ANATOMICAL AND BIOCHEMICAL BASES OF RESISTANCE IN BANANA TO YELLOW SIGATOKA LEAF SPOT DISEASE

By

V. K. SAIRA BANU

THESIS

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Department of Plant Pathology

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DECLARATION

I hereby declare that the thesis entitled **"Anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

Vellanikkara
29-10-2001

Saira 29/10/2001

V. K. SAIRA BANU

Dr. S. ESTELITTA
Associate Professor (Plant Pathology)
Communication Centre,
Kerala Agricultural University,
Mannuthy

CERTIFICATE

Certified that the thesis, entitled **"Anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease"** is a record of research work done independently by **Miss.V.K. SAIRA BANU** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara
29-10-2001

Dr. S. ESTELITTA
Chairperson,
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Miss.V.K.SAIRA BANU**, a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Pathology**, agree that the thesis entitled **"Anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease"** may be submitted by Miss.V.K.SAIRA BANU in partial fulfilment of the requirement for the degree.

31/12/01

Dr.S.ESTELITTA
(Chairperson, Advisory Committee)
Associate Professor(Plant Pathology)
Communication Centre
Kerala Agricultural University
Mannuthy

31/12/2001

Dr.Koshy Abraham
Associate Professor & Head
Department of Plant Pathology
College of Horticulture
Vellanikkara

31/12/01

Dr.A.Augustin
Associate Professor(Biochemistry)
AICRP on M&AP
College of Horticulture
Vellanikkara

31/12/01

Dr.K.Aravindakshan
Associate Professor
Department of Pomology & Floriculture
College of Horticulture
Vellanikkara

31/12/01

Dr.M.Balasundaran
(Scientist EI)
Scientist –in-charge
Division of Pathology
Kerala Forest Research Institute
Peechi,Thrissur
(External Examiner)

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Saira Banu V.K
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Dedicated to my beloved parents and sisters

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Introduction

INTRODUCTION

Banana is an important fruit crop of Kerala State where its cultivation extends around 80,640 hectares. It is cultivated as a pure crop on commercial basis as well as inter crop in homesteads. The most important commercial variety is Nendran [*Musa* (AAB) 'Nendran'] which covers around 38 per cent of total area under banana in Kerala.

The productivity of banana in Kerala is very low compared to other States of India. There are several constraints to the production of banana in Kerala, of which diseases play an important role. Yellow sigatoka leaf spot caused by *Mycosphaerella musicola* Leach (*Pseudocercospora musae* Zimm.) Deighton is the most important and widespread disease in the rainy season through out the banana growing regions. In Kerala, where rainfall and relative humidity are high, the leaf spot disease is a serious threat to the crop. Screening of 144 varieties of banana against yellow sigatoka disease revealed that varieties viz., Chenkadali, Sanna Chenkadali, Pisang Lilin, Paka, Tongat, Adakkakunnan and Thiruvananthapuram were tolerant to the disease (KAU, 1978). Almost all commercial varieties in Kerala are susceptible to this disease causing 30-50 per cent reduction in the yield (KAU, 1989). Evaluation of germplasm under field and artificial inoculation showed that the varieties viz., Manoranjitham, Thiruvananthapuram, Pisang Lilin and Sanna Chenkadali were highly resistant to leaf spot (Estelitta *et al.*, 1991).

Most of the varieties coming under the AAB group belong to the resistant and moderately resistant categories. The commercially important varieties coming under this group are mainly Nendran, Palayankodan and Poovan, of which

Palayankodan and Poovan are found moderately resistant and Nendran is susceptible.

The first symptom of the disease was noticed on the third or fourth leaf from the centre as light yellow or brownish green indistinct linear markings lying parallel to the veins and then turned to characteristic spots. In advanced stage leaves have large dead patches around the margin as well as numerous necrotic spots of varying size. Rapid destruction of leaf lamina is the serious feature of the disease. Symptoms are also observed on the bunches.

The nature of resistance reaction is largely determined by the initial stimulus provided by the parasite and its secretions and the ability of the host to respond (Barnett, 1959). The plant characteristics which give them resistance to the disease might be morphological or biochemical acting directly or indirectly on the parasite (Goodman *et al.*, 1986). The components of resistance may be present in the host before it comes into contact with the pathogen agent, but in many cases resistance mechanisms are induced in the host in contact with the pathogen. The defence mechanisms which actually arrest pathogen penetration or development include, the hyper sensitive response, physical barriers, antimicrobial proteins and metabolites such as phytoanticipins and phytoalexins (Collinge *et al.*, 1996).

In this context, an insight into the anatomical and biochemical bases of resistant, moderately resistant, susceptible and highly susceptible group of *Musa* spp. to yellow sigatoka leaf spot disease will be much useful in breeding programmes. Comparison of the changes in anatomical and biochemical parameters in resistant and susceptible cultivars of *Musa* spp. before and after infection with *M. musicola* will give added information on the host pathogen interaction.

The present study was thus aimed at analysing the anatomical and biochemical bases of resistance in banana groups viz. resistant Manoranjitham [*Musa* (AAA) 'Manoranjitham'], moderately resistant Thiruvananthapuram [*Musa* (AAB) 'Thiruvananthapuram'], susceptible Nendran [*Musa* (AAB) 'Nendran'] and highly susceptible Grand Naine [*Musa* (AAA) 'Grand Naine'] to yellow sigatoka leaf spot.

Review of Literature

REVIEW OF LITERATURE

Yellow sigatoka leaf spot caused by *Mycosphaerella musicola* Leach (*Pseudocercospora musae* Zimm.) Deighton is an important disease of banana causing considerable reduction in yield. The disease was originally recorded in Java in 1902 by Zimmermann. In Fiji, it became notorious in 1913 in the sigatoka valley, hence the name 'Sigatoka disease'. Rangaswamy and Kolandaiswamy (1962) reported the sigatoka leaf spot disease of banana caused by *Cercospora musae* Zimm. occurring in the Annamalai area of Madras State in India. There is no prophylactic and suitable control measures to tackle the disease and almost all commercial varieties in Kerala are susceptible to this disease. But varieties like Manoranjitham (AAA) and Thiruvananthapuram (AAB) are found resistant to leaf spot. So, a comparison of the anatomical and biochemical parameters in susceptible and resistant varieties of *Musa* spp. will possibly give added information on host pathogen interaction. With this intention, an effort is made in this review to bring together facts, interpretations and theories of anatomical and biochemical defence mechanisms in host pathogen interaction.

2.1 Symptomatology of yellow sigatoka leaf spot disease

Symptomatology of yellow sigatoka leaf spot disease of banana were reported by many authors.

Philpott and Knowles (1913, 1921) reported that many affected plants had not more than three green leaves and in the final stage, stalks were seen from which the bunch had fallen and possessing no leaves. The stalk had decayed at about the places where it emerged from the crown of the plant. The bananas were green and angular, being about half fill or less and when broken across the pulp characteristic yellow appearance of nearly mature fruit can be seen.

The evident feature of this disease was the presence of a profusion of small discrete spots on the leaf lamina of older leaves, with area of 'scorched' or brown leaf tissue. Though the two youngest leaves or heart-leaf of an actively growing plant were usually free from evident spotting line and also from minute specks, spots or lesions, these leaves were most susceptible to pathogen (Wardlaw, 1934, 1938, 1939). The first indication of infection which occurs on the third or fourth leaf from the centre and some times on the second leaf showed the appearance of light yellow or brownish-green, indistinct linear markings, 1-2 mm or more in length lying parallel to the veins and later form characteristic spot. These spots increase slightly in size, forming dark, muddy brown to black linear-oblong or elliptic areas up to one cm in length and about one third of this in width and usually seen on fourth to sixth leaf.

According to Stahel (1937), the very first symptoms were seen 15 to 17 days after inoculation with conidia, present in the minute yellow-green speckles or streaks, so 22 to 24 days after inoculation these speckles became streaks, the leaf having a slightly rusty appearance. From 24th to 27th days, the spots showed brown necrotic colour. The spot became readily visible to naked eye, about 28 days after inoculation. A week or more after the brown spot stage, these spots coalesced and the characteristic pale grey-buff sigatoka spot was formed. The relationship of spot development to leaf position ie., from the centre of the crown depended on the rate of growth of the fungus and on the rate at which new leaves emerged at the crown.

Bunches with slight leaf spot infection have approximately normal fruits (Wardlaw, 1939). The effect of sigatoka leaf spot on fruit physiology, size of bunches and ripening abnormalities were noticed.

Leach (1941) showed that the exposed under surface of the unfurling heart leaf (candela) was the major target for both conidia and ascospores and that little infection occurred on the upper surface of the leaves.

Merchan (1985, 1987) reported that under favourable conditions for infection and incubation the first spot became visible 35 days after the appearance of heart leaf. The average time for the 'latent period' during first production cycle of the spot was 82 days, for the second one 96 days. On leaves during the dry season first spot was detected 140 days after leaf emergence.

2.2 Source of resistance to *M. musicola*

Suharban (1977) reported that varieties like Vennettumannan and Chirapunchi were found least susceptible to sigatoka leaf spot.

The varieties viz., Chenkadali, Sanna chenkadali, Pisang Lilin, Paka, Tongate, Adakkakunnan and Thiruvananthapuram were found tolerant to sigatoka leaf spot disease (KAU, 1978).

With very few exceptions all wild *Musa acuminata* were considered to be resistant or highly resistant to both *M. musicola* and *M. fijiensis*. Several sub species of wild *Musa acuminata* have high levels of resistance to black sigatoka and have been used in crosses designed to upgrade the bunch qualities in resistant bred diploids (Rowe, 1984).

Eight accessions of the sub-species collected in Papua New Guinea by Jones and Montcel (1988) were found resistant to *M. fijiensis*, whereas *Musa balbisiana* has always been found to be highly resistant to both species.

Montcel (1989) reported that *Musa acuminata* spp. *banksii* was found highly susceptible to *M. musicola*. Diploids (AA) and Triploids (AAA) considered to be highly susceptible to *M. musicola*. There is wide spread susceptibility among the edible AA, AAA and AAB cultivar but the presumed parents of these cultivars, *Musa acuminata* and *Musa balbisiana* were in general resistant.

More than 20 accessions of sub species of *Musa acuminata* were maintained in the germplasm bank of Brazil. Almost all of them remain free from yellow sigatoka in all seasons (Shepherd, 1989).

Estelitta *et al.* (1991) screened the banana germplasm against sigatoka leaf spot disease both in the field and artificial inoculation revealed that the varieties Pisang Lilin, Sanna chenkadali, Manoranjitham and Thiruvananthapuram were tolerant/resistant to the leaf spot.

2.3 Cultural and morphological characters of *M. musicola*

2.3.1 The pathogen

The pathogen was originally observed and described in the conidial stage (*Cercospora musae*) by Zimmermann in 1902. The perfect stage of ascomycetous fungi was reported by Leach, 1941. Calpouzos, 1955 had demonstrated the presence of spermagonia in constant association with *Cercospora musae* on the buff - grey spot centres. Later conidial stage of *Cercospora musae* was renamed as *Pseudocercospora musae* (Zimm.) Deighton (Deighton, 1976).

2.3.2 Morphological characters of *M. musicola*

Parham (1935) found that certain irregular rounded bodies of varying size which occurred in cultures of *Cercospora musae* was the immature perithecia and that *M. musicola* might be the perfect stage. Leach (1941) demonstrated the

presence of ascospore stage of *C. musae* the organism then renamed as *M. musicola*.

Stahel (1937) reported that conidia and spermagonia were stages of the same fungus *M. musicola*. Meredith and Butler (1939) observed that ascospores of *M. musicola* were two celled and yielded conidia of *C. musae* in inoculated plant.

Calpouzos (1955) found that ascospores of *M. musicola* were not produced in the culture. He observed spermagonia with spermatia in pure culture and other structures like immature ascocarps.

Mulder and Holliday (1974) reported that ascospores released from leaves were hyaline, two-celled with one cell bigger than the other with a slight constriction at the septum. The size ranged from 13.2-19.8 x 3.3-4.9 µm with an average of 15.9 x 4.0 µm. Germination took place 24 h after discharge and was bipolar. Germ tubes were straight to slightly curved.

2.3.3 Cultural characters of *M. musicola*

Calpouzos (1955) described the procedure for isolating both mass and single conidial cultures. Four days were required for the development of minute colony that was visible to the naked eye. He also reported that approximately the same heterogeneous morphological character of wild type fungal colonies tend to develop on quite different media at any particular temperature. Evidence was presented to show that wild isolates were a mixture of sporulating and sterile mycelia, the stable lines of which could be isolated by selective subculturing. Definite proof of the identity of the fungus was lacking due to failure of the development of the perfect stage of the fungus in culture. Small numbers of conidia were occasionally obtained on banana leaf extract agar, usually from young colonies of three to six days old.

Stover (1976) studied the distribution and cultural characteristics of the pathogen causing banana leaf spot. Only *M. musicola* was present in Central and South America except in case of Honduras where *M. fijiensis* var. *difformis* was also present. Single ascospore culture work showed *M. fijiensis* var. *difformis* to form a separate distinct group. Cultural variation among single ascospore isolations was much greater in the former group than in the latter. The former produced two main types of cultures on mycophyl agar, a dark grey or grey brown colony with a cremate edge (DGB) and a pale grey and pink colony (PGP). The DGB cultures produced more conidia when first isolated and with time became unstable yielding PGP cultures.

Suharban (1977) found that profuse mycelial growth of *Cercospora musae* was observed on PDA, PSA and banana leaf extract medium, but no sporulation obtained.

Natural (1989) noticed that conidia were produced by only one isolate of *M. musicola* grown on PDA, CGYEA, CzYEA and SmGYEA after 14-21 days incubation at 22-25°C under continuous light. Isolate one to eight failed to produce conidia. Conidia of isolate nine were hyaline to cylindrical with a round base, unthickened hilum, oblong smooth-walled, 5-7 septa with dimensions 16.5-82.5 x 1.6-3.3 µm.

2.4 Seasonal influence on disease incidence

In north Queensland, the greatest intensity of infection occurs during wet summer months. The incidence is less during the winter months because of lower temperature and humidity. After penetration of inoculum into a susceptible leaf, the time of appearance of initial symptoms were dependent on the temperature and intensity of infection (Pont, 1960).

Brun (1963) reported a close correlation between incubation time and subsequent development of sigatoka leaf spot under artificial inoculation on banana leaves in Guinea. Calpouzos *et al.* (1963) showed the importance of rainfall dates in forecasting sigatoka leaf spot disease of banana. Heavy rains at several places were followed by disease out breaks, while dry spells were not.

Mourichon and Zapater (1990) suggested that yellow and black sigatoka diseases displayed seasonal dynamics determined by variations in temperature and precipitation throughout the year, because the reproductive structures develop by cross-inoculation, a process enhanced by the presence of moisture on the leaves.

Rapid spread of yellow sigatoka leaf spot disease was stimulated by rainy, humid and warm weather (Muharam and Subijanto, 1991).

The development of yellow and black sigatoka disease was maximum at temperatures of the order of 25 to 28°C with high relative humidity and wet leaves (Jacome and Schuh, 1992).

Porras and Perez (1997) reported that *M. musicola* adapted better to temperatures below 15°C, but development was affected more above 30°C. Plotez and Sauco (1998) noticed that yellow sigatoka was serious during the periods of high rainfall.

Torres *et al.* (2000) noticed that precipitation is a determinant parameter for the appearance of infection of yellow and black sigatoka diseases. Temperature and relative humidity enhanced the development of the epidemic as temperatures of 20 to 35°C enabled ascospore and conidial germination.

2.5 Anatomical defence mechanism in plants

The capacity or ability of a plant to defend itself against a pathogen is governed by its genetic constitution and the environmental conditions under which the genes operate. The attributes of the hosts that reduce the chances of infection or the development of the pathogen are considered to be the defence mechanisms. Anatomical defence mechanism is one of the initial means by which plants defend against the attack of pathogen.

2.5.1 Epidermis as defence barrier

The thickness of the skin imparted by the epidermis including cuticle acts as a defensive barrier against the invading pathogen.

Cuticle acts as a chemical and physical barrier to the germination and penetration of the pathogen. The cuticle thickness was correlated with levels of resistance to fungi that penetrated directly into the tissues (Wang and Pinckard, 1973 and Bell, 1974).

In resistant and susceptible cultivar of potato inoculated with *Phytophthora infestans*, the pathogen penetrated the leaves of susceptible and resistant cultivar either through stomata or directly through the epidermis. In susceptible host, fungus spread through out the tissue intercellularly and transcelluarly, whereas in resistant host it remained confined to the site of infection. During penetration by the fungal hyphae, host cell walls became more pliable and soft which resulted in deformed shape of the susceptible cells (Hohl and Suter, 1976).

Jorgensen and Mortensen (1977) reported that infection in epidermal cells of barley by *Erysiphe graminis* resulted in the germination of less conidia in the resistant cultivars than the susceptible cultivars.

Penetration of a host cell by the pathogen depends on softness of cell wall. Soft walled cells could be penetrated easily, where as in hard walled cells penetration could be achieved by enzymatic secretion of the pathogen at the site of infection (Agrios, 1978).

Kuch and Khew (1980) observed stomata in the lower epidermis of black pepper. Leaves inoculated with *Phytophthora capsici* had the largest number of sporangia on the lower epidermis followed by mesophyll layer and the upper epidermis. Mature leaves had the largest number of sporangia in the lower epidermis and the mesophyll, while old and young leaves had less number of sporangia in these tissues.

In rust resistant groundnut genotypes, the structural defence mechanisms observed by Mayee and Apet (1995) were thicker epidermis-cum-cuticle, more number of trichomes on abaxial surface of leaves, low frequency smaller stomata and compact palisade tissue. There are intense callus deposits around the site of infection in the resistant genotypes whereas susceptible genotypes had less intense callus deposits aloof the site of infection.

Trujillo *et al.* (1997) carried out anatomical studies of leaf blades of four cultivars of *Musa*, with several levels of resistance to yellow sigatoka disease caused by *M. musicola*. A single abaxial epidermal layer with rectangular, thin walled cells, hypodermis with two or three layers of cells with very thick walls and several layers of palisade and spongy mesophyll and high stomatal density on the abaxial epidermis were observed in the cultivars. A very thick hypodermis with a low stomatal density and a large number of phenolic crystals in the cells were observed in the clones exhibiting resistance.

Hermoso *et al.* (1997) studied the foliar anatomy of the somaclonal variant of Musa, CIEN BTA-03, resistant to yellow sigatoka. In comparison with the mother plant, the somaclonal variant CIEN BTA-03 showed an increase in the number of hypodermal layers, a greater thickness of the epidermal outer wall, a higher density of vascular bundles, lower number of stomata and an increase in leaf thickness.

Mahajan and Gill (1998) studied the relationship between stomatal density of cauliflower to downy mildew (*Perenospora parasitica*) in resistant and susceptible lines. A positive correlation was found between the disease and stomatal density. Sporulation and stomatal count were greater on the dorsal surface of the leaves. They noticed more number of stomata on leaves of the susceptible genotypes than the resistant lines.

Grewal *et al.* (1999) studied twenty six varieties/lines of *Triticum aestivum*, *Triticum durum* and *Tritium secale* with known levels of resistance/susceptibility to karnal bunt to determine the role of hairs and stomata in imparting resistance to disease. In bread wheat, the resistant lines had significantly less stomata on glumes and rachis than that of susceptible lines. However, no significant differences in stomatal frequency were observed between susceptible and resistant cultivars of *T. durum* and *T. secale* respectively.

Prepenetration anatomical barriers of 22 muskmelon genotypes against downy mildew (*Pseudoperenospora cubensis*) were investigated by Inder *et al.* (1999) and found that epidermis and cuticle thickness on both adaxial and abaxial leaf surfaces was significantly greater in the resistant genotypes than susceptible ones. The significant correlation of disease resistance with stomatal and trichome size and frequency indicated their importance in determining resistance.

Dagade (1999) reported that in *Piper* spp. the immune genotype *Piper colubrinum* was characterised by compact arrangement of cells and small epidermal, mesophyll and spongy parenchyma cells, thick lower epidermis with large cells and less number of stomata / unit area in the leaves. The susceptible genotype Panniyur 1 with thin palisade and collenchyma tissues, small vascular bundles and thin lower epidermis with small cells and more number of stomata / unit area. The tolerant genotype Kalluvally exhibited some what intermediate values between the immune and susceptible ones.

2.5.2 Mesophyll as defence barrier

Nirula (1944) reported the histopathology of stored betel vine leaves infected by a bacterium. The entry of the bacterium was through the cut ends of the petiole and in the advanced stages of infection destroyed mesophyll cells and disorganised xylem vessels got disintegrated.

In the early stages of infection of betel vine, the bacterial leaf spot pathogen was confined to the epidermis but subsequently to the spongy and palisade parenchyma cells. The parenchyma cells were slightly enlarged after the infection and assumed yellow colour and disintegrated (Asthana and Mahmud, 1945).

Jennings and Ullstrup (1957) reported that southern leaf blight of corn caused by *H. maydis* and *H. carbonum* ramified rapidly through susceptible parenchyma without invading the vascular bundles, whereas in the resistant inbred, *H. maydis* was effectively impeded by the chlorenchymatous tissues.

The mode of penetration of the *Cladosporium fulvum* race 1 in the susceptible, resistant and immune varieties of tomato was the same. In the susceptible variety, the leaf tissues were extensively colonised by the mycelia, but

in the resistant variety, mycelia development was very slow. The infected cells of the resistant variety became necrotic or exhibited changes like reduced starch content in the chloroplasts and accumulation of extra cellular material in the cell wall. Fungal development was restricted to few cells of mesophylls in immune variety. Host cells surrounding the fungus showed extensive deposition of callus like material (Lazorovits and Higgins, 1976).

Rohringer *et al.* (1979) studied the stem rust resistance of wheat and found that the Sr 6 temperature sensitive allele confers resistance only in mesophyll cells and the epidermal cells being susceptible did not exhibit any necrotic symptoms.

Jain *et al.* (1982) conducted histopathological studies in betel vine leaves and reported that the bacterial leaf spot pathogen invaded xylem, collenchyma and parenchyma cells.

Abraham (1986) studied the histopathological changes in betel vine leaves infected with *Xanthomonas campestris* pv. *betlicola* and observed that the bacteria entered through the stomata and disintegrated the spongy and palisade parenchymatous cells, leaving the xylem vessels unaffected.

Vasquez *et al.* (1989) reported that the highly susceptible variety Grand Naine had the highest stomatal density on the abaxial surface compared to the highly resistant variety Pelipita.

Maximum thickness of spongy parenchyma tissue was found in the susceptible varieties of groundnut to the tikka leaf spot pathogen compared to moderately susceptible varieties. The resistant varieties had thicker epidermis, palisade tissues and thinner spongy parenchyma (Kaur *et al.*, 1992).

2.5.3 Vascular system as defence barrier

Xanthomonas sp. of bacterium was reported in the vascular bundles and parenchymatous cells of infected betel vine leaves (Kotwal, 1978).

There was no correlation between vascular bundles or percentage of larger vascular bundles with mean leaf spot size in *Dactylis glomerata* on inoculation with *Stagnospora arenaria.* In resistant varieties, the proportion of small vascular bundles with two girders were greater than susceptible inoculated leaves which had higher lignin content than control indicating that lignin like compounds were synthesized during infection process (Sherwood and Berg, 1991).

Histopathology of four foliar pathogens (*Ramulispora sorghi, Gloeocercospora sorghi, Colletotrichum graminicola* and *Puccinia purpurea*) were studied by Kalappanavar and Hiremath (1998) in both resistant and susceptible sorghum varieties and reported that the infection of *R. sorghi, G. sorghi, C. graminicola* and *P. purpurea* caused severe damage to leaf tissues of the susceptible genotypes, but in the resistant genotypes the vascular bundles were partially infected.

2.5.4 Epicuticular wax deposition

Vasquez *et al.* (1989) reported that the susceptible banana variety Grand Naine possessed thinner deposition of wax on the adaxial and abaxial surfaces of leaves compared to the resistant variety Pelipita.

Wheat genotype 3336 showed a high level of resistance to the spot blotch pathogen (*Dreschslera sorokiniana*). Scanning electron microscopy of leaf surfaces revealed the presence of plate like wax particles distributed all over the leaf epidermis which were more condensed on the guard cells. Germination of

conidia of *Cochliobolus sativus* was maximum on susceptible Agra local showing hyphal penetration through stomata, whereas conidial germination on 3336 was very low in which the germ tubes showed papillate structures (Das *et al.*, 1999).

Singh *et al.* (1999) evaluated the susceptibility and resistant reaction in rapeseed mustard against *Alternaria brassicae* and found that epicuticular wax was one of the most important factors to be considered for improving resistance to Alternaria blight.

2.6 Biochemical studies

Walker and Stahmann (1955) reported that, in many histological studies resistance generally was not only associated with anatomical differences among the host plants, but also more directly to the biochemical or physiological aspects which might explain in part or at least the bases of resistance.

By biochemical conditions and reactions, the host inactivate the pathogen or kills it before the infection spreads and the disease became serious. Although biochemical mechanisms may be present in the plant before infection, more commonly, biochemical defence mechanisms develop in response to pathogenic activities. In the later case, the host cytoplasm or the cell walls react to pathogenic activities through abnormal metabolic process (Cruickshank, 1963; Bell, 1981).

Swain (1977) reported that disease results whenever the vital functions of plants were disrupted by a biotic stimulus and several quite often changes took place in the primary metabolism of the affected cells. Further interactions between host and pathogen determined biochemical dynamics of parasitism and pathogenesis (Cruickshank, 1980).

In the induced synthesis of these compound (phytoalexins) the speed and the magnitude of the accumulation appeared to determine the resistance of some cultivar- parasite combinations (Bailey and Mansfield, 1982). One defence mechanism conferring disease resistance of plants was the presence of constitutive or inducible antimicrobial compounds at the site of penetration of the pathogen (Ebel, 1986). Therefore physiological or biochemical bases of resistance is more important for resisting invasion by plant pathogens.

2.6.1 Phenols in defence mechanism

Chemicals possessing an aromatic ring bearing a hydroxyl substituent called phenolic substances showed antifungal, antibacterial and antiviral activities. In number of cases, resistant varieties have been reported to contain more phenolics (Vidhyasekaran, 1975) but in susceptible reactions enough phenolics are not synthesised (Howell *et al.*, 1976). Phenolics in high concentrations are found toxic to plant cell themselves (Tepper and Anderson, 1984). Hence phenolics will normally be present in small quantities only in plants and these quantities may not be sufficient to suppress the development of pathogen. But in many plant pathogen interactions, the synthesis of phenolics was activated after infection and high amount of phenolics were synthesised which repidly suppressed the pathogen development (Vidyasekaran, 1990). Phenolic compounds act as hydrogen acceptor/donor in oxidation - reduction reactions to form quinones which were involved in resistance of plants due to their higher toxicity to microorganisms.

2.6.1.1 Total phenol

Jayapal and Mahadevan (1968) reported that *Musa balbisiana* variety Kattuvazhai resistant to *Cercospora musae* was characterised by slightly more total phenol than the susceptible *Musa paradisiaca* var. *monthaniana*. There was an

increase in phenolic compounds in response to fungal infection in the resistant variety, while in Monthan leaves, *C. musae* inoculation led to rapid degradation of phenolic compounds.

Matern and Kneusel (1988) explained two way strategy of phenols in plants viz., rapid accumulation of phenols at the infection site and activation of de novo synthesis of specific defence such as phytotoxins or other stress related compounds.

Phenols in plants may act as an allelopathic compound or plant growth regulators (Siqueira *et al.*, 1991) or as an antibiotic (Nicholson and Hammerschmidit, 1992).

Increase in levels of total phenols were associated with resistance response of capsicum against *Phytophthora capsici* (Jeun and Hwang, 1991 and Lizzi *et al.*, 1995), chilli against bacterial wilt (Markose, 1996) and chilli and brinjal against *Ralstonia solanacearum* (Paul, 1998).

Sindhan *et al.* (1996) reported pre-infection higher levels of total phenols in flag smut resistant wheat varieties as compared to susceptible ones. Reduction in post infection concentration was observed in the both susceptible and resistant varieties, maximum being in susceptible ones.

Dagade (1999) estimated the amount of total phenol in different genotypes of *Piper* spp. and reported high content of total phenol in *Piper colubrinum* which is immune to foot rot disease and low content in the susceptible genotype Panniyur 1. The tolerant genotype Kalluvally expressed medium values for total phenols.

Bera *et al.* (1999) noticed a higher level of total phenol content in cercospora resistant genotypes of groundnut.

In cauliflower, in relation to downy mildew Mahajan (1999) indicated that disease incidence was significantly and negatively correlated with total phenol.

Singh (2000) noticed that resistant and moderately resistant cultivars of *Brassica* spp. against downy mildew and white rust contained a higher amount of total phenols than the susceptible cultivar at all stages of growth.

2.6.1.2 Ortho dihydric (OD) phenol

Jayapal and Mahadevan (1968) reported that resistant variety *Musa balbisiana* Colla variety Kattuvazhai contained more OD phenols than the susceptible Monthan variety (AAB). Inoculation of *C. musae* resulted in a rapid increase in total phenols in resistant leaves. Soon after inoculation, it gradually decreased with limitation of the lesion. But in susceptible variety, a rapid decrease in OD phenol was noted after inoculation.

Mukherjee and Kundu (1973) reported that the monomeric dihydroxy phenols have some antibiotic and enzyme denaturing activity.

Increase in dihydroxy phenols and decrease in ascorbic acid content were observed in rice inoculated with a virulent strain of *Pyricularia oryzae* (Sridhar and Ou, 1974). Addy (1976) reported that resistant varieties of apple to *Erwinia amylovora* leached dihydroxy phenols more rapidly than susceptible varieties.

The enzymes viz., polyphenol oxidase and peroxidase, oxidised the colourless dihydroxy phenols to give the coloured ortho quinones, while certain dihydroxy phenols got conjugated with each other or glucose hydroxyl group to form tannins, both form constituent of plant melanins (Beckman *et al.*, 1974). The

tannins and ortho quinones, the oxidised compounds of OD phenols were toxic to micro organisms (Hunter, 1978).

Bell (1981) suggested that, some pathogenic fungi reduced plant dihydroxy phenols to tetralones as a mechanism of inhibiting melanisation and overcome resistance provided that higher levels of dihydroxy phenols must be released and oxidised effectively for resistance expression. Also, leaching of dihydroxy phenols from vacuoles into cytoplasm and inter cellular spaces determine the level of resistance.

Abraham (1986) reported pronounced increase in OD phenol, total phenol in resistant *Piper betle* cultivar inoculated with bacterial leaf spot pathogen. Levels of OD phenol and total phenols were high in *Alternaria solani* inoculated plants than in healthy plants (Veermohan *et al.*, 1994).

Guleria *et al.* (1998) reported that there was a significant increase in the level of ortho-dihydroxy phenolic content in resistant cultivars of pea against powdery mildew.

Dagade (1999) observed a higher content of OD phenol in tolerant pepper cultivar Kalluvally than the immune cultivar *Piper colubrinum* to foot rot disease. The susceptible genotype Panniyur 1 contained lower amount of OD phenol.

2.6.2 Sugars in defence mechanism

Plant tissues with low sugar content become more susceptible to some diseases and less susceptible to others (Horsfall and Diamond, 1957). Raghunathan *et al.* (1959) reported that banana leaves in relation to Gleosporium infection showed higher susceptibility characterised by more soluble sugars.

Reduction in sugar content in infected plants might account for synthesis of poly phenols (Niesh, 1964). Easwaran (1967) noticed a high quantity of reducing sugar in sorghum variety susceptible to bacterial wilt disease than in the moderately susceptible varieties. Inoculation of pathogen led to reduction of sugars in both varieties.

Analysis of reducing sugars in banana leaves revealed higher amounts of reducing sugars in resistant leaves of variety Kattuvazhai while the susceptible variety, Monthan contained lower quantities. Inoculation of *Cercospora musae* led to a decrease in the reducing sugars in both the varieties. Non-reducing sugars were found more in resistant, variety Kattuvazhai and less decreased in the inoculated tissues (Jayapal and Mahadevan, 1968).

Higher levels of reducing and non-reducing sugars was observed in leaves of bacterial leaf blight susceptible rice variety than the tolerant one (Reddy and Sridhar, 1975).

Abraham (1986) attributed the resistance of betel vine cultivars to bacterial leaf spot pathogen showing higher levels of reducing, non-reducing and total sugars. On inoculation, the non-reducing sugar content was found more in susceptible cultivars.

Bhardwaj *et al.* (1986) reported significant decrease in levels of sugars and total phenols in leaves and fruits of capsicum plants susceptible to *Phytophthora nicotiana* var. *nicotianae*.

Veermohan *et al.* (1994) reported decreased photosynthetic efficiency and content of chlorophylls, reducing, non-reducing and total sugars and starch content in capsicum leaves infected with *Alternaria solani*.

Sindhan *et al.* (1996) found higher quantities of total and reducing sugars in flag smut resistant varieties of wheat as compared to susceptible ones. On inoculation there was reduction in sugar content.

Paul (1998) reported higher levels of soluble sugars in bacterial wilt resistant varieties of chilli and tomato.

Singh *et al.* (1999) reported that in rape and mustard, reducing sugar content contributed considerable and significantly in disease resistance to alternaria blight.

Bera *et al.* (1999) reported a higher level of soluble sugars in Cercospora resistant genotypes of groundnut.

There was a positive correlation between reducing sugar and the downy mildew disease incidence in cauliflower (Mahajan, 1999).

2.6.3 Proline

Prasada *et al.* (1999) reported that free proline, aspartic acid, asparagine and gamma-amino butyric acid generally increased in response to water stress. Amino acid accumulation was seen more pronounced in roots than leaves.

Plant water relations, proline content and activities of pyrroline-5-carboxylate synthetase (PSCs) and proline oxidase (PO) were studied by Phutela *et al.* (2000) in *Brassica juncea* genotypes differing in drought response. Increase in the activity of proline biosynthetic enzyme PSCs of roots and leaves under water stress was highest in drought tolerant variety and lowest in less drought tolerant variety. Reduction in the activity of proline degrading enzyme (PO) due to stress was also highest in the leaves and roots of drought resistant variety.

Zheng and Li (2000) suggested that in response to drought and salinity stress many plant species including soyabean accumulated high levels of proline which is thought to function in stress adaptation.

Mastrangelo *et al.* (2000) analysed the proline accumulation and expression levels of drought related genes in durum wheat (*Triticum durum*) at flowering stage, comparing two varieties with different drought tolerance capacity. The drought stress induced a strong increase in proline content in the drought tolerant variety.

Ramanjulu and Sudhakar (2000) studied the free proline levels and activities of pyrroline-5-carboxylate reductase, proline oxidase and proline dehydrogenase during water stress in drought sensitive and drought tolerant cultivars of mulberry. During water stress, proline metabolism was altered and greater levels of proline was found in drought tolerant variety. These higher levels were due to both increased rates of proline synthesis and less proline oxidation compared with drought sensitive cultivar.

Deka (2000) reported that leaf proline content increased in ten local cultivars of rice when subjected to water stress.

The effect of water stress on germination, seedling growth and organic solutes of different *Vicia faba* lines was studied by Tayeb and Hassanein (2000). They also reported that the soluble sugar, soluble protein, free amino acid and proline content in the shoots and roots of all the tested lines progressively increased as the stress level increased.

2.6.4 Enzymes in defence mechanism

Enzymes are large protein molecules which catalyse all the interrelated reaction in a living cell. Enzymes viz., peroxidase and polyphenol oxidase play an important role in disease resistance.

2.6.4.1 Peroxidase

A close correlation between disease resistance and peroxidase activity was reported by many authors. Ten fold increase in peroxidase activity was observed by Mace (1964) in resistant varieties of wheat. Peroxidase involved in lignin synthesis, catalyses the oxidation of phenolics into more toxic quinones (Kosuge, 1969).

Lizzi and Coulomb (1991) observed higher activity of the enzyme in resistant varieties when compared to susceptible.

Alcazar *et al.* (1995) reported low peroxidase activity in the intercellular fluid of susceptible capsicum cultivars than moderately susceptible and resistant cultivars to *Phytophthora capsici.*

In chilli (Markose, 1996) and brinjal (Paul, 1998) higher activity of peroxidase in conjugation with polyphenol oxidase and OD phenol was attributed to resistant varieties against bacterial wilt pathogens.

In cluster bean, healthy leaves of resistant varieties to alternaria blight exhibited high polyphenol oxidase and peroxidase activity with high phenolic compounds in comparison to susceptible ones at 65 days after sowing. Peroxidase activity was several times higher as compared to polyphenol oxidase activity and

pronounced increase in response to infection in both resistant and susceptible varieties (Saharan *et al.*, 1999).

Dagade (1999) studied the enzyme activities in *Piper* spp. and found higher activities of peroxidase and polyphenol oxidase enzyme in immune *Piper colubrinum*, medium in tolerant Kalluvally and lower in susceptible variety Panniyur 1.

2.6.4.2 Polyphenol oxidase

Polyphenol oxidase in combination with peroxidase and orthohydroxy phenol forms quinones that are toxic to the microorganisms.

Williams (1963) and Brown and Smart (1966) reported that peroxidase and polyphenol oxidase were important from the point of symptom expression and perhaps disease resistance. The production of quinones as a result of increased activity of these enzymes might prove toxic to the pathogen, or the host tissue or both and thus produced the browning or necrotic symptoms.

Muller and Beckman (1976) indicated that activities of polyphenol oxidase and peroxidase were more important since the former could add hydroxyl group during synthesis of dihydroxy phenols in plastids and the later was responsible for the oxidation of phenols to melanin in the cell walls of roots and hypocotyls of cotton seedlings.

Variation in the activities of peroxidase and polyphenol oxidase was observed in susceptible and resistant potato plants infected with *Phytophthora infestans* and *Erwinia carotovora* var. *atroseptica* (Bobes *et al.*, 1987).

In response to injury, activities of polyphenol oxidase and peroxidase were increased in the fusarium wilt resistant tomato plants (Genetile *et al.*, 1988).

Goy *et al.* (1992) reported resistance observed in tobacco hybrid *Nicotiana glutinosa* x *Nicotiana deloneyi* against TMV, tobacco necrosis necrovirus, *Ralstonia syringae* pv. *syringae, Ralstonia syringae* var. *tabaci, Perenospora tabacina, Perenospora parasitica* and *Thielaviopsis basicola* due to higher levels of polyphenol oxidase, peroxidase, chitinase and ß-1,3-glucanase.

Gupta *et al.* (1995) reported changes in peroxidase, polyphenol oxidase and catalase due to alternaria leaf blight infection in *Brassica* spp. The specific activity of polyphenol oxidase remained higher while that of peroxidase remained lower in tolerant *Brassica corinata* and *B. napus* when compared with susceptible *B. juncea* and *B. campestris*. In response to infection, activity of both the enzymes increased comparatively at much faster rate in the susceptible species. Polyphenol oxidase activity was higher at initial stages of plant growth in all species which dropped markedly at later stages.

Trandafirescu *et al.* (1999) noticed a direct relationship between the increase in content of polyphenol oxidase and the resistance of cultivars to *Stereum purpureum.*

Khirbat and Jalali (1999) reported that the specific activity of polyphenol oxidase remained higher in the resistant genotypes of chickpea (*Cicer arietinum*) in response to inoculation from 6 to 10 days with *Ascochyta rabiei* than the susceptible genotype.

In rice, there was a strong genetic correlation between polyphenol oxidase activity and resistance to bacterial blight (Chen *et al.*, 2000).

2.6.5 Protein

Enhanced protein synthesis appears to be a universal phenomenon in compatible host pathogen interactions.

De novo synthesis of new proteins was also reported (Tani and Yamamoto, 1979, DeWit and Bakkar, 1980). When the protein synthesis is inhibited by introducing inhibitors such as blasticidin S, puromycin and cycloheximide, resistance of the varieties break down. This clearly suggested that the protein synthesis was an important factor in disease resistance. The synthesised proteins might not be inhibitory to the pathogens. They mostly activate the synthesis of defence chemicals. The preformed existing proteins might not be involved in the disease resistance process (DeWit and Bakkar, 1980; Gabriel and Ellingboe, 1982).

Leach *et al.* (1983) detected entirely new protein which was different from pathogenesis related proteins (PR proteins). This protein could not be found in healthy tissues and appeared when lipopolysaccharide of *Ralstonia solanacearum* was infiltrated into the leaves of tobacco cultivar. The accumulation of new protein as well as the increase in relative content of atleast two other proteins were correlated with the appearance of resistance to bacterial multiplication in tobacco tissues.

Hanudin (1987) reported that tomato plants with high protein levels were more susceptible to infection by *R. solanacearum* than plants with lower levels.

Biochemical properties associated with rust and powdery mildew resistance in pea were studied by Chander (1989) revealing that the healthy leaves of powdery mildew and rust resistant lines of peas had more total nitrogen and protein, while another study by Chander (1994) in chillies indicated that the resistant chilli line contained less total nitrogen and true protein.

Total protein and soluble protein were high in okra cultivars resistant to yellow vein mosaic virus. After inoculation the total proteins increased in both resistant and susceptible cultivars, to a greater extent (Ahmed *et al.*, 1994).

Malhotra and Singh (1994) found negative correlation between coefficient of disease index of fusarium wilt in tomato and total protein.

Ganguly (1995) reported that *Rhizoctonia solani* resistant rice variety had higher levels of protein than susceptible cultivar tested. Post infection increase appeared to be associated with disease resistance.

Markose (1996) reported that in the resistance variety of chilli, Ujwala contained higher protein content than the susceptible variety and the increase in protein content due to infection was noticed in all plant parts of Ujwala.

Bera *et al.* (1999) noticed that a higher level of acid proteins was maintained in the leaves of *Cercospora* resistant genotypes of groundnut.

Trandafirescu *et al.* (1999) found a direct relationship between the decrease in content of amino acids and crude protein in the leaves and the resistance of six apricot cultivars by *Stereum purpureum*.

Sharma and Kaul (1999) assayed the total proteins and soluble proteins in the leaves of resistant and susceptible apple cultivars during scab challenge or pathogenesis. A higher amount of total and soluble protein was seen in the young expanding leaves of apple associated with the susceptible cultivars. The protein increased after inoculation and during pathogenesis.

2.6.6 Toxins

Many *Cercospora* spp. that are plant pathogenic produce phytotoxic metabolites (Balis and Payne, 1971; Fajola, 1973; Mumma *et al.*, 1973; Assante *et al.*, 1977; Sasaki *et al.*, 1981; Daub, 1982).

Meredith *et al.* (1970) noted the accumulation of dark melanin like or yellow or pink pigments in agar beneath and round the colony in many cultures of *M. musicola,* these pigments also eventually disappeared.

Non pigment producing isolates of species of *Cercospora* were reported by Assante *et al.* (1977) and Fajola (1978). They also reported that PDA was the most commonly used medium for *Cercospora* species and is also suitable for toxin production.

The non production of pigment in other media could be attributed to the failure of the medium to meet the requirements of the species (Shaw, 1981).

By thin layer chromatography (TLC), Natural (1989) found that pigment production was highly correlated with toxin production *in vitro*. TLC of concentrated culture extracts of all isolates grown on all media revealed that toxin was present only when an isolate produced pigment in culture. Toxin was recovered only from one isolate grown in PDA, CGYEA, CzYEA and SmGYEA. He also reported that the medium that produced the best resolution of toxic bands was PDA followed by SmGYEA. The medium which produced the largest bands on TLC plates was considered to be the medium which best supported toxin production.

Trujillo *et al.* (1997) investigated the effects of selection for resistance to yellow sigatoka disease (*M. musicola*) in plants and callus tissue of Venezuelan banana clones showing several degrees of susceptibility to the disease. Inoculation media derived from the pathogenic fungus that caused the disease and from the homogenised pathogen were tested. After inoculation with the asexual phase media, calluses and plants showed similar survival percentages for both pathogenic and homogenised media at the two concentrations tested. When inoculated with

sexual phase media, calluses and plants presented consistent differences in survival percentages, depending on the type and concentration of selective media used. Sexual phase was the most suitable for selection, while the homogenised fungus was the most efficient selective agent.

Harelimana *et al.* (1997) suggested that *Mycosphaerella fijiensis* toxins might not be involved in either the initiation of the infection or the hyper sensitivity reaction of very resistant cultivars. However, they may serve as secondary determinants in pathogenicity, thus contributing to the spread of lesions in cultivars that are partially resistant to black sigatoka. The effect of toxins on chlorophyll fluorescence using electron microscopy indicated that chloroplasts may be the sites of early action by the toxins. However, the heterotrophy of tissues *in vitro* does not allow screening with these toxins.

Materials and Methods

MATERIALS AND METHODS

The present investigations were carried out at the Department of Plant Pathology and Biochemistry Laboratory of the College of Horticulture, Kerala Agricultural University, Vellanikkara during 2000-2001. The study was aimed at analysing the reaction of four different groups of banana to yellow sigatoka leaf spot disease. Four different groups of banana viz., resistant (Manoranjitham, AAA), moderately resistant (Thiruvananthapuram, AAB), susceptible (Nendran, AAB) and highly susceptible (Grand Naine, AAA) varieties (Plate 1) to yellow sigatoka leaf spot disease (Plate 2a and 2b) were subjected to investigation. The details regarding the experimental materials and methodology adopted for conducting various aspects of the study are presented in this chapter.

3.1 Seasonal influence on disease incidence

The influence of climatic factors on leaf spot disease intensity were studied in different periods at three months interval (April 2000, July 2000, October 2000, January 2001). The intensity was recorded as per Gauhls modification of Stovers sigatoka severity scoring system (Orgeda, 1998).

$$\text{Infection index} = \frac{\Sigma nb}{(N-1)T} \times 100$$

where,

n = Number of leaves in each grade

b = grade

N = Number of grades used in the scale (7)

T = Total number of leaves scored

Plate 1. Different varieties of banana

Manoranjitham (AAA)

Thiruvananthapuram (AAB)

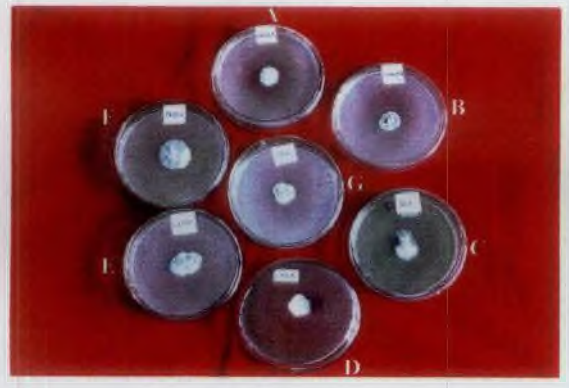
Nendran (AAB)

Grand Naine (AAA)

Plate 2.

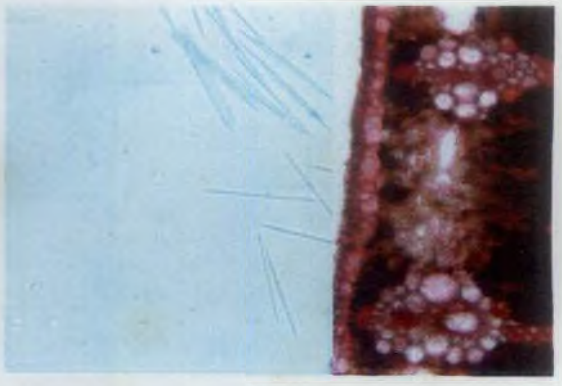
2a. Field view of yellow sigatoka leaf spot disease

2b. Symptoms of yellow sigatoka leaf spot disease



2c. Growth of *Mycosphaerella musicola* on different media incubated at 22°C-25°C under continuous light for five weeks

A. CGYEA **B.** SmGYEA **C.** BnDA
D. MEA **E.** CzYEA **F.** PGYEA
G. PDA



2d. Conidia of *Mycosphaerella musicola* released from the yellow sigatoka infected leaves of banana (× 400)

Meteorological data on rainfall, temperature and relative humidity were also recorded.

3.2 Isolation and maintenance of the pathogen

The pathogen causing yellow sigatoka leaf spot of banana was isolated as per the procedure described by Natural (1989). Dried and fresh leaf samples showing various stages of disease development were collected. Infected leaf samples were cut into small pieces and surface sterilised using 0.1 per cent mercuric chloride solution and allowed to dry. Isolations were made by ascospore discharge and spore pick technique.

3.2.1 Ascospore isolation

Infected leaf bits, about 2 cm^2 were stapled on a 9 cm diameter filter paper. These were immersed in sterile distilled water for 5 min. and then placed in the lid of a sterilised petridish with the leaf bits directly above 3 per cent water agar and incubated. The petri dishes were examined hourly for 24 h for released ascospores. The discharged ascospores were then picked up and streaked on Potato Dextrose Agar (PDA) in a petri dish.

3.2.2 Spore pick technique

Infected leaves of about 5 cm^2 were placed inside sterile petridishes lined with moist filter paper. After 48-72 h of incubation, the conidia from conidiophores were picked up from the lesions with a very thin needle dipped in sterile water. Conidia were then streaked on PDA in petri dishes. The plates were periodically checked for contamination and after two weeks of incubation, pure culture of the pathogen was transferred on to fresh media. This was done by

crushing a colony in 2-3 ml of sterile distilled water and streaking a loopful of the suspension on fresh PDA slants and petri dishes. Pure cultures were maintained on PDA slants and petri dishes incubated at 22 - 25°C under continous light.

3.3 Extraction of toxic metabolite and pathogenicity test

3.3.1 Extraction of toxic metabolite

Extraction of toxic metabolite was conducted as per the procedure of Natural (1989). Isolate of *M. musicola* was grown in PDA in a petridish for 30 days. The culture media and mycelia were cut in to small pieces and homogenized in a blender for 3 min. The homogenized mixture was allowed to stand in the refrigerator overnight and filtered through a Buchner funnel fitted with a filter paper to remove the agar and mycelial fragments. The homogenate was extracted with fresh cooled acetone until the extract became colourless. The solvent was allowed to evaporate under a fan at room temperature. The solvent free dry residue was dissolved in a small amount of acetone and used for pathogenicity test.

3.3.2 Pathogenicity test

Leaf puncture method (Sugawara *et al.* 1986) was used for pathogenicity test.

The concentrated culture extract was applied to the surface of banana leaf in a 5 µl droplet. The punctured leaf surface was sealed with parafilm in a petridish containing moistened filter paper and incubated for 72 h and observed for necrotic lesions.

3.4 Cultural and morphological characters of *M. musicola* Leach

The method suggested by Natural (1989) was followed for studying the cultural and morphological characters of *M. musicola* Leach.

3.4.1 Cultural characters of *M. musicola*

Isolate of *M. musicola* was grown on different agar media viz., potato dextrose agar (PDA), potato glucose yeast extract agar (PGYEA), synthetic medium plus glucose yeast extract agar (SmGYEA), Czapek's yeast extract agar (CzYEA), coconut glucose yeast extract agar (CGYEA), malt extract agar (MEA) and banana dextrose agar (BnDA). The media composition were given below.

Table 1. Chemical composition of media

Sl. No.	Media	Composition	(g/l)
1.	Potato dextrose agar (PDA)	Sliced potato	200
		Dextrose	20
		Agar	15
2.	Potato glucose yeast extract agar (PGYEA)	Sliced potato	200
		Glucose	20
		Yeast extract	2
		Agar	15
3.	Synthetic medium glucose yeast extract agar (SmGYEA)	$NaNO_3$	1
		K_2HPO_4	1.9
		$MgSO_4.7H_2O$	0.5
		KCl	0.5
		Glucose	20
		Yeast extract	1
		Agar	15
4.	Czapek's yeast extract agar (CzYEA)	$NaNO_3$	3
		K_2HPO_4	1
		$MgSO_4.7H_2O$	0.5
		KCl	0.5
		$FeSO_4.7H_2O$	0.01
		Sucrose	30
		Yeast extract	1
		Agar	15
5.	Coconut glucose yeast extract agar (CGYEA)	Coconut water	500 ml
		Glucose	20
		Yeast extract	2
		Agar	15
6.	Malt extract agar (MEA)	Malt extract	15
		Glucose	30
		Agar	15
7.	Banana dextrose agar (BnDA)	Banana leaf	200
		Dextrose	15
		Agar	20

3.4.2 Morphological characters of *M. musicola*

From the pure culture of *M. musicola* Leach, 0.4 mm disc of mycelium was cut and transferred to seven agar media and incubated for 30 days. Three replications were kept for each medium. Colony colour, colony diameter and pigments produced in media were observed and compared. Mycelial and conidial characteristics of the fungus were noted.

3.5 Anatomical and biochemical studies

3.5.1 Collection of sample

For anatomical and biochemical studies samples were collected from healthy leaves of plants grown at College of Horticulture, Vellanikkara and diseased leaves from germplasm collection of banana at Banana Research Station, Kannara. Leaves from seven-month-old plants were used for the study.

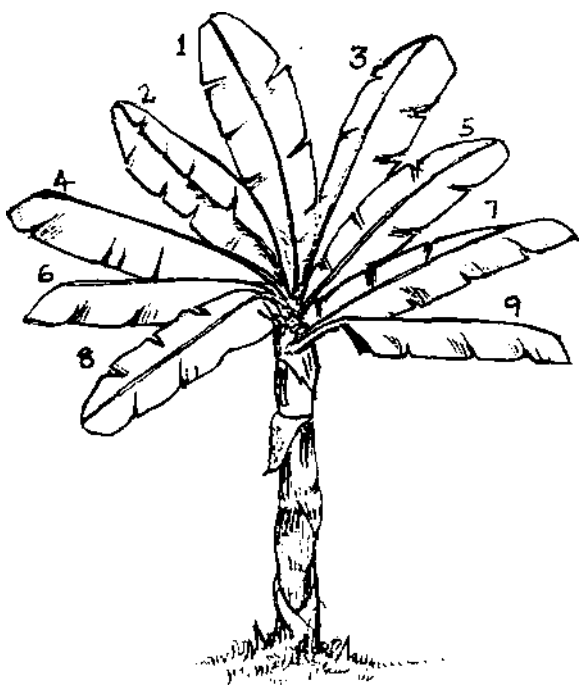
3.5.2 Anatomical studies

Anatomical studies were conducted as per the procedure of Vasquez *et al.* (1989).

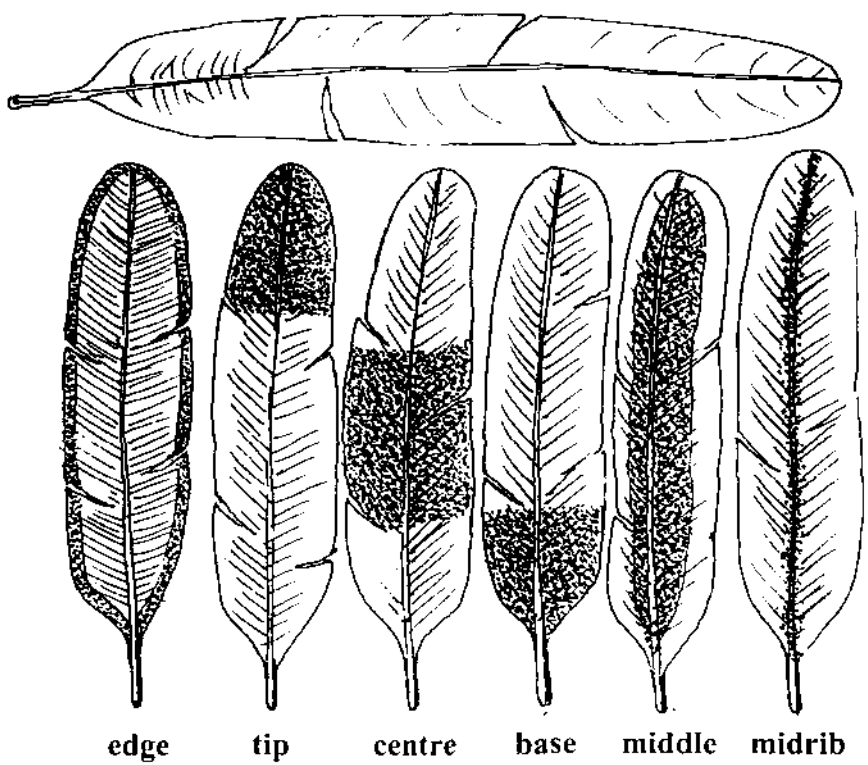
Healthy and diseased leaves from seven month old plants of four different groups of banana viz. resistant, moderately resistant, susceptible and highly susceptible to *M. musicola* Leach were used for anatomical studies using the procedure reported by Vasquez *et al.* (1989). From each variety, samples were taken from six portions of the third leaf lamina viz. the edge, the tip, the centre, the middle, the base and the midrib (Fig. 1). Observations on number of stomata/mm^2, stomatal opening during day and night hours and size of stomatal pore on the adaxial and abaxial surfaces, cuticle and epiderm thickness on the upper and lower epidermis, number and size of vascular bundle, thickness of spongy and palisade

Fig. 1. Leaf morphology studies

Seven month old plant



Leaf in position 3



Different portions of leaf lamina

tissues and epicuticular wax deposition were taken. Observations were taken under an ocular micrometer calibrated with stage micrometer for various parameters.

3.5.2.1 Number of stomata/mm²

The procedure reported by Rajeevan (1985) were followed for finding the number of stomata per mm². From each of the six portions, ten samples were taken at random for the preparation of surface replicates. The adaxial and abaxial surfaces of the healthy and diseased leaves were smeared with an adhesive, 'Quick fix' to facilitate easy removal of the peels and stained with safranin. Stomatal count was taken under a compound microscope with 40 X magnification. The stomatal index was indicated as number of stomata per/mm².

3.5.2.2 Stomatal opening during day and night hours

Healthy and diseased leaves from different groups of banana were collected during day (10 AM) and night hours (10 PM) from seven-month-old plants. Peels were taken using an adhesive 'Quick fix' and observed for stomatal opening.

3.5.2.3 Study of nature and arrangement of different layer of cells

Transverse hand sections were taken, stained with safranin and temporary slides were prepared and examined under compound microscope. Four different groups of banana were compared with regard to structural defence barriers and nature and arrangement of each layer of cells.

3.5.2.4 Epicuticular wax deposition

Procedure reported by Vasquez *et al.* (1989) was followed with a slight modification for the study on epicuticular wax deposition.

Replicate samples of leaves from four different varieties were fixed in FAA [formaldehyde (37%)], acetic acid; alcohol (95%), water (1.0-0.5-5.0-3.5%) for two days and then dehydrated in a series of ethyl alcohol solutions of ascending concentrations (50, 70, 80, 90, 95, 98 and 98 per cent) for 120 min. in each concentration. The samples were then dried in a Hitachi Critical point dryer, mounted with double-sided tape on aluminium stands, coated with gold for 2.5 min. under an acceleration current of 20 MA, and observed under a Hitachi S-530 scanning electron microscope (SEM).

3.5.3 Biochemical studies

Healthy and diseased leaves from seven month old plants of different groups of banana viz. resistant, moderately resistant, susceptible and highly susceptible were subjected to biochemical analysis for determining the total phenol, OD phenol, reducing and non reducing sugars, proline, enzymes (peroxidase and polyphenol oxidase), protein and toxic metabolite of *M. musicola* as per standard procedures. Three replications were maintained for all the analysis. Two types of sampling was adopted for the analysis.

For each group, first sample was taken from three parts of the lamina viz., the edge, the centre and the midrib of the leaf lamina considering the fact that disease spreads from edge of the leaf lamina to centre portion. Second type of sample was taken from the tip, the middle and the basal portions of leaf lamina assuming that disease starts from tip of leaf lamina and spreads to basal portion.

3.5.3.1 Estimation of total phenols and OD phenol

The samples from six parts of leaf lamina were separately cut in to small pieces and were crushed in distilled water and made up to 100 ml and the supernatant were used for analysis. The total phenol content was calculated with a

factor obtained from a standard curve prepared with catechol and was expressed as mg g^{-1} of sample.

$$\text{mg of phenols per g of sample} = \text{Factor} \times \frac{\text{Absorbance}}{\text{Volume made}} \times \frac{\text{Dilution (ml)}}{\text{Weight of sample (mg)}} \times \frac{1000}{1}$$

3.5.3.1.1 Total phenol

Total phenol was estimated by the method of Sadasivam and Manickam (1996). The intensity of blue colour developed was read at 650 nm in a spectrophotometer.

3.5.3.1.2 Ortho dihydric phenol

Arnow's method was followed for the estimation of ortho dihydric phenol (Mahadevan and Sridhar, 1986). The absorbance of the pink solution was read in a spectrophotometer at 515 nm.

3.5.3.2 Estimation of sugars

Reducing and non reducing sugars of the samples were determined as per the procedure of Mahadevan and Sridhar (1986). Dinitrosalicylic acid (DNS) reagent was used for estimation of sugars. Using glucose as a standard, values were expressed in mg g^{-1} of sample.

3.5.3.2.1 Reducing sugars

Two ml of methanol extracts of 200 mg samples were evaporated in a water bath for five min. Three ml of aliquot of the extract and 3 ml of DNS reagent were added to the test tube. The extract was heated in a boiling water bath, after

adding one ml of 40 per cent Rochelle salt and allowed to cool. The intensity of dark red colour was read at 575 nm in a spectrophotometer.

3.5.3.2.2 Non reducing sugar

One ml of alcohol extract of leaf sample was taken in a test tube and evaporated the content to dryness in a water bath. After adding one ml of distilled water and one ml of 1N H_2SO_4, it was hydrolysed for 30 min at 49°C. The tubes were then allowed to cool and added two drops of methyl red indicator to it. By adding 1N NaOH, the content was neutralized and the volume was made up to 10 ml. Five ml of the content was taken and by following DNS method with appropriate reagent blank as described for estimation of reducing sugar, the non reducing sugar present in the hydrolysate was estimated.

3.5.3.3 Estimation of proline

The procedure reported by Sadasivam and Manickam (1996) was followed for the estimation of proline in the sample.

Extracted 0.5 g sample in 10 ml of 3 per cent aqueous sulphosalicylic acid and filtered. Two ml of the filtrate, 2 ml of glacial acetic acid and 2 ml of acid Ninhydrin reagent were added in a test tube. The tubes were heated in a boiling water bath for 1 h and terminated the reaction by placing the tubes in ice bath. Added 4 ml toluene to the reaction mixture and separated the toluene layer. The intensity of red colour was read at 520 nm in a spectrophotometer. Pure proline was used as a standard in a similar way and a standard curve was prepared.

3.5.3.4 Estimation of enzyme activities

3.5.3.4.1 Estimation of peroxidase enzyme activity

Peroxidase activity was assayed by the method suggested by Sadasivam and Manickam (1996). Guaiacol was used as the substrate and the rate of

formation of guaiacol dehydrogenation product was assessed as a measure of the peroxidase activity.

$$\text{Guaiacol} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Oxidised guaiacol} + 2H_2O$$

a) Preperation of enzyme extract

Extracted one g tissue in three ml of 0.01 M phosphate buffer (pH 7.0) by grinding in chilled mortar and pestle. The homogenate was centrifuged at 18000 rpm for 15 min in a refrigerated centrifuge at 5°C. The supernatant liquid collected was used as the enzyme source.

b) Estimation of peroxidase activity

Spectrophotometer was set at 436 nm with 3.1 ml phosphate buffer. Guaiacol 50 µl (240 mg of guaiacol in 100 ml of water), enzyme extract (30 µl), hydrogen peroxide 30 µl (0.14 ml of 30 per cent hydrogen peroxide in 100 ml water) and phosphate buffer (3 ml) were taken in a cuvette and mixed well and spectrophotometer was set at zero. When the absorbance of the sample had increased by 0.05, started the stop watch and noted the time required in minutes (Δt) to increase the absorbance by 0.1.

Peroxidae enzyme activity on units/litre of extract

$$= \frac{3.18 \times 0.03 \times 1000}{6.39 \times 1 \times \Delta t \times 0.03} = \frac{498.65}{\Delta t}$$

3.5.3.4.2 Estimation of polyphenol oxidase enzyme activity

Polyphenol oxidase enzyme activity was assayed by the method suggested by Malick and Singh (1980).

$$\text{Catechol} \xrightarrow[\text{Oxidase}]{\text{Catechol}} \text{Quinone}$$

Buffer solutions used for the extraction and assay were 0.1 M monobasic and dibasic sodium phosphate (pH 6.0).

Catechol 0.01 M dissolved in 100 ml of phosphate buffer (pH 6.0) was used as the substrate.

a) Preparation of enzyme extract

Enzyme extract was prepared by grinding 1g tissue in 3 ml of phosphate buffer in a mortar and pestle. Resulting supernatant liquid was used for the enzyme assay.

b) Estimation of polyphenol oxidase activity

Three ml of buffered catechol and 0.5 ml phosphate buffer (pH 6.0) were taken in a cuvette and the absorbance was adjusted to zero in spectrophotometer at 495 nm. Three ml of phosphate buffer and 0.5 ml enzyme extract was added to the cuvette and the absorbance was noted. Then 0.5 ml of enzyme extract and 3 ml of buffered catechol were added to the cuvette, mixed immediately and noted the changes in absorbance for every 30 sec upto 5 min. The changes in aborbance values per minute were plotted and linear phase of curve was drawn on the basis of OD value.

3.5.3.5 Estimation of protein

Lowry's method was followed for the estimation of protein (Lowry *et al.*, 1951).

The extract prepared for the peroxidase enzyme assay was used for the estimation of protein. 0.1 ml of extract was taken and made up to 1 ml with distilled water. Then added 5 ml of reagent (alkaline copper solution). After 10 min., 0.5 ml of Folin-Ciocalteau reagent was added and mixed well. Then kept in the dark for 30 min. Blue colour developed and the solution was read at 660 nm in a spectrophotometer. A standard graph prepared with bovine serum albumin (BSA) was used for calculating the amount of protein in the sample.

3.5.3.6 Toxic metabolite of *M. musicola*

Extraction, thin layer chromatography and bioassay of toxic metabolite were done by the method of Natural (1989).

3.5.3.6.1 Extraction of toxic metabolite

The method followed for extraction of toxic metabolite is described in 3.3.1. The pathogen *M. musicola* was grown in seven media viz. PDA, PGYEA, SmGYEA, CzYEA, CGYEA, MEA and BnDA.

3.5.3.6.2 Thin layer chromatography (TLC)

The solvent free concentrated acetone extracts of *M. musicola* grown on different media were applied to pre-coated TLC plates (20 cm x 20 cm x 0.25 mm) by means of a microlitre syringe at 20 µl per sample per spot. The plates were developed using ethyl acetate:methanol (1:1 v/v) solvent. Thin layer

chromatograms were observed under UV light at a wavelength of 366 nm and Rf values for the bands were calculated using the formula

$$Rf = \frac{\text{distance travelled by the spot}}{\text{distance travelled by the solvent}}$$

3.5.3.6.3 Bioassay

The solvent free concentrated extracts of *M. musicola* grown on PGYEA were tested on tissue cultured Nendran plants. Samples of 20 µl were injected into the petioles of the leaves of three months old tissue cultured banana. These plantlets were incubated at 22-25°C under continuous light and observed for the development of necrotic lesions on leaves.

3.5.4 Statistical analysis

The statistical analysis of data was done as a Completely Randomised Design (CRD) using MSTAT C (Freed, 1986) package available at the Central Computer Facility, Department of Agricultural Statistics, College of Horticulture, Vellanikkara.

Results

RESULTS

The results of the study on the anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease are presented in this chapter.

4.1 Seasonal influence on disease incidence

Incidence of sigatoka leaf spot disease was recorded at three months interval during July 2000, October 2000, January 2001 and April 2001. Maximum disease incidence was recorded during July 2000 followed by October 2000, April 2001 and January 2001 (Table 1a).

Relationship of weather parameters and disease incidence was analysed. Maximum and minimum temperature were seen negatively correlated with disease incidence and positively correlated with relative humidity (morning and evening) and rainfall (Table 1b and Fig. 2).

4.2 Isolation and maintenance of the pathogen

Pathogen was isolated both by the ascospore isolation and spore pick technique. The culture was maintained on PDA at 22-25°C under continuous light. Koch's postulates were established in susceptible banana variety Nendran.

4.3 Cultural and morphological characters of *M. musicola*

4.3.1 Cultural characters of *M. musicola*

Growth of *M. musicola* was studied in different media at different intervals to determine the best media for culturing the pathogen.

Maximum growth of *M. musicola* was observed in the media PGYEA (mean 1.983 cm) followed by CzYEA (1.893 cm), CGYEA, BnDA and PDA

Table 1(a). Infection index and meteorological data at three months

Period	Maximum temperature (Mean)	Minimum temperature (Mean)	Relative humidity (morning) (%)	Relative humidity (evening) (%)	Rainfall (mm) (Mean)	Infection index (%)
July 2000	28.8	21.9	93	70	354.0	48.1
October 2000	30.7	22.7	91	68	262.2	34.2
January 2001	32.6	23.2	71	41	0.0	21.38
April 2001	34.2	24.7	88	63	243.1	26.76

Table 1(b). Relationship of weather parameters with yellow sigatoka leaf spot disease of banana

Sl.No.	Weather parameters	Correlation coefficient (r)
1	Maximum temperature	-0.738**
2	Minimum temperature	-0.591**
3	Relative humidity (morning)	0.719**
4	Relative humidity (evening)	0.719**
5	Rainfall	0.777**

** Significant at 1% level

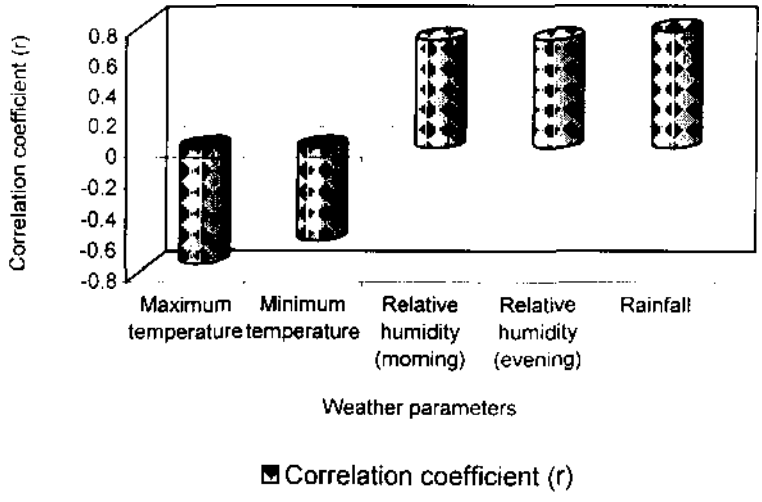


Fig. 2. Relationship of weather parameters with yellow sigatoka leaf spot disease of banana

during a period of five weeks (Table 2 and Plate 2c). The growth of *M. musicola* in the media PGYEA and CzYEA were on par. The growth of *M. musicola* in the media CGYEA and BnDA were also on par. Growth in media, BnDA and PDA were significantly different. Growth of *M. musicola* was less in MEA and SmGYEA

Conidia were produced sparsely on BnDA after seven- ten days incubation at 22-25°C under continuous light. Sporulation was not obtained on the other media tried. Red pigmentation was observed in all the media tried. As colonies aged, pigmentation disappeared.

4.3.2 Morphological characters of *M. musicola*

Ascospores were hyaline, two celled with one cell bigger than the other and with a slight constriction at the septum. The size of ascospores ranged from 1-18 µm x 3.6-4.2 µm. Germination of ascospores took place 24 h after discharge and was bipolar. Germ tubes were straight to slightly curved.

Conidia were olivaceous brown, straight to variously curved, cylindric with a round base, smooth walled, unthickened hilum with dimensions of 40-65 µm x 1.9-3 µm (Plate 2d).

Colonies grown from ascospore discharge and spore pick technique became visible 5-7 days after incubation on PDA at 22-25°C under continuous light. Aerial mycelia were observed initially white later turning olivaceous gray. Colonies were compact, hemispherical, raised with irregular zonation, a non-circular margin and a velvet surface. The central portion became darker than the periphery, which later turned olivaceous gray with maturity. Hyphal cells were dump-bell shaped. Ascospores and conidial characters confirmed with the descriptions of *M. musicola* as described by Natural (1989).

Table 2. Growth of *M. musicola* on different media at five weeks interval

Media	Ist Week (cm)	IInd week (cm)	IIIrd week (cm)	IVth week (cm)	Vth week (cm)	Mean (cm)
PDA	0.533	1.100	1.467	1.850	2.133	1.417
PGYEA	1.000	1.433	2.300	2.550	2.633	1.283
SmGYEA	0.233	0.767	0.967	1.100	1.367	0.887
CzYEA	0.850	1.400	1.800	2.450	2.967	1.893
CGYEA	0.667	1.267	1.700	2.333	2.867	1.763
BnDA	0.933	1.100	1.933	2.200	2.633	1.793
MEA	0.267	1.000	1.200	1.400	1.633	1.100
Mean	0.640	1.152	1.624	1.983	2.319	

CD for comparing media = 0.1515
CD for comparing weeks = 0.086
CD for comparing Media x Weeks = 0.229

4.4 Anatomical studies

The study of anatomical features was carried out to determine the nature of resistance mechanism in four different groups of banana.

4.4.1 Leaf anatomy of healthy leaves of *Musa* spp.

The different anatomical parameters observed are presented below.

4.4.1.1 Upper epidermis

The upper epidermis of leaf consists of an outer layer of cuticle, stomata and epidermal cells. The four different groups of banana exhibited slight variations in anatomical characters of upper epidermis.

4.4.1.1.1 Cuticle

The cuticle thickness showed some variations in the four different groups of banana observed. Thickest cuticle was observed in the resistant variety Manoranjitham (AAA) (Table 3 and Plate 3) as compared to the susceptible variety Grand Naine (AAA).

4.4.1.1.2 Epidermis

The size of the epidermal cells was small, ovoidal to slightly elongated in shape. Hypodermis was seen beneath the upper epidermis and comprising of laterally elongated cells and irregularly arranged few cells.

Transverse section of leaf of the resistant variety Manoranjitham (AAA) showed well defined epidermal cells on the upper epidermis. In the moderately resistant variety Thiruvananthapuram (AAB) (Table 4), the epidermal and hypodermal layers composed of closely arranged cells. Similar observations were

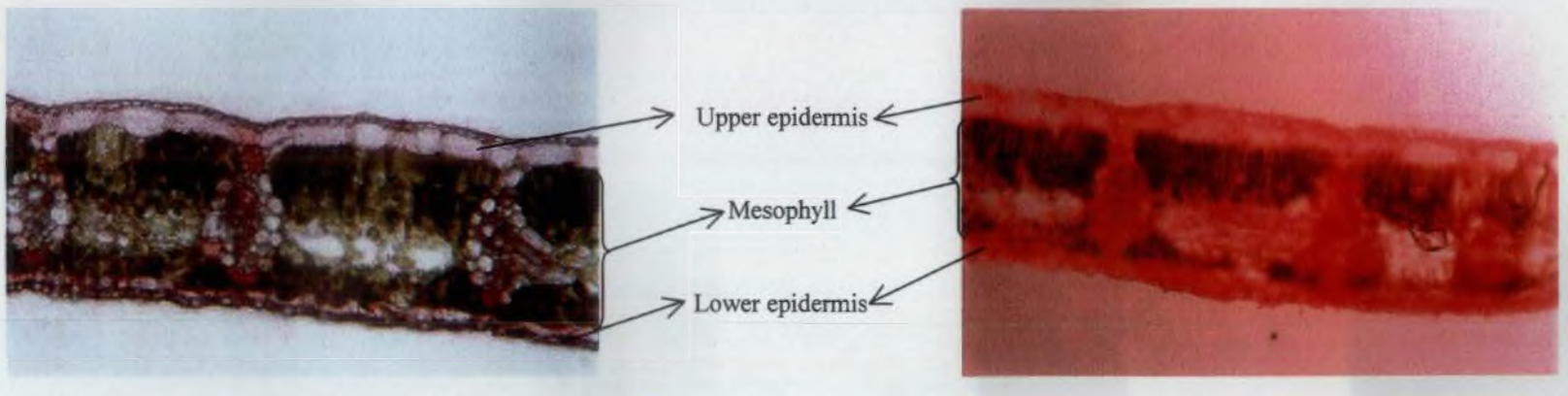
Table 3. Histo-pathological studies in different portions of leaf in resistant variety - Manoranjitham (AAA) [a]

Parameters		Units	Portions of leaf											
			Healthy						Diseased					
			E	C	T	M	B	Mean	E	C	T	M	B	Mean
I	**Upper epidermis**													
◗	Cuticle thickness	µm	4.50	4.60	4.50	4.60	4.50	4.54	4.60	4.80	4.50	4.60	4.50	4.57
◗	Epidermis thickness	µm	30.00	30.00	30.00	30.00	45.00	33.00	45.00	30.00	45.00	30.00	45.00	39.00
◗	Epidermal cell size	μm^2	60.00	53.00	60.00	60.00	60.00	58.60	60.00	60.00	60.00	60.00	60.00	60.00
II	**Mesophyll**													
●	No. of vascular bundles	Number	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	6.00	5.00	5.20
●	Vascular bundle size	μm^2	2025.00	2700.00	2250.00	2551.00	2310.00	2367.20	2025.00	1800.00	1800.00	2160.00	1800.00	1917.00
●	Distance between bundles	µm	150.00	150.00	150.00	180.00	195.00	165 .00	150.00	150.00	150.00	150.00	165.00	153.00
●	Spongy tissue thickness	µm	75.00	90.00	90.00	75.00	60.00	78.00	135.00	90.00	120.00	120.00	105.00	114.00
●	Palisade tissue thickness	µm	105.00	75.00	105.00	75.00	120.00	96.00	90.00	75.00	70.00	75.00	90.00	80.00
III	**Lower epidermis**													
◆	Lower epidermis thickness	µm	18.00	15.00	15.00	15.00	15.00	15.60	18.00	15.00	15.00	15.00	15.00	15.60
◆	Lower epidermal cell size	μm^2	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
◆	Cuticle thickness	µm	4.14	4.14	4.14	4.14	4.13	4.14	4.12	4.13	4.14	4.14	4.13	4.13

E - Edge, C - Centre, T - Tip, M - Middle, B - Base

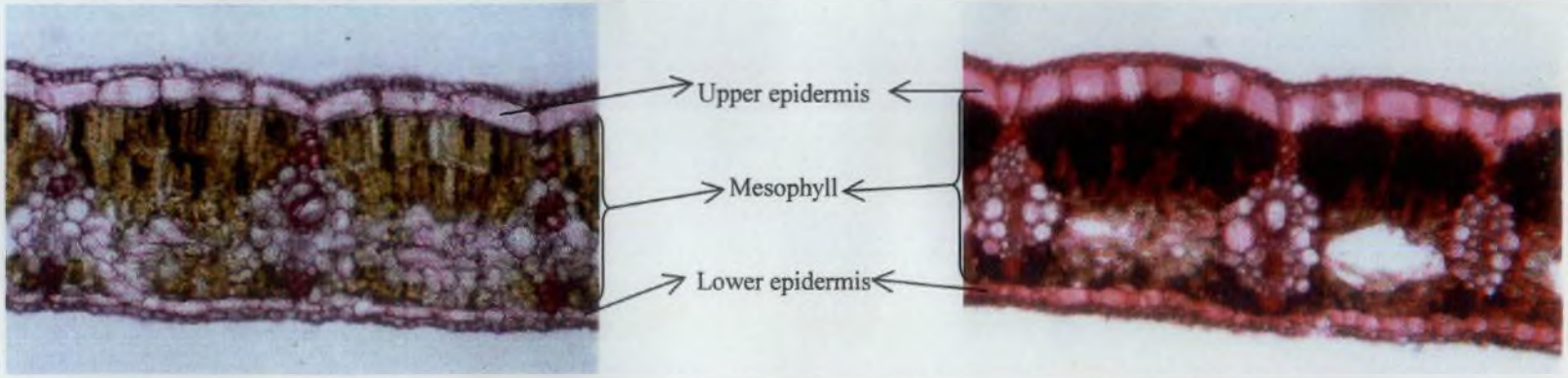
a - Average of 10 samples per leaf

Plate 3. Leaf anatomy of *Musa* spp. showing upper and lower epiderm and mesophyll (× 100)



Manoranjitham (AAA)

Thiruvananthapuram (AAB)



Nendran (AAB)

Grand Naine (AAA)

Table 4. Histo-pathological studies in different portions of leaf in moderately resistant variety - Thiruvananthapuram (AAB) [a]

Parameters		Units	Portions of leaf											
			Healthy						Diseased					
			E	C	T	M	B	Mean	E	C	T	M	B	Mean
I	**Upper epidermis**													
◗	Cuticle thickness	µm	4.50	4.58	4.50	4.51	4.50	4.52	4.50	4.59	4.50	4.55	4.50	4.53
◗	Epidermis thickness	µm	30.00	30.00	30.00	30.00	22.50	28.50	30.00	30.00	30.00	30.00	45.00	33.00
◗	Epidermal cell size	μm^2	45.00	45.00	45.00	60.00	45.00	48.00	75.00	48.75	60.00	60.00	45.00	57.75
II	**Mesophyll**													
●	No. of vascular bundles	Number	5.00	5.00	5.00	5.00	4.00	4.80	5.00	5.00	5.00	5.00	5.00	5.00
●	Vascular bundle size	μm^2	1950.00	2400.00	1800.00	2520.00	1800.00	2094.00	1125.00	2100.00	1350.00	2880.00	1575.00	1806. 00
●	Distance between bundles	µm	153.80	178.00	225.00	178.00	177.00	182.36	195.00	135.00	195.00	165.00	195.00	177.00
●	Spongy tissue thickness	µm	75.00	90.00	89.00	155.00	84.99	98.80	105.00	170.00	120.00	125.00	105.00	125.00
●	Palisade tissue thickness	µm	60.00	150.00	75.00	120.00	84.99	98.00	90.00	135.00	90.00	120.00	90.00	105.00
III	**Lower epidermis**													
◆	Lower epidermis thickness	µm	18.00	15.00	15.00	15.00	15.00	15.60	18.00	15.00	15.00	15.00	15.00	15.60
◆	Lower epidermal cell size	μm^2	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
◆	Cuticle thickness	µm	4.10	4.20	4.10	4.20	4.12	4.10	4.10	4.13	4.10	4.12	4.10	4.11

E - Edge, C - Centre, T - Tip, M - Middle, B - Base

a - Average of 10 samples per leaf

recorded in the case of susceptible variety Nendran (AAB) and highly susceptible variety Grand Naine (AAA).

The thickness of upper epidermis also showed variations in different varieties studied. Epidermal thickness was observed more in resistant variety Manoranjitham (AAA) and less in Nendran (AAB) (Table 5).

The size of the epidermal cells was found to be maximum in the highly susceptible variety Grand Naine (AAA) when compared to other varieties and minimum in moderately resistant variety Thiruvananthapuram (AAB) (Table 6).

4.4.1.1.3 Stomata

Stomatal index and number of stomata/microscopic field in the adaxial surface of the leaves were observed more in the highly susceptible variety Grand Naine (AAA) and less in the resistant variety Manoranjitham (AAA) (Table 7).

Size of stomata was observed maximum in the highly susceptible variety Grand Naine (AAA) and minimum in the resistant variety Manoranjitham (AAA).

No marked variation was seen in the stomatal pore width in different portions of the leaves of different varieties studied.

4.4.1.2 Mesophyll

Mesophyll region consisted of vascular bundles, spongy tissues and palisade tissues. In the resistant variety Manoranjitham (AAA), the palisade, spongy and lower mesophyll cells were seen with abundant chloroplasts. Moreover, vascular bundles were found well oriented with large xylem vessels and phloem cells. But in the moderately resistant variety Thriuvananthapuram (AAB)

Table 5. Histo-pathological studies in different portions of leaf in susceptible variety - Nendran (AAB) [a]

Parameters		Units	Portions of leaf											
			Healthy						Diseased					
			E	C	T	M	B	Mean	E	C	T	M	B	Mean
I	**Upper epidermis**													
◗	Cuticle thickness	µm	4.50	4.56	4.50	4.50	4.50	4.50	4.57	4.50	4.50	4.50	4.50	4.50
◗	Epidermis thickness	µm	18.75	30.00	23.74	30.00	30.00	26.50	30.00	24.00	22.50	24.00	39.38	27.98
◗	Epidermal cell size	μm^2	42.75	67.50	49.99	67.50	60.00	57.55	48.75	75.00	60.00	75.00	60.00	63.75
II	**Mesophyll**													
●	No. of vascular bundles	Number	5.00	5.00	4.00	5.00	4.00	4.60	5.00	5.00	4.00	6.00	4.00	4.80
●	Vascular bundle size	μm^2	1260.00	1440.00	1350.00	1800.00	1181.25	1406.00	1026.60	1800.00	1125.00	1800.00	1350.00	1420.32
●	Distance between bundles	µm	255.00	138.80	240.00	135.00	221.30	198.02	156.30	204.00	195.00	202.50	270.00	205.56
●	Spongy tissue thickness	µm	90.00	63.00	70.00	64.50	73.50	72.20	63.75	90.00	87.00	88.50	75.00	80.85
●	Palisade tissue thickness	µm	105.00	105.00	120.00	105.00	90.00	105.00	127.05	97.50	94.50	96.00	82.50	99.60
III	**Lower epidermis**													
◆	Lower epidermis thickness	µm	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
◆	Lower epidermal cell size	μm^2	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
◆	Cuticle thickness	µm	4.00	4.00	4.10	4.10	4.00	4.04	4.10	4.00	4.00	4.10	4.10	4.06

E - Edge, C - Centre, T - Tip, M - Middle, B - Base

a - Average of 10 samples per leaf

Table 6. Histo-pathological studies in different portions of leaf in highly susceptible variety - Grand Naine (AAA) [a]

Parameters		Units	Portions of leaf											
			Healthy						Diseased					
			E	C	T	M	B	Mean	E	C	T	M	B	Mean
I	**Upper epidermis**													
◗	Cuticle thickness	µm	4.20	4.15	4.18	4.20	4.13	4.17	4.15	4.20	4.18	4.20	4.20	4.19
◗	Epidermis thickness	µm	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
◗	Epidermal cell size	μm^2	75.00	75.00	75.00	45.00	60.00	66.00	63.75	60.00	60.00	45.00	60.00	57.75
II	**Mesophyll**													
●	No. of vascular bundles	Number	4.00	5.00	4.00	5.00	4.00	4.40	5.00	5.00	5.00	5.00	4.00	5.00
●	Vascular bundle size	μm^2	1800.00	2100.00	1575.00	2851.00	1350.00	1935.24	1800.00	2100.00	1575.00	1520.00	1575.00	1714.00
●	Distance between bundles	µm	255.00	195.00	232.50	225.00	195.00	220.50	150.00	195.00	165.00	180.00	210.00	180.00
●	Spongy tissue thickness	µm	75.00	90.00	90.00	75.00	105.00	87.00	120.00	120.00	120.00	120.00	165.00	127.00
●	Palisade tissue thickness	µm	150.00	135.00	150.00	135.00	105.00	135.00	120.00	120.00	120.00	120.00	150.00	126.00
III	**Lower epidermis**													
◆	Lower epidermis thickness	µm	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
◆	Lower epidermal cell size	μm^2	21.26	21.00	20.63	21.00	21.00	21.00	35.63	37.50	36.00	36.00	36.26	36.28
◆	Cuticle thickness	µm	3.75	3.80	3.75	3.75	3.75	3.76	3.75	3.80	3.75	3.75	3.75	3.76

E - Edge, C - Centre, T - Tip, M - Middle, B - Base

a - Average of 10 samples per leaf

Table 7. Amphistomatic descriptions on leaves of *Musa* spp. [a]

Musa spp.	Parameters	Units	Surface of leaves	Portions of leaf											
				Healthy						Diseased					
				E	C	T	M	B	Mean	E	C	T	M	B	Mean
Manoranjitham (AAA)	Stomata/ microscopic field	Number	Ad	37.50	37.50	37.50	50.00	50.00	42.50	37.50	50.00	50.00	50.00	50.00	47.50
			Ab	162.50	150.00	150.00	150.00	138.00	150.10	137.50	150.00	150.00	150.00	150.00	147.50
	Stomatal index/sq.mm	Number	Ad	4.00	3.00	3.00	4.00	4.00	3.60	3.00	4.00	4.00	4.00	4.00	3.80
			Ab	13.00	12.00	12.00	12.00	11.00	12.00	11.00	12.00	12.00	12.00	12.00	11.40
	Stomatal size	μm^2	Ad	450.00	450.00	337.50	337.50	450.00	405.00	379.60	337.50	450.00	450.00	337.50	390.92
			Ab	295.30	295.30	295.30	295.30	295.30	295.30	337.50	337.50	393.80	337.50	295.30	340.32
	Stomatal pore width	µm	Ad	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
			Ab	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Thiruvananthapuram (AAB)	Stomata/ microscopic field	Number	Ad	50.00	50.00	50.00	50.00	37.50	47.50	50.00	50.00	50.00	50.00	37.50	47.50
			Ab	175.00	187.50	187.50	187.50	125.00	173.00	187.50	187.50	187.50	187.50	137.50	177.50
	Stomatal index/sq.mm	Number	Ad	4.00	4.00	4.00	4.00	3.00	3.80	4.00	4.00	4.00	4.00	3.00	3.80
			Ab	14.00	15.00	15.00	15.00	11.00	14.00	15.00	15.00	15.00	15.00	11.00	14.20
	Stomatal size	μm^2	Ad	450	450	337.5	450	450	427.50	450	450	450	450	450	450.00
			Ab	295.30	450.00	360.00	450.00	337.50	389.56	295.30	295.30	295.30	295.30	295.30	295.30
	Stomatal pore width	µm	Ad	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
			Ab	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94

E - Edge, C - Centre, T - Tip, M - Middle, B - Base Ad - Adaxial Ab - Abaxial

a - Average of 10 samples per leaf

Contd.

Table 7. Continued [a]

Musa spp.	Parameters	Units	Surface of leaves	Portions of leaf											
				Healthy						Diseased					
				E	C	T	M	B	Mean	E	C	T	M	B	Mean
Nendran (AAB)	Stomata/ microscopic field	Number	Ad	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
			Ab	188.00	213.00	213.00	188.00	175.00	195.00	263.00	213.00	213.00	213.00	188.00	218.00
	Stomatal index/sq.mm	Number	Ad	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
			Ab	15.00	17.00	17.00	15.00	14.00	16.00	19.00	17.00	17.00	18.00	15.00	16.00
	Stomatal size	μm^2	Ad	450.00	393.80	450.00	393.80	393.80	416.28	450.00	393.80	450.00	393.80	450.00	427.52
			Ab	393.80	393.80	295.30	393.80	293.30	354.00	393.80	293.30	295.30	295.30	295.30	314.60
	Stomatal pore width	µm	Ad	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
			Ab	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Grand Naine (AAA)	Stomata/ microscopic field	Number	Ad	62.50	62.50	50.00	50.00	50.00	55.00	62.50	75.00	62.50	62.50	50.00	62.50
			Ab	200.00	200.00	200.00	200.00	200.00	200.00	200.00	213.00	213.00	213.00	213.00	210.00
	Stomatal index/sq.mm	Number	Ad	5.00	5.00	4.00	4.00	4.00	4.40	5.00	6.00	5.00	5.00	4.00	5.00
			Ab	16.00	16.00	16.00	16.00	16.00	16.00	16.00	17.00	17.00	17.00	17.00	16.80
	Stomatal size	μm^2	Ad	450.00	450.00	393.80	450.00	450.00	438.76	50.00	450.00	450.00	450.00	450.00	450.00
			Ab	295.30	393.80	393.80	315.00	295.30	338.64	393.80	295.30	450.00	450.00	295.30	376.88
	Stomatal pore width	µm	Ad	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
			Ab	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94

E - Edge, C - Centre, T - Tip, M - Middle, B - Base Ad - Adaxial Ab - Abaxial

a - Average of 10 samples per leaf

phloem cells. But in the moderately resistant variety Thriuvananthapuram (AAB) the palisade cells consisted of well oriented chloroplasts. Inter cellular spaces were comparatively less in the spongy mesophyll tissues. Large proportion of chloroplasts in mesophyll cells and larger vascular bundles were present in the healthy leaves of the susceptible variety Nendran (AAB). Similar observations were also recorded in the highly susceptible variety Grand Naine (AAA).

4.4.1.2.1 Vascular bundle

More number of vascular bundles were seen in the resistant variety Manoranjitham (AAA) as compared to highly susceptible variety Grand Naine (AAA). Large size of vascular bundle was observed in the resistant variety Manoranjitham (AAA) when compared to other varieties and smaller size in the susceptible variety Nendran (AAB).

Vascular bundles were seen distantly placed in the highly susceptible variety Grand Naine (AAA) and closely spaced in the resistant variety Manoranjitham (AAA).

Thickness of the spongy tissues were found more in the moderately resistant variety Thiruvananthapuram (AAB) as compared to the other varieties. Thicker palisade tissues were observed in the highly susceptible variety Grand Naine (AAA).

4.4.1.3 Lower epidermis

The lower epidermis of the leaf consists of a single layer of lower epidermal cell, stomata and an outer layer of waxy cuticle.

The lower epidermis consisted of more number of stomata compared to

the upper epidermis. The lower epidermis was much thinner compared to the upper epidermis. Cuticle thickness was also less in the lower epidermis.

The thickness of lower epidermis was high in the resistant variety Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB) as compared to susceptible and highly susceptible varieties viz., Nendran (AAB) and Grand Naine (AAA) respectively.

The susceptible variety Grand Naine (AAA) had the maximum cell size for the lower epidermis compared to other varieties. Cuticle was thicker in resistant variety Manoranjitham (AAA) and thinner in Grand Naine (AAA).

In the lower epidermis, stomata were seen abundant in the highly susceptible variety Grand Naine (AAA) as compared to the resistant variety Manoranjitham (AAA) (Plate 4). Stomatal size was observed maximum in the moderately resistant variety Thiruvananthapuram (AAB) followed by susceptible variety Nendran (AAB) and the size was observed minimum in the case of resistant variety Manoranjitham (AAA). Stomatal pore width remains the same in all the varieties studied.

4.4.1.4 Stomatal opening

Observations on stomatal opening revealed that stomata were found opened during day time and closed during night hours both in the case of healthy and diseased leaves of all the *Musa* spp. under investigation.

4.4.1.5 Epicuticular wax deposition

Epicuticular wax deposition on the abaxial surface of leaves were observed with an Hitachi S-530 scanning electron microscope. Patterns of epicuticular wax differed between varieties (Plate 5). The highly resistant and

Plate 4. Stomata on the abaxial surface of leaves of *Musa* spp. (× 400)

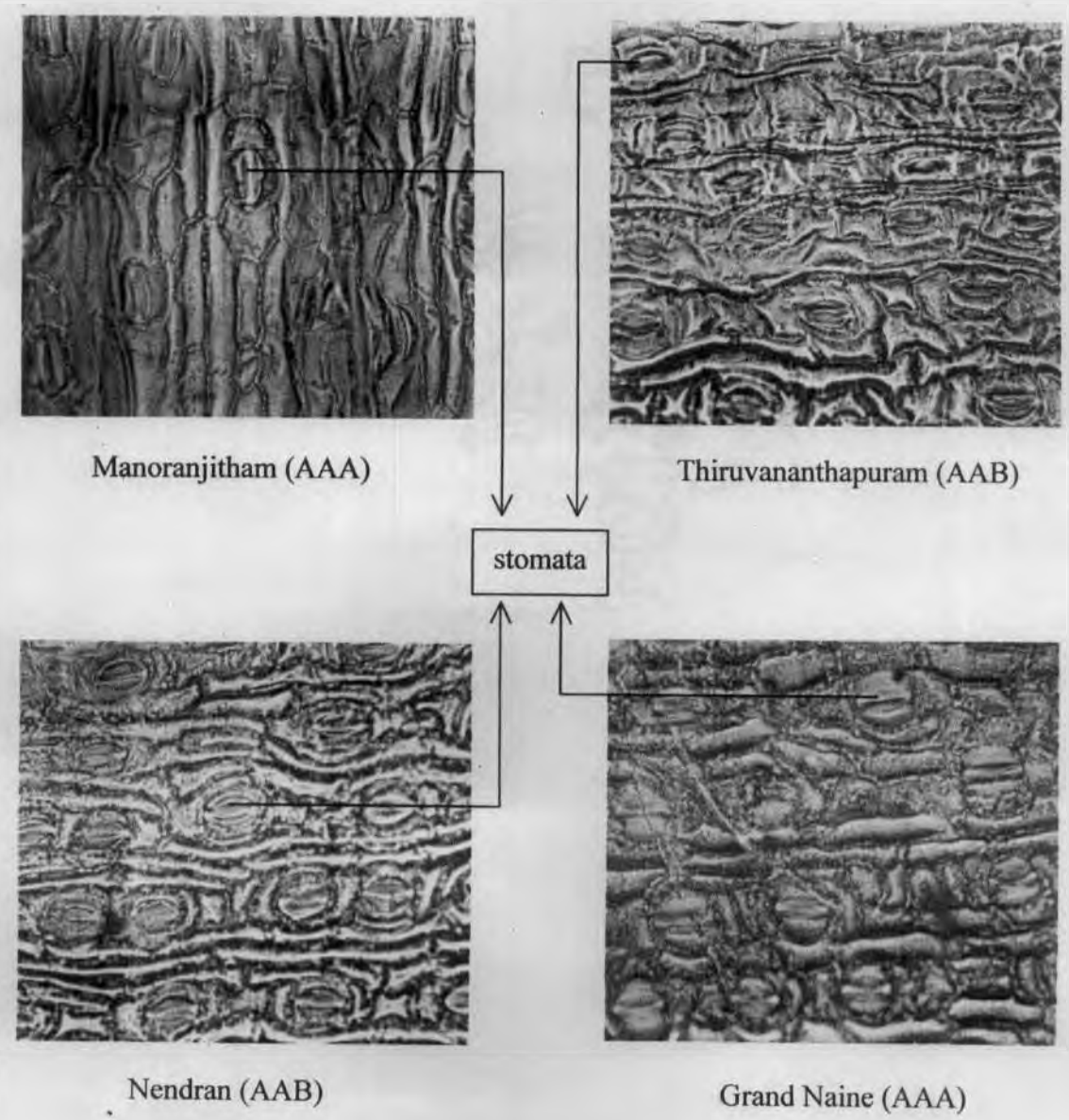
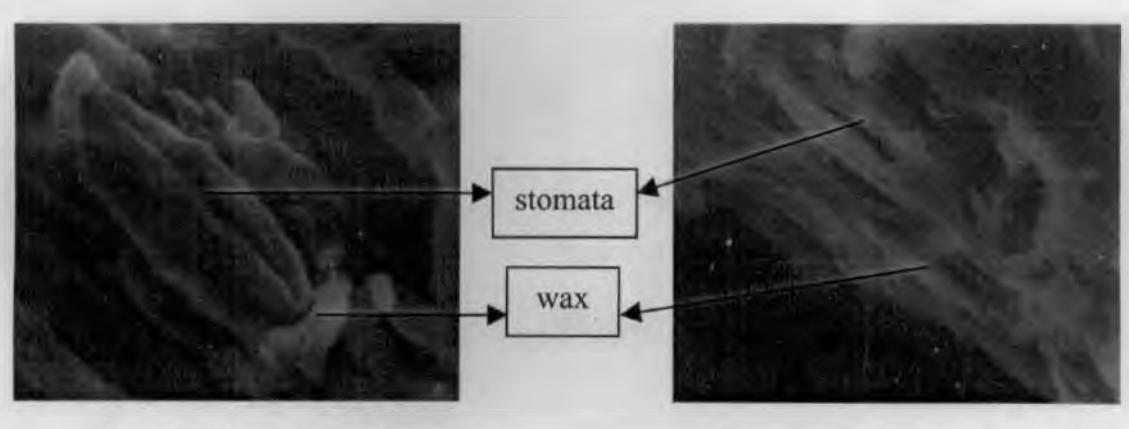
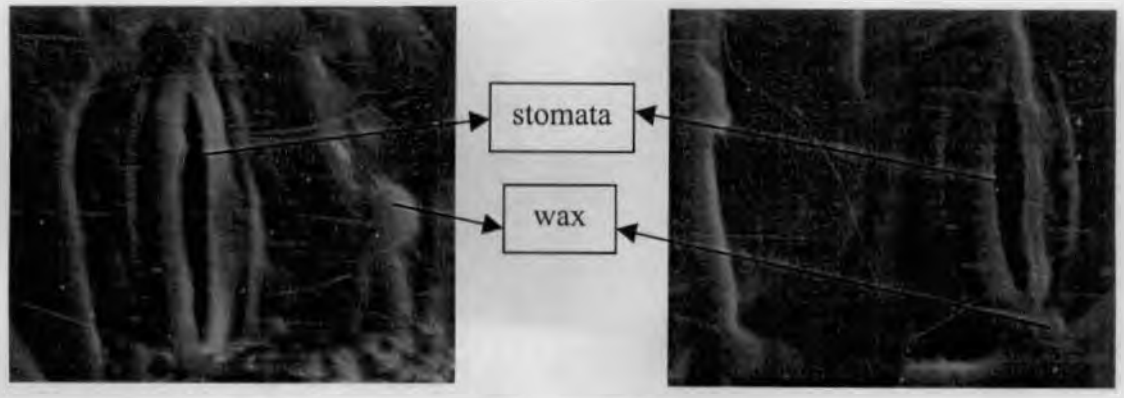


Plate 5. SEM microphotographs of abaxial surface of leaves of *Musa* spp. showing wax deposition and stomata (× 1500)



Manoranjitham (AAA) Thiruvananthapuram (AAB)



Nendran (AAB) Grand Naine (AAA)

moderately resistant varieties like Manoranjitham (AAA) and Thiruvananthapuram (AAB) showed thicker patterns of wax deposition while, thinner patterns of wax deposition were seen on the susceptible and highly susceptible varieties like Nendran (AAB) and Grand Naine (AAA).

4.4.2 Comparison of anatomical parameters of the healthy and yellow sigatoka infected leaves of *Musa* spp.

4.4.2.1 Upper epidermis

In the upper epidermis, no significant structural difference was noticed due to infection with the pathogen in all the banana varieties studied.

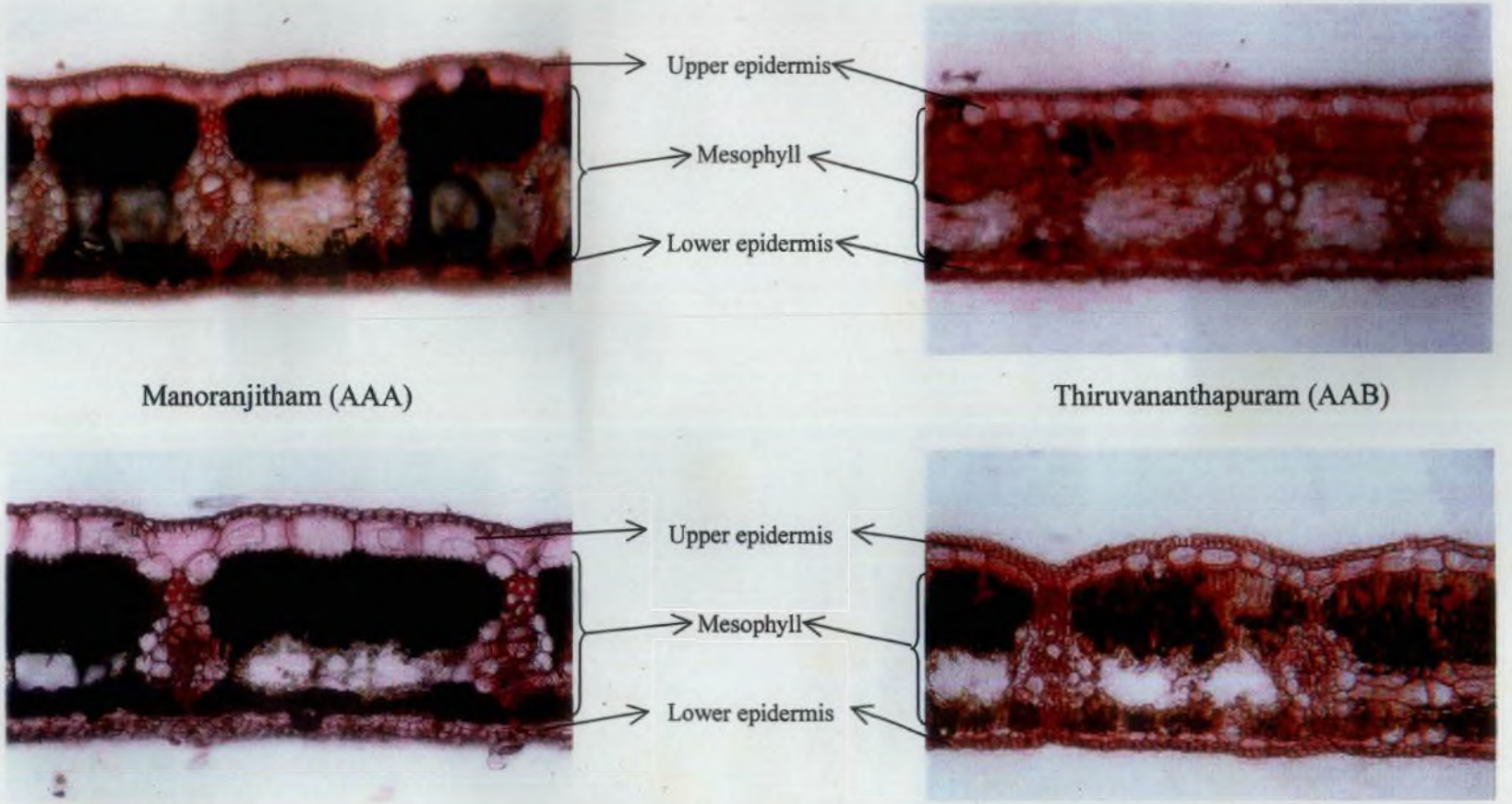
4.4.2.2 Mesophyll

Deeply stained cells of palisade mesophyll and lower mesophyll indicated less degree of necrosis of these tissues in the variety Manoranjitham (AAA). The vascular elements however appear quite healthy with large xylem vessels and turgid phloem cells. Large air spaces were seen in the spongy mesophyll which accounts for cellular deformity in this region (Plate 6).

In the variety Thiruvananthapuram (AAB), palisade mesophyll showed deep brownish colour due to necrosis of cells. Partial disintegration of cell walls and chloroplasts were noticed. Large air spaces were seen in the spongy mesophyll cells depicting disintegration. Partial necrosis of xylem vessels as well as phloem tissue were observed.

Majority of the mesophyll cells viz., palisade, spongy as well as lower mesophyll appeared brownish as against dark greenish chloroplasts of the healthy leaves of the variety Nendran (AAB). Complete disintegration of chloroplasts and cell walls of mesophyll cells were noticed. Necrotic, comparatively smaller vascular bundles were also seen in the infected leaf tissues.

Plate 6. Leaf anatomy of *Musa* spp. showing upper and lower epiderm and mesophyll infected with yellow sigatoka leaf spot (× 100)



Manoranjitham (AAA)

Thiruvananthapuram (AAB)

Nendran (AAB)

Grand Naine (AAA)

The infected leaves of the variety Grand Naine (AAA) showed ruptured areas in palisade and spongy mesophyll cells. The cell walls were indistinguishable and the chloroplasts appeared diffused in the palisade mesophyll cells. Structural deformity was also evident in the vascular bundles.

4.5 Biochemical studies

4.5.1 Biochemical characteristics of healthy leaves of *Musa* spp.

Biochemical studies of four different groups of banana viz., resistant, moderately resistant, susceptible and highly susceptible were carried out and were described in this part.

4.5.1.1 Estimation of total and OD phenols in healthy leaves of *Musa* spp.

4.5.1.1.1 Total phenol

Analysis of variance showed significant difference for total phenol content in the four different groups of banana studied (Table 8 and Fig.3). The resistant variety Manoranjitham (AAA) possessed higher content of total phenol which was 30 per cent higher than that of the moderately resistant variety Thiruvananthapuram (AAB).

The different portions of leaf analysed also showed significant variation in the content of total phenols. The phenol content in edge portion of the leaf was higher than the other portions studied. Comparing the varieties and portions of leaf, tip portion of the resistant variety Manoranjitham (AAA) showed maximum phenol content and minimum in the midrib portion of Nendran (AAB).

4.5.1.1.2 Ortho dihydric phenol

The content of OD phenol differed significantly between the resistant variety Manoranjitham (AAA) and other varieties studied (Table 9 and Fig.4). The

Table 8. Total phenol content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	24.267	24.567	15.047	25.133	22.933	19.333	21.88
Thiruvananthapuram (AAB)	19.827	12.900	9.803	18.123	14.460	13.277	14.732
Nendran (AAB)	22.533	22.500	3.600	23.600	21.367	18.100	18.617
Grand Naine (AAA)	19.933	17.533	13.123	16.700	18.000	16.100	16.898
Mean	21.64	19.375	10.393	20.889	19.19	16.702	

CD ($P<0.05$) for comparing varieties = 1.191
CD ($P<0.05$) for comparing different parts of leaf = 0.601
CD ($P<0.05$) for comparing varieties and parts = 1.202

Table 9. Ortho dihydric phenol content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	2.452	1.806	0.812	2.616	2.084	1.896	1.944
Thiruvananthapuram (AAB)	2.133	1.597	1.045	1.985	1.523	1.583	1.645
Nendran (AAB)	2.418	1.593	0.524	1.995	1.746	1.898	1.696
Grand Naine (AAA)	2.382	1.314	1.376	1.913	1.950	1.566	1.75
Mean	2.346	1.577	0.939	2.127	1.826	1.736	

CD ($P<0.05$) for comparing varieties = 0 .122
CD ($P<0.05$) for comparing parts of leaf = 0.122
CD ($P<0.05$) for comparing varieties and parts = 0.243

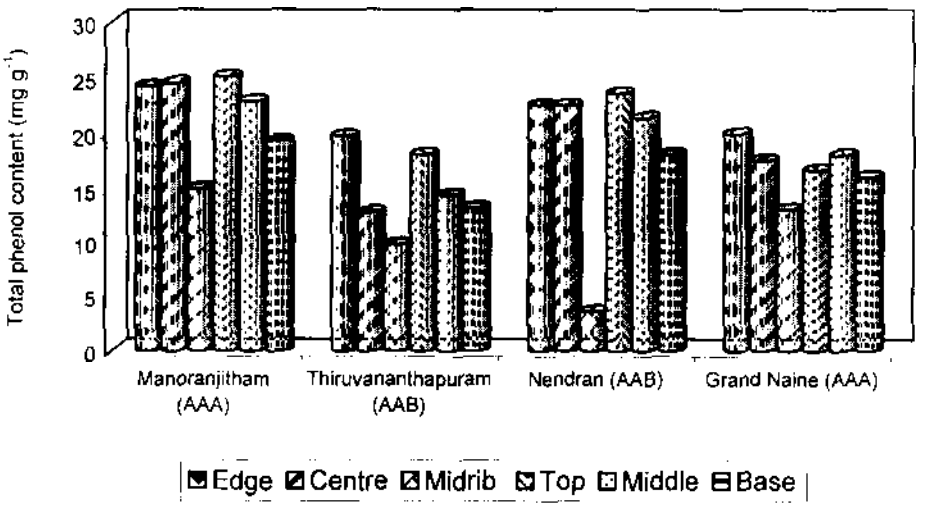


Fig. 3. Total phenol content (mg g^{-1}) in different portions of leaf in *Musa* spp.

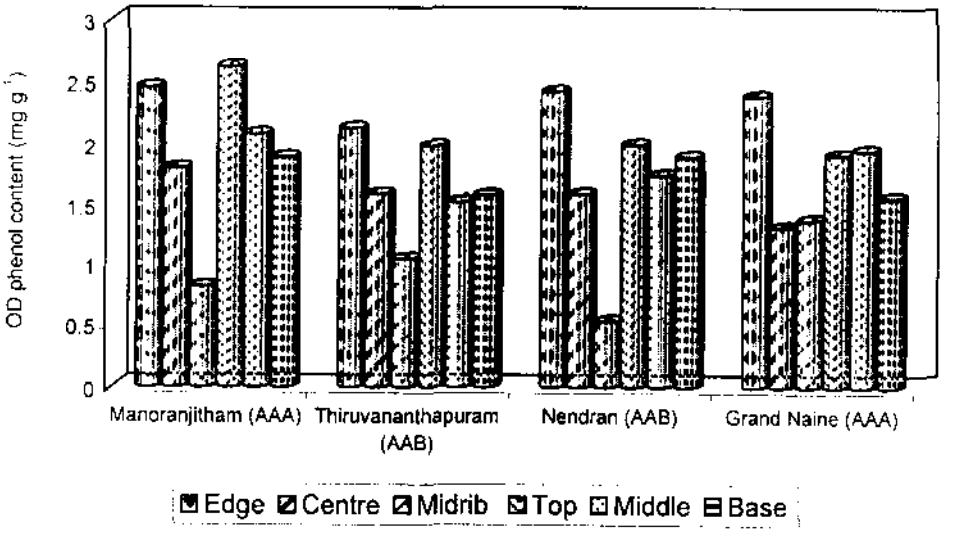


Fig. 4. Ortho dihydric phenol content (mg g^{-1}) in different portions of leaf in *Musa* spp.

resistant variety Manoranjitham (AAA) showed maximum content of OD phenol. The quantity of OD phenol in the highly susceptible variety Grand Naine (AAA), the susceptible variety Nendran (AAB) and the moderately resistant variety Thiruvananthapuram (AAB) were on par.

Among the different portions of leaf taken, higher OD phenol content was observed in the edge portion and lower in the midrib portion. When the varieties and portions of leaf were compared, tip portion of leaf of Manoranjitham (AAA) recorded the highest OD phenol content and lowest in the midrib of Nendran (AAB).

4.6.1.2 Estimation of sugars in healthy leaves of *Musa* spp.

4.6.1.2.1 Reducing sugar

Data presented in Table 10 and Fig.5 revealed that the contents of reducing sugar differed significantly in all the four different groups of banana studied. The highly susceptible variety Grand Naine (AAA) had maximum content of reducing sugar which was 98 per cent more than that of the resistant variety Manoranjitham (AAA).

Among the different portions of leaf studied, edge portion of the leaf possessed highest content of reducing sugar and lowest in the midrib. Comparing the varieties and portions of leaf, the edge portion of the highly susceptible variety Grand Naine (AAA) possessed higher content of reducing sugar and lower in the midrib portion.

4.6.1.2.2 Non reducing sugar

When the different groups of banana and leaf portions were analysed, significant differences were noticed in non reducing sugar content. Higher content

Table 10. Reducing sugar content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	3.233	2.807	2.751	3.036	2.882	2.096	2.801
Thiruvananthapuram (AAB)	4.961	5.512	3.441	3.984	5.122	3.872	4.482
Nendran (AAB)	5.223	4.953	1.840	5.809	8.051	3.984	4.977
Grand Naine (AAA)	7.393	5.790	1.356	6.549	6.016	6.085	5.531
Mean	5.202	4.765	2.347	4.844	5.518	4.009	

CD ($P<0.05$) for comparing varieties = 0.108
CD ($P<0.05$) for comparing parts of leaf = 0.190
CD ($P<0.05$) for comparing varieties and parts = 0.381

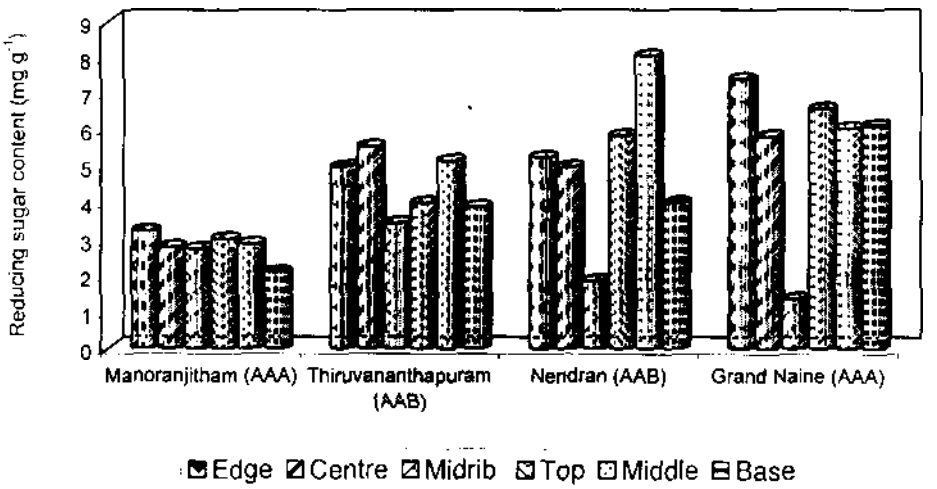


Fig. 5. Reducing sugar content (mg g^{-1}) in different portions of leaf in *Musa* spp.

of non reducing sugar was found in the resistant variety Manoranjitham (AAA) and low in the moderately susceptible variety Nendran (AAB) (Table 11 and Fig. 6).

From the different portions of leaf analysed, centre portion of the leaf showed higher content of non reducing sugar and lower in the midrib portion. Among the varieties and leaf portions compared, the centre portion of the leaf of the resistant variety Manoranjitham (AAA) exhibited highest content of non reducing sugar and lowest in the midrib of susceptible variety Nendran (AAB).

4.6.1.2.3 Total sugar

The total sugar content also significantly differed in the four different groups of banana studied (Table 12 and Fig.7). Higher total sugar was observed in the resistant variety Manoranjitham (AAA) which was 172 per cent more than that of the susceptible variety Nendran (AAB).

Statistically there was significant difference among the portions of leaf analysed. Total sugar content obtained was higher from the centre portion of the leaf and lower in the midrib portion of leaf. Among the varieties and portions of leaf analysed, the centre portion of the resistant variety Manoranjitham (AAA) showed highest content of total sugar and lowest in the midrib portion of the susceptible variety Nendran (AAB).

4.6.1.3 Proline

The data related to proline content in the different groups of banana studied are presented in Table 13 and Fig.8. The proline content differed significantly in the susceptible variety Nendran (AAB) and other varieties. Higher quantity of proline was observed in the susceptible variety Nendran (AAB) which was 194 per cent more than that of the highly susceptible variety Grand Naine (AAA). The quantity of proline between the moderately resistant and resistant

Table 11. Non reducing sugar content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	14.164	29.897	2.086	19.391	20.183	17.001	17.12
Thiruvananthapuram (AAB)	18.157	14.123	2.572	6.785	15.084	13.560	11.713
Nendran (AAB)	8.036	6.126	0.825	4.272	3.936	1.966	4.193
Grand Naine (AAA)	12.407	9.105	1.356	3.431	2.172	9.774	6.374
Mean	13.191	14.813	1.710	8.470	10.344	10.575	

CD (P<0.05) for comparing varieties = 0 .276
CD (P<0.05) for comparing parts of leaf = 0.333
CD (P<0.05) for comparing varieties and parts = 0.665

Table 12. Total sugar content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	17.386	32.704	4.83	22.427	23.065	19.097	19.920
Thiruvananthapuram (AAB)	23.117	19.635	6.012	10.769	20.206	17.432	16.195
Nendran (AAB)	13.259	11.079	2.665	10.083	11.987	5.950	9.171
Grand Naine (AAA)	19.791	14.895	2.712	10.013	8.188	15.858	11.905
Mean	18.393	19.578	4.055	13.315	15.861	14.584	

CD (P<0.05) for comparing varieties = 0.366
CD (P<0.05) for comparing parts of leaf = 0.403
CD (P<0.05) for comparing varieties and parts = 0.806

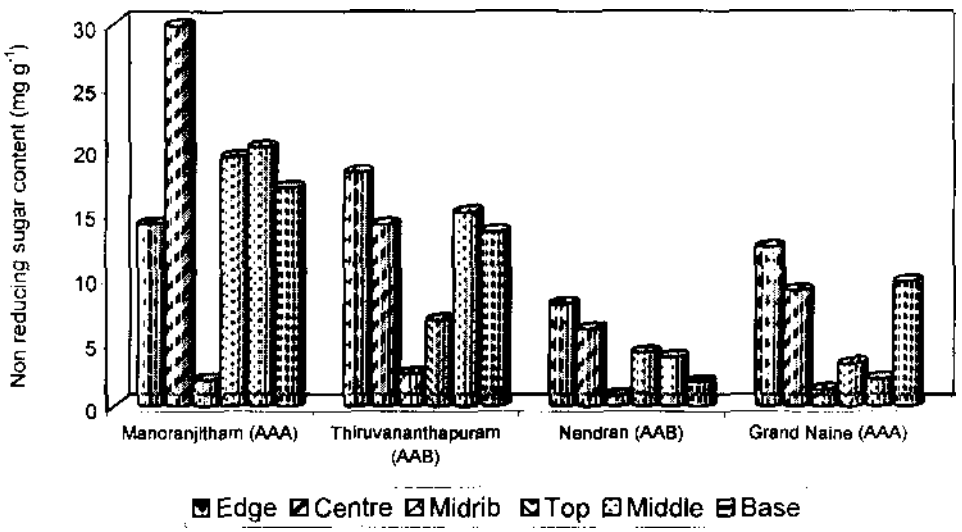


Fig. 6. Non reducing sugar content (mg g^{-1}) in different portions of leaf in *Musa* spp.

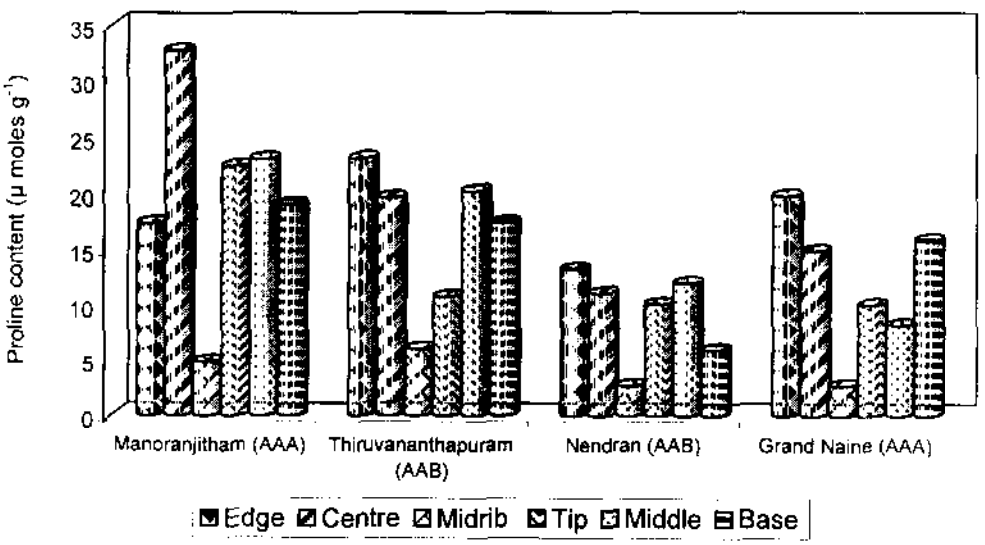


Fig. 7. Total sugar content (mg g^{-1}) in different portions of leaf in *Musa* spp.

Table 13. Proline content in different portions of leaf in *Musa* spp. (μ moles g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	4.395	3.046	0.173	1.557	3.183	4.087	2.740
Thiruvananthapuram (AAB)	3.948	3.188	1.437	2.249	3.879	2.700	2.900
Nendran (AAB)	4.156	4.155	0.226	2.492	4.467	4.017	3.252
Grand Naine (AAA)	1.212	1.212	0.173	1.558	2.077	0.900	1.483
Mean	3.428	3.341	0.502	1.964	3.401	2.926	

CD ($P<0.05$) for comparing varieties = 0 .160
CD ($P<0.05$) for comparing parts of leaf = 0.085
CD ($P<0.05$) for comparing varieties and parts = 0.169

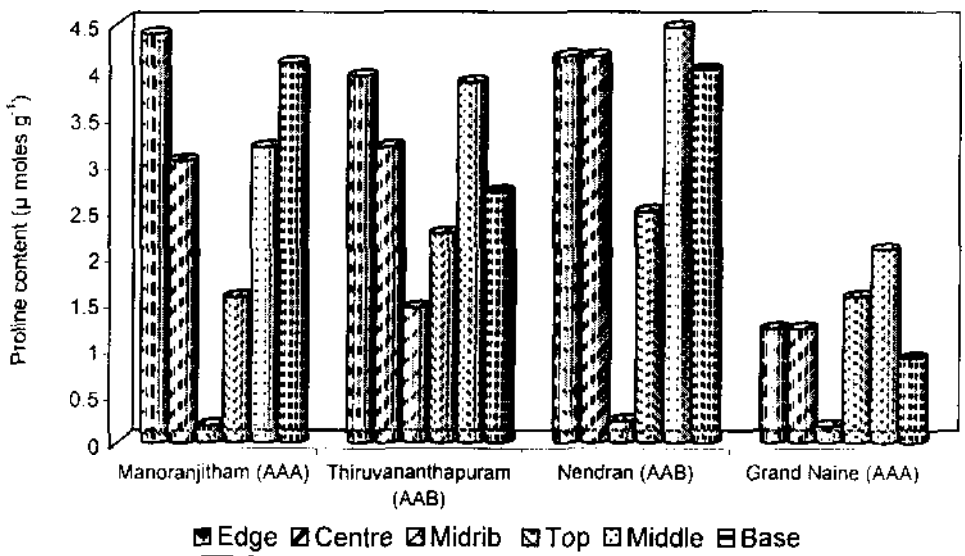


Fig. 8. Proline content (µ moles g^{-1}) in different portions of leaf in *Musa* spp.

varieties like Thiruvananthapuram (AAB) and Manoranjitham (AAA) were on par.

The different portions of leaf analysed showed significant variation in the proline content. Edge portion of the leaf had highest proline content and lowest in the midrib portion. Middle portion of the susceptible variety Nendran (AAB) showed higher proline content and lower in midrib portion of the resistant variety Manoranjitham (AAA) and highly susceptible variety Grand Naine (AAA).

4.6.1.4 Estimation of enzyme activities in healthy leaves of *Musa* spp.

4.6.1.4.1 Peroxidase

The activity of peroxidase enzyme differed significantly among the varieties and portions of leaf studied. The interaction between varieties x portions of leaf was also found to be significant.

The resistant variety Manoranjitham (AAA) showed higher enzyme activity which was 59 per cent more than that of susceptible variety Nendran (AAB) (Table 14 and Fig.9).

Among the different portions of leaf analysed the edge portion of the leaf showed higher activity and lower in the centre portion of the leaf. Edge portion of the leaf of moderately resistant variety Thiruvananthapuram (AAB) showed higher enzyme activity and lower in the basal portion of the susceptible variety Nendran (AAB).

4.6.1.4.2 Polyphenol oxidase

The activity of polyphenol oxidase was found to be higher in the susceptible variety Nendran (AAB) and lower in moderately resistant variety

Table 14. Peroxidase enzyme activity in different portions of leaf in *Musa* spp. (units litre^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	397.800	230.963	195.289	242.842	262.105	211.339	256.834
Thiruvananthapuram (AAB)	398.267	207.500	195.265	237.181	216.415	199.733	242.366
Nendran (AAB)	163.309	158.032	199.200	144.283	163.226	142.270	161.72
Grand Naine (AAA)	211.943	150.637	199.200	216.621	211.938	199.200	198.312
Mean	292.833	186.949	197.28	210.232	213.421	188.136	

CD ($P<0.05$) for comparing varieties = 1.951
CD ($P<0.05$) for comparing parts of leaf = 4.62
CD ($P<0.05$) for comparing varieties and parts = 9.293

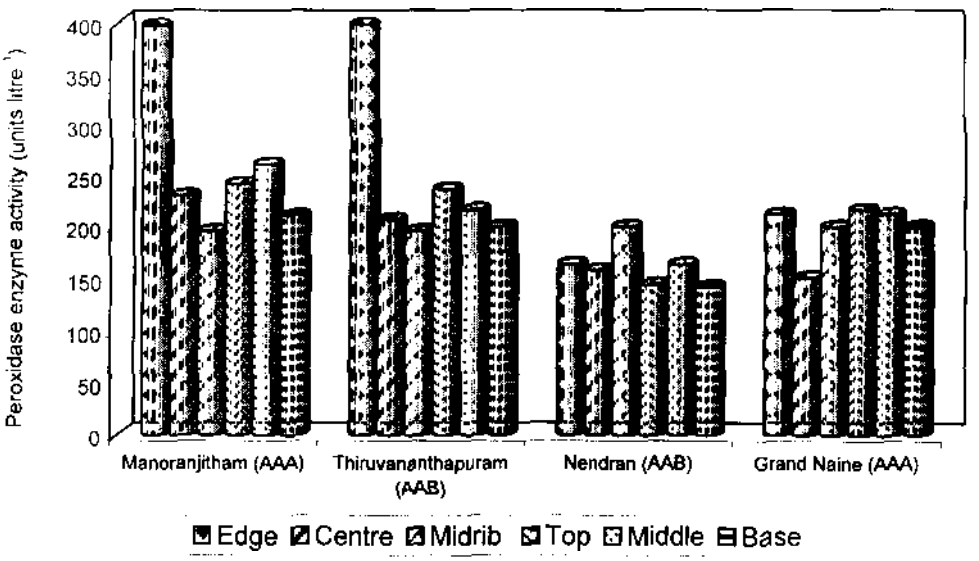


Fig. 9. Peroxidase enzyme activity (units litre^{-1}) in different portions of leaf in *Musa* spp.

Thiruvananthapuram (AAB) and differed significantly in all the varieties and portions of leaf analysed (Table 15 and Fig. 10).

Basal portion of the leaf showed higher activity of enzyme polyphenol oxidase and lower in the midrib portion of the leaf. Middle portion of the leaf of the susceptible variety Nendran (AAB) possessed higher activity of polyphenol oxidase and lower in midrib portion of the highly susceptible variety Grand Naine (AAA).

4.6.1.5 Protein

The quantity of protein differed in all the portions of leaf analysed (Table 16 and Fig.11).

Higher quantity of protein was observed in the resistant variety Manoranjitham (AAA) which was 22 per cent more than that of the highly susceptible variety Grand Naine (AAA). The quantity of protein between the variety Manoranjitham (AAA) and Nendran (AAB) were on par. The content of protein between the susceptible and moderately resistant varieties viz., Nendran (AAB) and Thiruvananthapuram (AAB) were also on par. Tip portion of the leaf exhibited higher amount of protein (17.613 mg g^{-1}) and lowest in the midrib portion analysed. Tip portion of the resistant variety Manoranjitham (AAA) had higher quantity of protein and lower in midrib portion of moderately resistant variety Thiruvananthapuram (AAB).

4.6.2 Estimation of biochemical parameters of healthy and yellow sigatoka infected leaves of *Musa* spp.

The study was undertaken to determine the biochemical changes seen in different portions of leaves infected with *M. musicola* in four different groups of banana.

Table 15. Polyphenol oxidase total enzyme activity in different portions of leaf in *Musa* spp. (OD value)

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	1.116	1.578	0.174	1.386	0.900	1.788	1.157
Thiruvananthapuram (AAB)	0.182	0.527	0.330	0.684	0.144	0.804	0.445
Nendran (AAB)	1.704	2.057	0.336	1.330	1.800	1.560	1.464
Grand Naine (AAA)	0.798	0.804	0.072	0.469	0.897	1.548	0.765
Mean	0.950	1.241	0.228	0.967	0.935	1.425	

CD ($P<0.05$) for comparing varieties = 0 .108
CD ($P<0.05$) for comparing parts of leaf = 0.087
CD ($P<0.05$) for comparing varieties and parts = 0.173

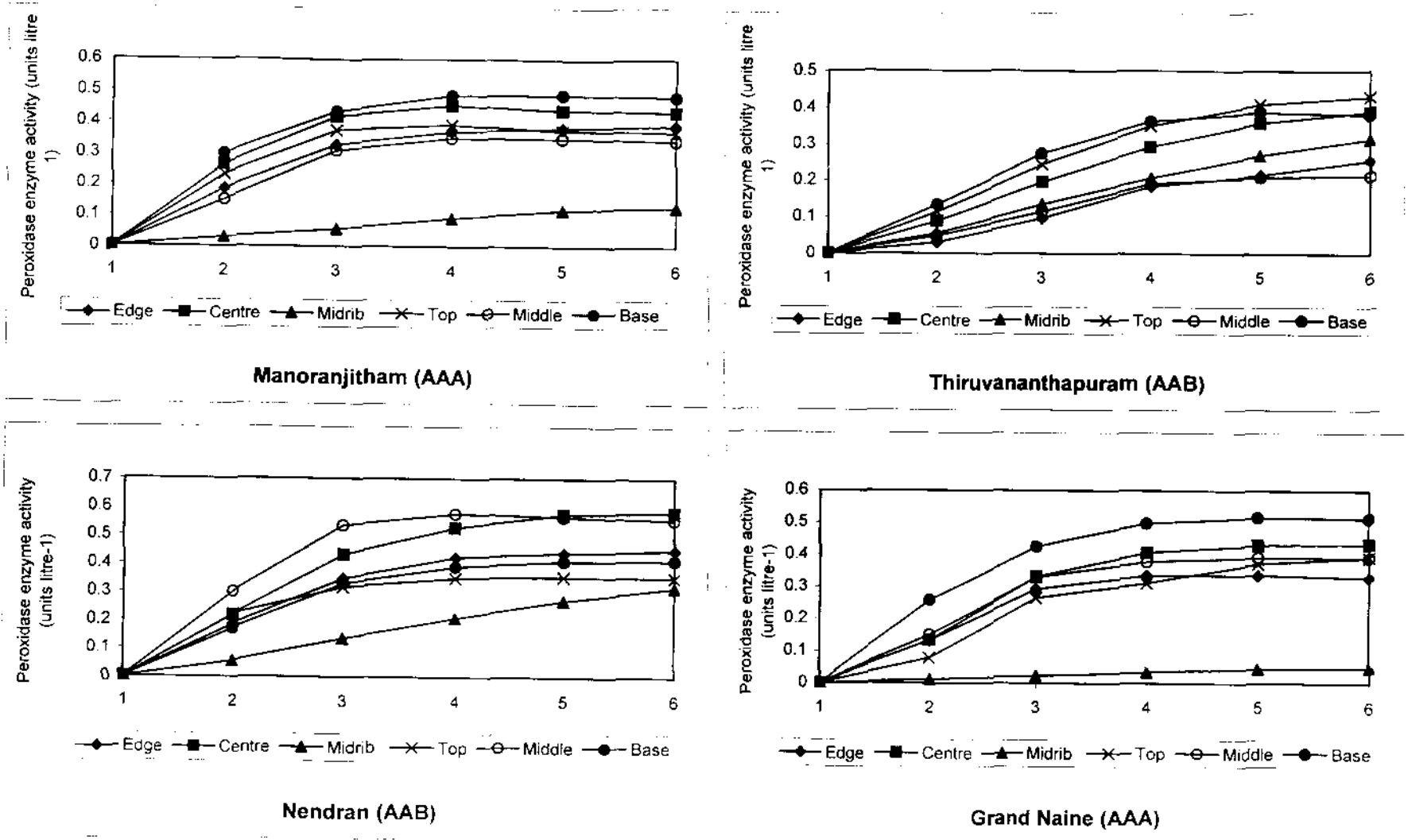


Fig. 10. Polyphenol oxidase total enzyme activity (OD value) in different portions of leaf in *Musa* spp.

Table 16. Protein content in different portions of leaf in *Musa* spp. (mg g^{-1})

Musa spp.	Portions of leaf						
	Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	17.199	14.931	6.575	21.849	14.04	15.272	14.982
Thiruvananthapuram (AAB)	13.393	17.145	5.768	16.714	14.445	17.541	14.168
Nendran (AAB)	17.199	15.175	7.561	17.670	15.931	12.851	14.296
Grand Naine (AAA)	11.903	11.502	6.901	14.917	14.013	14.283	12.253
Mean	14.923	14.703	6.716	17.613	14.607	14.987	

CD ($P<0.05$) for comparing varieties = 0 .808
CD ($P<0.05$) for comparing parts of leaf = 0.681
CD ($P<0.05$) for comparing varieties and parts = 1.362

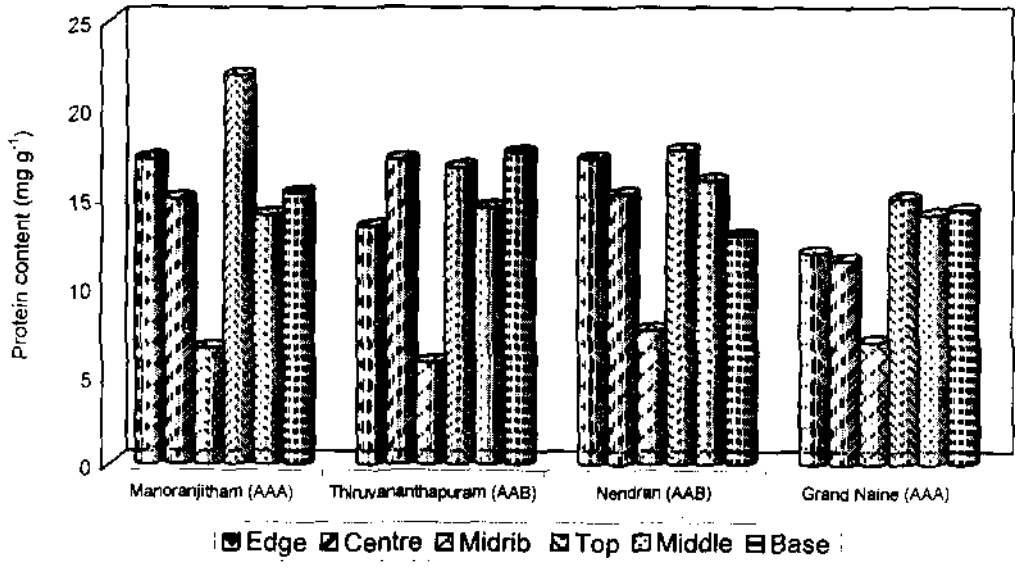


Fig. 11. Protein content (mg g^{-1}) in different portions of leaf in *Musa* spp.

4.6.2.1 Estimation of total and OD phenols in healthy and yellow sigatoka infected leaves of *Musa* spp.

4.6.2.1.1 Total phenol

Significant increase in total phenol content was observed on almost all portions of yellow sigatoka infected leaf in all the varieties except in the resistant variety Manoranjitham (AAA)(Table 17 and Fig. 12). The maximum increase in total phenol was observed in the susceptible variety Nendran (AAB) Which recorded 27 per cent more over the moderately resistant variety Thiruvananthapuram (AAB).

Out of the different portions of leaf, tip portion recorded higher quantities of total phenol comparing the other portions in different groups of banana studied. On infection, the tip portion of the leaf showed higher quantities of total phenol in susceptible variety Nendran (AAB).

4.6.2.1.2 Ortho dihydric phenol

The data on the OD phenol content on the healthy and diseased leaves are presented in Table 18 and Fig.13. Significant increase in OD phenol content was observed in all portions of the yellow sigatoka infected leaf in the four different groups of banana studied. Higher quantity of OD phenol was recorded in the resistant variety Manoranjitham (AAA) compared to highly susceptible variety Grand Naine (AAA).

Among the different portions of leaf analysed, higher quantity of OD phenol was observed in the tip portion of the leaf compared to midrib portion.

4.6.2.2 Estimation of sugars in different portions of healthy and yellow sigatoka infected leaves in *Musa* spp.

4.6.2.2.1 Reducing sugars

Analysis of variance showed significant difference in quantity of

Table 17. Total phenol content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	24.267	24.567	15.047	25.133	22.933	19.333	21.880
	D	22.233	16.600	20.807	24.033	17.433	18.293	19.900
Thiruvananthapuram (AAB)	H	19.827	12.900	9.803	18.123	14.460	13.277	14.732
	D	24.600	13.983	13.440	20.400	17.473	15.020	17.486
Nendran (AAB)	H	22.533	22.500	3.600	23.600	21.367	18.100	18.617
	D	26.667	22.733	3.640	27.400	28.533	24.400	22.229
Grand Naine (AAA)	H	19.333	17.533	13.123	16.700	18.000	16.100	16.898
	D	27.400	19.700	11.753	24.400	24.067	22.000	22.203
Mean	H	21.64	19.375	10.393	20.889	19.19	16.702	
	D	24.825	17.612	12.410	26.925	21.268	19.205	

H - Healthy: D - Diseased

CD ($P<0.05$) for comparing varieties = 0.762

CD ($P<0.05$) for comparing parts of leaf = 0.575

CD ($P<0.05$) for comparing varieties and parts = 0.813

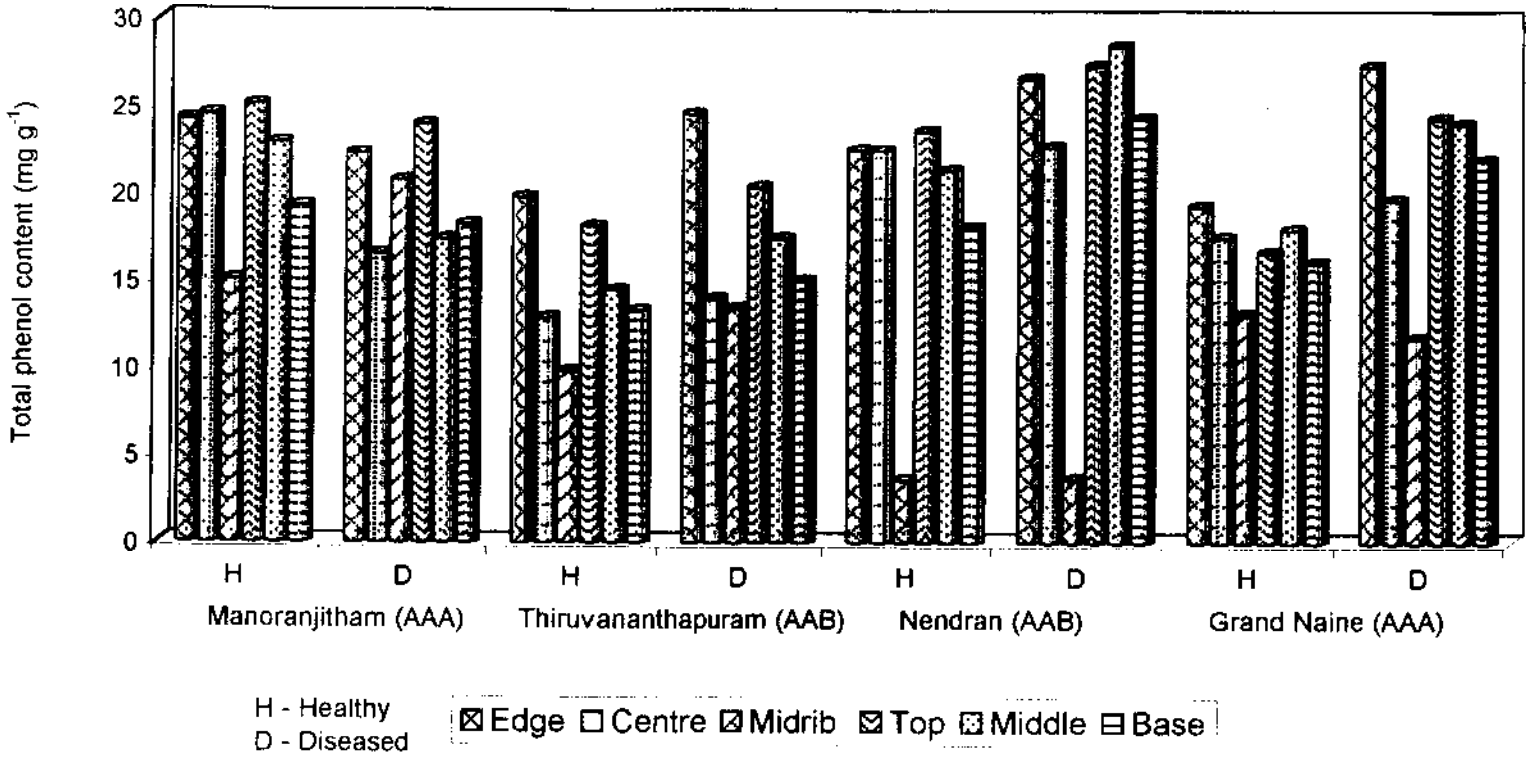


Fig. 12. Total phenol content in different portions of healthy and yellow sigatoka affected leaves of *Musa* spp. (mg g^{-1})

Table 18. Ortho dihydric phenol content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	2.452	1.806	0.812	2.616	2.084	1.896	1.944
	D	2.625	2.260	1.691	3.278	2.111	2.303	2.378
Thiruvananthapuram (AAB)	H	2.133	1.597	1.045	1.985	1.523	1.583	1.645
	D	3.033	1.253	1.327	2.556	2.414	1.793	2.063
Nendran (AAB)	H	2.418	1.593	0.524	1.995	1.746	1.898	1.696
	D	2.057	1.647	0.900	2.385	1.890	1.860	1.790
Grand Naine (AAA)	H	2.382	1.314	1.376	1.913	1.950	1.566	1.750
	D	2.041	1.475	1.271	1.995	1.650	1.650	1.680
Mean	H	2.346	1.577	0.939	2.127	1.826	1.736	
	D	2.439	1.659	1.297	2.553	2.016	1.902	

H - Healthy; D - Diseased

CD ($P<0.05$) for comparing varieties = 0.091

CD ($P<0.05$) for comparing parts of leaf = 0.056

CD ($P<0.05$) for comparing varieties and parts = 0.079

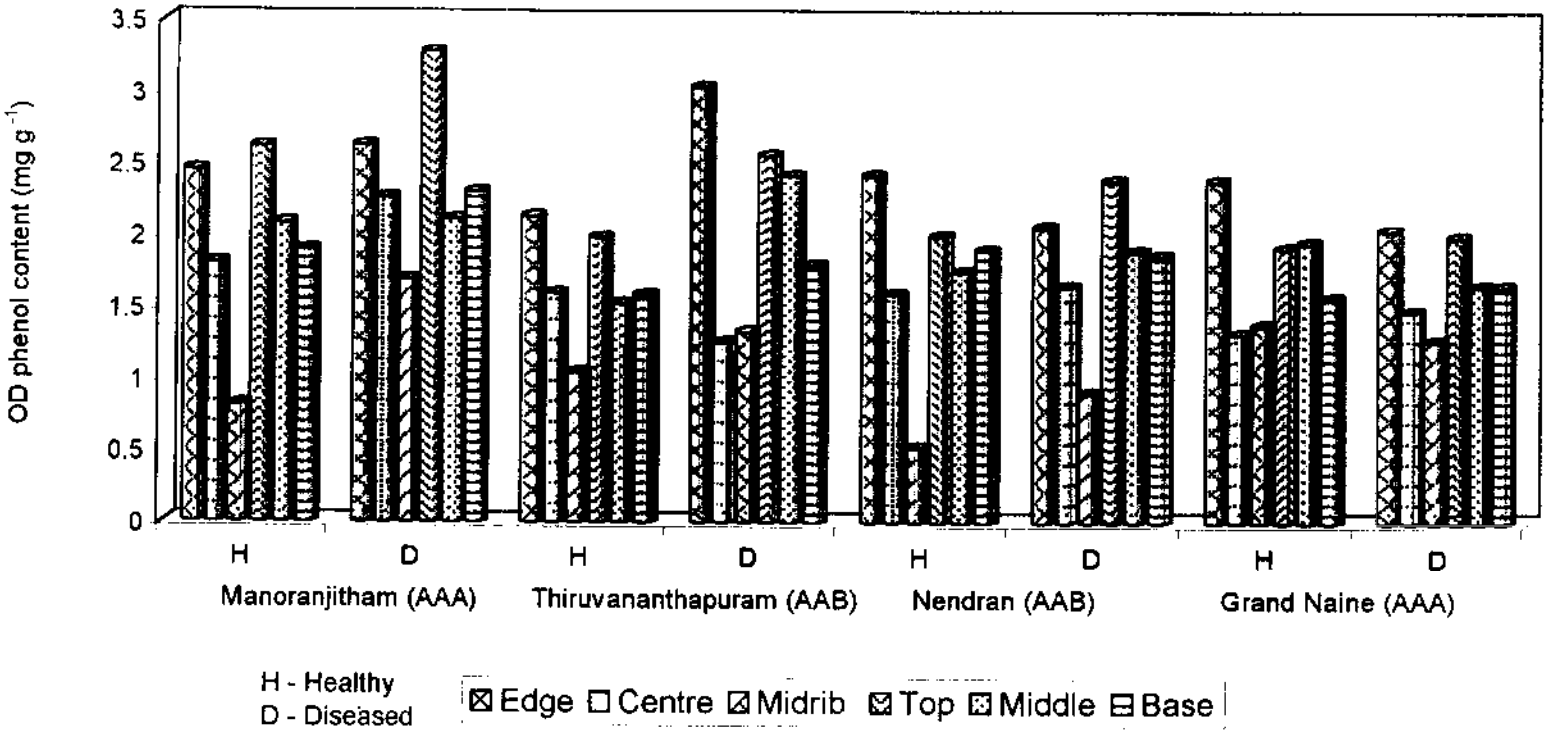


Fig. 13. Ortho dihydric phenol content in different portions of healthy and yellow sigatoka affected leaves of *Musa* spp. (mg g^{-1})

reducing sugars in both healthy and diseased leaves of the four varieties studied (Table 19 and Fig. 14).

An increase in the quantity of reducing sugar was noticed in almost all the varieties except the susceptible variety Nendran (AAB). Maximum increase in the reducing sugar content was noticed in the highly susceptible variety Grand Naine (AAA) which was 148 per cent more than that of the resistant variety Manoranjitham (AAA). Of the different portions of leaf analysed, higher quantities of reducing sugar was observed in the midrib portion of the leaf.

4.6.2.2.2 Non reducing sugar

Significant difference in non reducing sugar content was noticed in both healthy and diseased leaves in the four different varieties studied (Table 20 and Fig. 15). The quantity of non reducing sugar decreased in the diseased leaves of all the *Musa* spp. Maximum decrease was observed in the susceptible variety Nendran (AAB) and minimum in resistant variety Manoranjitham (AAA). Among the different portions of leaf analysed, the decrease was maximum in tip portion of the leaf than that of the edge portion.

4.6.2.2.3 Total sugar

Significant decrease in quantity of total sugar was observed in all portions of sigatoka infected leaves in all the *Musa* spp. studied.

The decrease in total sugar content was found higher in susceptible variety Nendran (AAB) which was 140 per cent less compared to resistant variety Manoranjitham (AAA) (Table 21 and Fig.16). The significant decrease in total sugar content was observed in all portions of leaf except in the midrib. Total sugar content was high in the tip portion and low in the edge portion of the leaf.

Table 19. Reducing sugar content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	3.233	2.807	2.751	3.036	2.882	2.096	2.801
	D	3.096	2.203	1.664	3.361	2.296	4.584	2.867
Thiruvananthapuram (AAB)	H	4.961	5.512	3.441	3.984	5.122	3.872	4.482
	D	6.416	6.592	2.953	6.326	4.481	2.961	4.955
Nendran (AAB)	H	5.223	4.953	1.840	5.809	8.051	3.984	4.977
	D	3.976	4.472	1.592	6.125	2.065	1.920	3.358
Grand Naine (AAA)	H	7.393	5.790	1.356	6.549	6.016	6.085	5.531
	D	8.168	7.607	0.857	7.008	6.336	6.984	6.160
Mean	H	5.202	4.765	2.347	4.844	5.518	4.009	
	D	5.414	5.218	1.766	5.705	3.794	4.112	

H - Healthy; D - Diseased

CD ($P<0.05$) for comparing varieties = 0.086

CD ($P<0.05$) for comparing parts of leaf = 0.103

CD ($P<0.05$) for comparing varieties and parts = 0.146

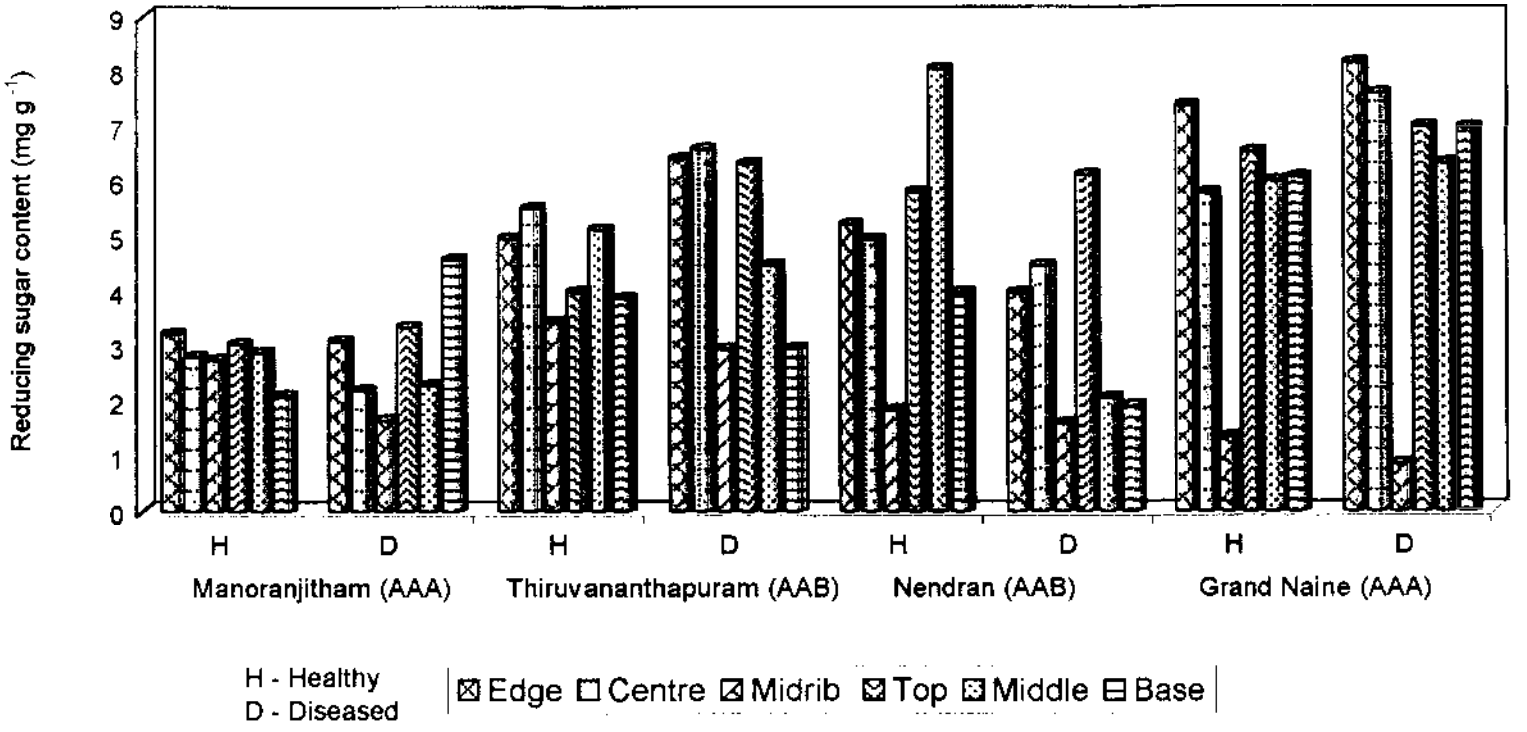


Fig. 14. Reducing sugar content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Table 20. Non reducing sugar content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	14.164	29.897	2.086	19.391	20.183	17.001	17.12
	D	15.831	17.067	3.865	9.645	17.934	16.523	13.477
Thiruvananthapuram (AAB)	H	18.157	14.123	2.572	6.785	15.084	13.560	11.713
	D	17.383	13.208	3.576	5.477	13.28	12.063	10.831
Nendran (AAB)	H	8.036	6.126	0.825	4.272	3.936	1.966	4.193
	D	5.090	3.545	0.239	3.113	7.118	1.727	3.472
Grand Naine (AAA)	H	12.073	9.105	1.356	3.431	2.172	9.774	6.374
	D	11.142	8.287	1.130	1.435	2.022	6.195	5.034
Mean	H	13.191	14.813	1.71	8.47	10.344	10.575	
	D	12.361	10.525	2.203	4.918	10.089	9.127	

H - Healthy; D - Diseased
CD (P<0.05) for comparing varieties = 0.315
CD (P<0.05) for comparing parts of leaf = 0.137
CD (P<0.05) for comparing varieties and parts = 0.193

Table 21. Total sugar content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	17.397	32.704	4.83	22.427	23.065	19.097	19.92
	D	18.927	19.270	5.518	13.006	20.230	21.107	16.342
Thiruvananthapuram (AAB)	H	23.117	19.635	6.012	10.769	20.206	17.432	16.195
	D	23.799	19.800	6.862	11.871	17.761	15.024	15.853
Nendran (AAB)	H	13.259	11.079	2.665	10.083	11.987	5.95	9.171
	D	9.066	7.828	1.831	9.237	9.182	3.647	6.799
Grand Naine (AAA)	H	19.799	14.895	2.712	9.980	8.188	15.858	11.905
	D	19.31	15.887	1.987	8.443	8.358	13.179	11.194
Mean	H	18.393	19.578	4.055	13.315	15.861	14.584	
	D	17.775	15.697	4.050	10.639	13.883	13.239	

H - Healthy; D - Diseased
CD (P<0.05) for comparing varieties = 0.120
CD (P<0.05) for comparing parts of leaf = 0.038
CD (P<0.05) for comparing varieties and parts = 0.054

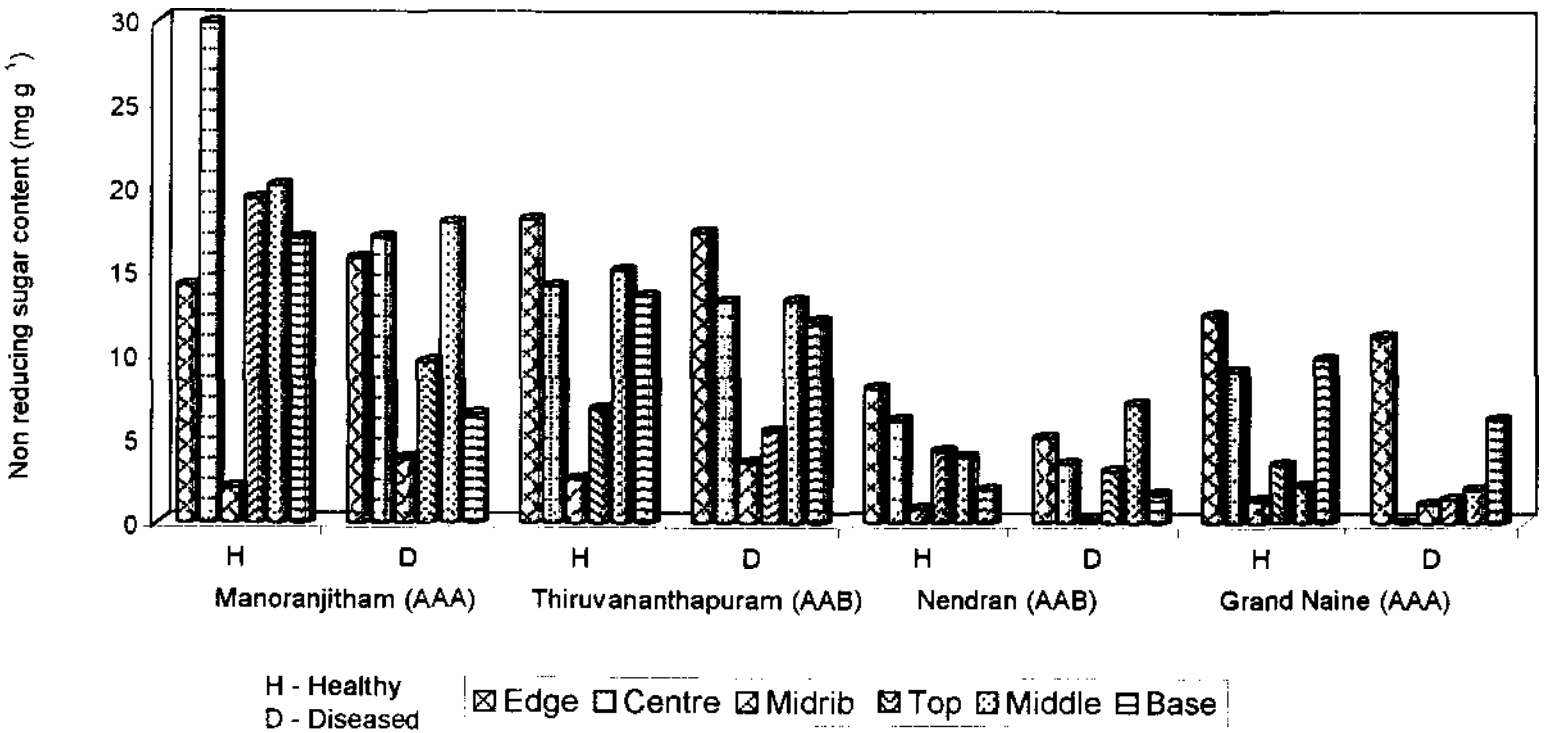


Fig. 15. Non reducing sugar content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

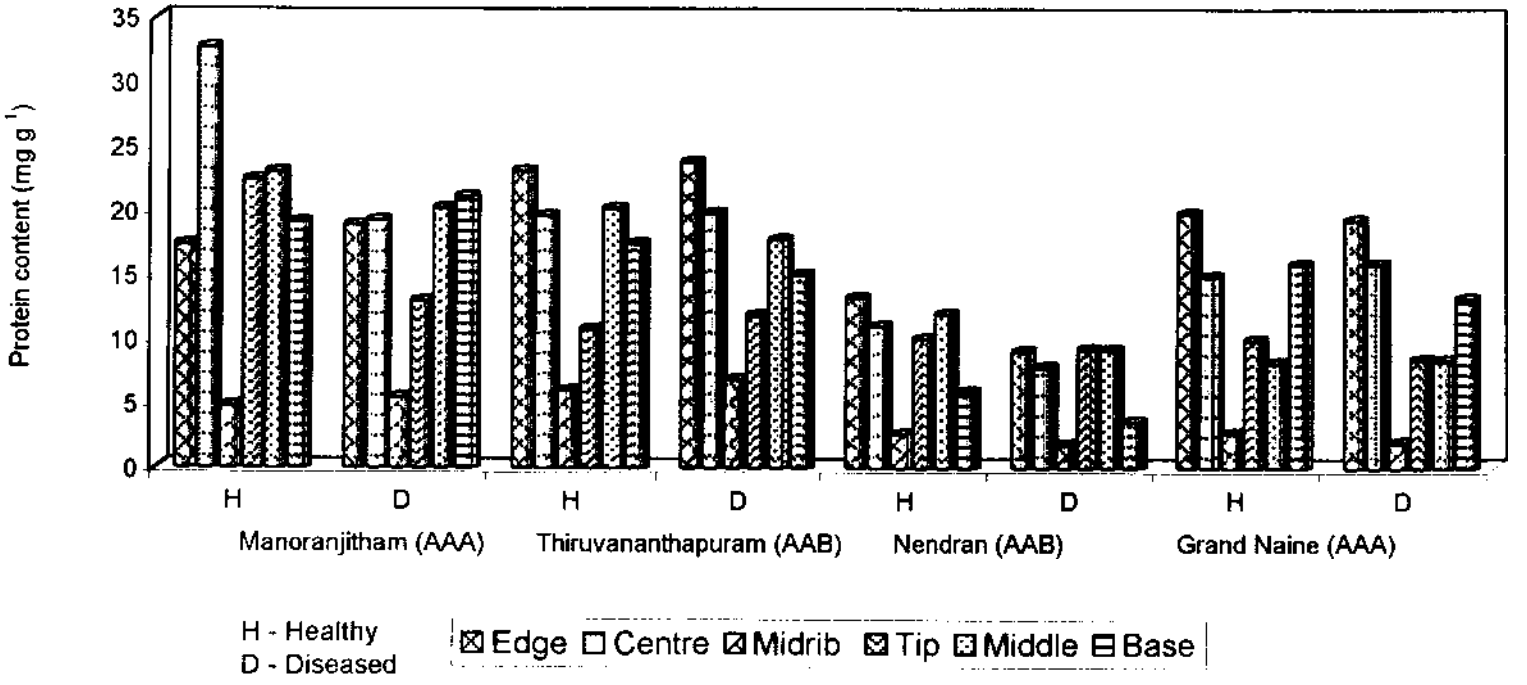


Fig. 16. Total sugar content (mg g^{-1}) in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.

4.6.2.3 Proline

Significant differences were observed for proline content in the four different groups of banana studied (Table 22 and Fig. 17).

Decrease in proline content was observed in the infected leaves of all varieties where as increase in quantity seen in highly susceptible variety Grand Naine (AAA). The decrease was found to be more in resistant variety Manoranjitham (AAA) compared to highly susceptible variety Grand Naine (AAA). Depletion of proline content was observed in all portions of leaf in all the varieties studied. The decrease was found more in midrib portion of the leaf compared to edge portion.

4.6.2.4 Enzymes

4.6.2.4.1 Peroxidase

Data presented in the Table 23 and Fig.18 showed significant differences in peroxidase activity in different varieties and different portions of leaf analysed. The activity of peroxidase was increased in all the portions of infected leaf in susceptible variety Nendran (AAB) whereas decreased in resistant variety Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB). Peroxidase enzyme activity was found less in the moderately resistant variety Thiruvananthapuram (AAB) and resistant variety Manoranjitham (AAA). The activity of peroxidase was observed maximum in susceptible variety Nendran (AAB) compared to other three varieties studied. Peroxidase activity was observed high in all portions of leaf and found maximum in the edge portion and minimum in the midrib portion of leaf.

4.6.2.4.2 Polyphenol oxidase

The OD values compiled in Table 24 and Fig.19 showed an increase in enzyme activity in infected leaves of all the varieties studied. Higher enzyme

Table 22. Proline content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (μ moles g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	4.395	3.046	0.173	1.557	3.183	4.087	2.740
	D	0.485	0.450	0.831	0.867	0.450	0.540	0.604
Thiruvananthapuram (AAB)	H	3.948	3.188	1.436	2.244	3.879	2.700	2.900
	D	0.519	0.312	0.537	0.657	0.346	1.766	0.690
Nendran (AAB)	H	4.156	4.155	0.226	2.492	4.467	4.017	3.252
	D	3.463	1.558	0.173	1.974	0.519	0.935	1.437
Grand Naine (AAA)	H	1.212	2.975	0.173	1.558	2.007	0.900	1.483
	D	1.558	1.558	0.416	3.636	3.184	2.978	2.222
Mean	H	3.428	3.341	0.502	1.964	3.401	2.926	
	D	1.506	0.970	0.489	1.784	1.125	1.555	

H - Healthy; D - Diseased
CD ($P<0.05$) for comparing varieties = 0.120
CD ($P<0.05$) for comparing parts of leaf = 0.038
CD ($P<0.05$) for comparing varieties and parts = 0.054

Table 23. Peroxidase enzyme activity in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (units $litre^{-1}$)

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	397.800	230.963	195.289	242.842	262.105	211.339	256.834
	D	368.920	226.365	216.522	158.365	249.000	237.143	242.719
Thiruvananthapuram (AAB)	H	398.267	207.500	195.265	237.181	216.415	199.733	242.366
	D	216.521	216.36	166.033	150.909	216.521	230.163	199.419
Nendran (AAB)	H	163.309	158.032	99.200	144.283	163.226	142.270	161.72
	D	415.000	415.000	211.917	433.044	433.042	332.333	373.389
Grand Naine (AAA)	H	211.943	150.637	199.533	216.621	211.938	199.200	198.31
	D	195.296	195.296	332.00	207.483	196.061	226.359	225.416
Mean	H	292.833	186.949	197.28	210.232	213.421	188.136	
	D	298.934	263.256	231.618	237.45	273.656	256.500	

H - Healthy; D - Diseased
CD ($P<0.05$) for comparing varieties = 14.509
CD ($P<0.05$) for comparing parts of leaf = 2.283
CD ($P<0.05$) for comparing varieties and parts = 3.228

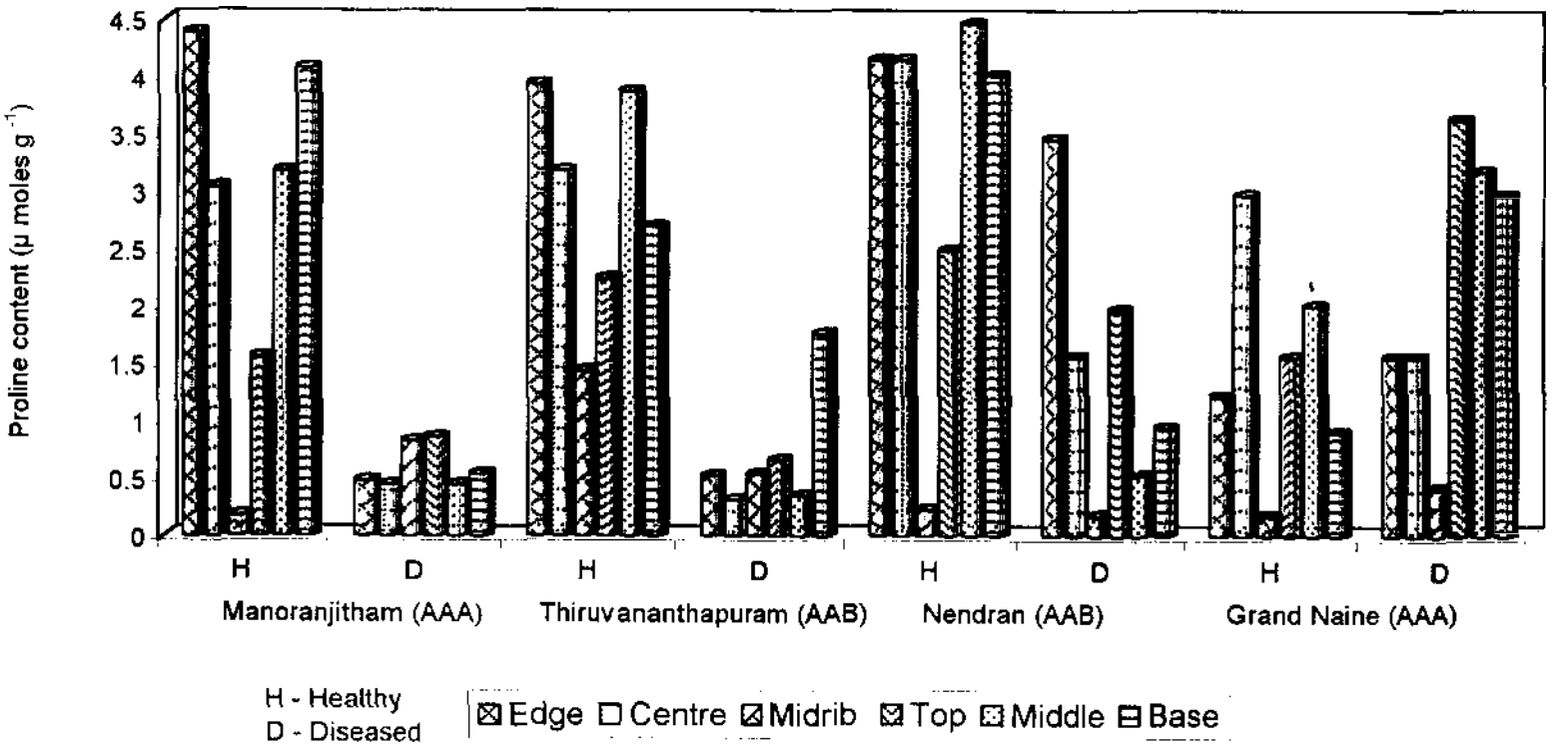


Fig. 17. Proline content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.(µ moles g^{-1})

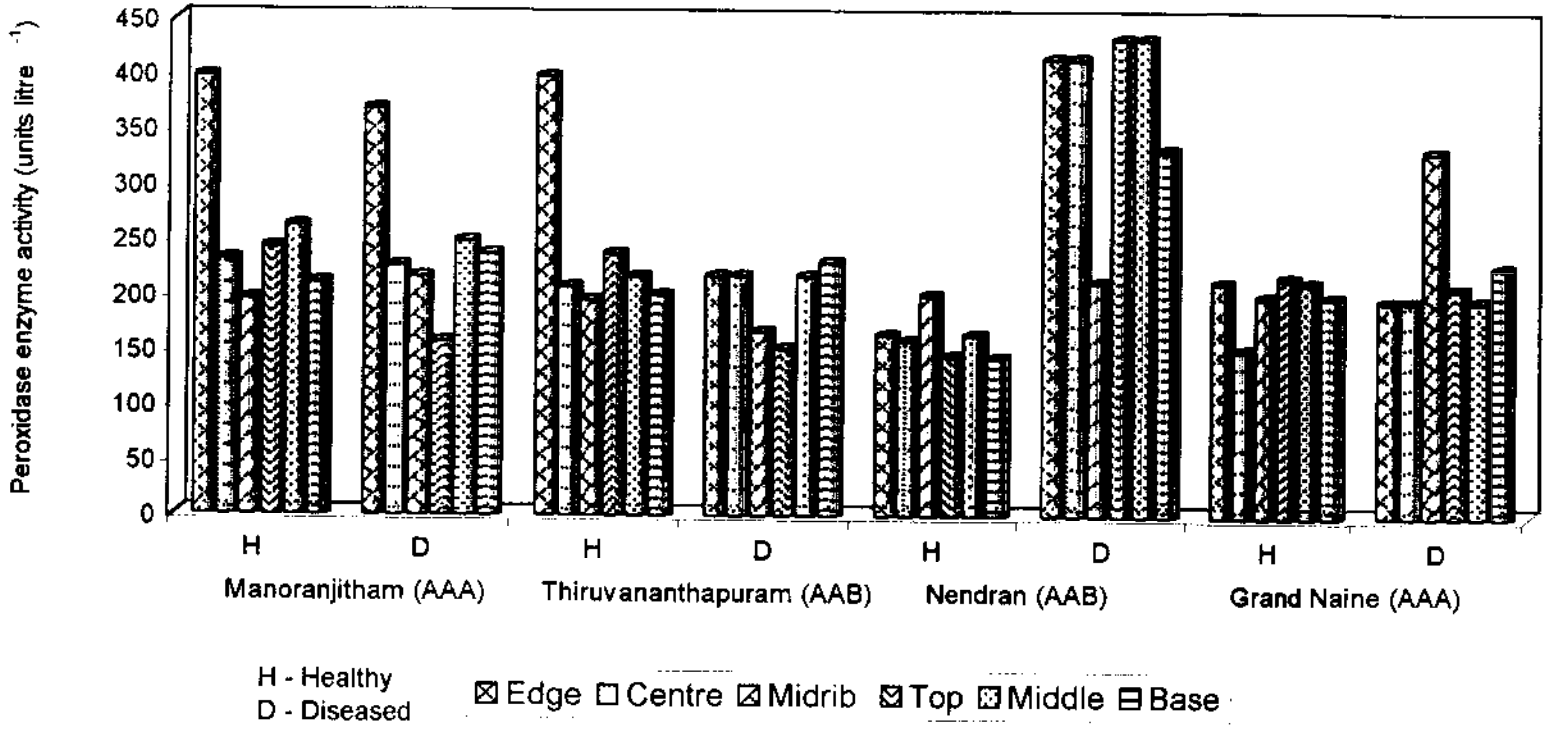


Fig. 18. Peroxidase enzyme activity (units litre^{-1}) in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.

Table 24. Poly phenol oxidase total enzyme activity in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	1.116	1.578	0.174	1.386	0.900	1.788	1.157
	D	1.536	2.142	0.122	2.520	1.607	2.479	1.734
Thiruvananthapuram (AAB)	H	0.182	0.528	0.078	0.684	0.144	0.804	0.445
	D	0.228	0.870	0.192	0.582	0.301	1.236	0.568
Nendran (AAB)	H	1.704	2.057	0.336	1.330	1.800	1.560	1.464
	D	1.908	2.430	0.186	1.830	1.896	2.226	1.746
Grand Naine (AAA)	H	0.798	0.804	0.072	0.468	0.900	1.548	0.765
	D	1.350	1.278	0.276	1.056	1.320	1.284	1.094
Mean	H	0.950	1.241	0.228	0.967	0.935	1.425	
	D	1.255	1.680	0.194	1.497	1.281	1.806	

H - Healthy; D - Diseased

CD ($P<0.05$) for comparing varieties = 0.118

CD ($P<0.05$) for comparing parts of leaf = 0.040

CD ($P<0.05$) for comparing varieties and parts = 0.057

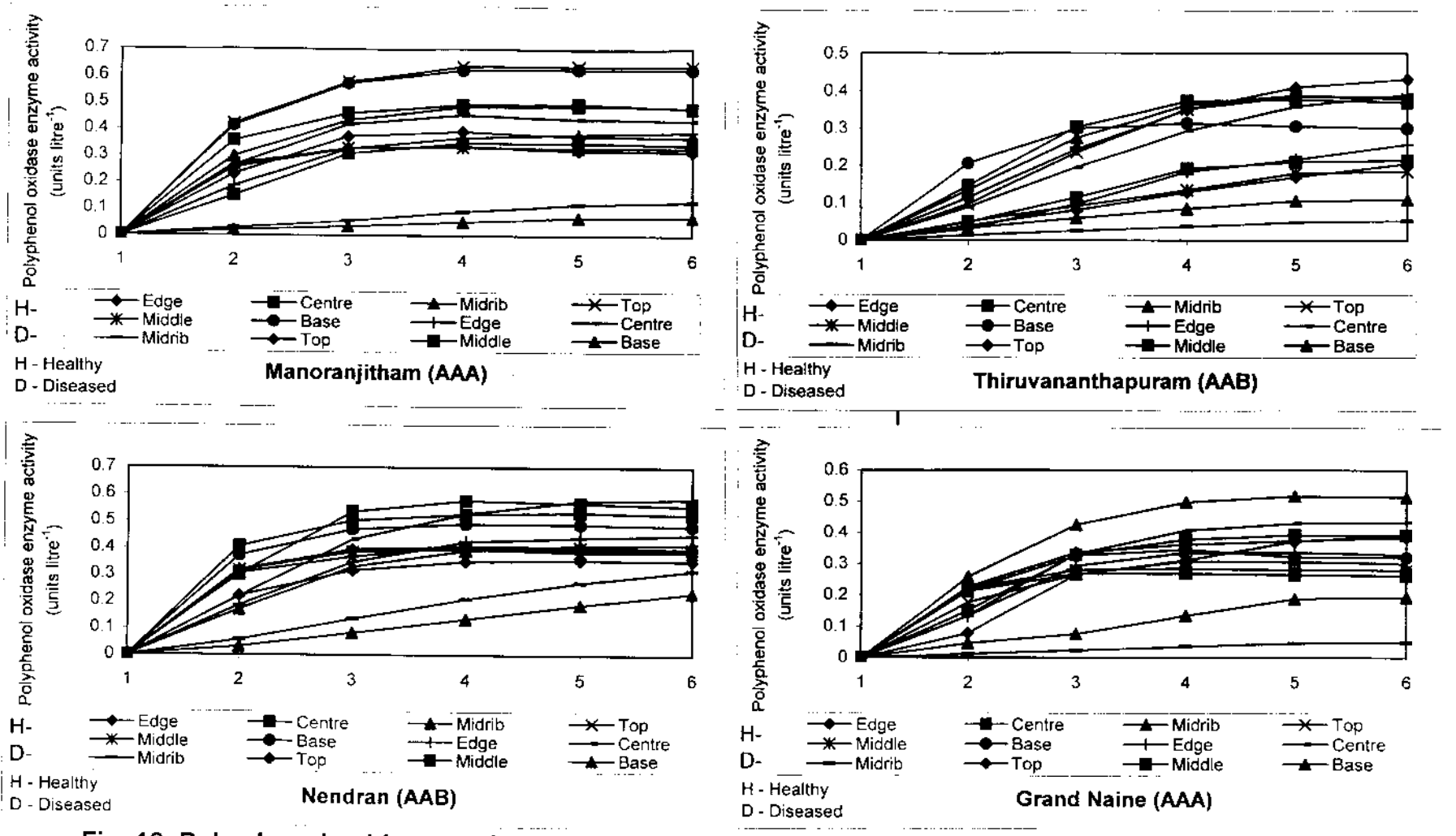


Fig. 19. Poly phenol oxidase total enzyme activity (OD value) in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.

activity was observed in the susceptible variety Nendran (AAB) and lower in moderately resistant variety Thiruvananthapuram (AAB). After infection, all portions of leaf recorded an increase in enzyme activity whereas a decrease was observed in the midrib portion. The enzyme activity was found high in the basal portion and low in midrib portion of the leaf.

4.6.2.5 Protein

The protein content differed significantly among healthy and diseased leaves of four different groups of banana. The interaction between varieties and portions of leaf was also found significant (Table 25 and Fig.20).

On infection, the protein content was depleted in diseased leaves of almost all the varieties except in susceptible variety Nendran (AAB). Maximum quantity of protein in the diseased leaves was found in Nendran (AAB) and minimum in Grand Naine (AAA). Maximum content of protein in the diseased leaves was found in the tip (118%) compared to the midrib portion.

4.6.2.6 Comparative evaluation of different media which produced toxic metabolite of *M. musicola*

4.6.2.6.1 Toxic metabolite

Thin layer chromatograms developed in ethyl acetate:methanol (1:1 v/v) of concentrated culture extracts of seven media in acetone gave only one band of differrent Rf values (Plate 7).

Rf values obtained for different media

PDA	PGYEA	SmGYEA	CzYEA	CGYEA	BnDA	MEA
0.679	0.721	0.715	0.673	0.667	0.382	0.636

The different media were produced different secondary metabolites and have different Rf values for TLC.

Table 25. Protein content in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp. (mg g^{-1})

Musa spp.		Portions of leaf						
		Edge	Centre	Midrib	Tip	Middle	Base	Mean
Manoranjitham (AAA)	H	17.199	14.931	6.635	21.815	14.04	15.272	14.982
	D	17.389	10.462	10.855	20.682	12.550	13.108	12.253
Thiruvananthapuram (AAB)	H	13.393	17.145	5.768	16.714	14.445	17.541	14.168
	D	13.109	15.234	6.278	13.554	13.681	12.609	12.254
Nendran (AAB)	H	17.199	15.175	7.561	17.670	15.931	12.851	14.296
	D	20.021	17.469	6.534	21.816	21.330	13.932	16.850
Grand Naine (AAA)	H	11.903	11.502	6.901	14.918	14.013	14.283	12.253
	D	11.340	10.435	8.478	14.033	10.935	13.473	11.449
Mean	H	14.923	14.703	6.716	17.613	14.607	14.987	
	D	15.465	13.345	8.036	17.521	14.626	13.280	

H - Healthy; D - Diseased

CD ($P<0.05$) for comparing varieties = 0.643

CD ($P<0.05$) for comparing parts of leaf = 0.348

CD ($P<0.05$) for comparing varieties and parts = 0.493

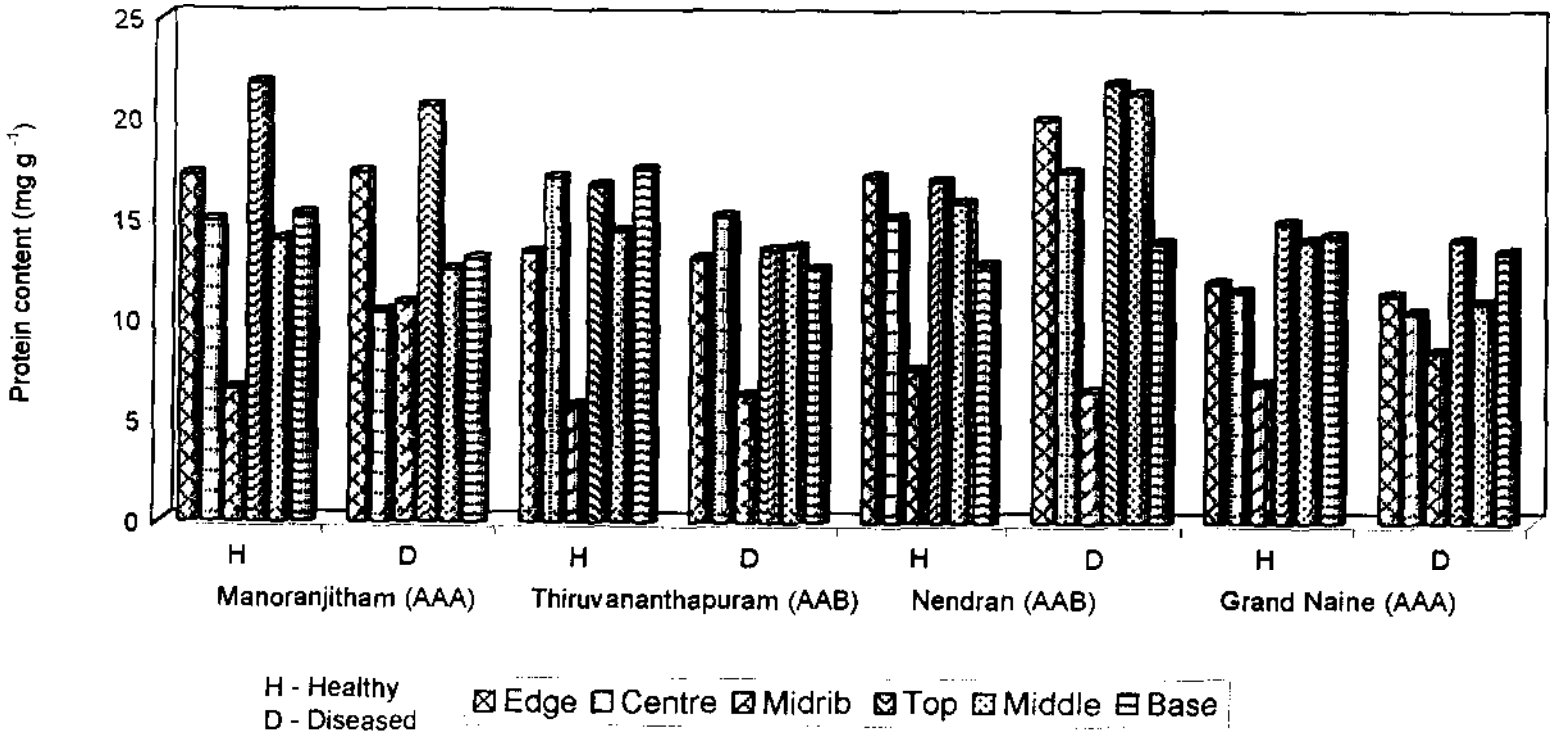


Fig. 20. Protein content (mg g^{-1}) in different portions of healthy and yellow sigatoka infected leaves of *Musa* spp.

Plate 7. Thin layer chromatogram (TLC) of concentrated culture extracts of *Mycosphaerella musicola* on seven media

A. PDA **B.** PGYEA **C.** MEA
D. SmGYEA **E.** CzYEA **F.** CGYEA
G. BnDA

4.6.2.6.2 Bioassay

Leaves of tissue cultured banana plants of cultivar Nendran showed necrosis in leaves 24 h after treatment with toxic metabolite of *M. musicola*.

DISCUSSION

Yellow sigatoka leaf spot incited by *Mycosphaerella musicola* Leach is a serious threat to banana cultivation through out the banana growing regions of the world causing heavy economic loss to the crop. The disease was first reported in Java in 1912 by Zimmermann. In Fiji, it became notorious in 1913 in the sigatoka valley, hence the name 'Sigatoka disease'. In India, the sigatoka leaf spot disease of banana caused by *Cercospora musae* Zimm. was reported first in the Annamalai area of Madras State (Rangaswamy and Kolandaiswamy, 1962).

Many workers have studied different aspects of banana viz., the intensity of sigatoka disease, economic loss and its control measures. However, information on the anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease from India is scanty. Hence the present investigation was undertaken with a view to throw light on anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease.

Studies on the seasonal influence of disease incidence revealed that intensity of disease varied with seasons. Maximum incidence of disease was observed during July 2000 because of heavy rainfall and high relative humidity prevalent at that period. Moreover, this is the peak period of monsoon showers which coincides with the reproductive phase of the plant influencing the physiological changes and making the plant more vulnerable to infection. This is often enhanced by the presence of moisture seen on the leaves through out the season favouring the ascospore development and germination. Decrease in disease incidence during January, 2001 is due to high temperature and low relative humidity prevalent at that period which coincides with the vegetative phase of the plant. This condition is quite unfavourable for the spore germination and dispersal in contrast to the condition of monsoon season. The findings of Mourichon and

Zapater (1990), Ploetz and Sauco (1998) and Torres *et al.* (2000) were also in line with these observations.

Ascospores of *M. musicola* were hyaline, two celled with one cell bigger than the other and with a slight constriction at the septum. Conidia of *M. musicola* were hyaline to olivaceous brown, straight to variously curved, cylindric with a round base, smooth walled, unthickened hilum with 5-6 septa. The size and shape of conidia were conformed to the description of *M. musicola* (Mulder and Holliday, 1974).

Initially, aerial mycelia were whitish and later turned whitish gray colonies which were compact, raised, hemispherical with irregular zonation, a non-circular margin with a velvety surface. Hyphal cells of *M. musicola* were dumb-bell shaped. Poor sporulation of *M. musicola* were observed on banana dextrose agar after seven to ten days incubation at 22-25°C under continuous light. The cultural characters of *M. musicola* resembled with those reported by Meredith and Butler (1939), Stover (1976) and Natural (1989).

Pigmentation of the fungal culture was observed on all agar media tried. Maximum growth of *M. musicola* was reported in the media PGYEA and CzYEA followed by CGYEA and BnDA. Minimum growth was observed in MEA and SmGYEA. Meredith (1970) noted the accumulation of dark, melanin - like yellow or pink pigments in agar beneath and around the colony. Natural (1989) also reported that pigmentation of *M. musicola* was observed in media viz., PDA, CGYEA, CzYEA and SmGYEA and occurred 7-14 days after incubation and pigmentation disappeared when the colonies were matured.

Anatomical studies on leaves of *Musa spp.* revealed that there existed variation among the four different groups of banana with regard to various parameters studied. The resistant and susceptible groups of *Musa* spp. also showed

differences in the various anatomical features.

The resistant variety Manoranjitham (AAA) was characterised by thickest cuticle and epidermis on the adaxial surface. Also possessed thinner spongy and palisade tissues, maximum number of large and closely spaced vascular bundles, thickest lower epidermis and thicker pattern of epicuticular wax deposition on abaxial surface and less number of stomata per unit area both on the adaxial and abaxial surfaces of leaves.

The moderately resistant variety Thiruvananthapuram (AAB) possessed thicker cuticle, thinner and small epidermal cells on the adaxial surface, thicker spongy and thinner palisade tissues, more number of large and closely placed vascular bundles, thickest lower epidermis and thicker pattern of epicuticular wax deposition on the abaxial surface and less number of stomata per unit area both on the adaxial and abaxial surfaces of leaves.

The susceptible variety Nendran (AAB) was characterised by medium thickened cuticle, thinner with intermediate sized epidermal cells on the adaxial surface, thinner spongy and palisade tissues, less number of small and distantly placed vascular bundles, thinner lower epidermis and faint pattern of epicuticular wax deposition on the abaxial surface and more number of stomata both on the adaxial and abaxial surfaces of leaves.

The highly susceptible variety Grand Naine (AAA) characterised by thinner cuticle, thickened and large sized epidermal cells on the adaxial surface, less number of small and distantly placed vascular bundles, thicker spongy and palisade tissues and faint pattern of epicuticular wax deposition on the abaxial surface and more number of stomata both on the adaxial and abaxial surfaces of leaves.

Since, the entry of the pathogen is mainly through lower epidermis, the thicker epidermis present in the resistant variety restrict the entry of the pathogen. Regarding the stomatal density, the fungus or pathogen penetrates only through stomata, whereas the high stomatal density leads to greater susceptibility to the disease. Like wise, the low stomatal density limited the entry of the pathogen which could be responsible for its higher degree of resistance to the disease. The thickness of lower cuticle, size and number of vascular bundles also help to check the further spread of pathogen in the leaf tissues.

The thicker lower epiderm with fewer number of stomata and dense patterns of epicuticular wax deposition prevent the entry of pathogen in resistant varieties viz., Manoranjitham (AAA) and Thiruvananthapuram (AAB) whereas the thinner lower epiderm with more number of stomata and light pattern of epicuticular wax deposition make the varieties viz., Nendran (AAB) and Grand Naine (AAA) more susceptible to the pathogen. The large and closely placed vascular bundles with increased number were also responsible for imparting resistance whereas small and distantly placed vascular bundles with less numbers favour the susceptibility.

Similar structural differences in leaf anatomy were observed by Kaur *et al.* (1992) in groundnut against tikka leafspot, Mayee and Apet (1995) against rust disease in groundnut and Dagade (1999) in *Piper* spp. against foot rot of pepper.

The presence of epicuticular wax was also involved in host response to the fungus. Under field conditions, the primary leaf and the leaf in position one (leaves with incomplete wax deposition) are generally more susceptible to the disease than older leaves (Vasquez *et al.*, 1989).

The present findings were supported by Singh *et al.* (1999) and reported that epicuticular wax deposition on leaf contributed significantly to disease resistance in rape and mustard against *Alternaria brassicae*.

Comparison of anatomical parameters of healthy and yellow sigatoka infected leaves of *Musa* spp. was made to study the anatomical changes in leaves due to pathogen infection.

Palisade and spongy mesophyll areas showed necrosis after infection with the pathogen in all the four different groups of banana studied. The vascular bundles were seen healthy in the resistant variety whereas partial necrosis was seen in the moderately resistant variety. But in the case of susceptible and highly susceptible varieties necrotic vascular bundles were observed.

So, it can be concluded that the necrotic vascular bundles might be responsible for the further spread of the pathogen in the leaf tissues where the tissues were extremely colonized by the mycelium after infecting the susceptible varieties. The healthy vascular bundles present in the resistant variety Manoranjitham (AAA) after infection with the pathogen may be responsible for its resistance, since the mycelium development was very slow and also the fungal development was restricted to few cells of mesophyll.

Similar findings were also reported by Lazorovits and Higgins (1976) in the susceptible and resistant varieties of tomato.

Defense mechanisms are the attributes of the hosts that reduce the chances of infection or the development of the pathogen. Biochemical defense mechanism is more important and common for resisting the invasion by plant pathogens. The biochemical defense mechanism may consist of the presence or absence of a particular chemical substance or a group of substances in a host plant,

which interferes with the growth and multiplication of the pathogen. By biochemical reactions and conditions the host inactivates the pathogen or its toxins or kills it before infection. Several chemicals of plant origin act as defensive barrier against the pathogen. Therefore it is imperative to know the role and functions of preformed biochemical compounds viz., total phenol, OD phenol, sugars, proline, enzymes like peroxidase and polyphenol oxidase and protein in different groups of banana. The total phenol content showed slight variation in the four different groups of banana studied. The resistant variety Manoranjitham (AAA) possessed higher quantity of total phenol than the other three varieties. The higher content of total phenol observed in the resistant variety compared to moderately resistant and susceptible varieties suggested the role of total phenol in disease resistance.

The role of phenolics in imparting resistance in host plants against the pathogen have been reported by many scientists. Phenolics are well known antifungal, antibacterial and antiviral compounds which inhibit fungal spore germination and production of enzymes (Vidhyasekaran, 1975), mycelial growth (Tourneau *et al.*, 1957), fungal enzymes (Vidyasekaran, 1975) and toxin production by pathogens (Tamari and Kaji, 1955).

In the present investigations the resistance obtained in the resistant variety Manoranjitham (AAA) may be due to higher content of phenol and the susceptibility of Grand Naine (AAA) may be due to lower phenol content. Similar findings on the higher content of total phenol in resistant varieties had been reported in Kattuvazhai against *Cercospora musae* (Jayapal and Mahadevan, 1968 and Salle *et al.*, 1989).

OD phenols play an important role in disease reactions and are easily oxidised by enzymes like polyphenol oxidase and peroxidase to form *ortho*

quinones (Bell, 1981). But high levels of dihydroxy phenols are desirable for resistance and but the phenols still must be released and oxidised effectively.

The present findings point to the fact that the higher quantity of OD phenol present in the resistant variety Manoranjitham (AAA) may be responsible for its resistant disease reaction. Moreover the higher content of OD phenol present in the resistant varieties may be due to its ability to with stand stress conditions. The highly susceptible variety Grand Naine (AAA) recorded lower quantity of OD phenol in all the portions except in the midrib portion studied. The break down of phenolic compounds was rapid in the infected cultivar while in the resistant one, the degradation of phenolic compounds was little.

Accumulation of phenolic compounds including OD phenols in host parasite interaction is a general phenomenon of disease resistance where as the rate of accumulation and break down of phenolic compounds determine the degree of disease resistance (Farkas and Kiraly, 1962 and Mahadevan, 1966).

Jayapal and Mahadevan (1968) also reported that high quantities of OD phenol was observed in the leaves of Kattuvazhai which is resistant to *Cercospora musae* than the susceptible variety Monthan.

Higher quantity of OD phenol had been reported in resistant varieties of chilli against bacterial wilt by Markose (1996) and in tomato by Paul (1998).

Co-existence of phenols and sugars results in glycolysation forming phenolic glycosides which are more soluble in cell sap and thus involved in disease resistance reaction more effectively (Walker, 1975).

Sugars are precursors for synthesis of phenolics, phytoalexins, lignin and callus and have an important role in defence mechanisms of plants

(Vidhyasekaran, 1975). Horsfall and Diamond (1957) assigned a major role for sugars in disease resistance.

The susceptible and highly susceptible varieties like Nendran (AAB) and Grand Naine (AAA) had significantly higher quantity of reducing sugar compared to moderately resistant and resistant varieties like Thiruvananthapuram (AAB) and Manoranjitham (AAA). The highly susceptible variety Grand Naine (AAA) possessed significantly higher content of reducing sugar in all the portions of leaf except in the midrib portion. Lower amount of reducing sugar was present in the leaves of moderately resistant variety Thiruvananthapuram (AAB) and minimum in the resistant variety Manoranjitham (AAA) except in the midrib portion of leaf. The higher quantity of reducing sugar present in the leaves of susceptible and highly susceptible varieties might be responsible for the pathogen development and hence the susceptibility. But the resistant and moderately resistant varieties had lower quantity of reducing sugar content compared to the susceptible varieties.

Raghunathan *et al.* (1959) reported that banana leaves infected with gloeosporium disease had more soluble sugars than the resistant varieties.

Sugars especially reducing sugars are the most suitable and preferred nutrient for the pathogen. In the present investigation, since the resistant variety Manoranjitham (AAA) had lower amount of reducing sugar, chances of development and spread of the pathogen was less when compared to highly susceptible Grand Naine (AAA). This implies that the rate of pathogen multiplication would be less in these resistant varieties. In contrast, the susceptible varieties had higher amount of reducing sugar which made possible for the fungus to build up its high level of inoculum.

The resistant and moderately resistant varieties possessed sufficiently higher content of non reducing sugar in all the portions of the leaf studied. The content of non reducing sugar was lower in all the portions of susceptible and highly susceptible varieties. The higher quantity of non reducing sugar present in the resistant varieties might be responsible for the resistant mechanisms against the pathogen. The resistant varieties possessed higher concentrations of non reducing sugars which could inhibit pathogens by blocking enzyme synthesis (Bateman and Millar, 1966). Jayapal and Mahadevan (1968) noticed that more quantity of non reducing sugar was present in the leaves of leaf spot resistant variety Kalluvazhai than the susceptible variety Monthan to *Cercospora musae* Zimm.

The maximum total sugar content was observed in resistant variety Manoranjitham (AAA) followed by moderately resistant variety Thiruvananthapuram (AAB) and highly susceptinble variety Grand Naine (AAA) and minimum in susceptible variety Nendran (AAB). Among the different portions of leaf analysed, centre portion of the leaf possessed higher quantity of total sugar compared to other portions. Similar observations was made by Jayapal and Mahadevan (1968) in banana leaves affected by *Cercospora musae*.

Amino acids serve as the corner stones for the synthesis of proteins and some are essential for the production of toxins (Tanaka, 1963) and the synthesis of phenolics, phytoalexins and lignin (Vidhyasekaran, 1990).

Proline is a basic amino acid found in higher percentage in basic proteins. A several fold increase in the proline content was reported under physiological and pathological stress conditions.

The proline content differed significantly in all the portions of leaf in the four different groups of banana studied. Higher quantity of proline was noticed in the leaves of the susceptible variety Nendran (AAB) and minimum in the highly

susceptible variety Grand Naine (AAA). The quantity of protein in the resistant and moderately resistant varieties viz., Manoranjitham (AAA) and Thiruvananthapuram (AAB) were on par. Considering the above facts higher quantity of proline in the susceptible variety Nendran (AAB) may be due to its response to drought and salinity stress. This higher quantity of proline accumulated in the susceptible variety Nendran (AAB) will help the plant to withstand the drought and salinity stress. The lower quantity of proline present in the highly susceptible variety Grand Naine (AAA) may be responsible for its susceptibility to drought and salinity stress.

Zheng and Li (2000) suggested that in response to drought and salinity stress many plant species including soyabean accumulation of high content of proline occurred which is thought to function in stress adaptation.

Host enzymes like polyphenol oxidase and peroxidase are frequently correlated with expression of disease resistance. These enzymes oxidise phenolics to quinones and these quinones are more fungitoxic than phenolics. Hence, sometimes the increased activity of these enzymes may be responsible for disease resistance.

Peroxidase is a key enzyme involved in many biochemical pathways including the phenol and lignin metabolism and it oxidises phenolics into quinones which are more fungitoxic.

Higher activity of peroxidase was observed in all portions of leaves in the resistant varieties like Manoranjitham (AAA) and Thiruvananthapuram (AAB) except in the midrib portions of the leaf. The highly susceptible variety Grand Naine (AAA) possessed medium values for the activity of peroxidase enzyme, except in the midrib portion. Lower activity of peroxidase enzyme was observed in the susceptible variety Nendran (AAB) except in the midrib portion. The

susceptible and highly susceptible varieties possessed higher activity of peroxidase enzyme in the midrib portion of the leaf compared to the resistant varieties.

From the above findings it is clear that higher levels of peroxidase enzyme activity present on all portions of leaves of the resistant varieties of *Musa* spp. might be involved in defence mechanism either by forming toxic compounds with polyphenol oxidase and OD phenol or by promoting process of lignification or by melanin synthesis.

Thus in the present study, correlation between higher levels of total phenol, peroxidase and lower levels of OD phenol and polyphenol oxidase were obtained.

Alcazar *et al.* (1995) reported that peroxidase activity was found lesser in the intercellular fluid of susceptible capsicum cultivars than moderately susceptible and resistant cultivars to *Phytophthora capsici.* Higher activity of peroxidase in resistant host plants had been reported by Markose (1996) in chilli against bacterial wilt and by Paul (1998) in brinjal.

The enzyme, polyphenol oxidase catalyse the oxidative polymerisation of phenolic compounds to quinones and tannins more readily than any other enzymes. These quinones were found more fungitoxic and bactericidal (Vidyasekaran, 1990). The activities of these enzyme were important with regard to plant defence mechanisms against pathogen.

Higher activity of polyphenol oxidase was found in the susceptible variety Nendran (AAB) in almost all portions of leaves except midrib portion, followed by the resistant variety Manoranjitham (AAA). The lower activity of polyphenol oxidase was found in the moderately resistant variety Thiruvananthapuram (AAB).

Enzyme activities in different portions of leaves of different groups of banana were compared and the results revealed that the basal portion showed higher enzyme activity followed by the centre and the tip portion. Considering the air borne nature of the pathogen and main site of infection, enzyme activity in different portions of leaves play an important role in disease resistance.

Here in this study, higher polyphenol oxidase activity was seen in the susceptible variety Nendran (AAB). During the host pathogen interaction, toxin or some other related compounds of high concentrations were formed which were found more toxic to host cell themselves than pathogen (Tepper and Anderson, 1984). But in the case of resistant variety, the host pathogen interaction was less, where the synthesis of phenolics was activated after infection and high quantity of phenolics were synthesised which rapidly suppressed the pathogen development (Vidyasekaran, 1990). Phenolic compounds act as hydrogen acceptor/donor in oxidation reduction reaction to form quinones which are involved in resistance in plants due to their higher toxicity to pathogen (Barnett, 1959).

Higher levels of polyphenol oxidase in resistant varieties had been reported by Gupta *et al.* (1995) in *Brassica* species against alternaria leaf blight, Markose (1996) in chilli against bacterial wilt, Paul (1998) in brinjal and tomato against bacterial wilt.

Protein content was found higher in the resistant variety Manoranjitham (AAA) and susceptible variety Nendran (AAB) followed by moderately resistant variety Thiruvananthapuram (AAB). Lower quantity of protein was present in the highly susceptible variety Grand Naine (AAA). Among the different portions of leaves studied, protein content was obtained maximum in the tip portion followed by the basal and edge portions.

Higher level of protein present in the variety Manoranjitham (AAA) can provide enough protection against the pathogen. The higher content of protein in the variety Nendran (AAA) might be responsible for preventing it from higher susceptibiliy to the disease whereas lower content in Grand Naine decide its higher susceptttibility.. Enhanced protein synthesis appears to be a universal phenomenon in incompatible host pathogen (resistant) interaction. (DeWit and Bakkar, 1980, Ahmed *et al.*, 1994 and Jiang *et al.*, 1994). De novo synthesis of new proteins has also been reported by Tani and Yamamoto (1979) and Yamamoto and Tani (1982). The synthesised protein may not be inhibitory to the pathogen, but they may activate the synthesis of defence chemicals such as phenolics, lignins, phytoalexins etc.

The resistant varieties achieve the resistance response through several defence mechanisms and the events governing resistance are associated with regulatory loci in the host that control expression of the structural genes functioning in the resistance response. Activation of the appropriate regulatory loci would trigger the expression of many structural genes associated with resistance (Tepper and Anderson, 1984).

The phenols are considered to play an important role in host resistance. However, it seems that phenols as such are not directly involved in host resistance, it is the derivatives of phenols which act against the pathogen. Phenol accumulation often present mostly in the infection site and also in the surrounding zone. The accumulation of phenolics as an initial response to infection may show a general increase in host metabolism as well as accumulation of relatively non toxic secondary metabolites, which ultimately serve as precursors of compounds for resistance reaction (Nicholson and Hammer - Schmidit, 1992).

After pathogen invasion, the total phenol content showed a wide variation in all the portions of the leaf in the different groups of banana studied.

The content of total phenol was found increased in the varieties belonging to the same genome Thiruvananthapuram (AAB) and Nendran (AAB). In contrast, a decrease in the content of total phenol was observed in all the portions of leaf in the resistant variety Manoranjitham (AAA) except in the midrib portion of the leaf. The highly susceptible variety Grand Naine (AAA) showed a fluctuation in the total phenol content in different portions of the leaf analysed. In Grand Naine (AAA) the edge and midrib portions of the leaf showed a reduction in phenol content whereas the other portions of the leaf showed an increase in the total phenol content. The decrease in total phenol content after pathogen invasion in the resistant variety Manoranjitham (AAA) might be responsible for checking spot development. Tepper and Anderson (1984) noticed that phenolics in high concentrations are toxic to plant cell themselves and hence will normally be present in small quantities.

In moderately resistant variety Thiruvananthapuram (AAB) and susceptible variety Nendran (AAB) spot development increased rapidly after pathogen invasion and might be due to the increased amount of total phenol which was produced after infection. The decreased content of phenol in the midrib portion of the leaf after infection with the pathogen was observed only in the highly susceptible variety Grand Naine (AAA), whereas this variety showed an increased content of total phenol in all the other portions of leaf. The highly susceptible nature of the variety Grand Naine (AAA) may be due to the higher quantity of total phenol after infection with the pathogen. The higher concentration of phenol which are cytotoxic might be responsible for cell necrosis and cell death in Nendran (AAB) and Grand Naine (AAA) and thereby its hypersensitive reaction to the disease.

Markose (1996) reported higher quantity of total phenol in chilli varieties susceptible to bacterial wilt and Dagade (1999) in pepper cultivars susceptible to foot rot disease of pepper.

Orthodihydric (OD) phenol play an important role in disease resistance reactions. They are easily oxidised by enzyme viz., polyphenol oxidase and peroxidase and the resulting orthoquinones are highly reactive and toxic to pathogen and their enzymes (Mahadevan, 1966). Certain dihydroxy phenols get conjugated with each other or glucose hydroxyl group to form tannins, both form constituent of plant melanins (Beckman *et al.*, 1974; Mayer and Harel, 1979 and Bell, 1981). These tannins and ortho quinones have toxicity to microorganisms (Hunter, 1978).

After infection with the pathogen, the OD phenol content increased significantly in the resistant variety Manoranjitham (AAA). Maximum reduction in the OD phenol content after infection with the pathogen was observed in the highly susceptible variety, Grand Naine (AAA) followed by susceptible variety Nendran (AAB). In the moderately resistant variety, Thiruvananthapuram (AAB), a decrease in the content of OD phenol was observed in the centre portions of the leaf.

Similar to the present study Jayapal and Mahadevan (1968) reported that inoculation of *Cercospora musae* resulted in a rapid increase in the content of OD phenol in the resistant leaves of variety Kattuvazhai which gradually decreased with limitation of the lesion. Similarly flag smut resistant wheat varieties had higher content of OD phenol, but on inoculation with fungi its level increased further (Sindhan *et al.*, 1996).

Sugars are the preferred nutrients for the pathogen and they are the precursors for the phenol synthesis. The preferential utilisation of sugars by pathogen will result in non-production of pectic and cellulolytic enzymes to degrade the cell wall barrier (Vidhysekaran, 1990).

The amount of reducing sugar was found low in most portions of the leaf in the resistant variety, Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB) and susceptible variety, Nendran (AAB). In the highly susceptible variety, Grand Naine (AAA) the reducing sugar content increased in the leaf after infection.

The decrease in sugar content observed in resistant variety Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB) might be due to its conversion to polyphenols, since sugars are the precursors for phenol synthesis. Sugars are the preferred nutrient for the development of pathogen and the decrease in sugar content observed in resistant variety Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB) might be the reason for their resistance to the pathogen.

At the same time, the increase in reducing sugar content observed in the highly susceptible variety Grand Naine (AAA) after infection might be responsible for its higher susceptibility by providing sufficient nutrients for the pathogen.

Prasada *et al.* (1972) observed reduction in the total sugar in bacterial leaf blight resistant rice varieties inoculated with pathogen. Reduction in sugar content had been reported by Sindhan *et al.* (1996) in flag smut resistant wheat varieties. Dagade (1999) noticed that the reducing sugar content increased in the susceptible variety Panniyur 1 and found low in the tolerant/immune varieties like Kalluvally and *Piper colubrinum*.

The quantity of non reducing sugar decreased significantly in almost all portions of the leaf in all the varieties studied after pathogen infection. Higher quantity of non reducing sugar content was noticed in the resistant variety, Manoranjitham (AAA) followed by moderately resistant variety,

Thiruvananthapuram (AAB), highly susceptible variety, Grand Naine (AAA) and susceptible variety, Nendran (AAB). Comparing the different portion of yellow sigatoka infected leaves, the edge and the middle portions showed maximum values.

Increased sugar content may enhance the synthesis of phenolics (Vidhyasekaran, 1974) and other defense chemicals (Emmanouil and Wood, 1981). The co-existence of phenols and sugars results in glycolysation of phenols by sugars forming phenolic glycosides which are more soluble in cell sap and thus involved more efficiently in resistance expression (Walker, 1975). The carbohydrates may also be utilised for meeting the energy requirement of host plant due to increased respiration. The decrease in the sugar content observed in these varieties can be attributed to the fact that a major part of these sugars being shifted for polyphenol synthesis as reported by Niesh (1964).

Similar to the present investigation, Abraham (1986) observed that the resistance of betelvine cultivars to bacterial leaf spot pathogen was due to pre-inoculation higher levels of reducing, non reducing and total sugars and post-inoculation increase of non reducing sugar in susceptible cultivars. Dagade (1999) reported non reducing sugar content decreased in the foot rot infected leaves of the different varieties of *Piper* spp.

A reduction in the quantity of proline was observed in all the groups of banana analysed except in the highly susceptible variety, Grand Naine (AAA). The proline content was found high in healthy leaves of susceptible variety, Nendran (AAB) and resistant varieties viz., Manoranjitham (AAA) and Thiruvananthapuram (AAB) respectively. A drastic fluctuation was seen after the infection with the pathogen. The highly susceptible variety, Grand Naine (AAA) possessed minimum quantity of proline in healthy leaf. But the content of proline

was increased significantly after infection with the pathogen.

Increase in amino acid content may be due to denaturation of host protein or decreased protein synthesis. Synthesis of amino acid by the growth of the pathogen caused an increase in amino acid content and the decrease in amino acid content might be due to pathogen utilisation (Andel, 1965).

Chile and Vyas (1983) reported that amino acid content decreased in less susceptible cultivar of betelvine due to inoculation of phytophthora leaf rot pathogen.

All enzymes are protein in nature and catalyse all the inter related reactions in a living cell. Most of the plant pathogen secrete enzymes through out their existence or upon contact with a substrate.

Peroxidase catalyses the dehydrogenation of a large number of compounds like phenol, aliphatic acid and aromatic amines etc. By the action of this enzyme phenol was converted to quinones and they are found more fungitoxic than phenols.

After pathogen infection, the activity of peroxidase enzyme showed greater variation in different groups of banana. In the resistant variety Manoranjitham (AAA) and moderately resistant variety Thiruvananthapuram (AAB) the activity of peroxidase enzyme was less whereas the susceptible variety, Nendran (AAB) exhibited a drastic increase in the enzyme activity.

The higher activity of resistant variety, Manoranjitham (AAA) exhibit the role of enzyme in imparting resistance to *M. musicola*. The enzyme peroxidase promotes IAA activity which lead to cell wall thickening and cell multiplication and in conjugation with OD phenol and polyphenol oxidase form toxic compounds viz., lignin, tannin and quinone which are toxic to the pathogen. The very same

observations can be exemplified by lower content of OD phenol and polyphenol oxidase in diseased plants under the present investigation.

Higher activity of peroxidase in various host plants after infection can substantiate the present findings. Lizzi and Coulomb (1991) reported higher activities of peroxidase and catalase in capsicum leaves inoculated with *P. capsici* and accumulation of higher concentration of enzyme could be lead to the destruction of the pathogen. Paul (1998) also reported higher activity of peroxidase enzyme in brinjal under diseased condition.

Polyphenol oxidase is the key enzyme involved in disease resistance and its level depend upon shifting of sugars for its synthesis and its polymerization capacity depends on levels of dihydroxy phenol and peroxidase.

The activity of polyphenol oxidase enzyme was found high in most portions of the leaf after infection with the pathogen in the four different groups of banana studied. The susceptible variety, Nendran (AAB) possessed higher activity of polyphenol oxidase followed by resistant variety, Manoranjitham (AAA) and highly susceptible variety, Grand Naine (AAA). Less activity of polyphenol oxidase was observed in the moderately resistant variety, Thiruvananthapuram (AAB).

Steady increase in the activity of polyphenol oxidase enzyme indicated that there was continuous production of toxic compounds like quinone and promotion of plant growth by auxins and the steady decrease in enzyme activity may be due to inhibition in production of toxic compounds in course of plant growth.

In tolerant *Brassica carinata* and *B. napus* the specific activity of polyphenol oxidase remain higher while that of peroxidase remained lower as

compared to *B. juncea* and *B. campestris,* but after infection with alternaria leaf blight, activity of both enzymes increased comparatively at much faster rate in the susceptible species than resistant ones. Gupta *et al.* (1995) observed that the activity of polyphenol oxidase was considerably higher at initial stages of plant growth in all species which markedly dropped at later stages.

The findings from the present investigation point to the fact that there exists a negative correlation between peroxidase and polyphenol oxidase enzyme activities. In conclusion to the experiment conducted by Rich and Horsfall (1954) proved that susceptibility of fungi to the phenolics seemed to be related to the quantity of polyphenol oxidase contained in the material.

A reduction in the quantity of protein was observed in most portions of the leaf after infection with the pathogen in the four different groups of banana under observation. But the susceptible variety, Nendran (AAB) showed an increase in the amount of protein in all the portions of the leaf except in the midrib portion.

Trandafirescu *et al.* (1999) observed a direct relationship between the decrease in content of amino acids and crude protein in the leaves and the resistance of six apricot cultivars by *Stereum purpureum*. Sharma and Kaul (1999) noticed higher quantities of total and soluble protein content in young expanding leaves of apple in the susceptible cultivars to scab, where the protein content increased after inoculation and during pathogenesis.

Toxins are one of the important components of the sigatoka disease syndrome (Natural, 1989). Many *Cercospora* species that are plant pathogenic produce phytotoxic metabolites (Sasaki *et al.*, 1981, Daub, 1982).

M. musicola produced red pigments in all the media tried. The media that produced the largest bands on TLC plates was considered to be the best

medium which supported toxin production. For the sigatoka pathogens few studies of phytotoxins have been conducted (Molina and Krausz, 1987). Pigments in culture can be used for extraction of toxic metabolite. In the present study, PGYEA found to be the best media followed by SmGYEA, PDA, CzYEA and CGYEA which showed best resolution of toxic bands and greater production of toxic metabolites. Temperature and light were found to have very important role in the optimum production being found in cultures incubated at 22-25°C under continuous light.

PDA has been reported as the most commonly used media for *Cercospora* spp. and is also suitable for toxin production (Assante *et al.*, 1977, Fajola, 1978). CzYEA was observed to induce cercosporin production by *C. personata* (Venkataramani, 1967).

From these findings it is evident that histological and biochemical changes observed in *Musa* spp. can effectively be used for screening the varieties against yellow sigatoka leaf spot (*M. musicola*). Such information could be used in the selection of parents in further breeding programmes and also in micro propagation techniques.

Moreover, keeping in account of the biochemical changes obtained after fungal infection, possibility of evolving a management strategy for the control of the disease cannot be ruled out.

Summary

SUMMARY

Banana is the major fruit crop of Kerala State commonly and severely affected by Yellow sigatoka leaf spot disease (*Mycosphaerella musicola* Leach) causing a great loss to the yield. All varieties of commercial importance are susceptible to yellow sigatoka leaf spot. Though many studies have been conducted on banana, only little information is available on the anatomical and biochemical bases of resistance in this crop to yellow sigatoka leaf spot.

In this background, a research project was designed and carried out to study the anatomical and biochemical aspects of resistance in banana to yellow sigatoka disease. Based on the observations, it may be easy to locate or identify a resistant variety, which can be used in breeding programme as well as for the micropropagation techniques.

The studies included the seasonal influence on disease incidence at three months interval, cultural and morphological characters of *M. musicola*, anatomical and biochemical features of healthy and yellow sigatoka infected leaves.

The major findings of the study were as follows:

1. Incidence of yellow sigatoka leaf spot disease was recorded at three months interval and observed maximum in the peak monsoon month of July followed by October and April and minimum in January.

2. Meteorological data recorded and correlated with disease incidence showed a positive correlation with relative humidity and rainfall and negative correlation with temperature.

3. Pathogen *M. musicola* was isolated by ascospore isolation and spore pick technique.

4. *M. musicola* was grown on different media viz., potato dextrose agar (PDA), potato glucose yeast extract agar (PGYEA), synthetic medium plus glucose yeast extract agar (SmGYEA), Czapeck's yeast extract agar (CzYEA), coconut glucose yeast extract agar (CGYEA), malt extract agar (MEA) and banana dextrose agar (BnDA).

5. Maximum growth of *M. musicola* was observed in the media PGYEA andCzYEA followed by BnDA and CGYEA and minimum in SmGYEA.

6. Sparse growth of mycelium and poor sporulation were seen on BnDA seven to ten days after incubation at 22-25°C under continuous light. Sporulation was not obtained on any other media tried.

7. Red pigmentation was observed in all the media tried.

8. Hyphal cells of *M. musicola* were dumb bell shaped. Aerial mycelia were whitish and later turned grey. Colonies were compact, raised, hemispherical with irregular zonation, a non-circular margin and a velvety surface.

9. Ascospores of *M. musicola* were hyaline, two celled with one cell bigger than the other and with a slight constriction at the septum. Conidia were hyaline to olivaceous brown, straight to variously curved, cylindric with a round base, smooth walled, unthickened hilum with 5-6 septa.

10. The resistant variety, Manoranjitham (AAA) is characterised by thickest cuticle and epidermis on the adaxial surface and denser pattern of epicuticular wax deposition on the abaxial surface. Thinner spongy and palisade tissues, maximum number of large and closely placed vascular bundles, thickest lower epidermis and decrease in number of stomata/unit area on the abaxial and adaxial surfaces of leaves.

11. The moderately resistant variety, Thiruvananthapuram (AAB) possessed thicker cuticle, thinner and small epidermal cells on the adaxial surface and denser pattern of epicuticular wax deposition on the abaxial surface. Thicker spongy and thinner palisade tissues, more number of large and closely placed vascular bundles, thickest lower epidermis and decrease in number of stomata/unit area on the adaxial and abaxial surfaces of leaves

12. The susceptible variety, Nendran (AAB) was characterised by medium thickened cuticle, thinner with intermediate sized epidermal cells on the adaxial surface, faint pattern of epicuticular wax deposition on the abaxial surface. Thinner spongy and thicker palisade tissues, less number of small and distantly placed vascular bundles, thinner lower epidermis and maximum number of stomata/unit area on the adaxial and abaxial surfaces of leaves.

13. The highly susceptible variety, Grand Naine (AAA) was characterised by thinner cuticle, thicker and large sized epidermal cells on the adaxial surface and faint pattern of epicuticular wax deposition on the abaxial surface. Less number of small and distantly placed vascular bundles, thicker spongy and palisade tissues and maximum number of stomata/unit area on the abaxial and adaxial surfaces of leaves.

14. After infection with the pathogen, the resistant variety, Manoranjitham (AAA) showed slight disintegration of spongy mesophyll tissues and moderately resistant variety, Thiruvananthapuram showed partial disintegration of spongy mesophyll and necrosis of vascular bundles. The susceptible variety, Nendran (AAB) and highly susceptible variety, Grand Naine (AAA) showed complete disintegration of chloroplast and mesophyll with necrotic vascular bundles.

15. Stomata were found opened during day time and closed during night hours in both healthy and diseased leaves of all *Musa* spp. studied.

16. In the biochemical parameters studied, resistant variety, Manoranjitham (AAA) possessed higher quantities of total phenol, OD phenol, non reducing and total sugars, protein and peroxidase activity and intermediate values for proline and polyphenol oxidase enzyme activity and lower quantity of reducing sugar.

17. The moderately susceptible variety, Thiruvananthapuram (AAB) showed intermediate values for protein, reducing and non reducing and total sugars, activity of peroxidase and proline and lower quantity of total phenol, OD phenol and polyphenol oxidase activity.

18. The susceptible variety, Nendran (AAB) showed higher quantity of proline, protein, polyphenol oxidase activity and intermediate values for total phenol, reducing sugar and lower quantity of non reducing and total sugars, OD phenol and peroxidase activity.

19. The highly susceptible variety, Grand Naine (AAA) was characterised by higher quantity of reducing sugar and intermediate values for total phenol, non reducing and total sugars and lower quantities of proline, protein and OD phenol and peroxidase and polyphenol oxidase activities.

20. After infection with pathogen, higher quantity of OD phenol, reducing sugar and polyphenol oxidase activity was observed in the resistant variety, Manoranjitham (AAA) whereas total phenol, non reducing and total sugars, protein, proline and activity of peroxidase found decreased.

21. In moderately resistant variety, Thiruvananthapuram (AAB) the quantity of OD phenol, total phenol, reducing sugar and activity of polyphenol oxidase found increased while non reducing and total sugars, proline, protein and activity of peroxidase and polyphenol oxidase decreased.

22. The content of total phenol, OD phenol, protein. activity of peroxidase and polyphenol oxidase enzyme showed a marked increase in the susceptible variety, Nendran (AAB) while reducing, non reducing and total sugars and proline content found decreased.

23. The highly susceptible variety, Grand Naine (AAA) exhibited an increase in total phenol, reducing sugar, proline and activities of peroxidase and polyphenol oxidase and decrease in OD phenol, non reducing and total sugars and protein content.

24. Toxic metabolite was extracted from the culture of *M. musicola* and thin layer chromatograms were developed. The concentrated culture extracts of seven media gave only one band of different Rf values.

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ANATOMICAL AND BIOCHEMICAL BASES OF RESISTANCE IN BANANA TO YELLOW SIGATOKA LEAF SPOT DISEASE

By

V.K. SAIRA BANU

ABSTRACT OF THE THESIS

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Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA

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ABSTRACT

Banana is an important fruit crop of Kerala, which is commonly infected by yellow sigatoka leaf spot disease caused by *Mycosphaerella musicola* Leach.

Investigations on anatomical and biochemical bases of resistance in banana to yellow sigatoka leaf spot disease were carried out in the Dept of Plant Pathology and Biochemistry Laboratory of the College of Horticulture, Vellanikkara during 2000-2001.

Severity of sigatoka disease was observed maximum in the peak monsoon month of July (cool moist period) followed by October (warm moist period) and April (warm dry period) and minimum in January (cool dry period).

Maximum growth of *M. musicola* was obtained in the medium potato glucose yeast extract agar (PGYEA) and poor sporulation in the medium banana dextrose agar (BnDA). Red pigmentation was observed in all media tried.

The study revealed that the four different groups of banana differed significantly in the various anatomical and biochemical parameters.

The resistant variety, Manoranjitham (AAA) was characterised by thickest cuticle and epidermis with inter mediate sized epidermal cells on the adaxial surface and denser pattern of epicuticular wax deposition on the abaxial surface of leaves. Thinner spongy and palisade tissues, maximum number of large and closely placed vascular bundles, thickest lower epidermis and decrease in number of stomata/unit area on the adaxial and abaxial surfaces of leaves.

The highly susceptible variety, Grand Naine (AAA) showed thinner cuticle, thickened and large sized epidermal cells on the adaxial surface and faint pattern of epicuticular wax deposition on the abaxial surface of leaves. Less number of small and distantly placed vascular bundles, thicker spongy and palisade

tissues and maximum number of stomata /unit area on the adaxial and abaxial surfaces of leaves.

After pathogen infection the resistant variety Manoranjitham (AAA) showed partial disintegration of spongy mesophyll tissues and the highly susceptible variety Grand Naine (AAA) showed complete disintegration of chloroplast and mesophyll with necrotic vascular bundles.

Stomata was found opened during day time and closed during night hours in both healthy and diseased leaves.

In the biochemical parameters studied, the resistant variety, Manoranjitham (AAA) possessed higher quantities of total phenol, OD phenol, non reducing and total sugars, protein and peroxidase activity and intermediate values for proline and activity of polyphenol oxidase and lower quantity of reducing sugar.

The highly susceptible variety, Grand Naine (AAA) was characterised by higher quantities of reducing sugar and intermediate values for total phenol, non reducing and total sugars and lower quantity of proline, protein, OD phenol and peroxidase and polyphenol oxidase enzyme activities.

After pathogen infection, higher quantity of OD phenol, reducing sugar and activity of polyphenol oxidase enzyme was observed in the variety Manoranjitham (AAA) where the content of total phenol, non reducing and total sugars, proline, protein and activity of peroxidase enzyme decreased. The highly susceptible variety, Grand Naine (AAA) exhibited higher quantities of total phenol, reducing sugar, proline and higher activities of peroxidase and polyphenol oxidase enzymes and lower quantities of OD phenol, non reducing and total sugars and protein.

The concentrated culture extracts of *M. musicola* in seven different media gave different Rf values.