http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (8): 1277-1280, 2000

Morphological Basis of Resistance in Lentil (*Lens Culinaris* Medik.) Against Ascochyta Blight

S.T. Sahi, M.A. Randhawa and S.M. Khan
Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Abstract: The lentil lines belonging to resistant group had higher number of hair both on dorsal and ventral side of the leaves and as such had more total number of hair and more leaf area covered by them. The number of stomata and leaf area covered by them was more in case of susceptible group as compared with the resistant one but the area of stomata, guard cells and stomatal aperture was greater in case of resistant group than the susceptible one.

Key words: Morphology, lentil, leaf hair, stomata, stomatal aperture, ascochyta blight

Introduction

Lentil (*Lens culinaris* Medik.) is the second most important rabi pulse crop of Pakistan only next to chickpea. It contains 28.6 percent protein, 3.1% ash, 4.6% crude fiber, 44.3% starch, 36.1% amylose, 63.1% total carbohydrates and 420 Cal. 100 g^{-1} gross energy (Bhatty and Wu, 1974). Moreover, lentils are lower in anti-nutritional factors such as haemagglutinins, oligosaccharides and favogens as compared with most of other legumes. Although they contain tannins in the seed coat, but not in the cotyledons (Vaillancourt *et al*., 1986). The high level of protein together with a lower level of anti-nutritional factors and a shorter cooking time than most of other pulses, make lentil very suitable for human consumption (Williams *et al*., 1993).

In 1998-99, lentil was grown over an area of 3.404 million hectares in the world, 35.25 percent being occupied by India, 5.88% by Bangladesh and 1.91 percent by Pakistan, the total share in world production by these countries being 29.55, 5.46 and 1.24% respectively (Anonymous, 1997a). Lentil is the second most important pulse crop of Pakistan after chickpea. In 1998-99, lentil was grown over an area of 65 thousand hectares with an average yield of 571 Kg ha^{-1} only (Anonymous, 1997b). This is extremely low as compared to other countries of the world e.g. 736 Kg ha^{-1} in India, 814 Kg ha^{-1} in Bangladesh, 728 Kg ha^{-1} in Syria and 1370 Kg ha^{-1} in USA (Anonymous, 1997a).

The low yield in Pakistan may be attributed to continuous cultivation of cultivars with low yield potential and excessive vegetative growth, narrow adaptability, low stability of yield and susceptibility to stress conditions (Rajput and Sarwar, 1989) and inadequate nitrogen nutrition. One of the most important stresses is damage by diseases. The common diseases of lentil in Pakistan are blight and rust. Blight is caused by *Ascochyta lentis* (Bond. and Vassilj.) and may cause considerable losses in yield especially under cool and moist conditions. Lentil blight can be managed through a number of means, the cheapest and most practicable being the use of resistant varieties. Although resistance to lentil blight is not scarce in the existing lentil cultivars yet the phenomenon of resistance is not clearly known, due to lack of studies on the morphological and biochemical basis of resistance.

A number of morphological characters are likely to contribute some role in host-parasite interaction. Such studies have been reported in case of chickpea (Randhawa, 1994). Similarly, Reddy and Khare (1984) reported the effect of some morphological characters of lentil in resistance to rust, but they need to be further investigated in case of lentil-*Ascochyta lentis* interaction.

Thus, it seems imperative to undertake studies on the determination of morphological characters of lentil which may possibly play a role in resistance against *Ascochyta lentis*.

Materials and Methods

Seven advanced lines each of the reaction group (resistant and susceptible) were sown in the field during the growing season of 1997-98. The test lines were sown in single row subplots, 3 meter long with 30 cm and 3-cm row to row and plant to plant distance respectively with four replications in group balanced block design. Green plant tops were collected randomly from all lines of the two reaction groups growing in the field. In order to be uniform, fifth compound leaf from the top was selected for microscopic studies on different structural parameters (density and size of hair, stomatal population, stomata and stomatal aperture size and thickness of leaf cuticle).

Density and Length of Hair: Leaflets were removed from the leaf and placed under binocular stereoscopic microscope (WILD M3B Heerburg, Switzerland) for counting the density and size of hair on their dorsal and ventral side in an area of 5.5 mm^2. To get a more precise picture four observations were recorded from the same leaflet.

Removal of Leaf Cuticle: 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 glass microslide, 1″x3″ size (in a small drop of safranine mixed well with two drops of Hoeyer's mounting medium). In this way permanent mounts were prepared for all the test lines of resistant and susceptible reaction groups.

Density and area of stomata, stomatal aperture and guard cells: The slides were examined under a compound microscope (Zeiss, Germany) at 80X to determine the stomatal population in a specific area (1.52 mm^2). The size of stomata, guard cells and stomatal aperture was determined at 320x. The thickness of hair at basal and distal end was also determined at 320x. Camera lucida drawing of typical stomata for each test line was drawn in order to calculate the correction factor.

Measurement Technique: Observations on length and width of the stomata were recorded under a research microscope (Zeiss, Germany) at 320X. The multiplication of length and width of a stomata (observed area) was multiplied by 0.7 on the calculative assumption that it would be nearest to the actual area (corrected area). The area of stomatal aperture was also calculated using the same formula. Area of guard

cells was determined as:

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

The data collected were analyzed following Steel and Torrie (1980), Gomez and Gomez (1984), Petersen (1989a, 1989b) and using computer programme MstatC. To elucidate the data, graphs, bar diagrams etc. were prepared using computer programme HG4.

Results

The data on the hair density on dorsal and ventral surface of leaves, their total, number of stomata, area of stomata, guard cells and stomatal aperture, percent leaf area covered by hair and stomata, of both the susceptible and resistant lines of lentil is given in Table 1. The analysis of variance of these data is given in Table 2.

Hair density on dorsal side of the leaf: The two reaction groups were at par statistically. However, the differences between the lines/cultivar within both the reaction groups were highly significant. The range of hair density on dorsal surface of the leaf was 17.25 to 53.00 (highly variable) with average being 36.23. Within susceptible group this range was 17.25 to 46.75 while within resistant group it was 23.75 to 53.00, being slightly higher than the former one.

Hair density on ventral side of leaf: Table 2 indicated highly significant difference between the two reaction groups. The differences among the lines/cultivar within the two reaction groups were also highly significant. The range of number of hair on ventral surface of the leaves was 26.50 to 87.75. In case of susceptible and resistant lines/cultivar, the range was 26.50 to 62.00 and 33.50 to 87.75, respectively.

Total number of hair on leaf: On the basis of analysis of variance of the data (Table 2), highly significant difference was observed between the two reaction groups. Similarly, the lines/cultivar within both, susceptible and resistant reaction groups, were also different from each other at a highly significant level. Variation in the total hair density was 44.00 to 140.80 being 44.00 to 106.80 and 57.25 to 140.80 in susceptible and resistant groups, respectively.

Number of stomata per unit area: Table 2 also revealed highly significant difference between the two reaction groups. Lines/cultivar within resistant group also displayed highly significant differences but the differences between the lines within susceptible group were non-significant. The stomatal density varied from 21.25 to 85.00 within the lentil entries, as a whole. In susceptible group the range was 55.00 to 85.00 and in resistant group, it was 21.25 to 67.25.

Area of Stomata: The area of stomata was significantly larger (Table 2) in the lines/cultivar of resistant group than those of susceptible group. But the differences between the lines/cultivar within both the groups were non-significant. The area of stomata ranged from 461.8 to 654.6 μm^2. In the susceptible group, the range was 461.8 to 548.5 while the range was 518.3 to 654.6 in resistant group.

Area of Guard Cells: Table 2 indicated a non-significant difference between the two groups with regard to area of guard cells. Moreover the differences between the Lines/cultivar within susceptible and resistant group, were also non-significant. The overall range of the area of guard cells was 212.0 to 299.3. In case of susceptible lines, the range was 212.0 to 262.6 while in the resistant lines/cultivar, the range was 234.7 to 299.3.

Area of Stomatal Aperture: Area of stomatal aperture was significantly larger in case of resistant lines/cultivar as compared with the susceptible ones (Table 2). But the differences in the size of stomatal aperture between the lines/cultivar of susceptible and resistant group were non-significant. The area of stomatal aperture ranged from 31.60 to 56.40. This range within the susceptible group was 31.60 to 44.48 and resistant group, 36.40 to 56.40.

Leaf area covered by hair: Table 2 represented non-significant difference between the two reaction groups with regard to percent leaf area covered by hair. But the differences between the lines/cultivar within susceptible and resistant group were highly significant. percent leaf area covered by hair ranged from 6.30 to 25.28% leaf area covered by hair in the lines within susceptible group ranged from 6.30 to 17.84 and within resistant group, from 8.96 to 25.28.

Leaf area covered by stomata: The leaf area covered by stomata was statistically larger in susceptible as compared with the resistant group (Table 2). The lines/cultivar within resistant group differed from each other significantly. But the lines within susceptible group did not differ from each other significantly. The range of percent leaf area covered by the lines/cultivar was 0.76 to 2.77. The range in the lines within susceptible and resistant groups was 1.70 to 2.77 and 0.76 to 2.36, respectively.

Correlation between morphological characters: The correlation matrix between different morphological characters is given in Table 3. In both the groups, highly significant correlation was observed between hair density on dorsal side of the leaves and total number of hair (r values 0.967 and 0.959 for susceptible and resistant group, respectively). Similarly, highly significant correlation was observed between hair on dorsal side of the leaves and leaf area covered by the total hair (r values 0.977 and 0.970 for susceptible and resistant groups, respectively). Highly significant correlation was calculated between hair on ventral side and total hair (r values 0.964 and 0.986 for susceptible and resistant group, respectively). But slightly lower degree of correlation was observed between the hair of ventral side of the leaves and area covered by total hair (r values 0.808 and 0.791, respectively). The correlation between the hair on dorsal side and ventral side of the leaves was more significant (r value 0.899) in case of resistant group as compared with susceptible group (r value 0.865).

Frequency of stomata was significantly correlated with percent leaf area covered by them for both the groups (r values 0.950 and 0.971 for susceptible and resistant group, respectively). Similarly, area of stomata was also significantly correlated with area of guard cells (r values 0.950 and 0.967 for susceptible and resistant group, respectively). On the other hand, correlation between area of stomata and stomatal aperture was non-significant in case of both the groups (r values 0.090 and 0.334 for susceptible and resistant group, respectively) meaning that the size of stomatal aperture is not affected by the size of stomata. All other correlation values were non-significant, either positive or negative.

Table 1: Mean number of hair on dorsal (A), ventral (B), dorso-ventral (C) sides of leaf, number of stomata (D), area of stomata (E), mean area of guard cells (F), area of stomatal aperture (G), percent area covered by hair (H) and percent area covered by stomata (I) for reaction groups and various lines/cultivar of lentil

Group	Lines/ Cultivar	A	B	C (A+B)	D	E	F	G	H	I
	91549	17.50 c*	26.50 d	44.00 e	74.25 ^{NS}	505.7 ^{NS}	221.9 ^{NS}	35.61 ^{NS}	6.30 b*	2.47 ^{NS}
	93523	42.75 ab	42.00 c	84.75 d	64.50	548.5	262.6	31.60	16.95 a	2.32
S	93536	39.00 b	62.00 a	98.50 b	55.00	461.8	212.0	36.51	14.40 a	1.70
	93564	38.75 b	48.50 b	87.25 cd	58.75	522.6	237.1	39.00	17.66 a	2.02
	93566	42.50 b	49.00 b	91.50 c	55.75	533.0	249.9	39.30	16.80 a	1.99
	95560	46.75 a	60.00 a	106.80 a	85.00	495.1	225.2	37.30	17.84 a	2.77
	96504	17.25 c	26.75 d	44.00 e	64.25	535.3	248.8	44.48	6.76 b	2.26
	Mean	34.93	44.96	79.54 6	5.36	514.59	236.79	37.69	13.82	2.22
	LSD	4.248	5.406	6.112	21.83	78.41	37.78	10.95	4.213	0.83
	94503	27.50 cd	35.00 e	62.50 de	41.25 b	637.0 ^{NS}	296.2 ^{NS}	47.59 ^{NS}	11.22 cd	1.75 b
	94506	33.25 c	59.50 c	92.75 c	44.25 b	585.1	271.4	37.97	10.37 cd	1.71 b
	95509	53.00 a	70.25 b	123.30 b	44.75 b	559.1	264.4	38.63	25.28 a	1.65 b
R	95513	31.75 c	38.25 e	70.00 d	67.25 a	552.0	260.1	42.15	14.75 bc	2.36 a
	95515	23.75 d	33.50 e	57.25 e	52.00 b	518.3	234.7	55.69	8.96 d	1.79 ab
	95527	40.50 b	47.50 d	88.00 c	21.25 c	541.0	237.0	36.40	18.90 b	0.76 c
	Masoor-93	53.00 a	87.75 a	140.80 a	29.50 c	654.6	299.3	56.40	25.01 a	1.27 b
	Mean	37.54	53.12	90.64	42.89	578.15	266.15	44.98	16.36	1.61
	LSD	6.697	6.575	12.18	10.86	98.62	54.48	19.17	4.689	0.58

*Means sharing similar letters in the same column do not differ from each other at p = 0.05

Table 2: Analysis of Variance for number of hair on dorsal (A), ventral (B), dorso-ventral (C) sides of leaf, number of stomata (D), area of stomata (E), area of guard cells (F), area of stomatal aperture (G), percent leaf covered by hair (H) and percent leaf covered by stomata (I) for reaction groups and various lines/cultivar of lentil

Source of Variance	Degree of Freedom	Mean Squares								
		A	B	C (A+B)	D	E	F	G	H	I
Replications	3	47.40^{NS}	102.42*	251.97*	91.07^{NS}	2918.80^{NS}	123.92^{NS}	286.60^{NS}	10.50^{NS}	0.231^{NS}
Reaction Group	1	95.16^{NS}	928.29**	1727.16**	7065.02**	56549.34*	12068.14^{NS}	744.24*	90.32^{NS}	5.130**
Error (a)	3	30.02	8.62	12.21	26.64	2481.24	1271.82	48.27	11.76	0.089
Lines within Susceptible Group	6	603.81**	817.70**	2570.45**	473.41^{NS}	3482.21^{NS}	1300.46^{NS}	62.27^{NS}	104.30**	0.496^{NS}
Error (b_1)	18	8.18	13.24	16.93	215.98	2785.47	646.86	54.35	8.04	0.315
Lines/Cultivar within Resistant Group	6	553.12**	1666.24**	3945.74**	887.99**	10251.14^{NS}	2605.49^{NS}	282.08^{NS}	187.04**	0.974**
Error (b_2)	18	20.33	19.59	67.17	53.45	4407.09	1344.72	116.52	10.00	0.151
Error ($b = b_1+b_2$)	36	14.25	16.42	42.05	134.71	3596.28	995.79	85.44	9.02	0.233
Total	55									

Table 3: Correlation between different morphological characters of lentil lines/cultivar susceptible (s) and resistant (R) to Ascochyta Blight

		A	B	C	D	E	F	G	H	I
S	A	-								
R										
S	B	0.865	-							
R		0.899								
S	C	0.967	0.964	-						
R		0.959	0.986							
S	D	-0.062	-0.091	-0.066	-					
R		-0.474	-0.445	-0.467						
S	E	-0.102	-0.554	-0.333	-1.102	-				
R		0.317	0.496	0.441	-0.337					
S	F	0.084	-0.364	-0.142	-0.227	0.950	-			
R		0.302	0.473	0.420	-0.128	0.967				
S	G	-0.425	-0.243	-0.338	-0.157	0.090	0.026	-		
R		-0.126	0.073	0.000	0.048	0.334	0.281			
S	H	0.977	0.808	0.927	-0.128	0.004	0.158	-0.362	-	
R		0.970	0.791	0.877	-0.426	0.259	0.242	-0.075		
S	I	-0.920	-0.262	-0.168	0.950	0.213	0.078	-0.129	-0.127	-
R		-0.455	-0.380	-0.417	0.971	-0.115	0.102	0.103	-0.426	

A = Hair density on dorsal side B = Hair density on ventral side
C = Total No. of hair D = Stomata population
E = Area of stomata F = Area of guard cells
G = Area of stomata aperture H = Percent leaf area covered by hair
I = Percent leaf area covered by stomata
Correlation Coefficient (r) = 0.754 (5 percent), 0.874 (1 percent)

Discussion

Morphological characters sometimes play an important role in contributing resistance towards some of the pathogens. This phenomenon is displayed by many plant-parasite interactions. Keeping this fact in view, it was thought worthwhile to study the role of the morphological characters of lentil in resistance against *Ascochyta lentis*, causing blight disease. To test this assumption studies were therefore, undertaken on different morphological characters of the lentil lines susceptible as well as resistant to the pathogen to work out the differences in the lentil lines with both susceptible and resistant reaction type, on the basis of morphology. These characters included the number of hair on dorsal and ventral surface of the leaves, number of stomata, size of stomata and guard cells, stomatal aperture and percent leaf area covered by hair and stomata.

For sake of counting the number of hair on both the sides of leaf, fifth compound leaf from the top was selected from all the lentil lines. This was based on a previous report (Pedersen and Morrall, 1994) that older leaves and leaves below 4-5th node are resistant to Ascochyta blight fungus.

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 lentil lines, was at par statistically in lentil lines of both the reaction groups. But the hair density on ventral surface of the leaves was significantly higher in case of resistant lentil lines as compared with the susceptible lentil lines. This was further supported by significantly higher hair density in case of resistant lentil lines on total basis. This larger hair population in case of resistant lines indicated some role in resistance to the pathogen. Also the percent leaf area covered by the hair was greater in case of resistant lines as compared with the susceptible lines. 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 spores held as clinging to the hair fail to establish a direct contact with the leaf surface. 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 to reach the leaf surface. Moreover, a thick mat of hair may render the leaf surface hydrophobic thus making the conditions rather unfavourable for the germination of *A. lentis* spore and subsequent infection. These results are in line with those of Randhawa (1994) who recorded significantly higher hairs in case of Ascochyta blight resistant chickpea cultivars. While Reddy and Khare (1984) could not find any significant difference in hair density in case of lentil cultivars resistant and susceptible to rust disease.

On the other hand, stomata population was significantly higher in case of susceptible lentil lines as compared with the resistant ones. These results are in line with those of Reddy and Khare (1984) who also observed higher stomatal density in the lentil cultivars susceptible to rust as compared with the resistant ones. On the other hand Randhawa (1994) reported similar stomatal density in case of chickpea cultivars with differential reaction to Ascochyta blight. As far as the presence of higher population of stomata in the susceptible lines is concerned, is rather considered to be so on the grounds that the rate of transpiration increases upon infection by the pathogen. The loss of phyton by rusts and blights is partly due to water loss. Thoughtfully, high number of stomata would be responsible for proportionately more loss of water in the one having more stomata. It may look out of place to mention here that a person suffering from cholera infection, sometimes collapse to death. Such a death is not due to the action/effect of causal bacterium but due to dehydration. Similar to that low loss of phyton in the resistant lines may be due to less amount of water transpired. Although, the infection due to *Ascochyta lentis* is reported to take place through direct invasion of the epidermal layer (Roundhill, 1995), yet there may be a possibility of infection through the stomata. If this is the case, then significantly higher number of stomata on the leaves of susceptible lentil lines in contrast to the resistant ones, supports this assumption. Although the area of stomata, guard cell and stomatal aperture was significantly higher in case of resistant lines, yet the percent leaf area covered by the stomata was significantly higher in the susceptible lentil lines primarily due to almost 50% more stomata than the resistant lines. Although the size of stomata, guard cells and stomatal aperture was higher in the resistant lines, yet the percent leaf area covered by the stomata was more in the susceptible lines meaning thereby, that vulnerable area by the pathogen was higher in the susceptible as compared to resistant lines. It may be assumed by this data that not size but the frequency of stomata may be accounted for resistance to the Ascochyta blight fungus. Another point in the favour of this conception is that the size of conidia of *Ascochyta lentis* is even much smaller than the smallest stomatal aperture recorded in both the reaction groups.

References

Anonymous, 1997a. Agricultural statistics of Pakistan. Govt. of Pakistan, Ministry of Food, Agriculture and Livestock, Economic Wing, Islamabad, pp: 50-51.

Anonymous, 1997b. FAO Statistical Yearbook 1997. Food and Agriculture Organization of United Nations, Rome, Italy, pp: 101.

Bhatty, R.S. and K.K. Wu, 1974. Determination of gross energy of cereals and legumes with a ballistic bomb calorimeter. Can. J. Plant Sci., 54: 439-441.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons, New York, USA., ISBN-13: 9780471870920, Pages: 680.

Pedersen, E.A. and R.A.A. Morrall, 1994. Effects of cultivar, leaf wetness duration, temperature and growth stage on infection and development of Ascochyta blight of lentil. Phytopathology, 84: 1024-1030.

Petersen, R.G., 1989a. Experimental Designs in Agriculture. Pakistan Agricultural Research Council, Islamabad, Pakistan, pp: 105.

Petersen, R.G., 1989b. Special Topics in Biometery. Pakistan Agricultural Research Council, Islamabad, Pakistan, pp: 68.

Rajput, M.A. and G. Sarwar, 1989. Genetic variability, correlation studies and their implication in selection of high yielding genotypes in lentil. Lens Newslett., 16: 5-8.

Randhawa, M.A., 1994. Role of some morphological and chemical characters of gram in resistance to *Ascochyta blight*. Ph.D. Thesis, Department of Plant Pathalogy, University of Agriculture, Faisalabad, Pakistan.

Reddy, R.R. and M.N. Khare, 1984. Further studies on factors influencing the mechanism of resistance in lentil (*Lens culinaris* M.) to rust (*Uromyces fabae* (Pers.) de Bary). LENS Newslett., 11: 29-32.

Roundhill, S.J., B.A. Fineran, A.L.J. Cole and M. Ingerfeld, 1995. Structural aspects of ascochyta blight of lentil. Can. J. Bot., 73: 485-497.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, Pages: 633.

Vaillancourt, R., A.E. Slinkard and R.D. Reichert, 1986. The inheritance of condensed tannin concentration in lentil. Can. J. Plant Sci., 66: 241-246.

Williams, P.C., W. Erskine and U. Singh, 1993. Lentil processing. LENS Newslatt., 20: 3-13.