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Morphological Characters of Chickpea Cultivars Related to Resistance Against Blight

S.M. IQBAL, C.A. RAUF†, N. AYUB‡ AND A. GHAFOOR
National Agricultural research Centre, Islamabad–Pakistan
†University of Arid Agriculture, Rawalpindi–Pakistan
‡Quaid.i.Azam University, Islamabad-Pakistan

ABSTRACT

Morphological traits *viz.* number of hairs on dorsal and ventral sides of leaves, number and size of stomata, guard cells and stomatal aperture of six chickpea cultivars consisting of two each resistant (NIFA-88, Dasht), tolerant (C-44, Punjab-91), and susceptible (C-727, ILC-263), and their relationship with *Ascochyta* blight resistance were investigated. No positive or negative correlation of these morphological traits was found.

Key Words: Chickpea; *Ascochyta rabiei;* Morphology; Resistance

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop after dry beans (*Phaseolus vulgaris* L.), and dry peas (*Pisum sativum* L.) in the world (FAO, 1988) but its average yield 623 kg ha^{-1} in Pakistan is very low as compared to its yield potential (Anonymous, 2000). Several environmental, agronomic and biotic factors constrain productivity of chickpea in the country. Among the biotic constraints, diseases are the most important. More than 50 pathogens attack chickpea in different parts of the world (Nene, 1980). Among them, *Ascochyta rabiei* (Pass.) Lab. is the most serious distributed on global basis (Nene, 1980). The disease has been reported to cause 50 to 70% yield loss (Malik, 1984).

The ideal, economical and feasible control of chickpea blight is the cultivation of resistant varieties. Efforts have considerably increased during the past to identify resistant sources and to breed resistant cultivars (Aziz, 1962; Grewal & Vir, 1974; Singh, 1978; Haq *et al*., 1981; Iqbal *et al*., 1989; 1994). Unfortunately, available chickpea germplasm has neither high nor stable resistance to all the prevalent races of *A. rabiei* (Singh *et al*., 1984). In general, pods are more susceptible than vegetative parts. Some lines show resistance in the vegetative stage against a wide range of isolates but are not resistant to pod infection (Singh & Reddy, 1991). Germplasm with resistance in the vegetative stage is available but none of them has resistance at vegetative and podding stage (Reddy *et al*., 1990).

Morphological characters of chickpea such as cuticle thickness, number of stomata and number of hairs per unit area of stem and leaf have been correlated with blight resistance (Ahmad *et al*., 1952; Hafiz, 1952). Harichand *et al*. (1988) reported no such correlation. Because of the discrepancy in the aforementioned reports, the present study was undertaken to establish the correlation among the morphological traits and chickpea blight resistance.

MATERIALS AND METHODS

Two each resistant (NIFA-88, Dasht), tolerant (C-44, Punjab-91) and susceptible (C-727 and ILC-263) chickpea cultivars were sown in the field during 1997-98 in single row sub-plots, 3 m long with 30 and 10 cm row to row and plant to plant distance, respectively, with three replications in a randomized complete block design. Green plant tops were collected randomly from all lines of the each reaction group. To maintain uniformity, fifth compound leaf from the top was selected for microscopic studies for different morphological parameters including density of hairs, stomatal population, stomata and stomatal aperture size and thickness of leaf cuticle.

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

Density and area of stomata, stomatal aperture and guard cells. For determination of frequency and size of stomata, the leaf cuticle was removed gently with the help of a scalpel and a pair of forceps (Randhawa, 1994). The cuticle layer was placed on a 1"x3"glass micro slide in a small drop of safranin mixed well with two drops of Hoeyer's mounting medium. Permanent mounts were prepared for all the test lines of resistant and susceptible reaction groups. The slides were examined to determine stomatal population in a specific area (1.52 mm) under a compound microscope (Zeiss, Germany) at 80X. Size of stomata, guard cells and stomatal aperture were determined at 320X. Length and width of stomata were recorded under research microscope (Zeiss, Germany) at 320X. Length and width of observed stomata was multiplied by 0.7 on the calculative assumption that it would be nearest to the

calculated area (corrected area). Area of guard cells was determined by the following formula:

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

The microphotographs of hairs and the stomatal units were taken with the help of research microscope (Leitz, Wetzler) fitted with Orthomat (Ortholux II) camera at 250X.

RESULTS

Number of hair on dorsal and ventral sides of the leaves were recorded under a stereoscopic microscope in an area of 5.5 mm^2. Data on the stomatal population were recorded under a compound research microscope at 80X in an area of 1.52 mm^2 using an ocular grid. The size of stomata, guard cells and stomatal aperture was determined under the same microscope at 320X using a linear ocular micrometer.

Significant differences among genotypes for number of hairs on both sides were observed (Table I). These two traits were significantly correlated (r=0.6421). The total number of hairs were also significantly correlated with both the components (Table III). The range of hair density on dorsal surface of the leaf was 19.48 to 31.30 (highly variable). Within susceptible lines, values were 19.48 to 26.94 while within resistant lines, it was 20.23 to 31.30. In moderately resistant germplasm, values were 22.69 to 26.91. These traits did not exhibit any effect on infection as this character was randomly scattered without influencing disease pattern. All the genotypes, irrespective of their reaction to disease, were different from each other for this trait as revealed by Duncan's Multiple Range Test. On an average, by this character, no role towards resistance against blight was found.

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

For number of stomata per unit area, significant difference between genotypes were observed (Table II), but no clear response was recorded for disease development. Number of stomata were significantly associated with area of guard cells. Stomatal density varied from 29.37 to 52.12. In susceptible lines, the variation was 37.25 to 48.97 and in resistant lines 41.63 to 52.12, while in moderately resistant lines, 29.37 to 31.27. These results indicate possible role of stomatal density towards resistance to *Ascochyta* blight fungus. The number of stomata were, therefore, inversely proportional to degree of resistance.

Data regarding the area of stomata revealed significant differences among cultivars (Table II). Area of stomata ranged from 374.7 to 474.5 for the genotypes. The values

Table I. Mean number of hairs on dorsal, ventral and dorso-ventral sides of leaves of the reaction groups of chickpea cultivars

	No. of hairs on dorsal side			No. of hairs on ventral side			No. of hairs on dorso-ventral side		
Cultivars	**R**	**MR**	**S**	**R**	**MR**	**S**	**R**	**MR**	**S**
NIFA-88	31.30 a	-	-	62.26 a	-	-	93.57 a	-	-
Dasht	20.23 d	-	-	51.96 ab	-	-	72.05 bc	-	-
C-44	-	22.69 c	-	-	37.73 bc	-	-	54.43 de	-
P-91	-	26.91 b	-	-	57.56 a	-	-	81.14 ab	-
C-727	-	-	26.94 b	-	-	37.34 bc	-	-	64.29 cd
ILC-263	-	-	19.35 e	-	-	30.16 c	-	-	49.64 e

Figures sharing the same letters are non-significant at 0.05%.; R= resistant, MR= moderately resistant, S= susceptible

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

	Area of guard cells (um^2)			Number of stomata (1.52 mm^2			Area of stomata (um^2)			Size of stomatal aperture (um^2)		
Cultivars	**R**	**MR**	**S**	**R**	**MR**	**S**	**R**	**MR**	**S**	**R**	**MR**	**S**
NIFA-88	286.5 a	-	-	41.63 b	-	-	396.5 d	-	-	73.23 ab	-	-
Dasht	238.4 b	-	-	52.13 a	-	-	374.7 f	-	-	67.87 b	-	-
C-44	-	239.7 b	-	-	29.37 d	-	-	456.4 b	-	-	65.25 b	-
P-91	-	273.2 a	-	-	31.27 d	-	-	474.5 a	-	-	79.60 ab	-
C-727	-	-	265.3 ab	-	-	48.97 a	-		384.5 e	-	-	79.65 ab
ILC-263	-	-	241.4 b	-	-	37.25 c	-		399.3 c	-	-	88.97 a

Figures sharing the same letters are non-significant at 0.05%.; R= resistant, MR= moderately resistant, S= susceptible

Table III. Correlation between different morphological characters of chickpea cultivars susceptible and resistant to *Ascochyta* blight

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

for area of stomata for resistant genotypes were 374.7 to 396.5 and for susceptible genotypes, 384.8 to 399.3. For moderately resistant genotypes, the values were 456.4 to 474.5. This indicated no response of stomatal area for disease development.

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

DISCUSSION

For counting the number of hairs on both the sides of leaf, 5th compound leaves from the top were selected from all the chickpea cultivars because older leaves below 4-5th nodes are resistant to *Ascochyta* blight fungus (Pedersen & Morrall, 1994). On the basis of data collected, it has been found that the hair density on the dorsal surface of leaves, though little bit higher in resistant lines, was statistically at par in lines of all the reaction groups. But the hair density on ventral surface of the lines was significantly higher in case of resistant lines as compared to susceptible ones. This was further supported by significantly higher hair density in case of resistant lines on total basis. Larger hair population in resistant lines indicated some role in the resistance to blight pathogen. Higher hair population, as a whole, may be contributory to resistance in some way or the other. It is assumed that the hair would help keep the spores away from the leaf and the spore held as clinging to the hair fail to establish a direct contact with the leaf. Hence, even if they germinate while clinging to the hair, the germ tube may not be long enough to cover the length of the hair. Earlier studies had indicated that resistant cultivars possessed larger number of hair on stem and leaves than susceptible types (Hafiz, 1952; Ahamd *et al*., 1952). Similarly resistant cv. E100Y (M) and pods of E100Y bear more hair than susceptible types (Harichand *et al.*, 1988). If glandular hairs are the site of malic acid secretion as suggested by Koundal and Sinha (1983), and if malic acid plays some role in governing resistance or susceptibility (Hafiz, 1952), then number of hair should have been a meaningful criterion related to disease reaction. On this basis, however, difference in hair number could only be related to disease reaction but it could not fully explain the phenomenon of resistance. The present studies do not give indications that the hair number could be a sound basis to differentiate between resistant and susceptible cultivars of chickpea and therefore, cannot be effectively utilized as a screening parameter for disease resistance. The data obtained in the present studies were at variance with that of Koundal and Sinha (1983) who showed direct relationship between the number of glandular hair, amount of malic acid secreted and enzymatic activity.

Reddy and Khare (1984) observed higher stomatal density in the lentil cultivars susceptible to rust as compared to resistant ones. Presence of higher population of stomata in the susceptible cultivars increased the rate of transpiration upon infection by the pathogen. In the present study, maximum number of stomata were observed in Dasht (resistant cultivar) and C-727 (susceptible cultivar) which indicated that the number of stomata has no role for the initiation of blight. Similarly, other parameters concerning with stomata like area of stomata, area of guard cells and size of stomatal aperture did not clearly exhibited any correlation with blight.

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