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Studies on Structural Mechanism of Resistance in Mulberry Against Two Important Foliar Diseases under Kashmir Conditions

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ABSTRACT

The leaf of mulberry (*Morus* spp.) constitutes the only food to silkworm (*Bombyx mori* L.). Like all other crops, it is prone to attack by a number of pests and diseases which result in reduced leaf yield and quality. Among other diseases leaf spot and powdery mildew are important and cause much loss to mulberry leaf. Besides other defence systems, the plants defend themselves against pathogens by means of structural characteristics which act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the host. Morphoanatomical features of some mulberry genotypes were studied in relation to incidence and intensity of leaf spot caused by *Cercospora moricola* (Cooke) and powdery mildew caused by *Phyllactinia corylea*. The present study was carried out on four years old mulberry trees of genotypes namely Goshoerami, Ichinose, Kairynezamigaeshi (KNG), Rokokuyaso, Chinese White, Tr-10 and one local genotype Chattatul Zangir for leaf spot and powdery mildew diseases. The Percent Incidence (PI) and Percent Disease Index (PDI) were calculated. Different anatomical features were studied as per standard laboratory methods. Results indicated that the anatomical features play an important role to hinder the infection and spread of the pathogens causing the diseases and hence can be used for rapid screening of mulberry genotypes for their resistance to these diseases.

Key words: Mulberry, leaf spot incidence, powdery mildew incidence, anatomical structures

INTRODUCTION

Mulberry, the only food to silkworm, is attacked by a number of diseases and pests which result in yield loss to a considerable extent (Tang *et al.*, 2005). The foliar diseases of mulberry are of major concern as they cause considerable loss to the main produce of mulberry; i.e., the foliage (Ghose *et al.*, 2010). In general, plants defend themselves against pathogens by means of structural characteristics which act as physical barriers and block the passage of the pathogen into the host. Anatomical barriers, though passive, give required protection to the plant till the induction of chemical resistance (Kulkarni and Deshpande, 2006). The anatomical barriers to pathogen penetration are very important in relation to the development of disease resistance, because they reduce the rate of infection progress. Certain features are present in plants which give them protection from infection of a pathogen. These include the amount and quality of wax and cuticle that cover the epidermal cells, the structure of the epidermal cell walls, the size, location and shape of stomata and lenticels. In addition to these, the presence of tissues made up of thick walled cells also prevents the advance of the pathogen. Anatomical characteristics (more number of Palisade layers, significantly higher thickness of epidermis cum cuticle, nature of cuticle and palisade tissue, comparatively thinner spongy parenchyma and significantly higher palisade proportion) are thought to act as structural barriers against the penetration and invasion by different pathogens (Sonibare *et al.*, 2006). Many workers have worked on various pathological aspects in mulberry (Fotadar *et al.*, 1998; Srikanthaswamy *et al.*, 1999; Chakraborty *et al.*, 2003; Raja, 2003; Ghosh *et al.*, 2003a; Tang *et al.*, 2005; Baiyewu *et al.*, 2005a, b; Ghose *et al.*, 2010). The present study was an attempt to study the anatomical features of seven mulberry genotypes, growing as trees, in relation to their response to leaf spot and Powdery mildew disease under rainfed conditions in Kashmir province of Jammu and Kashmir state.

MATERIALS AND METHODS

The present study was taken up on 4 year old mulberry trees raised at Central Sericulture Research and Training Institute, Pampore (J and K).; located at 30°12' north latitude and 75°35' east longitude and 2000 meters above the sea-level. Mulberry genotypes namely Goshoerami, Ichinose, Kairynezamigaeshi (KNG), Rokokuyaso, Chinese White, Tr-10 and one local genotype namely Chattatul Zangir, maintained under rainfed conditions, were taken up for the study. The experiment was laid out in a Randomized Block Design (RBD) with three replications for each genotype. Trees were raised at 9×8' spacing and number of plants per treatment per replication was kept uniformly 09. The plantation was maintained as per the package of practices recommended for temperate conditions of Kashmir (Ahsan *et al.*, 1990). Each year pruning was given only once, in the first week of June by cutting the branches right from the crown base. Regular observations were recorded during the two years with regard to Powdery mildew. During August, five branches from each plant from the three plants selected per treatment per replication were tagged. Disease scoring was done using the 0-5 rating scale as per FAO (1967):

Scale	Area of lamina infected (%)
0	Healthy leaf
1	01-05
2	06-10
3	11-25
4	26-50
5	51-100

The Percent Incidence (PI) and Percent Disease Index (PDI) were calculated by applying formula given by Wheeler, (1969):

$$\text{Percent incidence (PI)} = \frac{\text{Total No. of infected leaves}}{\text{Total No. of leaves observed}} \times 100$$

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of numerical rating*}}{\text{Total No. of leaves observed} \times \text{Max. grade}} \times 100$$

*The numerical values were obtained by multiplying the number of leaves with their respective grades. The data was recorded for two years and pooled.

For Anatomical studies, fully expanded leaves from three month old shoots were collected early in the morning. Soon after their collection, they were fixed in Formalin Acetic Alcohol (FAA). The material was kept in the fixative for 20 h. The fixed material was later transferred to 70% ethanol (C_2H_5OH) for preservation. For stomatal study and idioblast frequency lower and upper epidermal peels were taken as per the method of Pohl (1967) and Ghouse and Younis (1972). For the fixed material, dehydration, infiltration and embedding was done properly and thin sections (10 μm) cut mechanically with the help of a microtome. Standard procedures for staining and embedding were followed by Dwivedi and Singh (1990), Khasim (2002) and the sections mounted in DPX. Stomatal frequency was studied under (10×40) magnification. Idioblast frequency on the other hand was recorded under low magnification (10×10), so as to accommodate more number of idioblasts per microscopic field. Number per microscopic field in both the cases was counted and frequency calculated on millimeter basis. Microscopic measurements of various leaf tissues were made at 10 different locations in five cross sections, using ocular micrometer. Measurements were made at 10×40 magnification. The palisade and spongy proportion was calculated by Tiwari *et al*. (1986). Analysis of variance (ANOVA) was done by Singh and Choudhary (1977).

RESULTS AND DISCUSSION

During spring crop, the plants of all the seven genotypes were free from both leaf spot and powdery mildew. Symptoms appeared from September onwards. Leaf spot appeared earlier than powdery mildew. Leaf spot appeared in the form of black irregular necrotic spots with yellow halo margin. As the disease became severe, the spots enlarged and coalesced resulting in the formation of short holes. Severely affected leaves became yellowish and fell off pre-maturely. Powdery mildew appeared in the form of white colored powdery mass on the ventral surface of the leaves with slight yellowing on the concomitant dorsal surface. The leaves later on became yellowish, turn leathery and fell off pre-maturely.

Observations recorded on Percent Incidence (PI) and Percent Disease Index (PDI) in respect of leaf spot and powdery mildew recorded during both the years were pooled and analyzed and are furnished in Table 1.The percent incidence in respect of leaf spot was highest in Chattatul Zangir (34.85%), KNG showed 0.36% Tr-10, 6.66% and Chinese White 7.43%. Goshoerami, Ichinose and Rokokuyaso were free from this disease. The incidence of powdery mildew was maximum (74.45%)

Table 1: Response of mulberry genotypes to foliar diseases

	Leaf spot		Powdery mildew	
Genotype	DI	PDI	DI	PDI
Goshoerami	0.00	0.00	49.86	30.86
Ichinose	0.00	0.00	51.67	15.86
KNG	0.36	0.07	8.39	3.97
Tr-10	6.66	1.33	74.09	37.57
Chinese White	7.43	1.70	48.75	28.76
Rokokuyaso	0.00	0.00	74.45	46.50
Chattatul Zangir	34.85	18.92	42.44	18.89
General mean	7.04	3.15	49.95	25.87
CD at 5%	9.98	5.47	13.02	11.29

DI: Disease incidence, PDI: Percent disease index in Rokokuyaso which was closely followed by Tr-10 (74.09%) The values were significant over the remaining genotypes where it ranged from 8.39% in KNG to 51.67% in Ichinose. Goshoerami registered 49.86% disease incidence.

Out of the four genotypes affected by leaf spot, PDI was highest in Chattatul Zangir (18.92%) and the least (0.07%) in KNG, whereas PDI for powdery mildew was highest (46.50%) in Rokokuyaso which was followed by Tr-10 (37.57%) and the least in KNG (3.97%).

For leaf spot, the genotypes with maximum disease incidence also showed maximum PDI. In case of powdery mildew, the trend was also similar except in case of Ichinose where the PDI was very low as compared to the PI.

The observations recorded in respect of various foliar anatomical features are furnished in Table 2.

The upper epidermis was mostly single layered and the cells were longer than broad. The lower epidermis, too, was single layered and thinner than the upper epidermis. Trichomes were common but were most abundant in Chattatul Zangir. The thickness of upper Cuticle was maximum 11.38 μm in Goshoerami being significantly more than the other genotypes; whereas, the thickness was least (4.13 μm) in Chattatul Zangir. In the remaining genotypes, it ranged from 5.14 μm in Tr-10 to 7.98 μm in KNG. The thickness of lower cuticle, however, was maximum (5.98 μm) in Rokokuyaso being significantly higher than the other genotypes except Goshoerami (5.19 μm). The thickness was least (2.13 μm) in Tr-10. In Chattatul Zangir the thickness was (2.48 μm).

The upper epidermis in all the genotypes was thicker than the lower epidermis. The upper epidermis was thickest (24.74 μm) in KNG being statistically at par with that in Goshoerami (23.68 μm) and significant over the rest of the genotypes. The thickness was least (16.02 μm) in Chattatul Zangir. The lower epidermis on the other hand was thickest (13.32 μm) in Ichinose being statistically at par with that of KNG (12.75 μm) and significant over the remaining genotypes. The thickness of lower epidermis was least (7.38 μm) in Goshoerami.

The palisade thickness was maximum (73.38 μm) in Chattatul Zangir which was significantly higher than the rest of the genotypes. The thickness was least (57.55 μm) in KNG. The spongy tissue thickness was highest (97.29 μm) in Tr-10 being significantly higher than the remaining genotypes. The thickness was least (43.10 μm) in Chinese white. Palisade proportion was maximum (40.89%) in Chattatul Zangir, closely followed by Chinese white (40.70%), whereas it was the least in Ichinose with 31.60% palisade proportion. In the remaining genotypes it ranged from 32.26% in KNG to 37.27% in Rokokuyaso. Spongy proportion, on the other hand was maximum (49.16%) in Tr-10, whereas it was the least in Chinese white with only 29.48% Spongy proportion. In the remaining genotypes it ranged from 36.69% (Rokokuyaso) to 41.88% (Goshoerami).

The total leaf thickness was maximum (199.53 μm) in Tr-10 being statistically at par with that of Goshoerami (194.95 μm) and significant over the rest of the genotypes. The leaf thickness was least in Chinese white (146.36 μm). The stomata were present in the lower surface of the leaf and in no case stomata were observed on the upper surface. The Stomata frequency was maximum (710.66 mm^{-2}) in Rokokuyaso being non significant over that of Goshoerami and Chattatul Zangir and significantly higher than the other four genotypes. The frequency was least (431.14 mm^{-2}) in KNG. The Stomatal size was maximum (28.04×13.94 μm) in Chattatul Zangir. In the remaining, it was 20.78×10.83 μm in Goshoerami; 22.0×11.04 μm in Ichinose; 19.78×10.73 μm in KNG; 21.87×8.77 μm in Tr-10; 24.11×9.98 μm in Chinese white and 20.04×11.77 in Rokokuyaso.

Spring crops in respect of all the genotypes under study did not witness any symptom of leaf spot or powdery mildew. The symptoms appeared from first week of September because of a fall in

Table 2: Leaf anatomical features in 4 years old mulberry genotypes growing as trees

Genotype	Upper cuticle thickness (µm)	Lower cuticle thick-ness (µm)	Upper epidermal thickness (µm)	Lower epidermal thickness (µm)	Palisade parenchyma thickness (µm)	Palisade parenchyma proportion (%)	Spongy parenchyma thickness (µm)	Spongy parenchyma propor-tion (%)	Leaf thickness (µm)	Stomatal size (µm)	Stomatal frequency (mm^{-2})
Goshoerami	11.38	5.19	23.68	7.38	65.35	33.66	81.74	41.88	194.95	20.78×10.83	698.25
Ichinose	7.70	4.34	23.18	13.32	57.66	31.60	75.97	41.63	183.55	22.00×11.04	617.11
KNG	7.98	4.18	24.74	12.75	57.55	32.26	70.38	39.51	178.55	19.78×10.73	431.14
Tr-10	5.14	2.13	19.60	7.80	65.98	33.07	97.29	49.16	199.53	21.87×8.770	609.21
Chinese White	5.40	4.53	20.16	10.52	59.55	40.70	43.10	29.48	146.36	24.11×9.980	450.44
Rokokuyaso	5.92	5.98	22.88	7.55	62.18	37.27	61.17	36.69	166.27	20.04×11.77	710.66
Chattatul Zangir	4.13	2.48	16.02	9.83	73.38	40.89	70.54	39.26	179.60	28.04×13.94	696.05
General mean	6.81	4.18	21.47	9.88	63.09	35.63	71.46	39.66	178.26		601.84
CD at 5%	1.17	0.80	1.59	1.09	2.92	2.32	3.75	3.07	8.79		45.22

temperature. Leaf spot appeared earlier than powdery mildew. There was great degree of variation among the genotypes in terms of disease incidence which is in conformity with the studies conducted under tropical conditions (Sengupta *et al.*, 1990; Gangawar and Thangavelu, 1991; Ghosh *et al.*, 2003b). The genotypes Goshoerami, Ichinose and Rokokuyaso were totally free from leaf spot. The incidence was least (0.36%) in KNG and the highest (34. 85 %) in Chattatul Zangir. In the genotypes with low or no leaf spot i.e., Goshoerami, Ichinose, KNG and Rokokuyaso, the thickness of upper cuticle cum epidermis was more than 28 μm, whereas, it was least 20.15 μm in Chattatul Zangir with the maximum (34.85%) leaf spot incidence. The maximum incidence in case of Chattatul Zangir can be attributed to the lesser thickness of cuticle cum epidermis. The findings support the view that more thickness of cuticle cum epidermis acts as a structural barrier for the entry of the pathogens causing leaf spot. The thickness of the cuticle alone was also significantly more in the genotypes with no or low incidence of leaf spot. According to Juniper and Cox (1973), the epidermis and its cuticle constitute the first line of plant defence mechanism against the attack of pathogens. Similar conclusions were drawn by other workers (Mayee and Suryawanshi, 1995; Sarwar *et al.*, 1996). More incidence and intensity of leaf spot in Chattatul Zangir might also be due to increased Stomatal dimensions. The findings substantiate the observations reported earlier by Basru *et al.* (1985) and Mayee and Apet (1995) in ground nut varieties. Sukand and Kulkarni, 2006 also have found that ground nut genotypes resistant to *Puccinia arachidis* (Speg.) were characterized by significantly higher cuticle and epidermal cell thickness with lesser epidermal cells, besides less length, breadth and number of stomata. Further the genotypes followed the same trend as far as disease incidence and intensity are concerned. Regarding powdery mildew, again there was great variation among the genotypes both in terms of Disease Incidence (DI) and Percent Disease Index (PDI).Though powdery mildew appeared later than leaf spot, the incidence and intensity were higher than leaf spot. Incidence was least (8.39%) in KNG, whereas, in the remaining six genotypes, it ranged from 42.44% in Chattatul Zangir to 74.09 and 74.45%, respectively in Tr-10 and Rokokuyaso. The Percent Disease Index (PDI) for powdery mildew again was least (3.97%) in KNG, whereas, in Rokokuyaso, Tr-10 and Goshoerami, it was 46.50, 37.57 and 30.86%, respectively. The higher values of incidence and intensity may be attributed to higher spongy proportion. The least values registered in KNG may be attributed to the additive effect of stomata of smaller size and less frequency, thicker cuticle cum epidermis and less spongy proportion. Mahajan *et al.* (2003) have found that lesser thickness of spongy tissue which indirectly indicates lesser inter cellular spaces may have some role in conferring resistance to late blight infection in potato.

CONCLUSION

Mulberry genotypes though grown under similar conditions behave differently as for as the response to foliar diseases is concerned. Besides other intrinsic and extrinsic factors, foliar anatomy plays an important role in deciding the response of mulberry genotypes to different pathogens. KNG having the least attack by both the pathogens can be of use in breeding programmes aimed at developing genotypes resistant to these foliar diseases. This is of utmost importance in mulberry, since the leaf is the principal economic product. Mulberry diseases affect the leaf both quantitatively and qualitatively and the effects pass through all the rearing phases and result in less production and inferior quality of cocoons.

REFERENCES

Ahsan, M.M., K.L. Dhar and R.K. Fotedar, 1990. Package and practices of mulberry cultivation under temperate conditions. Indian Silk, 29: 7-12.

Baiyewu, R.A., N.A. Amusa and J.B. Idowu, 2005a. Leaf spot in mulberry plant (*Morus alba*) in the lowland humid tropics of Southwestern Nigeria. Plant Pathol. J., 4: 103-106.

Baiyewu, R.A., N.A. Amusa, G.O. Idowu and A. Smith, 2005b. The occurrence of leaf rust disease of mulberry plant (*Morus alba*) in lowland humid forest of Southwestern Nigeria. Plant Pathol. J., 4: 107-109.

Basru, K.K., S. Kaur and M. Dhillon, 1985. Anatomical and biochemical studies of the resistance and susceptibility of groundnut varieties Cercospora leaf spot. Ann. Biol., 1: 77-82.

Chakraborty, R., K. Neog, R. Das and R.K. Dutta, 2003. Seasonal incidence of major foliar diseases of mulberry in North East India. Indian. J. Seric., 42: 122-127.

Dwivedi, J.N. and R.B. Singh, 1990. Essentials of Plant Techniques. Scientific Publishers, India.

FAO, 1967. Crop losses due to diseases and pests. Food and Agriculture Organization, Rome.

Fotadar, R.K., A. Dhar and S.B. Dandin, 1998. Screening of mulberry genotypes against major fungal diseases and insect pests under field conditions. Indian J. Seric., 37: 40-43.

Gangawar, S.K. and K. Thangavelu, 1991. Occurance of diseases in Tamil Nadu, Indian. Phytopathology, 44: 545-549.

Ghose, L., F.A. Neela, T.C. Chakravorty, M.R. Ali and M.S. Alam, 2010. Incidence of leaf blight disease of mulberry plant and assessment of changes in amino acids and photosynthetic pigments of infected leaf. Plant Pathol. J., 95: 140-143.

Ghosh, L., M.S. Alam, M.R. Ali, A.M. Shohael, F. Alam and R. Islam, 2003a. Changes in some biochemical parameters of mulberry (*Morus sp.*) leaves after infected with leaf spot disease. J. Biological Sci., 3: 508-514.

Ghosh, L., N. Ahsan, S. Parvez, A.M. Swaraz, M.R. Khan, M.R. Ali and M.F. Alam, 2003b. Survey and evaluation of leaf spot disease in six varieties of mulberry (*Morus* sp). J. Biological Sci., 3: 1070-1075.

Ghouse, A.K. and M. Younis, 1972. Preparation of epidermal peels from leaves of Gymnosperms by treatment with hot, 60% HNO_3. Stain Technol., 47: 322-324.

Juniper, B.E. and G.C. Cox, 1973. The anatomy of the leaf surface: The first line of defence. Pest Sci., 4: 543-561.

Khasim, S.M., 2002. Botanical Micro-Technique: Principles and Practices. Capital Publishing Company, New Delhi, Pages: 27.

Kulkarni, M. and U. Deshpande, 2006. Anatomical breeding for altered leaf parameters in tomato genotypes imparting drought resistance using leaf strength index. Asian J. Plant Sci., 5: 414-420.

Mahajan, M., T.S. Thind and M. Dhillon, 2003. Structural variability amongst potato cultivars possessing varying grades of resistance to late blight. Indian Phytopathol., 56: 443-445.

Mayee, C.D. and A.P. Suryawanshi, 1995. Structural defence mechanism in groundnut to late leaf spot pathogen. Indian Phytopathol., 48: 160-165.

Mayee, C.D. and K.T. Apet, 1995. Structural defence mechanism in groundnut to rust pathogen. Indian Phytopathol., 48: 154-159.

Pohl, R.W., 1967. Controlled maceration of grass leaves in 40-80% nitric acid for preparation of epidermis for slides. Stain Technol., 42: 195-197.

Raja, S.A., 2003. Management of powdery mildew (*Phyllactinia corylea* (Pers.) Karst) of mulberry (*Morus* Sp.) using botanicals and biocontrol agents. M.Sc. Thesis, Sher-e-Kashmir University of Agricultural Sciences and Technology (Kashmir), Srinagar.

Sarwar, M., F. Jamil and N.A. Beig, 1996. Response of four chickpea varieties against Aschchyta blight and their biological studies. Pak. J. Bot., 28: 213-217.

Sengupta, K., P. Kumar, M. Baig and Govindaiah, 1990. Handbook of Pest and Disease Control of Mulberry and Silkworm. UN, New York, pages: 88.

Singh, R.B. and B.D. Choudhary, 1977. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India, pp: 53-59.

Sonibare, M.A., A.A. Jayeola and A. Egunyomi, 2006. Comparative leaf anatomy of *Ficus* Linn. species (Moraceae) from Nigeria. J. Applied Sci., 6: 3016-3025.

Srikanthaswamy, K., V.P. Gupta and J.P. Renkeswarappa, 1999. Incidence, severity and yield loss due to leaf spot of mulberry due to *Cercospora moricula*. Indian J. Seric., 38: 8-11.

Sukand, G. and S. Kulkarni, 2006. Studies on the structural and biochemical mechanism of resistance in ground nut to *Puccinia arachidis*. Indian Phytopathol., 59: 323-328.

Tang, M.A.K., K.A. Salam, M.A. Samad and N. Absar, 2005. Nutritional changes of four varieties of Mulberry leaves at infected with Fungus (*Cercospora moricola*). Pak. J. Biol. Sci., 8: 127-131.

Tiwari, S.P., N.P. Sukamaran and C.L. Khushu, 1986. Patho-coefficient analysis of leaf anatomical characters affecting frost injury in potato. Plant Breed., 97: 272-274.

Wheeler, B.E.J., 1969. An Introduction to Plant Diseases. Jhon Wiley and Sons Ltd., London, Pages: 254.