Studies on selected physiological and biochemical parameters responsible for resistance to spot blotch of barley (*Hordeum vulgare* L.)

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Abstract

The spot blotch caused by *Helminthosporium sativum* Pam., King and Bakke is attaining importance in Karnataka and Northern and Eastern parts of India, Hence, an attempt was made for a systematic study of the various physiological and biochemical factors which contribue to the resistance of the barley genotypes, The resistant genotypes recorded less number of stomata, lesser length and breadth of the stomata when compared to susceptible genotypes. The histological studies indicated that the cuticular thickness, epidermal thickness were more in resistant genotypes than in susceptible ones. The resistant genotypes recorded less number of epidermal cells per millimeter (mm) compared to susceptible ones. From the biochemical studies, it has indicated that resistant genotypes had more amount of total sugar, reducing sugar protein, total phenols, amino acid and wax content than susceptible genotypes. These were decreased after infection and the decrease was more in susceptible genotypes than in resistant ones. In contrast to this non-reducing sugars were more in susceptible genotypes than in resistant ones.

Keywords: *Hordeum vulgare* L, *helminthosporium sativum,* resistance, susceptible

Introduction

Barley (*Hordeum vulgare* L.) a crop of industrial value ranks fourth among the major food grain crops after wheat, rice and maize in the world with regard to acreage and production. It can be grown over a wide range of latitude covering diversified agro-climatic conditions. Barley crop is gaining importance in India in view of its varied utility in food, feed and brewing industries. Barley has became an important crop due to its demand for manufacturer of alcoholic beverages and in ayurvedic medicines (Misra *et al.,* 1982). Barley grain contains 11.5 percent protein, 69.6 percent carbohydrates and 1.3 percent fat. Besides, barley is most dependable cereal under extreme conditions of drought, salinity and even frost. Hence, it has special significance to Indian agriculture in view of its ability to with stand drought.

In the recent years demand for malting barley from the southern region of the country has been increasing. A number of breweries have been functioning in southern India which requires large quantities of good malting type of barley for the production of malt. The malt barley grown in southern India has quality characters with that of northern India. (Swaminathan, 1990)

Among various diseases of barley, the spot blotch caused by *Helminthosporium sativum* Pam., King and Bakke is attaining importance in Northern and Eastern parts of India. As the occurrence of this foliar blight complex disease is reflected on considerable yield loss amounted to 16% in India, 20% in Nepal and 23% In Bangladesh (Dubin and Ginkel,1991; Saari, 1998).

In recent years, it is becoming increasingly evident that several natural and induced defense mechanisms operate in host plants against different diseases. One such defense mechanism is the presence of certain compounds inhibitory to the pathogen. Sometimes, the host plant is induced to synthesize these compounds on infection. Hence, an attempt was made for a systematic study of the selected physiological and biochemical factors which contribute to the resistance of the barley genotypes, which would help us to know the nature of resistance present in different genotypes and to evolve a suitable resistant genotype against this disease.

Materials and Methods

A field experiment was conducted by selecting eight barley genotypes viz., BH-645, PL-760, DWR-46 and DWR-28 (moderately resistant), K-729 and K-741 (moderately susceptible) and RD-2640 and RD-2653 (susceptible). Among them PL-760 and RD-2640 were six rowed and remaining were two rowed barley genotypes. All the genotypes were sown in two rows of one meter length under Randomized Block Design (RBD) with three replications. The observations were made on these lines for spot blotch at 50 and 80 DAS by following double digit scale (Kumar *et al.,* 1998). The leaves were sampled randomly and composite leaf sample was made from each entry for further studies.

In the morpho-physiological studies, observations on stomatal frequency and size (length and breadth) were taken at 50 Days after sowing (DAS) and 80 DAS by following the procedure given by Varadarajan and Wilson (1973). The histological observations such as, cuticular thickness, epidermal cell layer thickness and number of epidermal cells per mm were studied by following microtome technique (Jensen, 1962).

For biochemical studies, one gram of the tissue was weighed and made in to small pieces and plunged immediately in boiling ethyl alcohol (80%). Then, it was cooled and passed through double layered muslin cloth. The piece of the tissue was ground thoroughly in a morter with pestle with hot alcohol. Again it was passed through muslin cloth. The above procedure was repeated. The filtrate were polled and

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filtered through Whatman NO. 41 filter paper and made up to ten ml volume with alcohol. Then the extract was stored in a refrigerator at 4°C. The bio-chemicals like, total sugars, reducing sugars, non-reducing sugars, phenols, amino acids, were estimated. Sugars were estimated by Nelson's modification of Somogyi's method (Nelson, 1944). Phenols were estimated by Folin – ciocalteau reagent method (Bray and Thorpe, 1954), amino acids were estimated by Moore and Steins (1958) method. Further, protein estimation was also done by the method of Lowry *et al.* (1951). Wax estimation was done by the method described by Ebercon *et al.* (1977).

Results

Stomatal frequency on upper surface of the leaves

Among the eight genotypes tested at 50 DAS, the genotypes DWR-28 and DWR-46 were recorded less (129.63 and 130.14 / mm^2, respectively) number of stomata and these two genotypes were statistically on par with each other. The more number of stomata was recorded by K-741 (134.51 / mm^2) and similar results were also observed at 80 DAS. The resistant genotypes were recorded less number of stomata as compared to susceptible genotypes (Table 1).

Stomata frequency on lower surface

At 50 DAS, the genotypes DWR-46 and DWR-28 recorded minimum (158.03 and 158.15/mm^2, respectively) number of stomata and the genotypes K-729 recorded maximum (161.47/mm^2) number of stomata followed by RD-2653 (160.58/mm^2) and similar results were also observed at 80 DAS. The resistant genotypes were showed less number of stomata as compared to the susceptible genotypes (Table 1).

Length of the stomata on upper surface of the leaves

At 50 DAS, the length of the stomata was more in the genotypes K-741 (32.19 micrometer (µm) and it was least in DWR-28 (28.76 µm). At 80 DAS, the genotypes K-741 recorded maximum (32.69 µm) length and the genotypes DWR-28 recorded the least (29.85 µm). Nevertheless, there was slight increase in the length of stomata from 50 to 80 DAS. The length was more in susceptible genotypes than resistant ones (Table 1).

Length of the stomata on lower surface of the leaves

The genotype DWR-28 showed minimum (31.77 µm) stomatal length and maximum was found in K-729 (34.14 µm) at 50 DAS. The genotype K-729 recorded maximum (35.09 µm) stomatal length followed by K-741 (34.81 µm) and the least was in DWR-28 (32.84 µm) at 80 DAS. There was slight increase in the stomatal length from 50 to 80 DAS (Table 1).

Breadth of the stomata on upper surface of the leaves

At 50 DAS, the genotype DWR-28 recorded the least (15.59 µm) stomatal breadth followed by DWR-46 (16.05 µm). The maximum was recorded in RD-2653 (17.59 µm) followed by K-741 (17.34 µm). Whereas, at 80 DAS, the same genotype DWR-28 recorded the least (17.13 µm) stomatal breadth and the maximum was recorded by K-741 (18.53 µm) followed by RD-2653 (18.37 µm). There was slight increase in the breadth of stomata from 50 to 80 DAS (Table 1).

Breadth of the stomata on lower surface of the leaves

Among all the genotypes resistant genotypes showed least stomatal breadth compared to susceptible ones. At 50 DAS, the genotypes RD-2653 recorded maximum (17.65 µm) stomatal breadth followed by K-729 (17.53 µm). The minimum was recorded in DWR-46 (16.56 µm) followed by DWR-28 (16.69 µm). Whereas at 80 DAS, the genotypes RD-2653 recorded maximum (19.21 µm) stomatal breadth and the minimum was recorded in DWR-46 (17.04 µm) (Table 1).

Histological parameters

In order to know the difference between resistant and susceptible genotypes, cuticular thickness, epidermal cell layer thickness and number of epidermal cells per mm were studied (Table 1).

Cuticular thickness

Cuticular thickness in case of DWR-46 was significantly more (4.25 µm) as compared to other genotypes followed by DWR-28 (4.08 µm) and BH-645 (4.06 µm). The least cuticular thickness was recorded in K-729 (3.16 µm) (Table 1).

Epidermal Cell Layer thickness

The epidermal thickness was significantly more in case of DWR-46 (11.84 µm) followed by DWR-28 (11.47 µm). The least epidermal thickness was recorded in the genotype K-709 (9.15 µm) (Table 1).

Number of epidermal cells per mm

Number of epidermal cells per mm was significantly more in case of RD-2653 (35.45 / mm) followed by RD-2640 (35.24/ mm) and the least was recorded by DWR-28 (32.20 / mm) followed by DWR-46 (32.42/mm) (Table 1).

Total sugars

The amount of total sugars in different barley genotypes decreased as the age of the crop advanced (Table 2). At 50 DAS, the genotype DWR-46 recorded maximum (14.91 milligram (mg)/dry wt.) total sugar content followed by BH-645 (14.43 mg/dry wt.). At 80 DAS, the same genotype DWR-46 recorded maximum total sugar (12.96 mg/dry wt.) followed by DWR-28 (12.86 mg/dry wt.). The genotype RD-2640 recorded the lowest total sugar content both at 50 DAS (12.39 mg/dry wt.) and 80 DAS (10.34 mg/dry wt.). When per cent decrease was calculated, the susceptible genotypes showed maximum decrease compared to resistant genotypes.

Reducing sugars

The reducing sugar content differed significantly among the genotypes and stages of the crop growth (Table 2). At 50 DAS, the reducing sugar content was maximum in DWR-28 (12.85 mg/dry wt.) followed by DWR-46 (12.75 mg/dry wt.). The least was recorded in K-729 (10.25 mg/dry wt.). At 80 DAS, the genotype DWR-46 recorded maximum (11.98 mg / dry wt.) reducing sugar content followed by DWR-28 (11.62 mg/dry wt.), however the least was observed in RD 2653 (9.03 mg / dry wt.). There was significant decrease in the reducing sugar content from 50 to 80 DAS. When percent decrease was considered, the susceptible genotypes showed maximum decrease compared to resistant genotypes.

Table 1. Physiological parameters in different barley genotypes as influenced by *Helminthosporium sativum*

Genotypes	Stomatal frequency (mm²)				Stomatal length (µm)				Stomatal breadth (µm)				Histological parameter		
	Upper surface		Lower surface		Upper surface		Lower surface		Upper surface		Lower surface				
	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	Cuticular thickness (µm)	Epidermal cell layer thickness(µm)	No. of epidermal cell per mm
BH-645	130.56	131.12	158.39	158.92	29.77	30.14	32.06	33.48	16.35	17.45	16.89	17.26	4.06	11.24	32.83
PL-760	131.23	131.65	158.31	158.76	29.69	30.63	32.19	32.94	16.54	17.38	17.06	17.64	4.05	10.76	33.25
DWR-46	130.14	129.47	158.03	158.93	29.31	29.85	32.31	33.67	16.05	17.81	16.56	17.04	4.25	11.84	32.42
DWR-28	129.67	129.92	158.15	158.94	28.76	29.59	31.77	32.84	15.59	17.13	16.69	17.37	4.08	11.47	32.20
K-729	133.45	132.91	161.47	161.81	30.74	32.26	34.14	35.09	16.79	18.01	17.53	18.46	3.16	9.15	34.33
K-741	134.51	134.79	159.35	160.42	32.19	32.69	33.04	34.81	17.34	18.53	17.21	18.43	3.58	9.43	34.16
RD-2640	132.34	133.14	159.12	159.86	31.59	32.51	32.80	34.12	17.31	17.89	17.20	18.49	3.42	9.35	35.24
RD-2653	131.64	132.24	164.58	161.03	30.14	31.96	33.06	34.25	17.59	18.37	17.65	19.21	3.42	9.64	35.45
Mean	131.68	131.90	159.17	159.83	30.27	31.20	32.66	33.90	16.69	17.82	17.09	17.98	3.75	10.36	33.93
Source	S.Em±	CD (1%)	S.Em±	CD (1%)	S.Em±	CD (1%)	S.Em±	CD (1%)	S.Em±	CD (1%)	S.Em±	CD (1%)	S.Em.±	S.Em.±	S.Em.±
Genotype (G)	0.93	3.91	0.31	2.14	0.34	1.43	0.20	0.84	0.10	0.42	0.22	0.92	0.03	0.02	0.05
Days (D)	0.46	NS	0.25	NS	0.17	0.71	0.10	0.42	0.05	0.21	0.11	0.46	CD (1%)	CD (1%)	CD (1%)
GXD	1.31	NS	0.73	NS	0.48	NS	0.29	NS	0.15	NS	0.32	NS	0.12	0.09	0.20

Table 2. Biochemical parameters in different barley genotypes as influenced by *Helminthosporium sativum*

	Total sugar (mg/dry wt.)		Reducing sugar (mg /dry wt.)		Non-reducing Sugar (mg /dry wt.)		Total phenols (mg /dry wt.)		Proteins (µg / fresh wt.)		Amino acids (µ moles/ fresh wt.)		Wax (mg/dm^2)	
	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS	50 DAS	80 DAS
BH-645	14.43	12.61	12.10	11.20	2.33	1.41	2.05	1.64	8.31	7.94	7.15	6.84	0.74	0.70
PL-760	14.27	12.78	12.60	11.42	1.67	1.36	2.14	1.81	8.58	7.74	7.05	6.69	0.76	0.72
DWR-46	14.91	12.96	12.75	11.98	2.16	0.98	2.11	1.73	8.33	7.64	7.35	6.92	0.84	0.80
DWR-28	14.35	12.86	12.85	11.62	1.50	1.24	2.04	1.66	8.32	7.95	7.17	6.86	0.82	0.70
K-729	13.15	11.09	10.25	9.12	2.90	1.97	1.71	1.26	8.01	7.20	6.42	5.88	0.71	0.66
K-741	13.14	11.05	10.75	9.14	2.39	1.91	1.92	1.34	8.14	7.25	6.63	6.15	0.72	0.67
RD-2640	12.39	10.34	10.45	9.20	1.94	1.14	1.86	1.31	7.95	6.98	6.73	6.19	0.70	0.65
RD-2653	12.79	10.92	10.45	9.03	2.34	1.89	1.57	1.14	8.07	7.22	6.58	6.10	0.72	0.67
Mean	13.67	11.82	10.45	10.34	2.15	1.48	1.92	1.48	8.21	7.49	6.88	6.45	0.75	0.70
Source	S.Em±	CD (0.01)	S.Em±	CD (0.01)	S.Em±	CD (0.01)	S.Em±	CD (0.01)	S.Em±	CD (0.01)	S.Em±	CD (0.01)	S.Em±	CD (0.01)
Genotype (G)	0.16	0.48	0.10	0.31	0.04	0.16	0.02	0.08	0.05	0.16	0.05	0.21	0.100	0.040
Days (D)	0.08	0.24	0.05	0.15	0.01	0.04	0.01	0.04	0.02	0.08	0.02	0.08	0.006	0.020
GXD	0.23	NS	0.12	0.43	0.04	0.16	0.04	0.12	0.07	0.23	0.07	0.29	0.018	NS

Non-reducing sugar

The non-reducing sugar content differed significantly among the genotype, stages of the crop growth and interaction of genotypes and days. At 50 DAS, the genotype K-729 recorded maximum (2.90 mg/dry wt.) non-reducing sugar content (Table 2). The least was recorded in DWR-28 (1.50 mg/dry wt.). However, at 80 DAS, genotype K-729 showed maximum (1.97 mg/dry wt.) followed by K-741 (1.91 mg/dry wt.). The least was recorded by DWR-46 (0.98 mg/dry wt.) (Table 2). There was significant decrease in non-reducing sugar content among the genotype as the days increased from 50 to 80 DAS. The percent decrease in the non-reducing sugar content was more in resistant genotypes than susceptible once.

Total phenols

Total phenol content varied significantly among different genotypes, stages of crop growth and interaction of genotypes and days (Table 2). At 50 DAS, the total phenol content was maximum (2.14 mg/dry wt.) in the genotype PL-760 followed by DWR-46 (2.11 mg/dry wt.). Similar trend was noticed in 80 DAS. However, it was recorded in RD-2653 (1.14 mg/dry wt.). Between 50 to 80 DAS, there was significant decrease in total phenol content in all the genotypes tested and the maximum decrease was found in susceptible genotypes than resistant once.

Protein

From Table 2, it was found that, the protein content varied significantly among different genotypes at different crop growth stages and interaction effect of genotypes and days. The protein content in the barley genotypes at 50 DAS was more in the genotypes PL-760 (8.58 kg/fresh wt.) followed by DWR-46 (8.33 kg/fresh wt.). On the contrary at 80 DAS, DWR-28 and BH-645 genotypes recorded maximum (7.95 kg/fresh wt. and 7.94 kg/fresh wt., respectively) protein content. There was significant decrease in protein content from 50 to 80 DAS. The maximum decrease was found in susceptible genotypes than resistant once.

Total amino acids

Amino acid content varied significantly among the genotypes, stages of the crop growth and interaction of genotypes and days (Table 2). Among the barley genotypes at 50 DAS, the genotype DWR-46 recorded significantly maximum (7.35 k moles / fresh wt.) quantity of amino acids followed by DWR-28 (7.17 k moles /fresh wt.). The least was recorded in K-729 (6.42 k moles / fresh wt.). At 80 DAS, the same genotype DWR-46 recorded maximum (6.92 k moles/ fresh wt.) amount of amino acids and the least was recorded in K-729 (5.88 k moles / fresh wt.). Between 50 and 80 DAS, the quantity of amino acids decreased significantly and the percent decrease was maximum in susceptible genotypes than resistant once.

Wax: The wax content varied significantly among the genotypes and stages of crop growth. But the interaction found to be non-significant. It was found that, the wax content decreased from 50 to 80 DAS. At 50 DAS, the genotype DWR-46 recorded significantly maximum (0.84 mg / decimeter square (dm^2) wax content followed by DWR-28 (0.82 mg/dm^2). The least was recorded in RD-2640 (0.70 mg/dm^2). At 80 DAS, the same genotype DWR-46 recorded maximum (0.80 mg/dm^2) wax content followed by DWR-28 (0.78 mg/dm^2). The least was recorded by RD-2640 (0.65 mg/dm^2). The percent decrease was maximum in susceptible genotypes than resistant once.

Discussion

Helminthosporium sativum appeared to be the major pathogen on barley. Though Patil (1982) studied some aspects of this disease caused by *Drechslera sorokiniana* (Sacc.) Subram and Jain, but much needs to be carried out in Karnataka. So, with this background the present investigation was undertaken to know the selected physiological and biochemical parameters responsible for resistance to spot blotch of barley. The results obtained during the course of the investigation are discussed in this chapter as hereunder.

In almost all plants, stomata serve as avenues for the entry of various pathogens. If the stomatal number per unit area is less and opening is narrow, or if the stomata remained closed for most of the time, the pathogens that penetrate through stomata find it difficult to enter. The present investigation indicated that stomatal frequency was higher in susceptible genotypes than resistant ones. More number of stomata was recorded on abaxial surface than that of adaxial surface of the leaf. Similarly, the size (length and breadth) of stomata was also more in susceptible genotypes as compared to resistant ones. The mode of entry of *H. sativum* is mainly through stomata. Susceptible genotypes recorded higher frequency and size, which provided higher opportunity for penetration by the pathogen and resulted in high disease severity than resistant ones. Similarly, Yadav (1976) and Kalappanavar and Hiremath (2000a) reported that foliar disease resistant sorghum genotypes had stomata with shorter length and breadth and also the frequency of stomata were also less. Even in other crops like groundnut, the frequency and size of stomata were significantly lower in abaxial surface of the leaves of resistant genotypes against leaf spot and rust diseases (Mayee and Apet, 1995, Mayee and Suryawanshi, 1995 and Benagi, 1995). Thus, it appeared that the number and size of the stomata are important characters of the leaf in relation to resistance or susceptibility of the plant to many foliar pathogens.

The static anti-infection structures include thicker leaf cuticle, thicker epidermal cell layer and minimum number of epidermal cells per mm. The present investigation revealed that the number of epidermal cells per mm were less in resistant genotypes. Whereas, epidermal cell later expanded and cuticular thickness were more in resistant genotypes than susceptible genotypes. These mechanisms act as first line of defence barrier for invading pathogen along with epicuticular wax. These structures imparted resistance to host against the pathogen *H. sativum* which infected directly through the epidermal cell or cuticle. These results are in agreement with the findings of Yadav (1976), who reported that anthracnose resistant sorghum genotypes have thickened cuticle and hypodermis of midrib. Similar observations were also made by Mayee and Apet (1995) wherein, groundnut varieties resistant to leaf rust had thicker epidermis cum cuticle. Thus, histological parameters studied acted as a physical barrier for the entry of the pathogen there by imparting resistance to genotypes.

In recent years, it is becoming increasingly evident that several natural and induced defense mechanisms operate in host plants against different diseases. One such defense mechanism is the presence of certain compounds inhibitory to the pathogen. Sometimes, the host plant is induced to synthesize these compounds on infection. Analysis of biochemical's in selected and resistant and susceptible genotypes was carried out at two different stages to understand there role in resistance/susceptibility to barley genotypes

In general infections by some pathogens bring about lot of changes in the respiratory pathways and photosynthesis which is very vital processes occurring in the plant. This lead to a wide fluctuation in sugars in the plant pathogen interactions (Farkas and Kiraly, 1962; Klement and Goodman, 1967; Jaypal and Mahadevan, 1968). The disease reaction has been correlated with the sugar level in different crop plants.

The disease reaction has been correlated with the sugar level in different crop plants. Generally high level of total sugar and reducing sugars in the host plant are stated to be responsible for disease resistance.

Difference in sugar level at 50 DAS between resistant and susceptible genotypes was due to inherent character of the genotypes as there was less disease development in all the genotypes especially in susceptible genotypes. Subsequently the gap widened with respect to sugar content as well as disease development between resistant and susceptible genotypes. This indicated the sugar by the invaded pathogen for their nutrition. Such nutritional utilization of sugars by the invading pathogens has been reported earlier also (Thind *et al.,* 1977).

In the present investigation, the resistant genotypes of barley exhibited more amounts of total sugar and reducing sugar as compared to susceptible genotypes during the growth i.e., 50 and 80 DAS. Further, observations revealed that there was reduction in total, reducing and non-reducing sugars due to infection. These results are in conformity with the report of Ramdayal and Joshi (1968), in barley against leaf spot pathogen, Mandokhot *et al.* (1979) and Lavy and Cohen (1984) in case maize against turcicum leaf blight and Subramanyam *et al.* (1990) in wheat against *Exerohilion hawaiiensis.*

Among all the biochemical components of different hosts, phenol stand out as most important component in imparting resistance to several plant diseases. High concentration causes an instant lethal action by a general tanning effect while, low concentration causes gradual effect on the cellular constituents of the parasite. If the concentration does not occur in toxic level, the inhibition will be obviously slow. Besides, the pathogens readily detoxify low concentrations of the toxicant rather than high concentrations (Dasgupta, 1988).

A definite correlation exists between the resistance of plants to diseases and the state of their phenolic complex (Kosuge, 1969). Phenolic compounds must be liberated and converted from inactive forms, since they are fungistatic only in the free state (Friend, 1979).

In the present investigation, resistant genotypes recorded higher amount of phenols than susceptible ones both at 50 and 80 DAS. The high phenol content in resistant genotypes may be due to more sugar as it acts as precursor for synthesis of phenolics (Prabhu *et al.* (1984), who reported that the total phenolics were more in callus tissues of sorghum genotypes, which were resistant to downy mildew. The phenol content in different genotypes decreased with increase in age of the plant. However, the amount of phenol content was significantly more in resistant genotypes compared to susceptible ones. These results are in line with the findings of Malk *et al* (2000) who reported that there was more reduction in total phenols in susceptible genotypes than in resistant genotypes of mothbean following yellow mosaic virus infection Kalappanavar and Hiremath (2000) reported higher phenol content in multiple foliar disease resistant sorghum genotypes than susceptible ones. Based on these findings, it could be concluded that rapid accumulations of phenolic compounds occur in incompatible (resistant) host-pathogen interaction than the compatible (susceptible) ones.

The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis, particularly during incompatible reaction. Quantitatively speaking, the total protein synthesis is much enhanced in the tissue around the infected tissues. This additional protein is considered to be entirely of host origin (Dasgupta, 1988).With respect to protein content, mean soluble protein content was more in resistant genotypes than the susceptible genotypes of barley. The protein content decreased slightly from 50 to 80 DAS. The decrease may be due to degradative activity, which is more pronounced in diseases caused by facultative parasites as pointed out by Uritani (1971). The decrease in protein levels is probably mainly due to decreased uptake and metabolism of nitrogen as shown by Walters and Ayers (1980). In the present findings, resistant genotypes recorded more of soluble protein than the susceptible ones. These results are in agreement with the findings of Vidyasekaran (1972) and Kalappanavar and Hiremath (2000). Amino acids may act as inhibitory to the activity of pathogen or may act as precursor of various fungitoxic compounds, particularly phenolic, though schikimic pathway.

In the present investigation, resistant genotypes of barley recorded higher amount of amino acids compound to susceptible ones. These findings are inaccordance with Reddy (1984) and Kalappanavar and Hiremath (2000). It has been reported that higher amino nitrogen content in the resistant host plants helped in the breakdown of naturally occurring phenols to toxic products, which in turn inhibited the pathogen. The amino acid content decreased from 50 to 80 DAS. Low content of amino acids in susceptible genotypes may be due to inability of infected leaves to synthesize carbohydrates which are essential for synthesis of amino acids or it may be due to utilization of amino acids for nutritional purpose by the pathogens. Similar results were obtained by Tripathi and Chiranjeevi (1977) in sorghum genotypes infected with zonate leaf spot.

The natural protective covering of the epidermal cells of leaves of higher plants consists of the cuticle with its waxy coating. The waxy cuticle reduces the adherence of water and prevents formation of infection droplets. The waxy layer besides offering a mechanical barrier which cannot be rendered soluble by the enzymatic action of the germ tubes of fungi seems to contain substances which inhibit bacterial, fungal and insect attack (Chibnall and Piper, 1934). Cruickshank *et al.* (1977) have noted that the diterpenoids associated with cuticular wax may inhibit the germination of downy mildew spores which may delay the onset of epidemic.The present investigation revealed that, the resistant genotypes had higher wax content than that of susceptible ones, both at 50 and 80 DAS. Similar results have also been reported in paddy against brown spot (Gangopadhyay and Chattopadhyay, 1973), in groundnut leaf spot (Benagi, 1995) and in wheat against leaf blight (Nandagopal, 1995).

Hence, for the first time studies revealed that higher total sugar and reducing sugars, proteins, phenols, amino acids and epicuticular wax are the reasons for resistance in barley against spot blotch disease.

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