plants killed while some have broken & dry branches common	41-50	6	Moderately susceptible(MS)
7	Plants having mortality of 25% like lesions as in 5 with defoliated, broken and dry branches	51-75	7	Susceptible (S)
8	Plants having mortality of 50% like category 7	76-100	8	Highly susceptible (HS)
9	Plants having mortality of 100% like category 7	100 %	9	Highly susceptible (HS)

Table 2: *Disease severity rating of chickpea advance lines during 2018-19 and 2019-20.*

S. No	Entries	Type	Severity mean ± SE	Disease Rank/ class	S. No	Entries	Type	Severity mean ± SE	Disease Rank/ class
1	D-16001	Desi	6.2±0.7	MS	31	D-17024	Desi	4.2± 0.3**	MR
2	D-16003	Desi	6.3±1.0	MS	32	D-17025	Desi	7.3±0.9	S
3	D-16004	Desi	6.7±0.6	MS	33	D-17026	Desi	6.2±0.7	MS
4	D-16005	Desi	5.1±0.3	T	34	D-17027	Desi	6.1±0.9	MS
5	D-16006	Desi	7.7±0.9	S	35	D-17028	Desi	5.2±0.3	T
6	D-16009	Desi	6.2±0.7	MS	36	D-17029	Desi	7.1±0.7	S
7	D-16010	Desi	6.1±0.9	MS	37	D-17030	Desi	6.2± 0.3	MS
8	D-17001	Desi	4.1±0.6**	MR	38	D-17031	Desi	7.4± 0.6	S
9	D-17002	Desi	6.3±0.7	MS	39	D-17032	Desi	4.4± 0.3**	MR
10	D-17003	Desi	6.2±0.3	MS	40	Pb-2008	Desi	5.2± 0.6	T
11	D-17004	Desi	6.1±0.7	MS	41	K-15012	Kabuli	8.1± 0.3	HS
12	D-17005	Desi	4.0±0.9**	MR	42	K-16025	Kabuli	8.4± 0.7	HS
13	D-17006	Desi	7.2±0.7	S	43	K-16026	Kabuli	8.2± 1.0	HS
14	D-17007	Desi	7.0±0.7	S	44	K-16027	Kabuli	7.1± 0.3	S
15	D-17008	Desi	4.2± 0.8**	MR	45	K-16028	Kabuli	8.1± 0.7	HS
16	D-17009	Desi	4.4± 0.6**	MR	46	K-16029	Kabuli	8.3± 0.9	HS
17	D-17010	Desi	7.4±0.3	S	47	K-17011	Kabuli	8.1± 0.9	HS
18	D-17011	Desi	4.1± 0.7**	MR	48	K-17012	Kabuli	7.1± 0.3	S
19	D-17012	Desi	7.7±0.9	S	49	K-17013	Kabuli	8.0± 0.9	HS
20	D-17013	Desi	6.2±0.7	MS	50	K-17014	Kabuli	7.3± 0.6	S
21	D-17014	Desi	6.1±0.9	MS	51	K-17021	Kabuli	8.2± 0.7	HS
22	D-17015	Desi	7.2±0.7	S	52	K-17022	Kabuli	8.4± 0.3	HS
23	D-17016	Desi	7.1±0.6	S	53	K-17023	Kabuli	7.4± 0.6	S
24	D-17017	Desi	6.1±0.7	MS	54	K-17024	Kabuli	7.0± 0.3	S
25	D-17018	Desi	6.2±0.3	MS	55	K-17025	Kabuli	8.1± 0.9	HS
26	D-17019	Desi	6.2±0.7	MS	56	K-17027	Kabuli	7.2± 0.6	S
27	D-17020	Desi	7.2± 0.6	S	57	K-17028	Kabuli	8.1± 0.9	HS
28	D-17021	Desi	7.1± 0.3	S	58	K-17029	Kabuli	8.2± 0.6	HS
29	D-17022	Desi	6.2±0.3	MS	59	K-17030	Kabuli	8.0± 0.9	HS
30	D-17023	Desi	4.0± 0.6**	MR	60	K-17031	Kabuli	7.3± 0.6	S

HS: Highly susceptible; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible; SE: Standard error.

Similar findings were also observed by different researchers in Chick pea crop grown under Pakistani climate (Aslam *et al*., 2008; Khan *et al*., 2018). Data revealed that most of the investigated advance lines had high level of susceptibility to *Ascochyta* blight. All the genotypes were ranked for their reaction to the disease under controlled environmental conditions.

Results showed that none of the lines was resistant, 8 desi chickpea lines (D-17001, D-17005, D-17008, D-17009, D-17011, D-17023, D-17024 and D-17032) were found moderately resistant with disease severity rating of 4. Our findings agree to the previous findings of (Collard *et al*., 2003; Rashid *et al*., 2014; Shah *et al*., 2015) who also reported that desi types are more resistant to AB disease than kabuli types. It was also recorded that 3 entries (D-17005, D-170025 and a commercial variety Punjab-2008 were tolerant having disease severity rating of 5. While 17 lines behaved as moderately susceptible showing disease severity rating of 6 and the rest 32 lines were categorized as susceptible and highly susceptible with disease severity rating of 7-8. (Alam *et al*., 2003; Chaudhry *et al*., 2005; Shah *et al*., 2015; Gayacharan *et al*., 2020) also reported similar kind of results. The susceptible and highly susceptible genotypes have no utility in breeding programs however the genotypes with resistant to moderately resistant response may

be utilized for breeding program (Sahi *et al*., 2012; Sarwar *et al*., 2012; Pande *et al*., 2005). Based on this study, moderately resistant advance lines obtained from screening will be useful in future breeding programs for development of blight resistant chickpea cultivars.

Conclusions and Recommendations

From the present study it was concluded that no line was resistant to *Ascochyta* blight however eight chickpea desi lines were found moderately resistant while all other lines were susceptible to highly susceptible. Identified moderately resistant lines (D-17001, D-17005, D-17008, D-17009, D-17011, D-17023, D-17024, D-17032) may be exploited further in chickpea breeding program for development of AB tolerant chickpea commercial cultivars.

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Novelty Statement

Chickpea (*Cicer arietinum* L.) crop production now a days facing yield loss due to *Ascochyta blight* (*AB*) even which is an important leguminous crop but in the Faisalabad area it is the first study to evaluate its loss in chickpea crop.

Author's Contribution

Javed Anwar Shah designed and collected data and Dr. Azhar Iqbal analyzed the data and Muhammad Tariq Mahmood and Dr. Muhmmad Aslam they designed this experiment. Muneer Abbas prepared the first draft of the article. Ilyas Ahmad also finalized the draft after a careful reading.

Conflict of interest

The authors have declared no conflict of interest.

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