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COMPARATIVE ASSESSMENT OF DENSITY OF GLANDULAR HAIRS, POPULATION AND SIZE OF APERTURE OF STOMATA IN RESISTANT AND SUSCEPTIBLE CULTIVARS OF CHICKPEA TO *ASCOCHYTA* BLIGHT DISEASE

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Abstract

The comparative assessment of density of glandular hairs, population and size of stomatal aperture in chickpea cultivars resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) to *Ascochyta* blight revealed that the density of the glandular hairs on the ventral, dorsoventral sides and population and size of the aperture of stomata were highly significantly different among the four reaction groups. Similarly length of the glandular hairs and area of stomata were significantly different among the four reaction groups. In the resistant reaction group of cultivars, there were higher number of glandular hairs on the ventral, dorsoventral sides and higher number of stomata of leaf as compared to susceptible group. There was no appreciable difference among the population of glandular hairs on ventral side, length of non-glandular hairs and size of stomata of two reaction groups of resistant and susceptible cultivars. The resistant group had the smallest aperture than other groups.

Introduction

Chickpea (*Cicer arietinum* L.) is grown as a pulse crops in Pakistan and is one of the major source of protein in human diet. It also serves to replenish soil nitrogen and has occupied an important place in county's economy. In Pakistan, it is cultivated as a post monsoon winter crop in barani areas over 1073 million hectares with production of 842 tones per hectares and an average yield of 784 kg /ha (Anon., 2007). This is extremely a low yield as compared to the yield of many other chickpea growing countries of the world (Saxena & Singh, 1984). Besides several other factors, chickpea diseases play major role in reducing its yield in Pakistan. Chickpea blight caused by a fungus *Ascochyta rabiei* is a devastating disease which affects the production statistics of the crop (Nene, 1982; Kausar, 1965; Malik, 1984). The disease perpetuates through infected seeds and crop debris (Maiden, 1987). Infection during the pod formation stage often results in shrivelled and infected seeds (Nene, 1982; Singh & Sharma, 1998; Akeem, 1999). The chickpea cultivars differ in their degree of susceptibility and resistance against *Ascochyta* blight disease (Rahat *et al*., 1996; Ilyas *et al*., 1991; Ilyas *et al*., 1999). This paper reports comparative assessment of density of glandular hairs, number and size of stomatal aperture in susceptible and resistant cultivars of chickpea to visualize their role, if any, in resistance against chickpea blight disease.

Materials and Methods

In order to assess the density, size, type and morphology of hairs, and to determine the size, population of stomata and thickness of cuticle, 12 chickpea cultivars belonging to 4 reaction groups i.e., resistant(R), moderately resistant (MR), moderately susceptible

(MS) and susceptible (S) to *Ascochyta rabiei* infection and each group including one variety of each of three seed colour viz., white, brown and black (Table 1) were sown in the field experimental area of the Department of Plant Pathology, University of Agriculture Faisalabad.

Collection of green plant tops of adult plants at mid pod stage was made at random from different cultivars growing in the field. In order to maintain uniformity, seventh compound leaf from the top was decided, arbitrarily to be taken for microscopic study for the purpose of observations on density, size, type and morphology of hair, size and population of stomata and thickness of leaf cuticle. Leaflets were removed from the leaf and placed under binocular stereoscope (WILD M3B Heerbrugg, Switzerland) for determination of the parameters of hair and stomata. The cuticle of the leaflet was removed gently with the help of a scalpel and a pair of a forceps. Since the ordinary dissection scalpel could not help, a new scalpel was fabricated for this purpose as follows:

A sharp V-shaped edge of a new 7 O'clock shaving blade was cut with the help of a scissor. This removed edge was then firmly held in a pair of forceps. The edge of the piece of cuticle was loosened from the underlying tissue with the help of this sharp edged-scalpel to some extent. With the help of another pair of forceps it was peeled off while still under stereoscope and placed on a slide in a drop of lactophenol. Upon covering with a coverslip, a semipermanent mount was prepared for further studies.

Observations on various morphological characters were recorded with the help of a research microscope (Zeiss, Germany). The observations on the density of glandular hair, ordinary hair, number of stomata, size of stomata and size of the stomatal aperture of the leaf cuticle of the cultivars were recorded with the help of microscope.

Hair density and size: Glandular hair were recorded from both the dorsal and ventral sides of a leaflet with the help of stereoscope from a microscopic field at the magnification of lOx/2lx 40x, of an area of 10.625 mm^2. For this purpose the leaf was exposed as such by placing it on the micro slide. Each time the leaflet selected was taken from the seventh leaf from top of a branch of each variety. Four observations from different places were recorded from the same leaflet for the sake of uniformity and convenience.

Removal of chickpea leaf cuticle: In order to record the observations on number and size of stomata and size of its aperture, a simple technique was evolved for the removal of leaf cuticle. The sharp pointed end of specially fabricated scalpel was gently scratched against the leaf under the stereoscope by free hand to an extent that a part of the cuticle was seen separated from the lower tissues. It was then, pulled apart gently by holding in a pair of forceps. The cuticle so peeled off was then placed in lactophenol mounting medium on a micro slide.

In this way, temporary mounts were prepared in lactophenol medium for further use. In these studies, observations on number of hair (glandular and ordinary), number of stomata per unit area, size of stomata and length and breadth of stomatal aperture of the cultivars were recorded. Collections for the purpose of the observations on stomata size and its aperture were made in early hours of the day when stomata are known to be open as a general principle.

Table 1. Characters of the cultivars.

S. No.	Variety	Reaction	Seed colour
1.	W 184	R	White
2.	NEC 1256	R	Brown
3.	AUG 970	R	Black
4.	ILC 1256	MR	White
5.	CM 72	MR	Brown
6.	ICC 6304	MR	Blake
7.	ILC 2548	MS	White
8.	C 727	MS	Brown
9.	AUG 679	MS	Black
10.	White Bulk	S	White
11.	AUG 1117	S	Brown
12.	AUG 918	S	Black

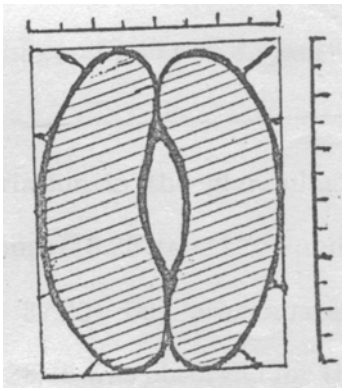

Fig. 1. A stoma with its guard cells representing barred area to be 70 percent of 12x8 divisions of the stomatal drawing.

Density and area of stomata and stomatal aperture: The collection of leaf samples of the 12 cultivars was made during the first week of March when the plants were in mid pod stage. Observations on the size and number of stomata, and the size of stomatal aperture from the dorsal side of a leaf were recorded in quadruplicate from an area of the field of microscope using 8x ocular 40x objective piece equalling to approximately 0.1521 sq. mm (1,52,114 sq. microns). The size was recorded by noting the length and width of stomata.

Measurement technique: Observations on length and width of the stomata were recorded under a research binocular microscope (Zeiss, Germany) with a magnification of 8x ocular and 40 x objectives. The multiplication product of length and width of a stoma was multiplied by 0.7 on the calculative assumption that it would be nearest to the actual area. The areas of stomatal aperture were also calculated using the same formula. The area of both the entities was subjected to statistical analysis. Hence, calculations of the area of stomata and stomatal opening were exercised with the help of Fig. 1 which helped in the description and calculation of the area.

The barred area appears to be 2/3 i.e. 66.6% for a sharp angled spindle-shaped body. But an oblong body having round margins would cover a little over than 66.6%. Hence, it was supposed to be 70% of 12 x 8 divisions as shown in Fig. 1.

Results and Discussion

Glandular hair on dorsal side of leaf: The average glandular hair count ranged from 13.25 to 37.25 for the 12 cultivars (Table 2) with an average of 25.17 hairs per 10.62 mm^{-2} area. There was a wide range of variation in the glandular hair count of cultivars of the resistant reaction group (13.25 to 34.75) and those of the susceptible reaction group (15.50 to 37.25) but a comparatively narrow variation of 16.25 to 30.50 and 25.00 to 28.75 was observed for the MR and MS groups, respectively. The 4 reaction groups had the mean number of hair within a range of 24.42 to 26.92. Hence, the groups were at par with one another, suggesting that there ought to be hardly any role in resistance of the glandular hair on the dorsal side of the leaves. Likewise a wide range of the glandular hairs count within the individual groups also points out towards the same conclusion.

Glandular hair on ventral side of leaf: The average glandular hair count varied from 22.75 to 70.00 for the 12 cultivars (Table 3) with an average of 43.25 hairs $Unit^{-1}$ area. There was a wide range of variation in the glandular hair count of the cultivars of the susceptible reaction group (22.75 to 51.00), followed by resistant (50.00 to 70.00) and MR groups (30.50 to 50.00) but a comparatively narrow variation of 35.00 to 48.75 was observed for the MS group. The 4 reaction groups had the mean number of hair within a range of 36.92 to 57.33 Here the resistant group had significantly more density over the other 3 reaction groups i.e., MR, MS and S which had only slight differences in the number of hairs $unit^{-1}$ area. Hence, in the resistant reaction group, there was more number of glandular hairs on the ventral side. There was, therefore, a clear indication of the role of glandular hair population to resistance and it was considered to be positively correlated with resistance. Moreover, on the basis of this assumption, the number of glandular hair in MR group should have been higher than that of the MS group which is reverse although non-significant according to these results.

Total glandular hair on dorso-ventral sides of leaf: The average glandular hair count ranged from 38.25 to 104.75 for the 12 cultivars (Table 4) with an average of 62.25 hairs $Unit^{-1}$area. There was wide variation in the glandular hair count of the cultivars of the resistant reaction group (63.25 to 104.75), susceptible reaction group (38.25 to 77.25) and the MR group (46.75 to 63.50) but a comparatively narrow variation of 60.00 to 77.50 was observed for the MS group. The 4 reaction groups had the mean number of hair within a range of 57.67 to 81.75. Here the resistant group had significantly more density of the glandular hair over the other 3 reaction groups. The susceptible group had the least number (57.67) of glandular hair $unit^{-1}$area.

Data collected and analyzed for the role of glandular hair for both the dorsal and ventral surfaces of the leaf indicate some role of glandular hair population in resistance and it was considered to be directly proportional to resistance. The studies conducted by various workers on morphological aspects of chickpea proved that the resistant cultivars had more density of hair $unit^{-1}$area. But this could not be proved in the present studies, rather contradictory data were recorded. The present study is broad-based and more comprehensive as it includes quite a handful number of cultivars (12), belonging to 4 reaction groups and representing all the 3 seed colours in each reaction group. Hence, one is forced to believe that the present findings are more logical and reliable, at least on papers. This is indirectly supported by earlier studies (Hafiz, 1952) in which the fungus took 96 hours for penetration in the resistant stock while 24 hours in the susceptible cultivar. It is thus logically possible that under natural conditions, a cultivar with higher density of hair may escape the infection because the conditions conducive for infection may not continue to prevail for days together. Similar results were also reported in the past in India (Chand *et al.,* 1988).

Table 2. Number of glandular hairs on dorsal side of leaf for reaction groups and various cultivars of chickpea (per 10.62 sq.mm).

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	34.75 a	-	-	
2	25.25 b	-	-	-
3	13.25 c	-	-	-
4	-	16.25 c	-	-
5	-	28.00 b	-	-
6	-	30.50 ab	-	-
7	-	-	28.75 b	-
8	-	-	27.00 b	-
9	-	-	25.00 b	-
10	-	-	-	37.25 a
11	-	-	-	15.50 c
12	-	-	-	20.50 bc
Mean	**24.42 a**	**24.92 a**	**26.92 a**	**24.42 a**

Same letters in columns for two or more means indicate non-significant difference between them.

Table 3. Number of glandular hairs on ventral side of leaf for reaction groups and various cultivars of chickpea (per 10.62 sq.mm).

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	70.00 a	-	-	-
2	52.00 b	-	-	-
3	50.00 b	-	-	-
4	-	30.50 c	-	-
5	-	35.50 c	-	-
6	-	50.00 b	-	-
7	-	-	48.75 b	-
8	-	-	36.75 c	-
9	-	-	35.00 c	-
10	-	-	-	51.00 b
11	-	-	-	22.75 cd
12	-	-	-	37.00 c
Mean	**57.33 a**	**38.67 b**	**40.08 b**	**36.92 b**

Same letters in columns for two or more means indicate non-significant difference between them.

Length of glandular hair: The average length of glandular hair ranged from 319.92 to 469.00 µm for the 12 cultivars (Table 5) with an average of 400.75 µm. There was wide variation in the length of glandular hair of resistant reaction group (319.92 to 420.42), susceptible reaction group (361.80 to 469.00) and the MS group (346.73 to 442.20) but a comparatively narrow variation of 422.10 to 440.52 µm in the MR group. The mean length of glandular hair for the 4 reaction groups ranged from 381.34 to 431.59 µm. Hence, the MR group had the longest hair whereas the R-ones had the shortest length while the other 2 reaction groups had intermediate hair length. Hence, it could be inferred from these data that, contrary to the expectations, the length of hair did not have positive correlation with resistance.

Table 4. Number of glandular hairs on dorsoventral side of leaf for reaction groups and various cultivars of chickpea (per 10.62 sq.mm).

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	104.75 a	-	-	
2	77.25 b	-	-	-
3	63.25 bc	-	-	-
4	-	46.75 cd	-	-
5	-	63.50 bc	-	-
6	-	60.00 cd	-	-
7	-	-	77.25 bc	-
8	-	-	63.50 bc	-
9	-	-	60.00 cd	-
10	-	-	-	77.25 bc
11	-	-	-	38.25 d
12	-	-	-	57.50 c
Mean	**81.75 a**	**63.58 ab**	**67.00 ab**	**57.67 c**

Same letters in columns for two or more means indicate non-significant difference between them.

Table 5. Length (µm) of glandular hairs for reaction groups and various cultivars of chickpea.

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	319.92 a	-	-	
2	420.42 ab	-	-	-
3	403.67 ab	-	-	-
4	-	440.52 ab	-	-
5	-	432.15 ab	-	-
6	-	422.10 ab	-	-
7	-	-	346.53 b	-
8	-	-	358.45 b	-
9	-	-	442.20 ab	-
10	-	-	-	469.00 a
11	-	-	-	361.80 b
12	-	-	-	393.63 ab
Mean	**381.34 b**	**431.59 a**	**382.46 b**	**408.14 ab**

Same letters in columns for two or more means indicate non-significant difference between them.

Length of (non-glandular) ordinary hair: The average length of ordinary hair ranged from 224.45 to 335.00 µm for the 12 cultivars (Table 6) with an average of 276.76 µm. There were non-significant differences in the range of variation in the length of ordinary hair of the cultivars of the 4 reaction groups. The mean length of ordinary hair of the 4 reaction groups varied from 229.29 to 311.58 µm. The MS group had the longest hair whereas the R had the shortest hair length, while the other 2 reaction groups (MR and S) had intermediate hair length. Hence, it could be inferred from these data that the length of ordinary hair did not have any relevance with resistance.

Table 6. Length (µm) of ordinary hairs for reaction groups and various cultivars of chickpea.

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	308.20 a	-	-	
2	269.67 a	-	-	-
3	320.00 a	-	-	-
4	-	304.85 a	-	-
5	-	224.45 a	-	-
6	-	318.25 a	-	-
7	-	-	335.00 a	-
8	-	-	273.02 a	-
9	-	-	326.70 a	-
10	-	-	-	284.75 a
11	-	-	-	268.00 a
12	-	-	-	328.30 a
Mean	**229.29 a**	**282.52 a**	**311.58 a**	**293.68 a**

Same letters in columns for two or more means indicate non-significant difference between them.

Table 7. Number of stomata for reaction groups and various cultivars of chickpea.

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	39.00 bc	-	-	
2	44.50 bc	-	-	-
3	65.00 a	-	-	-
4	-	32.75 cd	-	-
5	-	26.00 d	-	-
6	-	36.25 c	-	-
7	-	-	58.50 a	-
8	-	-	39.25 bc	-
9	-	-	45.50 b	-
10	-	-	-	51.25 b
11	-	-	-	43.25 bc
12	-	-	-	4075 bc
Mean	**49.50 a**	**31.67 b**	**47.75 a**	**45.08 a**

Same letters in columns for two or more means indicate non-significant difference between them.

Number of stomata: The average number of stomata on the ventral side of the leaves ranged from 26.00 to 65.00 with an average of 43.50 for an area of 0.15 mm^{-2} (Table 7). There was wide variation in the number of stomata of the R group that ranged from 39.00 to 65.00 and comparatively narrower in cultivars of the MR group ranging from 26.00 to 36.25. The other 2 reaction groups, MS and S were placed in the intermediate position. The mean number of stomata in the 4 reaction groups ranged from 31.67 to 49.50. The minimum number was found in MR group and the maximum in the R group. The data did not point out any relevance between disease reaction and stomatal population.

Table 8. Area of a stoma for reaction groups and various cultivars of chickpea.

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	440.25 ab	-	-	
2	352.75 b	-	-	-
3	396.50 ab	-	-	-
4	-	457.25 ab	-	-
5	-	455.75 ab	-	-
6	-	437.00 ab	-	-
7	-	-	366.25 b	-
8	-	-	432.25 ab	-
9	-	-	482.25 a	-
10	-	-	-	370.00 b
11	-	-	-	392.00 ab
12	-	-	-	418.00 ab
Mean	**396.50 b**	**450.00 a**	**426.92 a**	**395.67 b**

Same letters in columns for two or more means indicate non-significant difference between them

Table 9. Size of stomatal aperture for reaction groups and various cultivars of chickpea.

Cultivars	G1 (R)	G2 (MR)	G3 (MS)	G4 (S)
1	92.50 ab	-	-	
2	53.95 c	-	-	-
3	81.17 bc	-	-	-
4	-	100.03 ab	-	-
5	-	119.30 a	-	-
6	-	117.05 a	-	-
7	-	-	58.75 bc	-
8	-	-	83.68 b	-
9	-	-	54.78 ab	-
10	-	-	-	65.65 bc
11	-	-	-	92.80 ab
12	-	-	-	80.92 b
Mean	**75.88 b**	**112.13 a**	**79.07 b**	**79.79 b**

Same letters in columns for two or more means indicate non-significant difference between them

Area of stomata: The average area of a stoma on the ventral side of the leaves ranged from 352.75 μm^{-2} to 482.25 μm^{-2} with an average of 417.27 μm^{-2} (Table 8). There was a wide variation in the area of stomata of the MS group (366.25 to 482.25 μm^{-2}) and comparatively narrower in the cultivars of the MR group (437.00 to 457.25) while the other 2 reaction groups (R and S) occupied the intermediate position. The 4 reaction groups had the mean area of the stomata within a range of 395.67 to 450.00 μm^{-2}. The average area of the stomata of leaf was lowest in the S group closely followed by the R group and highest in that of the MR group while the 4th reaction group, MS, was in close proximity with the MR group. The data did not suggest any relevance between disease reaction groups and area under stomata of leaf. No data on number and area of the stomata unit-' area of leaf were available for the sake of comparison with the present studies. The resistant reaction group had more stomata compared with susceptible reaction group although the difference among them was non-significant.

Area of stomatal aperture: The average area of stomatal aperture on the ventral side of the leaves ranged from 53.95 to 119.30 μm^{-2} with an average of 86.97 (Table 9). There was wide variation in the area of stomatal aperture of the R (53.95 to 92.50), MS (54.78 to 83.68) and S groups (65.65 to 92.80 sq., m) whereas a comparatively narrower variation in the MR group, ranging from 100.03 to 119.30 pm2 was observed. The 4 reaction groups had an area of stomatal aperture within a range of 75.88 to 112.13 m-2. The minimum area of the aperture of stomata was found in R group, closely followed by MS and the S groups and the maximum in the MR group. The data point out some role of the size of stomatal aperture to resistance but in the case of R group only, whereas it did not hold good for the MR group. However, the area of aperture was almost the same in the resistant and susceptible groups. No such information on area of the aperture of stomata was available with the author for the sake of comparison.

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