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RESEARCH ARTICLE

Assessment of adaptability in genetically diverse chickpea genotypes (Cicer arietinum L.) based on different physio-morphological standards under ascochyta blight inoculation

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Abstract

Globally biotic stresses are the major environmental destructive elements in crop plants and are major threats to adaptability of any crop. Ascochyta blight is responsible for massive losses in quality and quantity of chickpea crop. Chickpea genotypes collected from different research institutes were evaluated against chickpea blight in screenhouse. Genetic variability among chickpea genotypes was assessed on the basis of ability to adapt under epidemic form of ascochyta blight based on different standards like survival rate (SR), chlorophyll a (Chl-a), chlorophyll b (Chl-b), and β-carotenoids (BC) contents. Data collected were analyzed by analysis of variance and multivariate analysis (Biplot analysis). Significantly different levels of adaptability were noted in inoculated set of experiment which reflected the genetic differences, opportunity for selection and chances of improvement in genotypes. Comparatively adapted / resistant chickpea genotypes (CM-98, 1848, 6003, and 7050) showed little reduction in values of evaluating standards. Whereas, comparatively greater reduction in moderately resistant (1818, 6255, 6015 and 6028), moderately susceptible (7020, 7056, 810, 1019 and 4025) and poorly adapted / susceptible genotypes (1205 and 3022) was evident. The objective of study was to asses the extent of variability among chickpea genotypes regarding adaptability to ascochyta blight, mechanism of adaptability, to identify adapted / resistant sources and its utilization for further breeding program.
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Introduction

Chickpea is an important annual pulse crop. It is only cultivated species of genus Cicer. Chickpea ranks fifth in production among world pulses and is grown in 35 countries of the world. India, Turkey, Pakistan, Iran, Mexico, Myanmar, Ethopia, Australia, Spain and Canada are top ten chickpea producing countries. Globally it was cultivated on area of 11.55 million ha with production 10.46 million tons (Anonymous, 2011). It is a Rabi crop in Pakistan and is grown in rainfed areas of different districts. Pakistan is ranked 2nd to India with 1068 thousand ha and 523 thousand tons of production (Anonymous, 2010-2011). Chickpea contributes a lot in sustainability of agriculture and reduction in inputs because of N_2-fixation properties and best fitting in crop rotation.

Biotic stresses play a significant role in increasing losses regarding chickpea production throughout the world. In chickpea, ascochyta blight (Aschocyta rabiei L.) is very severe disease, responsible for massive reduction in quantity and quality. Ascochyta blight was identified for the very first time in Indo-Pak in 1911 (Butler, 1918). Severity of this disease increases in seasons with rainfall more than 350 mm (Nene and Reddy, 1987). Losses have been reported up to 100% under favorable epidemic conditions (Singh and Reddy, 1993; Alwawi et al., 2009). Ascochyta blight is the most severe foliar disease affecting all aerial parts including; leaves, branches, seeds and

pods (Pande et al., 2005). Rain splashes, stubbles of previous season and infected seeds originate the inoculum which can infect the crop throughout its life cycle depending on epidemiological conditions (Pande et al., 2005).

In Pakistan low yield per unit area is mainly due to non-availability of resistant germplasm to different biotic stresses especially Ascochyta rabiei L. Fungicide application, seed dressing, at least four years crop rotation and removal of stubbles are recommended practices in field to prevent the disease (Pulse Australia, 2011), which are not feasible especially in Pakistan due cost limitations and utilization of marginal lands for pulses cultivation.

Infected seeds and stubbles are sources of inoculation and pathogen dissemination by rain splashes (Pande et al., 2005) and to some extent by air. Amongst 50 different pathogens to which chickpea is susceptible, Ascochyta rabiei L. is the most severe. Symptoms of this disease appear within week after inoculation that is yellow or brown discoloration of leaf which has confusing resemblance with sclerotinia stem rot and pod rot, leaf miner insects, frost and hail damage, seed rots, drought damage, wireworm damage, heat stress damage, herbicide damage and seedling blights (PRRP, 2010). Chickpea yield ranged from 0 to 3600 kg/ha depending upon biotic and abiotic constraints and their management (Gan et al., 2007). Always there is a continue process of evolution of new pathotypes which make the resistant genotypes as susceptible (Pande et al., 2005). The causal pathogen of ascochyta blight is a fungus named as Didymella rabiei (Kovachevski) v. Arx. (anamorph: Ascochyta rabiei (Pass.) Labrousse) and is heterothallic in nature. Great genetic and pathogenic variability is induced by recombination in Ascochyta rabiei L. which renders the germplasm susceptible that might be resistance in earlier days. All chickpea growing areas including India, Pakistan, Iran, Turkey, Syria, Canada and some regions of U.S.A have found to harbor the pathogenic variability in ascochyta blight (Chongo et al., 2004). Resistance/high level of adaptability against ascochyta blight in chickpea lines is not resilient because of high variability of Ascochyta rabiei (Singh and Reddy, 1993). New pathotypes are evolved in ascochyta rabiei and virulence within pathogen population is increased due to partially resistance chickpea cultivars.

Therefore, it is necessary for a breeder to find out resistant resources by evaluation and identification from existing germplasm. The success of evaluation plan depends upon finding of significant differences among genotypes at allelic level on the basis of standards linked to resistance/adaptability and identification of resistant/adapted germplasm to be used in breeding program for the development of resistant/adapted variety. The objectives of present study were, to assess the extent of genetic variability present in local available chickpea germplasm regarding resistance/adaptation to ascochyta blight and to identify ascochyta blight resistant/adapted genotypes which can be used in further breeding program to develop chickpea blight resistant/adapted varieties.

MATERIALS AND METHODS:

The experiment was conducted in the screenhouse of the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad to evaluate 38 chickpea genotypes (CM-98, 1848, BITTAL-98, 6003, 7050, 1818, 6255, 6015, 6028, 7048, 504, 4004, 7005/1, 2009, Paidar-91, PB-2000, CH-8, 950130, 1283, 1032, PCH-15, 115, 6019, PB-2008, 117, 4025, 1019, 810, 7056, 7020, 3022, 1205, 627/11, 6010, 6005, 6103, 7059, 405) by following triplicated completely randomized design. Experiment was divided in to two subunits, one was treated as normal and second as inoculated with ascochyta blight inoculum. Recommended agronomic practices were carried out uniformly in both the experimental units. Data were recorded for evaluating standards like survival rate [(number of seedlings survived/total number of seedling) ×100)], chlorophyll-a [$0.999A_{663}$-$0.0989A_{645}$ (mg/100ml)], chlorophyll-b [$0.328A_{663+}1.77A_{645}$ (mg/100ml)], and carotenoids [$0.216A_{663}$-$1.22A_{645}$-$0.304A_{505}$+$0.452A_{453}$ (mg/100ml)]. Analysis of variances was computed to compare the genotypes by following Steel et al (1997). Data recorded for different morphological and physiological traits were statistically analyzed by using principal component analysis (Bi-plot analysis).

Preparation of Inoculum:

Pods infected from A. blight were collected from field and harvested seeds were placed on gram seed meal agar medium (Agar = 20g, Glucose = 20g, Gram seed meal = 20g, Sterilized water = 940 ml) in petri plates. Petri plates were incubated at 20 ± 2°C for 15 days (Ilyas and Khan, 1986). Colonies were picked and further propagated using single spore streak method (Phatak, 1986). Then inoculum was increased in volume by infecting fresh chickpea seeds with infected seeds. Almost 500g of chickpea seeds which had to be infected were soaked in tap water for 5-7 hours. The seeds were then surface dried and placed on the paper. Dried seeds were put in glass flasks. Flasks were sealed by placing cotton plug in neck of flask and covered with aluminium foil and then placed them in autoclave @ 15 psi pressure for 25-30 minutes. Flasks were inoculated by 8 mm sized mycelial plugs from pure culture. Streptomycin (30 mg/flask) was added in flask for reducing bacterial contamination and flasks were placed in incubator at 20 ± 5°C for two weeks for complete development of pycniospores (Ilyas & Khan, 1986). Inoculum from flasks was stirred in water to prepare spore suspension which was filtered through muslin cloth for purity. The filtrate was used for spray purposes as an inoculum to create epidemic conditions. The inoculum was prepared by

using the mass multiplication method devised by Ilyas and Khan (1986) and plants were artificially inoculated by spraying the spore suspension. High humidity was maintained as favorable conditions for the rapid disease development. Suspension of fungal spores (500 ml) was mixed in 1 litter water. Inoculum suspension was poured into hand sprayer and then sprayed uniformly on 1 set of experiment specified for inoculation manually. To enhance the intensity of disease, inoculum was sprayed 3 times with the interval of 10 days in each application.

After inoculation, epidemic conditions were set and maintained by applying temperature (20^oC-25^oC), humidity 80% and inoculum thrice after equal intervals. Epidemic conditions were maintained from the date of inoculum to the date of harvesting. Chickpea genotypes were ranked against aschocyta blight on the basis of symptoms produced by pathogen in inoculated set. Scoring was done by following scale of disease rating proposed by Pande et al (2011).

RESULTS:

Disease rating scale:

Disease rating scale presented in table-2 was used for rating chickpea genotypes and genotypic grouping as adapted/resistant to not adapted/susceptible was presented in table-3. Five genotypes were found as adapted/resistant (R), 19 as moderately adapted/ moderately resistant (MR), 12 as poorly adapted/moderately susceptible (MS) and 2 as not adapted/susceptible (S). Surprisingly no genotype was found to be highly susceptible and highly resistant among 38 studied genotypes according to rating scale used.

Survival rate (SR) %:

Results declared the presence of genetic variability at allelic level among chickpea genotypes regarding their responses to ascochyta blight which was a good reflector of genetics differences regarding extent of adaptability. Analysis of variance (ANOVA) revealed that genotypes showed highly significant differences at 5% level of significance for survival rate (SR) with broad range under A. blight prevalence (Table 1). BITTAL-98 had highest SR% (88) whereas CM-98, 1848, 6003, 7050, and Paidar-91 exhibited more than 80% SR under inoculated conditions. Most of the genotypes showed SR between 60 to 80%. Nine genotypes had survival rate in the range of 40 to 60% whereas; genotype 3022 showed SR even lower than 40% under disease prevailing circumstances (Figure 1).

Performance of chickpea genotypes regarding SR was evaluated by estimating percent decrease in SR under inoculated condition relative to normal condition. Least percent decline in SR was observed in case of BITTAL-98 (4.3%) whereas Paidar-91, 1848, 6003, 7050 and CM-98 showed less than 10% decline in SR. Nineteen genotypes were suffered up to SR decline range 10 to 30% and SR of 11 genotypes decreased from 30 to 50%. Genotypes 3022 and 6010 encountered more than 50% decline in SR compared to normal condition (Table 3).

Biplot graph for SR was presented in Figure 5. Every trait had been represented by one vector. SR(T1) and SR(T2) vectors represented SR under normal and inoculated conditions respectively. Scattered position of genotypes among all of four quadrants showed that all genotypes had variable responses for all traits under normal and inoculated environments (Figure 5). Genotypes with high variability and positioned more apart from the origin towards positive quadrants were recorded as well adapted to prevailing environments. Poorly adapted genotypes secured position close to each other towards negative quadrants. Vector lengths of SR(T1) and SR(T2) were almost same which reflected that both vectors had equal discrimination powers for the explanation of 38 accessions regarding SR. Genotypes BITTAL-98, CM-98, 1848, 6003, 7050, and Paidar-91 secured position farther from origin in positive quadrant therefore, grouped as well adapted with higher SR%. Overlapping cluster of chickpea genotypes in biplot graph explained the similar level of adaptability of genotypes under both environments. Genotypes 1032, 4004, 7056, 105, 4025, 627/11 and 3022 positioned in negative quadrant and opposite sides of environment vectors showed comparative poor adaptability/SR% (Figure 5).

Chlorophyll-a (Chl-a) contents:

Significance differences were observed among chickpea genotypes for chlorophyll-a contents subjected to A. blight inoculated environment (Table 1). Genotypes 3022 and CM-98 showed minimum (0.3029 mg/100ml) and maximum (2.7567 mg/100ml) chlorophyll-a contents respectively under inoculated condition (Figure 2). Among 38 chickpea genotypes, six entries carried chlorophyll-a contents with a range between 2 to 3 mg/100ml whereas, 26 entries showed chlorophyll-a contents ranging from 1 to 2 mg/100ml under inoculated environment. Rest of the six entries exhibited chlorophyll-a contents less than 1 mg/100ml under inoculated condition (Figure 2). Under normal condition chlorophyll-a contents of chickpea genotypes were higher with a range from 2.2083 (3022) to 3.0267 mg/100ml (BITTAL-98). Genotypic differences regarding chlorophyll-a contents under normal condition were naturally existing.

Percent decline in chlorophyll-a contents was studied to assess the relative performance and adaptability of chickpea genotypes to variable environments. In inoculated environment CM-98 and BITTAL-98 showed less than

10% decline in chlorophyll-a contents and 24 different chickpea genotypes suffered with a range from 10 to 40% reduction in chlorophyll-a contents. Decline ranging from 40 to 60% in chlorophyll-a contents was observed in six genotypes and 60 to 80% in 5 genotypes whereas one genotype (3022) showed decrease up to 86.3% in chlorophyll-a contents (Table 3).

Biplot graph was constructed for visual display of genotypic performance regarding chlorophyll-a contents. Normal and inoculated environmental conditions were represented by Chl-a(T1) and Chl-a(T2) vectors respectively. Chl-a(T1) vector had more discrimination power than Chl-a(T2) because of longer vector length. Genotypes secured scattered positions in all the four quadrants which reflected high level of versatility in genotypes regarding chlorophyll-a contents. BITALL-98 and CM-98 got positions distantly away from origin in the direction of environment vectors. Overlapping positions of the clusters in graph showed similar performance of genotypes. Genotype 3022 is farther away from origin and present in opposite direction of the environment vectors (Figure 6).

Chlorophyll-b (Chl-b) contents:

Differences among genotypes were significant for chlorophyll-b contents under inoculated conditions (Table 1). Minimum (0.1967 mg/100ml) and maximum (3.6306mg/100ml) chlorophyll-b contents were observed in case of genotype 3022 and CM-98 respectively in inoculated set of experiment. Under normal condition minimum chlorophyll-b contents (1.8490mg/100ml) were observed in genotype 627/11 and maximum (2.8133mg/100ml) in BITTAL-98 (Figure 3). Range of chlorophyll-b contents was higher under inoculated condition (3.6306 to 0.1967 mg/100ml) than under normal condition (2.8133 to 1.8490 mg/100ml). Out of 38 chickpea genotypes, 19 genotypes showed chlorophyll-b contents ranging from 2 to 4 mg/100ml, five genotypes exhibited chlorophyll-b with range from 1 to 2 mg/100ml while all other had less than 1 mg/100ml chlorophyll-b contents under A. blight prevalence. A range of differences in chlorophyll-b contents reflected the presence of genetic differences in chickpea genotypes under A. blight inoculation (Figure 3).

In case of CM-98 and 6003 chlorophyll-b contents increased under inoculated condition which proved the activation of their defense mechanism to prepare sufficient food to protect the plant from death. Nine genotypes suffered from less than 10% reduction in chlorophyll-b contents, 11 genotypes reduced their chlorophyll-b contents from 10 to 30%, 30 to 50% reduction in chlorophyll-b contents was observed in eight chickpea genotypes and rest of the genotypes showed reduction in chlorophyll-b contents from 60 to 82.4% under inoculated environment (Table 3).

In biplot graph vector Chl-b(T1) represented the normal environment and Chl-b(T2) explained the A. blight treated environment. Mainly three clusters were constructed on the basis of genotypic performance in biplot. Those which were positioned farther away from origin in positive quadrant carried genotypes with higher chlorophyll-b contents whereas, cluster with position near to origin in positive quadrant represented genotypes with intermediate chlorophyll-b contents. Genotypes with lower chlorophyll-b contents were placed in cluster positioned in opposite side of environment vectors and in negative quadrant (Figure 7).

β-carotenoid (BC) contents:

Analysis of variance showed the significant differences among chickpea genotypes for beta-carotenoids (Table-1). Genotypes 3022 and BITALL-98 showed minimum BC (0.2041mg/100ml) and maximum BC contents (0.7542mg/100ml) respectively. Six chickpea genotypes possessed BC contents with a range from 0.6 to 0.8mg/100ml, 13 genotypes showed BC contents ranging from 0.2 to 0.4mg/100ml and from 0.4 to 0.6mg/100ml was the range of rest of the genotypes. Minimum reduction in BC contents was observed in BITALL-98 (24.6%) whereas; maximum reduction (82.4%) was observed in case of 3022 in inoculated environment as compared to normal set of experiment (Figure 4). These results explained that chickpea genotypes were comparatively more sensitive to A. blight regarding BC contents than Chl-a and Chl-b contents.

In biplot graph B.C(T1) vector represented normal environment and B.C(T2) vector represented inoculated environment. Genotypes were dispersed all along the 4 quadrants of the graph that explained the presence of high level of variability among genotypes in respect of B.C. contents. BITALL-98 was positioned farther from origin in positive quadrants that presented it as carrier of higher BC contents whereas, 3022 proved as owner of lowest BC contents as was distantly located in opposite sides of the environment vectors in negative quadrants. All other genotypes were located in between BITALL-98 and 3022 genotypes so, the performance can be assessed by their distance from origin, direction regarding vectors and quadrants in which these are located (Figure 7).

Tables and Figures:

Table 1: Mean Squares with level of significance for different selected traits

Source of variation	DF	B.C	Chl-a	SR	Ch1-b
Replication	2	0.0005	0.0044	8.4	10.615
Genotype	37	0.0475**	0.957**	327.7**	2.2667**
Treatment	1	13.033**	63.221**	31472.8**	18.49**
Genotype × Treatment	37	0.0259**	0.208**	185.5**	0.6087**
Error	150	0.00002	0.002	1.2	0.213
Total	227				

Table 2: Disease rating scale developed by Pande et al., (2011)

Rating	**Symptoms**	**Resistant Class**
0	No symptoms	Immunes
1-2	A few scattered lesions (1-10% Infection)	Resistant
3-4	lesions are common on plants, Least damaging (10-25% Infection)	Moderately resistant
5-6	Lesions very common on plants Intermediate severity (25-50% Infection)	Moderatelysusceptible
7-8	Extensive lesions on all plants, Defoliation and dried branches, Few Plants are completely killed (50-75% Infection)	Susceptible
9	All plants and plant parts completely killed (75-100Infection)	Highly susceptible

Table 3: percent decrease in Survival rate (S.R), chlorophyll-a & b contents, β-carotenoids under A. b genotypic Grouping based on scoring scale.

Sr.No	Genotypes	S.R (%decrea	CHL-a (%decrease)	CHL-b (%decrease)	B.C (%decrease)	Genotype status	Disease rating
1	1283	20.6	32.0	9.4	51.0	MR	03
2	950130	22.0	37.8	12.7	43.0	MR	03
3	CH-8	21.0	37.4	9.7	43.9	MR	04
4	PB-2000	19.3	35.3	7.5	45.2	MR	03
5	Paidar-91	7.9	22.0	7.2	34.5	MR	03
6	7050	7.7	22.9	0.3	33.1	R	02
7	6003	6.9	19.9	-0.3	31.9	R	01
8	1848	9.6	17.2	6.5	29.1	R	02
9	CM-98	5.1	8.1	-11.4	43.5	R	01
1	BITTAL-98	4.3	9.3	9.4	24.6	R	01
1	1818	22.2	36.3	9.3	45.1	MR	03
1	6255	27.6	36.9	10.0	46.2	MR	04
1	6015	24.9	37.3	10.3	46.0	MR	03
1	6028	24.4	37.8	10.1	48.0	MR	03
1	7048	21.0	35.8	8.0	41.4	MR	03
1	504	20.7	36.7	10.4	45.8	MR	03
1	4004	21.2	35.6	12.3	44.3	MR	03
1	7005/1	21.7	34.5	10.8	49.0	MR	03
1	2009	32.0	35.2	12.2	48.4	MR	04
2	1032	21.3	38.7	21.2	41.0	MR	03
2	7020	27.5	42.0	57.3	73.0	MS	05

2	7056	31.3	42.5	54.3	68.1	MS	05	
2	810	30.6	41.4	59.0	54.1	MS	05	
2	1019	28.9	40.0	54.6	59.1	MS	05	
2	4025	36.3	34.9	52.9	55.2	MS	06	
2	117	27.6	34.0	53.4	54.5	MR	03	
2	PB-2008	28.6	34.3	49.8	51.3	MR	03	
2	6019	29.1	33.5	20.0	59.8	MR	03	
2	115	31.7	33.7	23.1	50.2	MR	03	
3	PCH-15	26.9	32.9	21.5	47.5	MR	03	
3	405	34.6	41.9	61.8	67.1	MS	05	
3	7059	42.1	48.5	56.4	75.0	MS	06	
3	6103	49.4	63.4	61.1	62.1	MS	06	
3	6005	38.2	76.2	66.3	73.4	MS	05	
3	6010	50.9	77.9	63.1	66.5	MS	05	
3	627/11	48.1	77.5	73.0	81.1	MS	05	
3	1205	49.2	79.6	67.8	69.4	S	07	
3	3022	53.3	86.3	82.4	76.8	S	08	

Figure 1: survival rate of chickpea genotypes under normal (light bar) and inoculated (dark bar) conditions

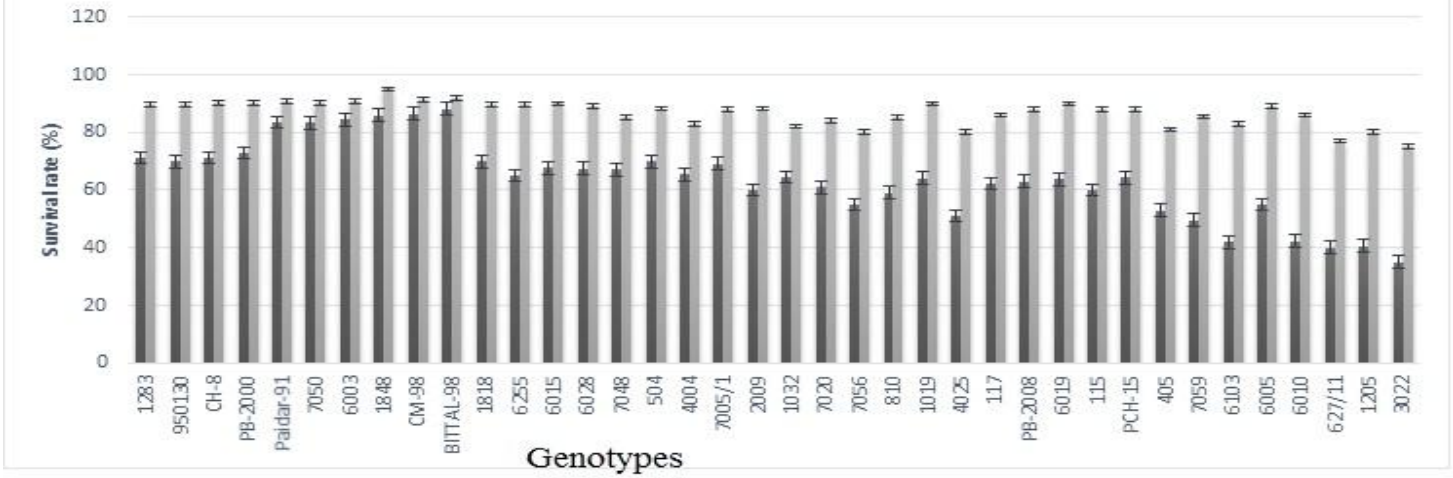


Figure 2: Chlorophyll a contents of chickpea genotypes under normal (light bar) and inoculated (dark bar) conditions

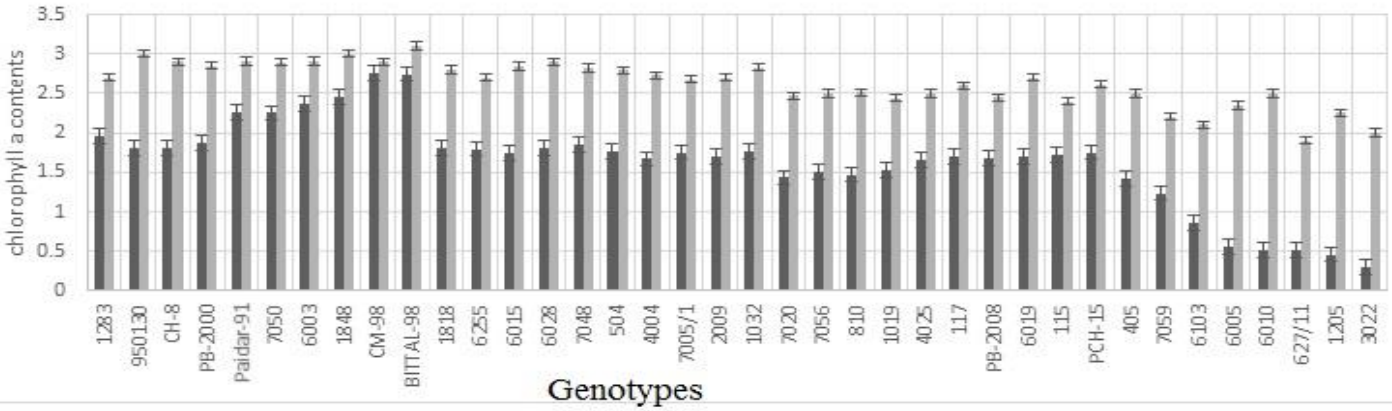


Figure 3: Chlorophyll b contents of chickpea genotypes under normal (light bar) and inoculated (dark bar) conditions

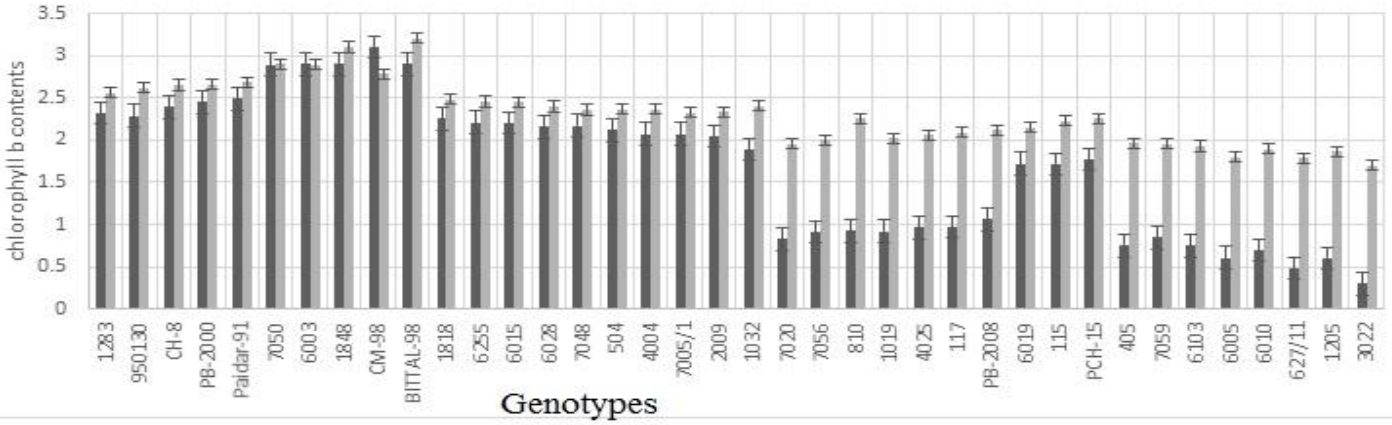
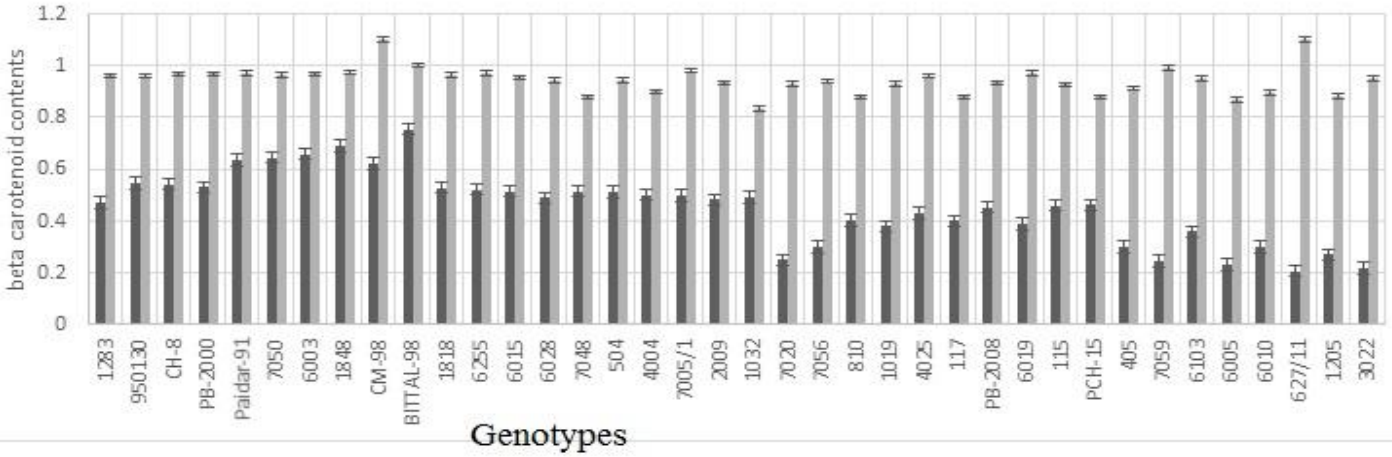


Figure 4: β-carotenoid contents of chickpea genotypes under normal (light bar) and inoculated (dark bar) conditions



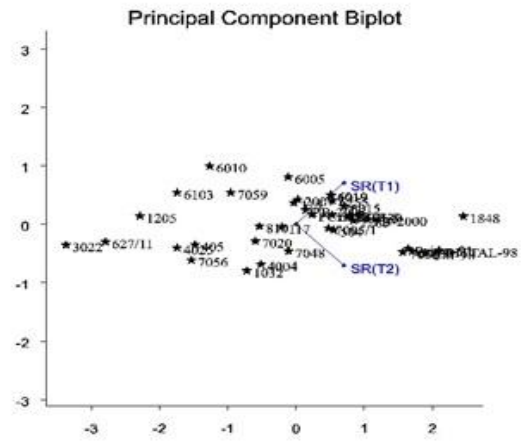


Figure 5: Biplot graph of 38 chickpea genotypes for survival rate under normal {SR(T1)} and A. blight inoculation {SR(T2)}

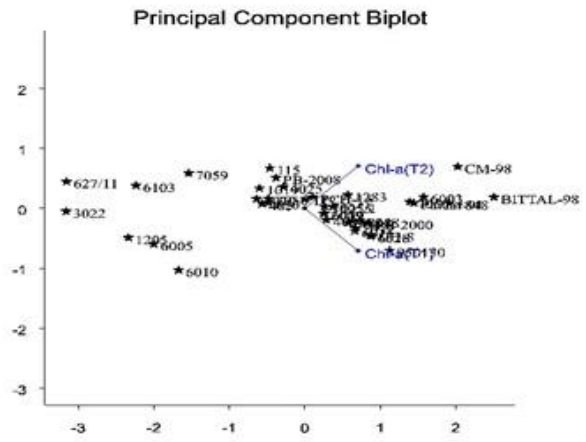


Figure 6: Biplot graph of 38 chickpea genotypes for chlorophyll-a contents under normal {Chl-a(T1)} and A. blight inoculation {Chl-a(T2)}

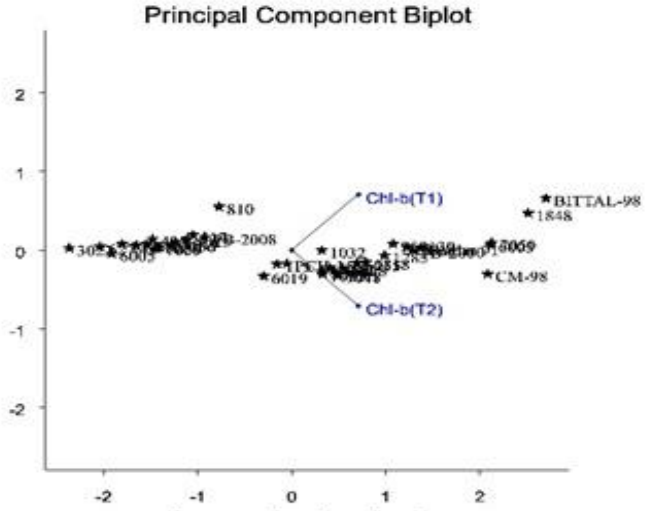


Figure 7: Biplot graph of 38 chickpea genotypes for chlorophyll-b contents under normal {Chl-b(T1)} and A. blight inoculation {Chl-b(T2)}

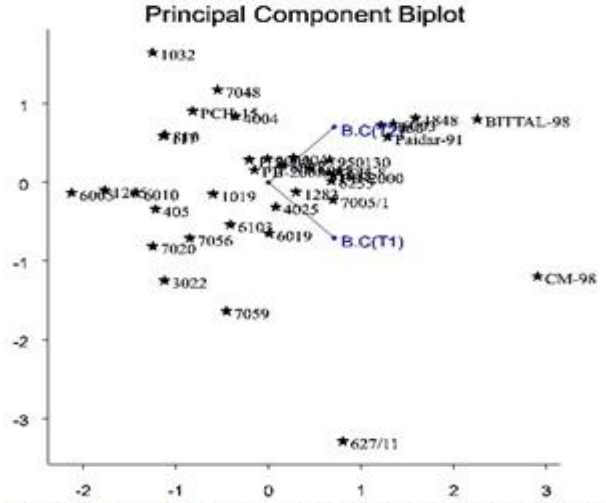


Figure 8: Biplot graph of 38 chickpea genotypes for chlorophyll-b contents under normal {Chl-b(T1)} and A. blight inoculation {Chl-b(T2)}

DISCUSSIONS:

Ascochyta blight symptoms were appeared after 10-15 days of inoculation. Disease intensity increased day by day and was at extreme after 20-25 days of inoculation on all aerial parts of the plants. Symptoms resulting from infected seeds produced brown lesions on the stems which quickly girdle the stems and caused lodging (Pande et al., 2005). Conidia and ascospores generated water soaked spots on leaves. Fungus might have damaged the chlorophyll and plant parts were blighted bounded by lesions. Extent of adaptability of plants was rated on visual basis by following scoring scale which explained the qualitative evaluation. Visual or qualitative evaluation based on scoring scale was also practiced by numerous researchers in their studies (Reddy & Singh, 1984; Reddy et al., 1984; Haware et al., 1995).

Inoculation lowered down the SR. Broad range of SR among chickpea genotypes under variable environments of normal and A. blight inoculation showed that there were differences in genetic makeup of

genotypes. Genotypes with stronger genetic make up for adaptability under A. blight stress environment exhibited higher SR as compared to genotypes with lesser SR. More reduction in SR reflected high level of susceptibility and lower level of adaptability whereas; lesser decline in SR depicted higher level of adaptability and lower level of susceptibility. Severity of stem lesions was a good indicator of extent of adaptability (Pande et al., 2005). Genotypes with lesser extent of adaptability/low survival rate were suffered from more stem lesions whereas; genotypes with high level of adaptability showed lesser symptoms of stem lesions. Vascular tissues of blighted stems were unable to transport inputs and remobilize photosynthates. Disconnection of input supply might damage aerial parts of plants and caused other allied complications.

Chlorophyll contents are always very important pigments in plants that impart the green color in them (Almela et al., 2000). Chlorophyll a and b are different regarding functional group, their color and stability (Steet & Tong, 1996). Chlorophyll contents (a & b) in chickpea genotypes reduced in response to A. blight environmental stress. These findings confirmed the results claimed by Gujar et al (1998) who declared that chlorophyll-a contents decreased in grapes susceptible to powdery mildew (Uncinula necator). The differences among genotypes regarding chlorophyll-a contents under inoculated environmental condition was due to presence of different alleles controlling this trait. Chickpea genotypes with higher chlorophyll-a contents under diseased environment possessed the strong genetic background supporting high level of adaptability and vice versa.

Yellowing of leaves which was determinant of chlorophyll degradation was included among primary disease symptoms in chickpea. Leaf or plant pigments completely destroyed when fungus formed the lesions on aerial plant parts. All chlorophyll dependent mechanisms especially photosynthesis was badly affected. Chlorophyll contents were reduced due to nematode infection (Siddiqui and Husain, 1992). In case of moderately and severely blighted leaves, the production of chlorophyll-a reduced significantly. The reduction in chlorophyll-a production was due to the pathogencity of A. rabiei or enhanced activity of chlorophyllase (Gaur, 2000). Results confirmed the finding of Gaur, 2000 who revealed that chlorophyll-b production reduced significantly in severely blighted leaves. Degradation of chlorophyll contents and inhibition of photosynthetic activity were ultimately responsible for plant death. It was reported that along with chlorophyll degradation numerous proteases were also found to be upregulated during leaf death in legumes (De Michele et al., 2009). Genes for chlorophyll biosynthesizing enzymes and photosystem assembling apparatus (cytochrome B6F complex, oxygen evolving complex, chlorophyll a/b binding complex, and electron transport system) were downregulated during leaf death (Chai et al., 2005). During leaf death several proteins were also degraded either through down regulation of protein biosynthesizing genes or up regulation of proteases (De Michele et al., 2009). Leaves become photosynthetically inactive as a result of chlorophyll degradation which changed their energy metabolism (De Michele et al., 2009).

Chlorophyll catabolism or degradation might be due to two reasons; first one is natural that happened during senescence especially during autumn while the second one is the result of pathogen infection, nutrient deficiencies and insect attack (Ni et al., 2002). Ni et al (2001) reported that chlorosis or leaf death under insect attack was different from natural leaf death. Chlorophyll contents and photosynthetic activity of the unattacked or undamaged leaves was increased to compensate the lost pigments in damaged region (Ni et al., 2002).

Responses of genotypes regarding chlorophyll a, b and carotenoids were almost the same as reported earlier (Biswal et al., 1994; Malkin & Niyogi, 2000). Reduction in carotenoid contents under A. blight prevalence increased severity of stress because carotenoids had very crucial role in plants. Carotenoids protect the photosynthetic apparatus from oxidative stress, harvest light to boost up photosynthesis, and are scavenger of reactive oxygen species (Biswal et al., 1994; Malkin & Niyogi, 2000). Carotenoids act as a shield for plants under different biotic stresses.

Activity of crop plants like wheat (Triticum aestivum), rice (Oryza sativa), grape (Vitis vinifera) and other crops reduced under fungal infections (Bassanezi et al., 2001; Bastiaans & Roumen, 1993; Moriondo et al., 2005; Robert et al., 2006). Our findings in chickpea are in corroboration with these research findings as chlorophyll a, b and carotenoids reduced in response of A. blight stress.

Conclusions and future prospects:

Resistance in identified genotypes might be due to broader diversification in genetic base. Genotypes with higher level of resistance at seedling stage can be used for evaluation of resistance level at reproductive stage. There is dire need for exploitation of marker assisted breeding to strengthen the chickpea breeding for resistance against A. blight. In recent scenario the exploitation, development and delivery of marker assisted breeding is sluggish in legumes that need to gain pace. There is need to understand the background mechanisms in parasite and host

relationship. Candidate genes for resistance against A. blight can be identified by thorough understanding of developmental processes in parasites and host (chickpea).

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