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Histological and histochemical changes in sunflower due to infection by *Erysiphe cichoracearum*

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Histological and histochemical changes due to infection by Powdery Mildew were investigated in resistant (KBSH 53) and susceptible(KBSH 44) sunflower hybrids . Highly susceptible hybrid KBSH 44 showed more damage to leaf structures than the resistant hybrid KBSH 53.KBSH 53 with the presence of thick and intact epidermal layer with cylindrical palisade and round spongy parenchyma cells.In diseased leaves of KBSH 53 nucleic acids in palisade and spongy parenchyma cells were medium (++) whereas, KBSH 44 showed less stain (+) indicating low level of nucleic acids. Diseased cells of resistant hybrid showed medium (++) and the susceptible hybrid showed low (+) total proteins by exhibiting light staining with MBB.

Key words: Powdery mildew, susceptible, resistant, histological, histochemical, sunflower

INTRODUCTION

Sunflower *(Helianthus annuus* L.) is an important oil seed crop belonging to the family Asteraceae.Karnataka with the largest share in sunflower area (8.08 lakh ha) and production (3.16 lakh tonnes) in India, is recognized as "Sunflower State" in the oilseeds scenario of the country.

Until recently, the major diseases limiting sunflower cultivation in India were *Alternaria* leaf blight,downy mildew, sunflower necrosis disease and occasionally rust. During the past 2–3 years, powdery mildew caused by *Erysiphe cichoracearum* has become a serious problem on sunflower in the major sunflower growing regions in Southern India. In India, the occurrence of powdery mildew was first reported on Mexican sunflower in the year 2008 (Baiswar *et al.* 2008) and subsequently on cultivated sunflower in 2009.

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Powdery mildew is worldwide in its distribution, but the greatest severity has been observed in tropical parts of the world, where it advances senescence of the plant at the flowering or post flowering stages (Zimmer and Hoes 1978; Gulya *et al.* 1997). A field survey on prevalence of powdery mildew at farmer's field in seven districts of Karnataka state, India which is the largest sunflower growing region recorded 30–74 % disease severity (Dinesh *et al.* 2010).In the present study histological and histochemical changes in resistant and susceptible sunflower hybrids due to infection by powdery mildew are investigated.

MATERIALS AND METHODS

Selection of resistant and susceptible genotypes (cultivars) of sunflower to Powdery Mildew

Reaction of sunflower genotypes viz., KBSH -1, KBSH-41, KBSH-2, KBSH-44 and KBSH-53 against

powdery mildew caused by *Erysiphe cichoracearum* DC. was carried out to identify the resistant and susceptible genotypes (cultivars) of sunflower to powdery mildew.

Each genotype was raised in earthern pots and artificial inoculation was done by transferring the inoculated under glasshouse conditions by dusting the conidia with the help of camel hair brush on 30 days old seedlings. The disease severity was recorded using 0 to 9 scale as given by Mayee and Datar (1986) on five marked plants of each genotype. The genotypes were further grouped based on reaction type as given by Khare and Lakpale (1997) (Table1).

Histopathological and histochemical studies

For the determination of morphological basis of resistance histopathological and histochemical studies and changes in cell permeability were estimated in the leaf samples of healthy and infected susceptible (KBSH 44) and resistant (KBSH 53) sunflower grown under glass house conditions.

The leaf bits (10x3 mm) were fixed in Carnoy's B fixative (ethyl alcohol : chloroform : acetic acid, 6:3:1) for 2 h and subjected to dehydration using series of 70, 80,90 per cent alcohol and absolute alcohol.Then the leaf bits were passed through alcohol : butanol mixtures (3:1,1:1 and 1:3) and pure butanol twice.

Paraffin were added successively and kept in hot air oven at 75ºC thus replacing the last traces of butanol with paraffin.

Then the molten wax with the leaf bits were poured into the paper boat and leaf bits were arranged properly before the wax gets solidify. After the entire block was cooled it was removed.

The microtome sections of 10 µm thickness were taken. Paraffin sections were arranged on the surface of a adhesive and warmed for proper stretching at 45 ºC. Then the slides were dried for 24 h in a dust free chamber.

Before staining, the slides with sections were kept in xylol for 5 minutes to deparaffinize and later passed through butanol, alcohol successively for 5 minutes in each grade. After staining the sections were dehydrated using alcohol butanol series cleared in three changes of xylol mounted in DPX for observations.

Staining

Total proteins

The sections were stained in mercuric bromophenol blue stain (Maiza *et al.,* 1953) in alcohol for 6 minutes and differentiated in tap water. Insoluble protein appears blue.

Total polysacharides

After hydration the sections were kept in 0.1 per cent toluidine blue stain (Feder and O'Brein.1968) for 10 minutes and washed in running water.

Histochemical assessment

All observations in any treatment were recorded from 5 sections originating from different leaf bits. Based on visual observations of the degree of histochemical reactions with specific reagents for various cellular compounds of the leaves, the qualitative grading was done as detailed below.

Rich : +++ Medium : ++ Low: + Absent/ Negligible : —

RESULTS AND DISCUSSION

Selection of resistant and susceptible genotypes (cultivars) of sunflower to powdery mildew

To select the resistant and susceptible genotypes among the cultivated ones for further use in the present investigation, hybrids of sunflower viz., KBSH-1, KBSH-41, KBSH-42, KBSH-44 and KBSH-53 were evaluated for their reaction to powdery mildew disease. The screening work was undertaken at ZARS, UAS, GKVK, Bengaluru under glass houseconditions.

The results obtained are presented in Table 2. Hybrid KBSH-53 recorded least powdery mildew severity of 4.2 per cent compared with other hybrids and was recorded as resistant to powdery mildew.

The highest disease severity (61 %) was recorded in hybrid KBSH-4 which was recorded as highly

susceptible to powdery mildew. Whereas, other hybrids namely KBSH-1, KBSH-41 and KBSH-42 showed disease severity ranging from 43 to 47 per cent and were found susceptible to powdery mildew.

Based on this, KBSH- 53 was recorded as resistant and KBSH -44 as highly susceptible genotypes and were used in all the future studies.

Table 1 : Key for assessment of disease severity

Scale	Description	Reaction
0	No symptom of powdery mildew on leaves.	Immune (I)
1	Small scattered powdery mildew specks covering 1 % or less leaf area.	Highly resistance (HR)
3	Small powdery lesions covering 1-10 % of leaf area.	Resistance (R)
5	Powdery lesions enlarged covering 11-25% of leaf area.	Moderately resistant (MR)
7	Powdery lesions coalesce to form big patches covering 26-50% of leaf area.	Susceptible (S)
9	Big powdery patches covering: 51% or more of leaf area and defoliation occur	Highly susceptible (HS)

Table 2 : Screening of sunflower hybrids against powdery mildew

Hybrid	Disease severity (%)	Reaction
KBSH 1	43	Susceptible
KBSH 41	45	Susceptible
KBSH 42	47	Susceptible
KBSH 44	61	Highly Susceptible
KBSH 53	4.2	Resistant

Histological studies

The microtome sections of healthy leaves of sunflower of resistant and highly susceptible hybrids to powdery mildew revealed the presence of thick and intact epidermal layer with cylindrical palisade and round spongy parenchyma cells. Whereas, the sections of diseased leaves of highly susceptible hybrid KBSH 44 showed more damage to leaf structures than the resistant hybrid KBSH 53. The epidermis was not intact or damaged.

Histochemical studies

The histochemical studies for localization of insoluble polysaccharides and proteins in different regions of healthy and diseased leaves were studied through microtome technique and the results are presented in Table 3.

Nucleic acids

The palisade and spongy parenchyma cells of healthy leaf showed rich (+++) nucleic acids which were prominent by dark stain while, in diseased leaves of resistant hybrid nucleic acids in palisade and spongy parenchyma cells were medium (++) whereas, KBSH 44 showed less stain (+) indicating low level of nucleic acids (Fig.1).

Proteins

The results revealed that, palisade and spongy parenchyma cells of healthy leaves were rich (+++) for mercuric bromophenol blue (MBB) indicating more concentration of total protein which was indicated by dark blue stain, while the diseased cells of resistant hybrid showed medium (++) and the susceptible hybrid showed low (+) total proteins by exhibiting light staining with MBB (Fig.2).

The studies on histological observations of diseased and healthy leaf tissues revealed that healthy leaves showed thick and intact epidermal layer with cylindrical palisade and parenchyma cells. Whereas, the diseased leaf microtome sections showed disintegrated upper and lower epidermis and not intact. The cells of leaves were completely destroyed, with no demarkation of palisade tissue and spongy parenchyma. The cells were irregularly distributed and disintegration of stomata and damaged cuticle were also observed. Increase in air space in infected leaf

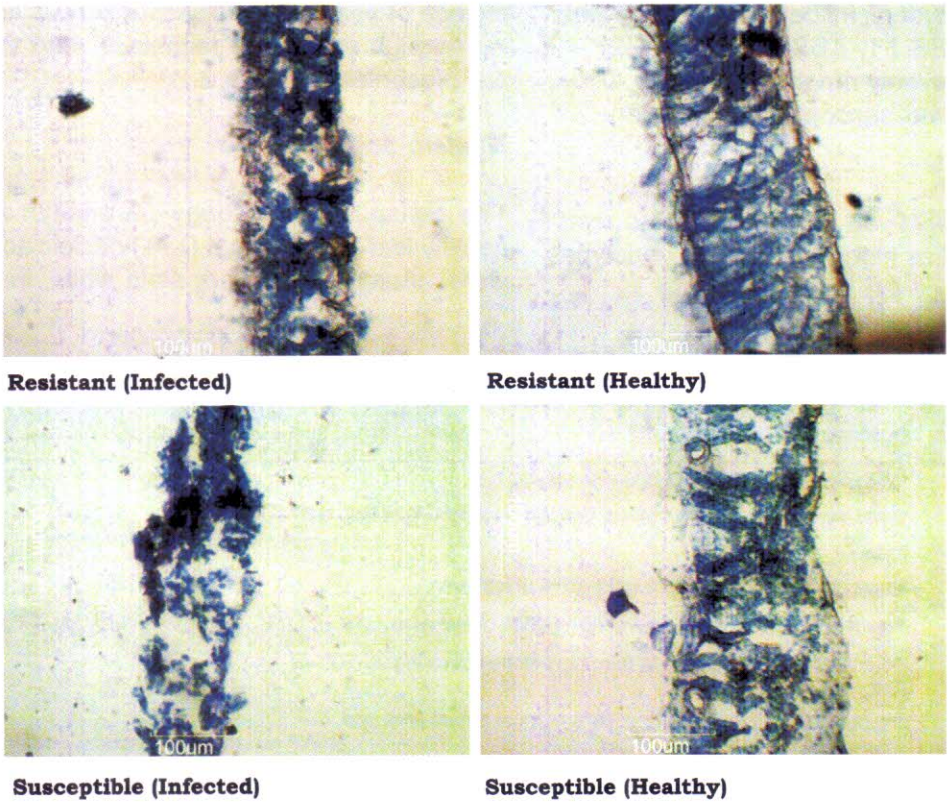

Fig. 1 : Histochemical changes in healthy and powdery mildew infected leaves showing localization of nucleic acids in resistant and susceptible hybrids of sunflower.

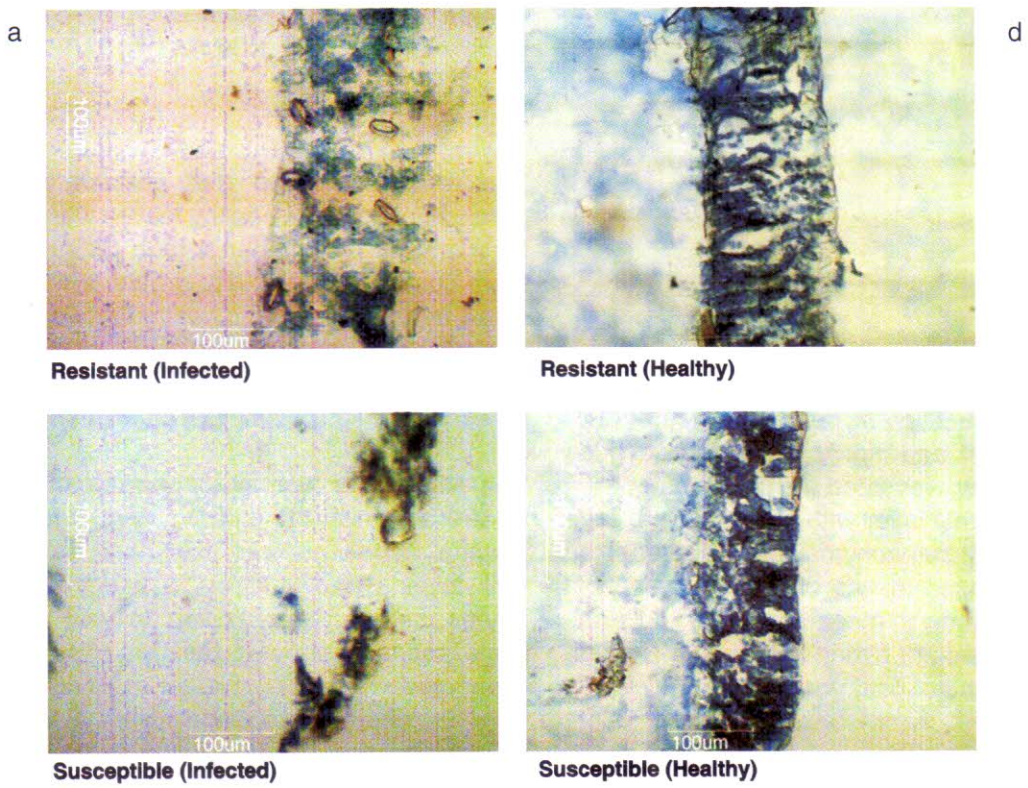

Fig. 2 : Histochemical changes in healthy and powdery mildew infected leaves showing localization of proteins in resistant and susceptible hybrids of sunflower.

Table 3 : Histochemistry of healthy and powdery mildew infected leaves of sunflower

Nucleic acids	Hybrid (Status)	Type of stain	Density of Nucleic acids		
			Epidermis	Palisade parenchyma	Spongy parenchyma
KBSH 53	Healthy	TB	+++	+++	+++
(Resistant)	Diseased	TB	++	++	++
KBSH 44	Healthy	TB	+++	+++	+++
(Susceptible)	Diseased	TB	+	+	+

Total Proteins	Hybrid (Status)	Type of stain	Density of Proteins		
			Epidermis	Palisade parenchyma	Spongy parenchyma
KBSH 53	Healthy	MBB	+++	+++	+++
(Resistant)	Diseased	MBB	++	++	++
KBSH 44	Healthy	MBB	+++	+++	+++
(Susceptible)	Diseased	MBB	+	+	+

LEGEND : +++ : Rich, ++ : Medium + : Low
STAINS : MBB : Mercuric Bromophenol Blue for total proteins.
TB :Toulene blue

tissue was attributed to disintegration of tissue. Similar type of observations were made by Prameela *et al.* (1990) in case of pigeon pea infected by powdery mildew, where infection results in reduction of leaf and epidermal cells thickness, palisade cell length, number of plastids and nucleic acid contents of leaves.

Histochemical studies revealed that the concentration of nucleic acids and proteins differed between healthy and diseased leaf tissues. Healthy tissues exhibited rich concentration of nucleic acids and proteins while, reduction of insoluble polysaccharides and proteins in diseased leaf tissues was observed during the present study under report. The pathogen depleted the host leaf tissues of these important metabolites due to fast degradation i.e., varied metabolism. The histochemical localization revealed gradual depletion of polysaccharides, proteins and nucleic acids from the pigeon pea host tissue infected by powdery mildew (Prameela *et al.*,1990) and from the groundnut host tissue infected by early and late leaf spot (Kaur and Dhillon, 1988 and Vijaykumar, 1990).

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