Short Communication

Contribution of Leaf Tissue Parameters for Leaf Rust Resistance in Bread Wheat (*Triticum aestivum* L.)

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Abstract

In an attempt to understand the contribution of morpho-physiological parameters of leaf tissue in leaf rust resistance, histological analysis was made on resistant as well as susceptible bread wheat genotypes. On average, the resistant genotypes had epidermal cell layer thickness of 17.93 µm, epidermal cell number of 36.20 per mm^2 and laminar thickness of 148.79 µm; while the corresponding susceptible genotypes had epidermal cell layer thickness of 15.50 µm, epidermal cell number of 33.40 per mm^2, and laminar thickness of 145.05 µm. All the three histological parameters had significant negative correlation with leaf rust score (Average Coefficient of Infection) values. The result revealed that resistant genotypes had thicker epidermis, more number of epidermal cells, and thicker leaf lamina than the susceptible genotypes. These leaf morpho-physiological traits might be useful for selection against leaf rust resistance in wheat.

Introduction

Host plant resistance is among several ways through which plants confer resistance to pathogens (Van der Plank, 1968). First line of defense of plants against invading pathogens is the surface barrier, which a pathogen must penetrate before causing infection. The structural features of epidermis and internal tissue patterns may greatly affect the ability of pathogens to invade it. Early evidence of the relationship between cuticle thickness and rust resistance was provided by Melander and Craigie (1927) who showed that young leaves of barberry, with their thin cuticle, were more susceptible to infection by *basidiospore* of *Puccinia graminis tritici* than older leaves in which the cuticles are thickened. The objective of this study, therefore, was to know the association of morpho-physiological structure of leaves and rust resistance in wheat with reference to leaf rust (LR) caused by *Puccinia triticina*

Materials and Methods

The experiment was conducted in 2002 at the experiment field station of the University of Agricultural Sciences, Dharwad, India. Leaf rust severity score was visually recorded as percent infection (Cobb scale) (Peterson *et al.* 1948) on five resistant and five susceptible bread wheat genotypes. Recording was made three times at every fortnight from tillering throughout green leaf stage. The data were later transformed into average coefficient of infection (ACI) by multiplying the percent infection with response value assigned to each infection type according to *Loegering scale* (Joshi *et al.* 1988) as shown in Table 1.

Table 1. Loegering Scale (Joshi *et al*, 1988)

Response value	Infection type	Description
0	0	No visible infection
0.2	R	Resistant, necrotic area, with or without minute *uredia*
0.4	MR	Moderately resistant, small *uredia* surrounded by necrotic area
0.6	M	Intermediate, variable sized *uredia*, with some necrosis or chlorosis
0.8	MS	Moderately susceptible, medium sized uredia with no necrosis, but possibly some distinct chlorosis
1.0	S	Susceptible, large *uredia* with neither necrosis nor chlorosis

Fresh leaves of adult plant were collected randomly from resistant and susceptible plants and cut into pieces of 2.0 cm length. The pieces were fixed in standard Formaldehyde-Acetic Acid-Alcohol (FAA) fixative solution (90 ml of 70% *alcohol*; 5 ml of *acetic acid* and 5 ml of *formalin*) for 24 hrs. Fixed leaves were washed thoroughly with 70 per cent alcohol and dehydrated using 80 percent, 90 percent and *absolute ethanol*. Then with *n-buthanol* and alcohol in the ratio of 1:3, 1:1, 3:1, and 1:0. The samples were left in each grade for a period of 3 hrs.

Paraffin wax with melting point of 58 to 60°C was added successively to the medium of pure *n-buthanol* containing dehydrated samples until the medium reached a saturation point at room temperature. The specimens were kept in oven at 60°C. Subsequent changes with fresh molten paraffin were given at every 4 hrs interval to replace even the trace of *n-buthanol*. The specimens were further embedded in paraffin wax (60°C) employing *paper boat* technique (Jensen, 1962) to prepare paraffin blocks.

The paraffin blocks were cut into ribbons of thin sections of 12 µm size with *EPMA* rotary *microtome* and placed on the slides smeared with gelatin adhesive. To facilitate flattening and stretching of the ribbon, slides were warmed over a weaning plate. The sections were then deparaffinized by passing the slides through *xylene* for 5 minutes, then transferred to 1:1 mixture of *xylene* and absolute alcohol for an other 5 minutes. Then, partially hydrated by passing through a series of *alcohol* of decreasing concentration as absolute, 95%, 70% and 50% for 5 minutes in each.

Staining was done using *1% Safranin* in absolute alcohol for 24 hrs. The slides were washed thoroughly with water, passed quickly through acidified 70% alcohol to destain, and then passed rapidly through 95% and absolute alcohol. Counterstaining was done with *Fast green* (0.5% solution in 50% *clove oil*, 50% alcohol) for 1-5 minutes. Excess stain was cleared by *clove oil* mixture. Three changes of *xylene* for 15 minutes each was attended, mounted and observed under compound microscope with the help of filar micrometer. Data were taken on epidermal cell layer thicknesses (µm), number of epidermal cells per mm and laminar thickness (µm).

Results and Discussion

The five selected resistant genotypes showed thick epidermis while the susceptible ones had relatively thin layers (Table 2). The significant ($P<0.01$) negative correlation ($r = -0.79$) between the trait and LR score revealed that there was strong association between epidermal thickness and LR resistance (Table 2). This is in line with previous reports of direct relationship between epidermal and laminar cell layer thickness and disease resistance in ground nut (Mayee and Ape, 1995), barberry species (Melander and Craigie 1927) and rice (Veeraraghavan 1983).

Table 2. Histological parameters in leaf of rust resistant and susceptible bread wheat genotypes

Genotypes	Histological parameters			Disease parameters	
	Epidermal cell layer thickness (µm)	Number of epidermal cells (mm^{-2})	Laminar thickness (µm)	Reaction Type	ACI[1]
Resistant group					
HD 2189	18.2	37.0	148.3	1R	0.2
GW-324	16.2	36.0	147.2	1R	0.2
DWR 247	17.2	35.0	146.5	0	0.0
Lal B. X GW 324	18.7	36.0	151.7	0	0.0
Pusa 4 X HD 2189	19.3	37.0	150.2	10S	10.0
Mean	**17.9**	**36.2**	**148.8**		
Susceptible group					
Lal Bahadur	15.0	34.0	143.5	60S	60.0
Sonalika	14.9	32.0	145.2	100S	100.0
Pusa 4	15.3	33.0	146.2	100S	100.0
Lal B X DWR 247	16.2	34.0	144.3	100S	100.0
Pusa 4 X NIAW34	16.1	34.0	146	80S	80.0
Mean	**15.5**	**33.4**	**145.04**		
LSD (5%)	**1.1**	**1.2**	**1.8**		
Correlation with ACI	**-0.793****	**-0.861****	**-0.736***		

*, ** - Significant at 5% and 1% probability levels, respectively
R – Resistant reaction
S – Susceptible reaction
LSD (5%) – Least significant difference at 95% confidence level
[1]ACI - Average coefficient of infection calculated by multiplying the percent infection with response value assigned to each infection as indicated in Table1(Example 1R = 1x0.2 = 0.2; 60S = 60x1.0 = 60.0)

As shown in Table 2, the highly resistant genotypes had the largest number of cells per mm^2 of epidermis. The susceptible genotypes on the other hand, had the least number of epidermal cells indicating the direct relationship between number of epidermal cells and ability to resist disease. The correlation coefficient between number of epidermal cells and ACI value (r = -0.86) was highly significant ($P<0.01$) indicating that increase in number of epidermal cells contributes to the resistance mechanism of the host. Kaur and Dhillon (1985) studied the structural characteristics of wheat leaves in relation to resistance against leaf rust pathogen and reported significantly higher number of *sclerenchyma* layers in resistant genotypes than in the susceptible ones.

The resistant wheat genotypes (parents) and their crosses had thicker laminar cell layer than their susceptible counterparts (Table 2) indicating the desirable contribution of the parameter towards leaf rust resistance. Significant negative correlation was observed between leaf laminar thickness and ACI of leaf rust indicating that there is positive relationship between leaf laminar thickness and rust resistance mechanism. The arrangement of epidermal cells and vascular tissues in the resistant genotypes were well compact (Plate 1) which may be used as a mechanical barrier for pathogen entry. The susceptible genotypes, however, had discontinuous and irregular epidermal cells (Plate 2) which suggests that the more intercellular spaces in the tissue of the susceptible genotypes may be used as avenue for easy entry for pathogens.

From this experiment, it can be concluded that leaf tissue parameters are important avenue through which the pathogens find entry to the host. Genotypes with thicker epidermis as well as over all leaf lamina and more number of epidermal cells usually have better resistance to pathogens. Field screening of genotypes supplemented with histo-pathological analysis will, therefore, be effective for developing rust resistant genotypes.

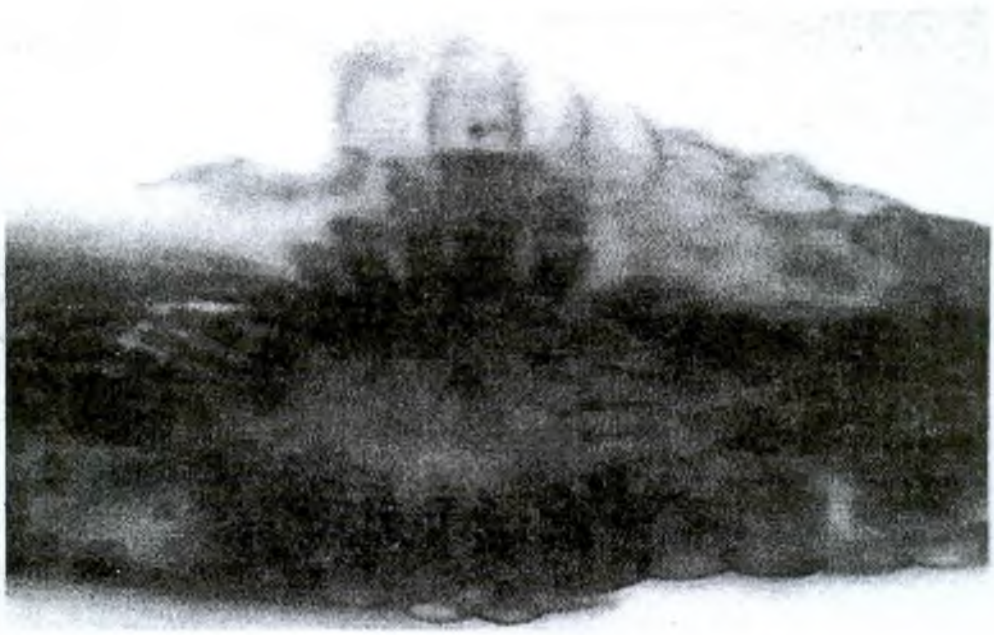

Plate 1. Section of leaf tissue of one of the rust resistant bread wheat genotypes (HD 2189) showing compact cellular arrangement (x40 magnification).

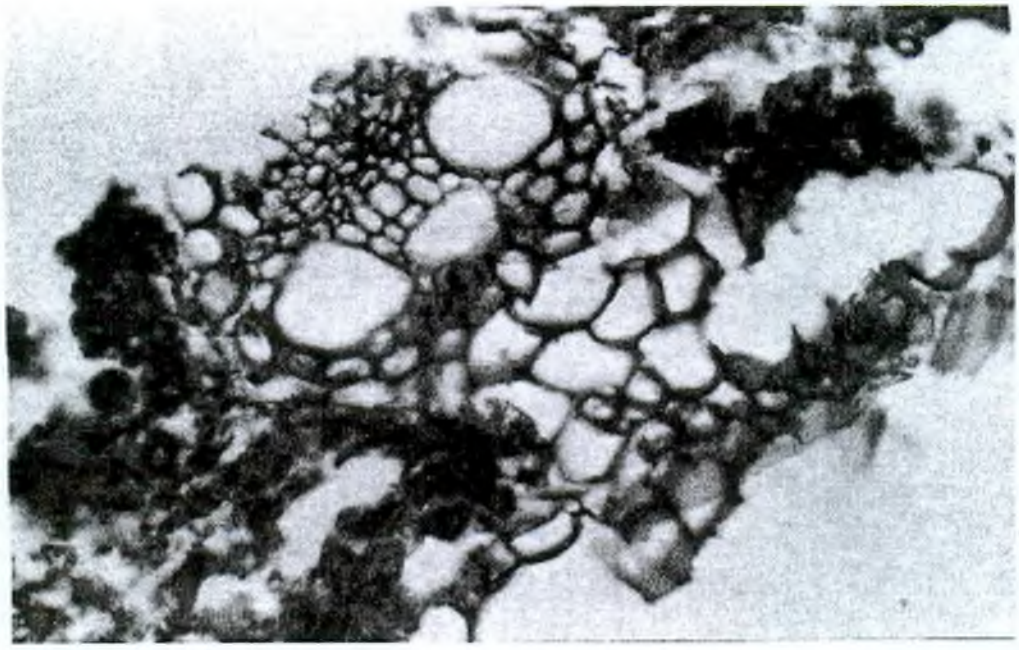

Plate 2. Section of Leaf tissue on one of the rust susceptible bread wheat genotypes (Pusa-4) showing wider intercellular spaces (x40 magnification).

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References

Jensen WA. 1962. *Botanical Histochemistry; Principles and Practices*. W.H. Freeman and Company, San Francisco. pp. 28-54.

Joshi LM, Singh DV and Srivastava KD. 1988. *Manual of Wheat Diseases*. Malhotra Publishing House, New Delhi. p.75.

Kaur J and Dhillon M. 1985. Structural characteristics of wheat leaves associated to resistance of leaf rust (*Puccinia recondita*) and stripe rust (*P. striiformis*). *Inidian Journal of Ecology* **12:** 46-50.

Mayee CD and Apet KT 1995. Structural defense mechanism in ground nut to rust pathogen. *Indian Phytopathology*, **48**(2)**:** 154-159.

Melander, LW and Craigie JH. 1927. Nature of resistance of *Barberries spp.* to *Puccinia graminis*. *Phytopathology* **17:** 95-114.

Peterson, RF., Campbell, AB and Hannah, AE 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26: 496-500

Van der Plank JE. 1968. Disease Resistance in Plants. Academic Press, New York. 210pp.

Veeraraghavan J. 1983. Relationship between leaf cuticle and resistance in rice to *Pyricularia orizae* Cav. *Indian Phytopathology* **36**(1)**:** 41-42.