APAU CENTRAL LIBRARY
Acc: No: D5741
Date: 4.9.99

MECHANISM OF RESISTANCE OF ACIDLIME(*Citrus aurantifolia* christm.swing.) CLONE (Tenali Selection) TO CANKER DISEASE (*Xanthomonas axonopodis pv.citri* (Hasse) Vaut.)

By

K. NARAYANA SWAMY, B.Sc., (Ag.)

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(PLANT PATHOLOGY)

ANGRAU
Central Library
Hyderabad
D05741

DEPARTMENT OF PLANT PATHOLOGY
SRI VENKATESWARA AGRICULTURAL COLLEGE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
TIRUPATI - 517 502 (A.P.)

DECEMBER, 1998

CERTIFICATE

Mr. K. Narayana Swamy has satisfactorily prosecuted the course of research and that the thesis entitled **"Mechanism of resistance of acidlime [Citrus aurantifolia (Christm. swing.) clone (Tenali selection) to canker disease (Xanthomonas axonopodis pv. citri (Hasse) Vaut.]"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: 14/12/98

(M. RAMASUBBA REDDY)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **"Mechanism of resistance of acidlime [citrus aurantifolia (Christm. swing.) clone (Tenali selection) to canker disease (xanthomonas axonopodis pv. citri (Hasse) Vaut.]"** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture** of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. K. Narayana Swamy** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. All assistance and help received during the course of the investigation have been fully acknowledged by the author of the thesis.

Chairman of the Advisory Committee
(M. RAMASUBBA REDDY)
Plant Pathologist
Citrus Improvement Project, Tirupati.

Thesis approved by the Student Advisory Committee

Chairman **(M. RAMASUBBA REDDY)**
Plant Pathologist

Member **(Dr. R.J. ANANDAM)**
Associate Professor
Dept. of Plant Pathology
S.V. Agricultural College
TIRUPATI - 517 502.

Member **(Dr. V. KRISHNA RAO)**
Associate Professor
Dept. of Plant Pathology
S.V. Agrl. College,
TIRUPATI - 517 502.

Member **(Dr. K. HARI BABU)**
Associate Professor
Dept. of Horticulture
S.V. Agricultural College
TIRUPATI - 517 502.

ACKNOWLEDGEMENTS

With immense pleasure, I wish to express my deep sense of gratitude and indebtedness to ***Sri M.Ramasubba Reddy****, Plant Pathologist, All India Co-ordinated Research Project on Citrus, Tirupati for suggesting the problem and for his kind guidance, constructive criticism, constant encouragement throughout the course of investigation and for preparation of the dissertation.*

My profound thanks are due to ***Dr.K.Sitaramaiah,*** *Professor and Head, Department of Plant Pathology, S.V.Agricultural College, Tirupati for his valuable suggestions given in the course of study.*

I wish to express my gratitude to ***Dr.R.J.Anandam*** *Associate Professor, Department of Plant Pathology, S.V.Agricultural College, Tirupati for his help and advice during the course of problem work.*

My sincere thanks to ***Dr.A.V.Ramana Reddy****, Associate Professor, Department of Plant Pathology, Agricultural College, Bapatla for his help and tended guidance during my research work.*

My deep sense of gratitude is due to ***Dr.K.Hari Babu****, Associate Professor, Department of Horticulture S.V.Agricultural College, Tirupati for his kind cooperation and generous help during my research work.*

It gives me immense pleasure in extending my deep sense of reverence and gratitude to the other members of the Department ***Dr.V.Krishna Rao****, Associate Professor,* ***Dr.S.V.Ramakrishna Rao****, Associate Professor,* ***Dr.N.P.Eswara Reddy,*** *Assistant Professor, Department of Plant Pathology, S.V.Agricultural College, Tirupati for their generous help and constant guidance rendered during my research work.*

I deeply acknowledge with thanks to ***Dr.P.Sreenivasulu,*** *Professor and Head, Department of Virology, S.V.University, Tirupati for allowing me to do most of my research work in their lab.*

It is a great pleasure to I record my profound regard, deepest admiration and heartful thanks to ***Dr.D.V.R.Sai Gopal,*** *Associate Professor, S.V.University, Tirupati for his patience, personnel interest, constant care and scholarly guidance.*

In effable is my gratitude and sincere thanks to ***Dr.G.J.Moses,*** *Senior Scientist, AICRP, Tirupati.* ***Dr.G.Subbi Reddy,*** *Horticulturist,* ***Mrs.M.Madhavi,*** *Junior Horticulturist* ***Dr.V.N.Sheshadri*** *Horticulturist and other members in AICRP, Tirupati.*

I proffer my sincere thanks to ***Dr.K.Balakrishna Reddy,*** *Associate Professor, Department of Plant Physiology, S.V.Agril. College, Tirupati,* ***Dr.Harinatha Naidu,*** *Plant Pathologist, RARS, Tirupati for their constant guidance and valuable suggestions during the course of investigation.*

I also extended my sincere thanks to non-teaching staff in the Department of Plant Pathology, in Virology, S.V.University, and in AICRP on Citrus, Tirupati for their help and co-operation during my research work.

I am indearth of words to express my unboundful gratitude and love to my beloved venerable parents Smt.Narayanamma and Sri Lakshmaiah for their unbounding love unparallel affection and dedicated efforts to educate me to this level without whose unstinted encouragement and invaluable moral support. It remains less even if I say a lot about them.

I express my love from the inner core of my heart to my endearing sisters Mrs Pramela and Mrs.Lakshmi, my uncle Sri Venkataramana, my aunty Smt. Savithri my cousin Sekhar, late Bhaskar, my late grand father Sri Venkataswamy, my grand mother Smt.Papamma, my aunty Mrs.Saroja, Smt.Mangamma, Smt.Venkatalakshmi,

Smt.Ramalakshmi, Smt.Venkatanaramma, Smt.Narayanamma, Smt.Lakshminarayanamma, Smt.A.Anasuyamma, Smt.late Narayanamma. My uncle Sri Sanjappa, Sri Chandraiah, Sri Narayanappa, Sri Krishnamurthy, Sri Narayanappa, Sri Narayana[2] and Sri Late Nagaiah.

I am very happy to acknowledge my friends in S.V.University Prasanna, Devendra, Devendra, Sarovar, Dayakar and other friends. My colleagues Rao, Tirumal, Ravi, Khayum, Lux, Vemana. Mathi Junior friends MK.Seenu, Shakthi, Shyam, Sundaram and Veni.

I express my heartfelt gratitude to beloved friends Chowdary, Kesavulu, unforgetable person Upendra, Case, Bala[2], Mohan, Hari[2], Jaffar, Subbu[2], Vijay, Shoban, Shyam, Anil[2], Kalai, Venkat, Vamsi, Sree Ramulu, Rayal,Ramakrishna, Jakka, Siddaiah, Murthy, Naresh. Govind, Boss, Junior friends Mouli, Humayun, Sudhakar, Bharat, Abbas, Guru and others.

I am greatefully acknowledge the Government of Andhra Pradesh for granting financial assistance during the period of my post graduation studies.

My immense gratitude to photographer Vissu and Narayana Reddy who helped to me in preparation of thesis.

K. Narayana Swamy

(K. NARAYANA SWAMY)

DECLARATION

I, Mr.K.Naryana Swamy, here by declare that the thesis entitled **"MECHANISM OF RESISTANCE OF ACID LIME (*Citrus aurantifolia* Christm. Swing.) CLONE (Tenali selection) TO CANKER DISEASE (*Xanthomonas axonopodis* pv. *Citri* (Hasse) Vaut.)"** submitted to Acharya N.G.Ranga Agricultural University for the degree of **Master of Science in Agriculture** is the original research done by me. I also declare that the material contained in this thesis has not been published earlier.

Date : 14/12/98

K. Narayana Swamy

(K.NARAYANA SWAMY)

CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATIONS

mm	:	millimeters
μ	:	micron
cm	:	centimeters
m	:	meter
mg	:	milligram
g	:	gram
kg	:	kilogram
μl	:	micro liter
ml	:	milliliter
L	:	liter
mm^2	:	square millimeter
ppm	:	parts per million
P.S.i	:	Per square inch
°C	:	degree celsius
rpm	:	revolutions per minute
nm	:	nanometer
M	:	molar
N	:	normality
df	:	dilution factor
OD	:	optical density
NDA	:	Nutrient dextrose agar
AICRP	:	All India Co-ordinated Research Project

ABSTRACT

Name of the Author	:	**K. Narayana Swamy**
Title	:	Mechanism of resistance of acidlime [Citrus aurantifolia (Christm. Swing.] Clone (Tenali Selection) to canker disease Xanthomonas axonopodis pv. citri (Hasse) Vaut.
Major advisor	:	**M. Ramasubba Reddy**
Submitted for the award of degree	:	Master of Science in Agriculture
Faculty	:	Agriculture
Department	:	Plant Pathology
University	:	Acharya N.G. Ranga Agricultural University
Year of submission	:	1998

In acidlime, bacterial canker is a major disease causing severe economic losses. Though effective management practices are available, they are not economical and cause pollution. The only economic and eco-friendly way to manage the disease is through host resistance. Screening of acidlime clonal selections viz., Tenali, Pramalini, Vikram, Chakradhar, Kasipentla, Mungilpattu, Local selections available at AICRP on citrus, Tirupati has indicated that Tenali selection is resistant and free from bark eruptions.

In the present investigation the mechanism of resistance in canker resistant Tenali acidlime clone was studied in comparison with other susceptible clones with particular reference to the pre-existing morphological and biochemical nature.

Since the bacterium causing citrus canker (Xanthomonas axonopodis pv. citri is known to enter through stomata; the frequency and size of stomatal aperture were studied in resistant Tenali clone in comparison with other susceptible acidlime clones. The cuticle and epidermal thickness in the resistant and susceptible acidlime clones were also studied to understand the nature of morphological resistance.

In Tenali clone the stomatal frequency (374.26 mm^{-2}) and size of aperture (2.3 μ) were significantly less than the susceptible acidlime clones. The cuticle thickness was more in the resistant Tenali clone (2.0 μ) than the susceptible acidlime clones. Thus there is morphological resistance mechanism in Tenali acidlime clone.

The involvement of biochemical nature of disease resistance was also investigated in different acidlime clone.

Higher quantities of total phenols, total sugars, reducing and non-reducing sugars, chlorophylls and lower quantities of ascorbic acid, nitrogen content were recorded in the resistant Tenali clone than in other susceptible acidlime clones. These results showed that there is biochemical resistance in Tenali clone compared to other susceptible acidlime clones. There was no significant difference in protein, amino acid and starch content in resistant Tenali clone over other susceptible acidlime clones.

The per cent disease index of canker on fruit, leaf and twig was very less in Tenali clone than other clones and it is free from bark eruption. Cumulative yield interms of fruit number and fruit weight was more in Tenali clone than in other acidlime clones. Other qualities like rind per cent, rind thickness, juice per cent and titrable acidity was similar in all clones under study.

INTRODUCTION

CHAPTER I

INTRODUCTION

Citrus occupies a place of considerable importance in the fruit economy of the country next to mango and banana. Citrus provide minerals, vitamins and other essential elements which are required for human health, besides being a rich source of vitamin C.

In India citrus is cultivated in an area of 3.86 lakh hectares with annual production of 28.22 lakh tonnes of fruits. In India mandarins, sweet oranges and acid limes are occupying 50, 20 and 10 per cent of total area respectively. In Andhra Pradesh, it is being cultivated in an area of 62876 hectares with annual production of 9.431 lakh tonnes of fruit (Directorate of Horticulture, 1995-96).

Acidlime (Kagzi lime) is a popular acid fruit and is commonly used for preparation of refreshing drinks, seasoning foods, pickles, commercial citric acid, calcium citrate, pectin, peel oil, cosmetics etc.

Acidlime is known to be affected by several diseases in Andhra Pradesh. Among them citrus canker caused by X. axonopodis pv. citri is a major bacterial

disease, causing considerable yield losses in acidlime and is responsible for drastic reduction in market value of acidlime besides reducing longivity of the trees.

Wherever acidlime is grown, it is invariably affected by canker to some extent or other. Though the disease is important throughout the year, it is more severe during rainy season and winter; while in summer months it is less severe. Sweet orange and Rangapur lime are severely affected by canker usually in the nursery stage, but with maturity incidence is reduced on these varieties under field conditions.

Acidlime is highly susceptible to canker disease but some of the acidlime clones (A clone is a group of plants produced from a single plant through asexual reproduction) are resistant to canker. Some of the acidlime clones released from Maharastra state and local clones of Andhra Pradesh under evaluation in AICRP on Citrus, Tirupati. Acharya N.G. Ranga Agricultural University research scientists have identified a cankerless acidlime clone with desirable horticultural traits from Tenali area of Guntur district in Andhra Pradesh and it is contemplated to study the mechanism of resistance; morphological, biochemical aspects of the cankerless clone in comparison with other clones. The particulars of which are furnished below.

Clonal selections	Source	State
Tenali	Tenali area, Guntur District	A.P.
Chakradhar Pramalini Vikram	Maharastra Agril. University	Maharastra
Kasipentla Mungilpattu	Chandragiri area, Chittoor District	A.P.
Local variety	Railway Kodur, Cuddapah District	A.P.

The present study is conducted with the following objectives.

Morphological Aspects.

1. Measurement of Epidermal, cuticle thickness in leaf and rind thickness in the fruit in cankerless clone of Tenali selection in comparison with other six clones.

2. Measurement of number of stomata, size of stomatal aperture in the leaf of cankerless Tenali clone in comparison to other six clones.

Biochemical aspects

1. Estimation chlorophyll a, chlorophyll b and total chlorophylls, total starch, total sugars, total proteins, total amino acids in leaf of Tenali selection in comparison to other six clones.

2. Estimation of total phenols, total ascorbic acid in the leaf and titrable acidity in the fruit of Tenali selection in comparison to other six clones.

3. Estimation of nitrogen content in leaves of Tenali in comparison to other six clones.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The literature available on the "Mechanism of resistance in citrus to canker disease" is being reviewed. Since the literature available on citrus canker, particularly morphological and biochemical nature of resistance is meagre, the information pertaining to fungal diseases and bacterial diseases of other horticultural and other broad leaved crops is reviewed in this chapter.

2.1 SYMPTOMATOLOGY

Symptoms under natural conditions on acidlime

Patel and Desai (1970) described the symptoms of citrus canker. The disease first appears as small, raised, translucent spots usually darker green than the surrounding unaffected tissue. Later, the epidermis over the spots becomes yellowish or whitish and ruptures to expose crator lined with spongy than coloured tissue.

Canker lesions about 15 cm in diameter, appear on the bark of mature, lignified branches and 5 to 7.5 cm in diameter on small branches. Infected twigs start drying from the tip and severe defoliation occurs.

The canker lesions on the fruit appear similar to those on the leaves, except that the yellow halo is usually absent and the crator like appearance is more conspicuous. The spongy rough eruptions may be scattered over the surface and several such eruptions may coalesce, forming irregular scurfy mass.

Reddy et al. (1981) reported the bark eruptions on stems and branches of acidlime. In the nursery, bark eruption malady starts as small, hard, brown and corky out growth at 1 to 10 cm height on stem. These eruptions increase in size and form vertical segments.

Reddy and Naidu (1986) reported the canker disease symptoms on roots of acidlime trees. The symptoms consisted of corky out growth. Canker incidence on roots decreased with increase in depth and radial distance of 120 cm on the roots (Plate 1).

2.2 MORPHOLOGICAL STUDIES

2.2.1 Stomatal Studies

Mc Lean (1921) observed grapefruit to be more susceptible than mandarin orange to canker (Pseudomonas citri) which was due to the fact that mandarin had less number of stomata and smaller stomatal openings.

Plate 1. Acid lime root with canker symptom

Tang (1958) reported that the leaves, shoots and fruits of citrus were most susceptible to infection by Xanthomonas citri at the stage when new stomata and lenticells are in process of formation. The relative susceptibility of four species were found to be correlated with the density and size of their stomata and was in the following descending order Citrus sinensis, C. aurantium, C. limon and Poncirus trifoliata.

Kishore and Chand (1972) investigated the relation of stomatal characters with resistant and susceptible Citrus spp. to canker disease and observed that the number of stomata, guard cells and stomatal apertures were found to be maximum in the susceptible Citrus aurantifolia and minimum in the resistant Citrus spp.

Chourasia et al. (1987) studied structural and biochemical nature of disease resistance in betelvine for bacterial leaf spot caused by X. campestris pv. beticola. They found that the stomatal number was negatively correlated with resistance.

Tomy-Philip et al. (1991) conducted research in mulberry genotypes against Cerotelium fici in 3 susceptible and 3 resistant genotypes. Anatomical studies revealed that in all the resistant varieties the

average thickness of the cuticle/epidermis was significantly higher; there were fewer stomata and palisade tissue was thicker than in susceptible varieties.

Danilova and Kotukhov (1991) studied resistance mechanism in Allium nutans against rust (Puccinia allii) pathogen. Their studies revealed that the resistant Kalbinskii form had the fewest stomata per unit area (41/mm^2) and least infection under culture (25.8%). The most susceptible group of forms (90-100% infection) had 84-87 stomata/mm^2. An intermediate group of forms had 46-61 stomata/mm^2 and 47.8-71.2 per cent infection.

Ramos et al. (1992) studied the stomata and leaf epidermis of 8 Lycopersicon spp. had revealed a relationship between frequency of stomata, stomatal size and some morphological leaf characteristics and resistance to bacterial leaf spot caused by X. campestris pv. vesicatoria. The resistant cultivar had less number of stomata and less number of bacterial lesions per unit leaf area. The length and width of stomata were correlated with frequency of stomata in adaxial and abaxial epidermis. The stomatal width was correlated with number of bacterial lesions per cm^2 and was less in resistant cultivar.

Pavlenko (1993) studied the mechanism of morphological resistance in Allium species against Peranospora. The resistant hybrids tended to have a reduced number of stomata/unit area. The epidermal cells adjacent to the stomatal guard cells seemed to cover the guard cells leaving only a sort of epidermal hollow visible from the leaf surface.

Pavlenko (1993) studied the morphological resistant mechanism in Allium species and hybrids against Peranospora destructor. Resistant plants had a well-developed cuticle, with pronounced layers and had a lower pectin content and higher content of phospholipids in the cell walls of the epidermal tissue than susceptible plants.

Pullaiah et al. (1994) reported the relationships between citrus resistance to (X. campestris pv. citri) stomatal frequency and stomatal aperture. They found that susceptible citrus species had significantly higher number of stomata and immune species showed narrow stomatal aperture and lower stomatal frequency.

Philip and Govindaiah (1996) studied the morphological resistant factors in mulberry against Cercospora moricola using the resistant genotypes Kaliakuttai and Belidevalaya and the susceptible

genotypes, Sujanpur-5 and ACC-155. Resistant genotypes had more wax, thicker surface layers, fewer stomata per unit area, a thick palisade layer and higher polisade index values. Susceptible genotypes were characterised by greater leaf area, less wax, thin surface layers, more stomata per unit area and a thin palisade layer.

Chowdhury et al. (1997) evaluated variation in structural characters of the 6 susceptible and resistant varieties of lentil plants against Stemphylium botryosum. The resistant plants had the thick cuticle and epidermal layer cortical layers was also higher than susceptible varieties. The resistant lines with their lower number of stomata.

2.2.2 Epidermis

Kheladze et al. (1984) studied resistance factors in varieties of pear against rust pathogen (Gymnosporangium sabinae). The leaf cuticle of resistant varieties was twice as thick as that of susceptible varieties; thickness of the upper epidermis was 5.5 F greater and that of palisade tissue was increased by 42.1 F.

2.2.3 Cuticle

Bassi et al. (1984) reported that the resistance mechanism of rose against Sphaerotheca pannosa. In leaves of the susceptible cultivar the upper epidermis had a thinner cuticle, while in those of the resistant cultivar there were large masses of electron-dense vacuolar material.

Xie and Leng (1990) studied resistance of apple cultivars against blotch pathogen Marssonia coronaria. Resistance was correlated with fruit cuticle thickness and nitrogen content.

Yang et al. (1992) conducted a study to determine the role of cuticle to defence Rhizoctonia solani in Rapeseed and mustard (Sinapis alba). As rape (Brassica napus) and mustard plants develop from 1 to 3 weeks of age, they become increasingly resistant to R. solani AG2-1 seedling root rot. Seven-day-old seedlings of S. alba cultivars were more resistant than rape cultivars. Mustard cultivars did not show an abvious cuticle layer at 1 week but at 3 weeks the presence of cuticle is seen and increase in resistance to R. solani. Removal of cuticle from 3 week old hypocotyls by chloroform treatment and a significant increase in

disease severity in both resistant and susceptible cultivars.

2.3 BIOCHEMICAL STUDIES

2.3.1 Phenols

Kishore and Chand (1975) investigated total phenols in resistant and susceptible citrus species to canker incited by X. citri and found that there were more total phenols in resistant species than in susceptible ones.

The levels of total phenols were found higher in the cultivars of muskmelon resistant to powdery mildew disease (Sphaerotheca fulginea) than in susceptible cultivars (Jindal et al., 1979).

Mishra et al. (1980) found that total phenols and O-dihydric phenols were higher in varieties of sorghum resistant to Colletotrichum graminicola than in susceptible varieties.

A biochemical study on phenolic content in Brassica juncea cultivars resistant and susceptible to Sclerotinia sclerotiorum infection revealed that the resistant cultivars contained higher levels of phenols than the susceptible ones (Rai et al., 1980).

Mohammed et al. (1981) observed that total phenols were less in the susceptible species of watermelon than in resistant varieties to Fusarium oxysporium f.sp. niveum.

Mahto et al. (1987) found phenol content was highest (278.26 mg/100 g) in the resistant rice variety, IR-20 to X. campestris pv. oryzae, while it was lowest (100 mg/100g) in the highly susceptible variety, Anand. The amount of phenols in the leaves of the 7 resistant varieties analysed varied considerably but was always higher than that present in moderately susceptible Bhagalpuri. A similar pattern occurred for the distribution of reducing and total sugars, which were highest in IR 20 and lowest in Anand. They concluded that high amounts of phenols and sugars keep the bacterial lesions small by generating a resistant reaction.

Siddiqua and Kashem (1993) reported that resistant rice variety (P-3) against sheath blight pathogen contained greater amounts of total phenol and soluble sugar than the susceptible varieties. Disease incidence had a significant negative correlation with phenol and sugar contents.

Satyanarayana et al. (1995) noticed the relationship between 5 biochemical compounds and charcoal

rot (Macrophomina phaseolina) resistance was investigated in the maize seeds of freshly harvested. Increased mean carbohydrate and sugar (reducing, non-reducing and total sugar) content was positively and significantly correlated with increased charcoal rot resistance.

2.3.2 Ascorbic Acid

Ragab et al. (1987) have reported the relationship between Ascorbic acid, chlorophyll, crude protein of barley against net blotch. Ascorbic acid concentration was twice as high in susceptible barley cv. Giza 117 than in resistant cv. C.I.10125. Healthy leaves of Giza 117 contained higher levels of chlorophyll a and chlorophyll b than cv. C.I.10125. The reduction of chlorophyll content is more in susceptible cultivar than resistant cultivar after inoculation with Pyrenophora teres.

Reddy and Khare (1988) conducted research on resistance mechanism of groundnut cultivars against rust. Ascorbic acid increased in the inoculated susceptible cultivar (Jyothi) and significantly decreased in the inoculated resistant cultivar (ICG 1697).

In the studies of Chen-Lifeng et al. (1997) winter wheat varieties were inoculated with a conidial

suspension of Fusarium graminearum (Gibberella zeae) JF-12 and ascorbic acid (ASA) the activities of ASA oxidase and ASA peroxidase in the head tissues sampled between 2 and 22 days after inoculation were assayed. The results showed that susceptible and moderately susceptible varieties (Ning No.6, Yangmai No.3 and Ning 8026) had higher concentration of ASA and lower activities of ASA oxidase and ASA peroxidase, while the resistant variety (Wangshuibai) had lower concentration of ASA and higher activities of ASA oxidase and ASA peroxidase in the inoculated heads compared with the controlheads (inoculated with water).

2.3.3 Nitrogen

2.3.3.1 Bacterial Pathogens. In the studies on resistance, susceptibility of rice to bacterial blight (Have and Kauffman, 1972) found more nitrogen in susceptible plants than the resistant plants.

Easwaran (1973) investigated total nitrogen in jowar varieties resistant and susceptible to Xanthomonas rubrisorghi. The total nitrogen content was found less in the resistant variety Co-19 than the susceptible Co-20.

Padmanaban et al. (1974) found higher concentration of protein nitrogen in species of citrus susceptible to canker disease (X. c. pv. citri).

2.2.3.2 Fungal Pathogens. Farkas and Kirally (1961) noticed that free amino nitrogen content of the leaves increased only in wheat leaves infected by a race of stem rust fungus which resulted in a susceptible reaction. Host-parasitic combinations which showed a totally resistant (hypersensitive) reaction exhibited no increased ammonia content, while resistant combinations yielded intermediate amounts of ammonia.

Rangaswami and Natarajan (1966) noticed more quantities of nitrogen in banana leaves susceptible to Diaghtoniella torulosa and Helminthosporium gibberosporium

Gupta et al. (1987) observed less nitrogen in the cultivars of sesamum resistant to Alternaria leaf spot disease than in susceptible varieties.

Luthra et al. (1996) reported that the resistant seeds of sorghum against leaf spot disease contained higher levels of reducing sugars and low amounts in nitrogen content.

2.3.4 Sugars

Chattopadhyay (1989) reported high levels of phenols in resistant rapeseed and mustered midos, RC 781, YRT 3 than susceptible Benoy, Composite 2, Varuna. Phenol

accumulation is greater in resistant ones. The total sugar content fell more rapidly in susceptible lines than resistant lines against Alternaria blight.

2.3.5 Proteins

Shree and Reddy (1986) reported that large amounts of total phenols, reducing sugars, free amino acids and proteins in resistant (CSH 6 and 148) lines than susceptible cultivars of sorghum against Helminthosporium.

Studies of Samiran-Gangopadhyay et al. (1986) revealed that high levels of total phenolics and soluble proteins observed in resistant (CM 600) variety of maize than susceptible (CM 102) against Rhizoctonia solani.

Raghuchander et al. (1988) reported the resistance mechanism of triticale cultivars against leaf blight fungus Bipolaris sorokiniana. Susceptible cultivars had lower levels of phenols, sugars and proteins than in resistant cultivars.

Angra-Sharma and Sharma (1994) conducted research on susceptible and resistant maize leaves infected by Helminthosporium maydis. Biochemical examination showed

high concentration of protein and phenolic compounds in the resistant variety compared with the susceptible host.

2.3.6 **Amino acids**

2.3.6.1 **Bacterial Pathogens.** The cotton cultivars resistant to Xanthomonas campestris pv. malvacearum, Verma and Singh (1974) found higher amounts of free amino acids than in the susceptible cultivars.

More quantities of free amino acids were found in varieties of chillies (Capsicum annum) resistant to Xanthomonas vesicatoria than in susceptible varieties (Thind et al., 1979).

Marimuthu and Kandaswamy (1983) observed that amino acids were more in the resistant varieties of mung line than in the susceptible varieties of Xanthomonas campestris pv. vignaradiata incitant of chilli bacterial leaf blight disease.

The comparative analysis of some biochemical and structural parameters revealed that betelvine cultivars resistant to X. campestris pv. beticola had more free amino acids (Chourasia et al. 1987).

2.2.6.2 **Fungal Pathogens.** Bisen (1975) noticed that free amino acids were more in the resistant varieties of

apple than in susceptible varieties to Aspergillus niger.

Thind et al. (1977) noticed that amino acids were less in the susceptible varieties of apple than in resistant varieties to Clathridium corticola.

Mogle and Mayee (1979) observed with pearlmillet downy mildew disease, that there were more amino acids in the resistant varieties than in susceptible varieties.

Singh and Prashar (1984) noticed that amino acids were more in the resistant varieties of pea than in susceptible varieties to Rhizopus stolonifer.

The cultivars of tobacco, Delcrest and Harrison special with moderate resistance to leaf spot caused by Helminthosporium spiciferm contained greater quantities of free amino acids than the susceptible C-304 and T-235, although there were no qualitative differences (Chauhan, 1987).

Agrawal (1990) reported healthy plants of the resistant cv. KPBR 80-1-4 had lower amino acid content than those of the susceptible Hy 3c or tolerant ICP 7065 pigeonpea plants. Following stem inoculation with P. drechsleri f.sp. cajani, amino acids decreased in

leaves and stems of Hy 3c and ICP 7065 but increased in KPBR 80-1-4.

In comparative studies made by Pullaiah et al. (1993) with 8 immune species of citrus and 2 (lime and orange) susceptible to canker, immunity was positively correlated to higher levels of phenols and amino acids and negatively correlated to nitrogen content.

2.3.7 Starch

Popova and Temirbekova (1981) found that the susceptible wheat varieties attacked with enzymo-mycotic exhaustion and observed depletion in starch and protein content. These changes are accompanied by infection by Alternaria, Helminthosporium, Fusarium, Cladosporium spp. and bacteria.

Bhatia and Thakur (1994) found lower starch content in highly susceptible Pennisetum glaucum cultivar NHB-3 against Sclerospora graminicola.

Pall (1994) found that there was decline in starch and ash contents in the seeds of blast infected Eleusine coracana.

Marik (1997) reported that the barley Luxor variety which is resistant against Erysiphae graminis had yielded well and contained good amounts of starch and proteins.

2.3.8 Chlorophylls

Gaponenko et al. (1984) found that the concentration of chlorophyll a and chlorophyll b per unit leaf area and the content of soluble protein in the leaves were higher in the high yielding variety - intensivnyi when compared to viner variety in barley.

Legenchenko et al. (1988) found a correlation between yield and ratio of flourescence intensities in barley at 735 and 695 nm that is the flourescence intensities of chlorophylls of the photosystem I and PS II, chlorophyll protein complexes respectively.

Agaev et al. (1988) found that the chlorophyll a, chlorophyll b ratio was closely correlated with winter hardiness and yield in the varieties of red clover mid late varieties accumulated a higher content of chlorophyll a and b and proved higher yielding and hardier.

Eid et al. (1991) found that application of K upto 100 kg K_2O has generally increased growth parameters, total yield and chemical constituents like chlorophyll a and b and carotenoids in garlic plants.

El-khateeb-M A et al. (1991) found that the application of GA3 increased leaf number, leaf fresh and dry weight, and the content of N, P and chlorophyll a and b and the yield of essential oil in Ruta graveolens (L.) plants.

Babu et al. (1993) conducted an experiment using hybrids and parents of Vigna radiata and found that the photosynthetic rate, chlorophyll a and b content, chlorophyll a/b ratio and leaf area of the hybrids were higher than those of respective parents. Hybrids out yielded their parents. The results suggested that the yield could be increased by breeding for improved chlorophyll concentration.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 LOCATION OF WORK

The investigations for this thesis work have been taken up during the period 1997-1998 at All India Co-ordinate Research Project (AICRP) on Citrus, S.V. Agricultural College and S.V. University, Tirupati, Andhra Pradesh which is located at 79°E longitude, 13°N latitude with an altitude of 182.9 m above mean sea level in the tropical belt of South India.

3.2 CLIMATE

Tirupati area falls under the southern agroclimatic zone of Andhra Pradesh. This zone is characterised by fairly hot summer and rainfall is received in two spells viz., South-West (June-September) and North East (October-January) monsoon periods. Normally, more rainfall is received during the retreating North-East monsoon period.

3.3 LABORATORY TECHNIQUES

The general laboratory techniques adopted were those described by Kirally (1974) and Riker and Riker (1936) with modification wherever necessary, for preparation of media, isolation of bacteria etc.

3.4 GLASSWARE USED

Corning make glassware viz., petridishes of 10 cm diameter for experiments with solid nutrient media and 15 cm long test tubes were used for maintaining stock cultures.

3.4.1 - Cleaning of glassware and sterilisation

Glassware like petridishes, test tubes, volumetric flasks, beakers, jars, burettes, pipettes and conical flasks were washed with concentrated cleaning solution prepared by adding 60 g of potassium dichromate into 300 ml distilled water and then adding into it 460 ml of concentrated sulfuric acid drop by drop, the glassware was washed with distilled water and dried. The glassware was sterilised by keeping in hot air over at 160°C for two hours.

3.5 STERILISATION OF MEDIA

Nutrient dextrose agar medium was sterilised by autoclaving for 15 minutes at 121.6°C in an autoclave at a pressure of 15 p.s.i.

3.6 MEDIUM USED FOR MAINTAINING THE CULTURES

Nutrient Dextrose Agar medium (NDA) in which the organism develops well was used for maintaining the cultures.

Compositions of Nutrient Dextrose Agar (NDA)

Peptone (Difco)	5.0 g
Beef extract (Difco)	3.0 g

Dextrose	5.0 g
Agar Agar	15.0 g
Distilled Water	1000 ml

The chemicals employed for all the experiments were of the analytical grade Analar (Glaxo).

3.6.1 pH of the Medium

The pH measurements were made by using BDH narrow range pH paper. The initial pH of the medium adjusted to about 7.0 before autoclaving by using 0.1N Hydrochloric acid or 0.1N Sodium hydroxide as the case may be. The pH after autoclaving was noted as 7.0.

3.7 ISOLATION OF THE PATHOGEN

Isolation of Xanthomonas axonopodis pv. citri was done by streak plate method on NDA from infected acidlime leaves. Before attempting isolation, leaf lesions were ascertained for canker by examining under the microscope in a drop of water for bacterial ooze from the cut ends.

For isolation, a few typical young lesions from diseased leaves were cut with a sterilized blade avoiding as much of the surrounding healthy tissue as possible. The cut pieces were surface sterilized with 0.1 per cent solution of Mercuric chloride for 30 seconds after which they were washed in two to three changes of sterile water. The pieces were then removed on to a sterilized slide and macerated with a

sterile glassrod in a drop of sterile distilled plate. Isolation was made from this suspension by streak plate method which is briefly described as follows. About 20 ml of melted NDA was poured in each of the several sterilized petridishes. The plates were inverted after the medium has set and were kept as such overnight. Streaking was done with a small loop of nicrome wire dipped only once in the bacterial suspension onto the above plates. The same loop was used to streak two or three more plates without heating. The plates were inverted and kept in the incubator at ±2 28°C. After an interval of 3 to 5 days, separate colonies were located on the surface of the last streaked plates which were picked up individually and transferred to NDA slants.

3.8 IDENTIFICATION OF THE BACTERIUM

The bacterium was identified by gram reaction and pathogenicity tests.

3.8.1 Gram Reaction

Staining was done according to Hucker's and Cohn's modification or the Ammonium oxalate crystal-violet stain (Bartholomow, 1962).

SOLUTION A

Crystal violet (90% dye content)	2.0 g
Ethyl alcohol	20.0 ml

SOLUTION B

Ammonium oxalate	0.8 g
Distilled Water	80.0 ml

Solution A and B were mixed

COUNTER STAIN

Safranin (2.5% in 95% alcohol)	1.0 g
Potassium iodide	2.0 g
Distilled water	300.0 ml

Foty eight hour old culture was suspended in sterile water. A smear of suspension from the above culture was prepared on clean glass slides which was dried and fixed by passing over a flame. The smear was covered with Hucker's ammonium oxlate crystal violet stain for one minute and then washed in tap water. Then it was flooded with gram iodine solution and allowed to remain for one minute and washed in tap water and blot dried. The smear was decolourised with alcohol until only faint violet colour remained. It was washed in tap water and blot dried. Then it was covered with counter stain safranin for 30 seconds and washed in tap water, drained, blotted and air dried and examined under oil immersion objective of a microscope. The bacterium looked pink red and hence it is gram negative. The bacterial cells were rod shaped.

3.9 INOCULATION STUDIES WITH TEST ACIDLIME CLONES

Pathogenicity tests were conducted on four months old acid lime seedlings of different clones in the glass house at AICRP on Citrus, Tirupati. The seedlings were free of natural infection.

The following acidlime clones were selected for the present study viz.,

Tenali, Pramalini, Vikram, Chakradhar, Kasipentla, Mungilpattu and Local acid lime clones. The selected acidlime clones were inoculated on 3rd young leaf from the tip and at 10 a.m. of the day with the bacterium for pathogenicity tests during August and September. Suitable controls with sterile water in the place of inoculum were maintained.

3.10 DETERMINATION OF STOMATAL FREQUENCY AND MEASUREMENT OF STOMATAL APERTURE

Five leaves of acidlime of a twig were numbered from the tip to downwards for testing. Third leaf is taken for measuring stomatal frequency. Smear a thin film of quick fix at different spots on under side of the leaf, allow it to dry and remove the impressions from the epidermal surface. Mount the epidermal impressions on slides in such a way that the lower side of the film should face upwards. Place a coverslip over it and count the number of stomata within the area of high power field of the microscope. Take three of such

readings from each film by selecting three random fields and calculated the average number. This gives the number of stomata within the area of field of vision. From it, calculated the number of stomata per mm^2 of leaf surface. This gives stomatal frequency.

In order to determine the size of the stomatal aperture, the occular micrometer is first standardised with the help of stage micrometer. For standardisation of occular micrometer, it is first placed inside the eye piece and the stage micrometer on the stage of a microscope. Then observed how many divisions of the occular micrometer (omm) coincide exactly with those of stage micrometer. Three such readings are taken and the average is determined.

The length and width of the stomatal aperture were measured by a calibrated occular micrometer and the size is expressed by the formula (Saradadevi, 1982).

Stomatal aperture = L X W

Where L : Length of stomata

W : Width of stomata

Calculation length and width

L = ½ X length divisions of omm correction factor = A

W = ½ X width divisions of omm X correction factor = B

$$\text{Stomatal aperture} = \frac{\pi}{4} \text{ X A X B} = \frac{\pi \text{ AB}}{4}$$

3.11 CUTICLE THICKNESS

Third leaf from tip of a twig is taken, very thin sections were taken from this leaves. This thin sections were placed on clean slide on a stage of microscope. The cuticle thickness is measured with occular micrometer which is fixed inside eyepiece. Three such readings are taken and the average is determined.

3.12 EPIDERMIS THICKNESS

Thin sections are taken from healthy acidlime clones. This sections are placed on clean slides and thickness is measured with occular micrometer under high power in compound microscope. Three such random readings are taken and the average is calculated.

3.13 RIND THICKNESS

Mature acidlime fruits are taken and the fruits are cut with blade, rind is removed from the slices. The thickness of the rind is measured with vernier caliperse.

3.14 BIO-CHEMICAL FACTORS : ANALYTICAL METHODS

Collection of leaf samples for analysis

Third leaf from the tip of a twig from actively growing acidlime plants of clonal selections was collected, thoroughly washed and used for analysis.

The leaves were oven dried for three consecutive days at 50°C and powdered in wareing blender.

3.15 ESTIMATION OF AMINOACIDS

The total amino acid content in leaves of acidlime clone were estimated as per the method of Stein and Moore (1948) in both susceptible and resistant varieties.

Leaf tissue (200 mg) was homogenized in 15.0 ml of 80.0 per cent ethanol followed by centrifugation at 2000 rpm (241 RGF) for 15 minutes. 0.1 ml of aliquot was taken from the supernatant and 0.5 ml of acetate cyanide buffer was added to it followed by 0.5 ml of ninhydrin solution. The mixture was heated on a water bath at 100°C for 15 min. Five ml of diluent was added into each tube after incubation and the contents of tubes were thoroughly stirred. The solution were allowed to cool and their absorbance was read at 570 nm in spectronic-20. The amount of total aminoacids was calculated by using a standard curve made from Leucine.

Various reagents described above were prepared as follows :

1. Stock Sodium cyanide : 0.01 M
2. Acetate buffer : 2700 g of Sodium acetate in 2.0 L of distilled water and 500 ml of glacial acetic acid, made up to the final volume of 7.5 L with distilled water (pH 5.3-5.4)
3. Acetate-cyanide buffer : 0.0002 M Sodium cyanide by acetate buffer, 20.0 ml of solution made upto 1.0 Lit with solution 2.
4. Nin-hydrin : 3.0 per cent (W/V) in Methylcellosolve (ethylene glycol, monomethyl ether)
5. Diluent : Isopropyl alcohol and water in 1:1 ration (v/v)

3.16 ESTIMATION OF NITROGEN

The nitrogen content in leaves of different acidlime clones were estimated as per the method of Mahadevan and Sridhar (1982) in both susceptible and resistant varieties.

One gram of leaf sample was taken in microkjeldahl flask and 3-4 ml of con.H_2SO_4 and 2-3 grams of catalstic mixture

were added. The contents were allowed for cold digestion over night and on the next day the contents were heated on hot plate till clear solution formed.

It was cooled and transferred into a microkjeldahl distillation flask with washings and the volume was made up to 100 ml. To this 10 to 15 ml of 40 per cent Sodium hydroxide was added. Before that, 25 ml of 4 per cent boric acid was taken in a 250 ml beaker and a few drops mixed indicator were added and kept under a condenser.

The distillation was continued till all the liberated ammonia was absorbed by the boric acid (this was tested with moist red litmus paper). The distillation was completed in - 10 minutes. The absorbed ammonia in the boric acid (Ammonium tetra borate) titrated against 0.1 N H_2SO_4 by adding drop by drop until the original pink colour appeared.

The reading was noted and the percentage of total nitrogen present in the leaf sample was calculated by using the following factor.

FACTOR

1 ml of 0.1N H_2SO_4 = 0.0014 grams of nitrogen.

3.17 ESTIMATION OF PHENOLS

The total phenol content in leaves of acidlime clones was estimated as per the method of Sadasivam and Manickam (1996) in both susceptible and resistant varieties.

From each sample weighed exactly 0.5 to 1.0 g of the sample and grind it with a pestle and mortar in 10 times volume of 80 per cent ethanol. Centrifuge the homogenate at 10000 rpm for 20 min. The supernatant was taken reextracted the residue with five times the volumes of 80 per cent ethanol, centrifuged and pooled the supernatant. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water (5 ml) pipette out different aliquots (0.2 to 2 ml) into test tubes. Made up the volume in each tube to 3 ml with water. Added 0.5 ml of Folin-ciocalteau reagent and after 3 min. added 2 ml of 20 per cent Sodium carbonate solution to each tube. Mixed thoroughly and placed the tubes in a boiling water for exactly one min. cooled and measured the absorbance at 650 nm against a reagent blank. Then the standard curve was prepared using different concentrations of catechol.

Preparation of reagents

a. Eighty per cent Ethanol was prepared by adding 80 ml of absolute alcohol and made upto 100 ml by using distilled water.

b. Twenty per cent Sodium carbonate : 20 grams of Sodium carbonate in 100 ml of distilled water.

3.18 ESTIMATION OF TOTAL SUGARS

The total sugar content of citrus leaves was estimated as per the method of Dubois et al., (1956) in different acidlime clones.

The supernatant obtained after homogenisation of tissue in 80 per cent ethanol was used for the estimation of total sugars.

1. Phenol 5% in distilled water
2. Sulfuric acid concentrated

To an aliquot (0.1 ml) taken from supernatant, 1.0 ml of phenol and 5.0 ml of H_2SO_4 were added slowly in the centre of the tube and the mixture was shaken thoroughly. The absorbance was read in Spectronic-20 at 490 nm.

The amount of sugar was calculated from a standard curve prepared with glucose.

3.19 ESTIMATION OF REDUCING SUGARS

The reducing sugars in leaves of acidlime clones were estimated as per the method of Mahadevan and Sridhar (1982) in both susceptible and resistant varieties.

Preparation of Sugar Extract

For estimation of reducing sugars, 0.5 g of powdered, dried material was taken in a 15 ml centrifuge tube and mixed with 10 ml of 80 per cent ethanol. The mouth of the tube was covered with glass marble and kept in a hot water both at 80°C for 30 minutes. The supernatant was filtered through Whatman No.41 filter paper into 50 ml beaker. This extraction was repeated thrice. The alcohol extract was evaporated on a water bath at 80-85°C until most of the alcohol was removed

(reduced the volume to about 3 ml). The volume was made upto 25 ml with distilled water.

Five ml of sugar extract was transferred to a 100 ml volumetric flask and the volume was made upto the mark with distilled water. Five ml aliquots of dilute sugar extracts were transferred to test tubes and tubes containing the glucose standards were kept in an ice-bath. To each tube, 10 ml of anthrone reagent was added slowly allowing the reagent to run down the side of test tube and stirred slowly with a glass rod. These test tubes were kept in boiling water bath for 7.5 minutes and then cooled immediately in an ice-bath. The absorbance was measured in Spectronic-20 at 647 nm. The quantity of reducing sugars was then correlated with the help of graph of glucose standards.

Non reducing Sugars

The amount of non-reducing sugars in leaves of different acidlime clones were obtained by subtracting the value of reducing sugars from total sugars. This value was multiplied with a constant value 0.93 to correct water taken up during hydrolysis (Khatri and Chenulu, 1969).

3.20 ESTIMATION OF CHLOROPHYLL

Chlorophyll estimation was done according to the method of Arnon (1949). The young healthy leaves were collected from different acidlime clones. Midribs were removed and leaves were cut into small pieces. One gram of leaf tissue is taken

separately and washed with tap water and followed by the distilled water. The samples were macerated in cold 80 per cent acetone and centrifuged at 3000 rpm for 15 minutes. The pellet was thoroughly washed thrice with 80 per cent acetone and the supernatant were pooled. The pellet were discarded and pooled supernants were made upto 25 ml with 80 per cent acetone.

The following formulae were used for total chlorophyll, chlorophyll a and chlorophyll b estimation (Arnon, 1949).

Total chlorophyll : [20.2 X O.D645) + (8.02 + O.D663) df

Chlorophyll a : [12.7 X O.D663) - (2.69 + O.D645)] df

Chlorophyll b : [(22.9 X O.D645) - (4.68 + O.D663)] df

Where O.D values in optical density and d.f. is dilution factor. The results were expressed as mg of chlorophyll/g fresh weight.

3.21 ESTIMATION OF PROTEINS

Protein content in leaves was estimated by method of Lowry *et al.*, (1951).

Preparation of reagents

a) Grinding buffer : pH 8.3

Tris (Hydrooxymethyl) methylamine : 1.57 g

Sucrose : 17.1 g

2-mercaptoethanol : 2.0 ml

Dissolved in 100 ml of distilled water

b) 0.1N Sodium hydroxide; 0.1N Sodium hydroxide was prepared by dissolving 400 mg of Sodium hydroxide in 100 ml of distilled water.

c) Alkaline copper sulphate

Solution A : 3 g of Sodium carbonate was dissolved in 100 ml of 0.1N Sodium hydroxide

Solution B :

i) 0.2 g of copper sulphate ($CuSO_4$) was dissolved in 10ml of distilled over

ii) 0.4 g of Potassium tartarate was dissolved in 10 ml of distilled water solutin (i) and (ii) were mixed in an equal volumes.

Alkaline Copper sulphate solution was prepared by mixing of 96 ml of solution A and 4 ml of Solution B.

d) 20% Trichloro acetic acid : 20 g of trichloroacetic acid was dissolved in 100 ml of distilled water.

e) 5% Trichloro acetic acid : 5 g of trichloroacetic acid was dissolved in 100 ml of distilled water.

f) Folin phenol reagent

10 ml of Folin and Ciocalteu phenol was diluted with 10 ml of distilled water. All the above reagents were freshly prepared for the protein estimation.

Procedure

One gram healthy leaves of different acidlime clones were taken separately and washed thoroughly with tap water followed by distilled water and blotted to dry in between filter paper folds. The midrib of the leaves was removed and leaf material was homogenized separately in a mortar at 4°C using grinding buffer (0.1 M Tris HCL, pH 8.3; 0.5 M sucrose and 0.5 per cent 2-mercaptoethanol) at the rate of 2 ml/gram. The homogenate was squeezed through muslin cloth and centrifuged at 10000 rpm for 10 min. The supernatant were collected separately and equal volume of 20 per cent trichloroacetic acid (TCA) was added each sample and kept for 2 hours at 4°C. The TCA precipitate was collected by centrifugation at 10,000 rpm for 10 min. The pellet was washed twice with 5 per cent TCA and thrice with ice cold solvent ether. The final protein pellet was dried under vaccum and solubilised in a minimal known volume of 0.1 N NaOH solution. Insoluble material was removed by centrifugation at 8000 rpm for 10 min and the soluble protein in the supernatant was estimated according to Lowry et al., (1951). 20 μl of protein obtained from different samples were taken and to each sample 5 ml of freshly prepared alkaline copper sulphate reagent was added. The samples were mixed well and the solution was allowed to stand for 10 min at room temperature. After 10 min incubation 0.5 ml of Folin phenol reagent was added to each sample and mixed thoroughly. After 30 min incubation the absorbance of the samples was read at

660 nm by using Spectrophotometer. Amount of protein (mg/g fresh leaf) was calculated by using bovine serum albumin (BSA) standard curve.

3.22 ESTIMATION OF ASCORBIC ACID

Ascorbic acid was estimated by colorimetric method Sadasivam and Manickam (1996).

Preparation of reagents

a. 4 per cent oxalic acid solution : 4 grams of oxalic acid was dissolved in 100 ml of distilled water.

b. 0.5 N sulfuric acid : 117.6 ml of conc. sulfuric acid was dissolved in 32.4 ml of distilled water.

c. 2% 2,4 dinitriphenyl hydrazine Reagent (DNPH) 2 grams DNPH was dissolved in 100 ml of 0.5 N H_2SO_4 by heating and later filtered through Whatman No.1 paper.

d. 10% Thiourea solution

   10 grams of thiourea was dissolved in 100 ml of distilled water.

e. 80% sulfuric acid

   80 ml of Sulfuric acid was dissolved in 20 ml of distilled water.

f. Bromine Water

   1 to 2 drops of liquor bromine was dissolved in 100 ml of cold distilled water.

f. Ascorbic acid stock solution

   100 mg of ascorbic acid was dissolved in 100 ml of 4 per cent oxalic acid solution.

Procedure

In Leaves

One gram of healthy leaves from different acidlime clones were ground by using mortar and pestle in 25 ml of 4 per cent oxalic acid solution. The extracts were centrifuged and collected the supernatant.

10 ml supernatants of different acidlime clone were transferred separately into a conical flask and bromine water was added in dropwise with constant mixing and made upto 25 ml with 4 per cent oxalic acid solution. Similarly 10 ml of ascorbic stock solution was converted into dehydroform by bromination.

0.1 ml of each brominated samples were pipette out and was made upto 3 ml by adding distilled water. One ml of DNPH reagent was added followed by 1 to 2 drops of thiourea to each tube. A blank was set as above with water in place of ascorbic acid solution. The contents of the tubes were mixed thoroughly and incubated at 37°C for 3 hours. After incubation the orange-red osazone crystals formed were dissolved by adding 7 ml of 80 per cent H_2SO_4. Absorbance at 540 nm was measured. The ascorbic acid content in the samples was calculated by using ascorbic acid standard curve.

3.23 ESTIMATION OF STARCH

The starch content was estimated by method of Mc Cready et al., (1950).

Preparation of reagents

a. 80% Ethanol : 80 ml of absolute alcohol was made upto 100 ml by using distilled water.

b. 0.2% Anthrone reagent : 200 mg of anthrone was dissolved in 100 ml of conc. H_2SO_4 and maintained in cold condition.

3. 52% Perchloric acid (PCA) : 52 ml of 60% PCA was made upto 60 ml by using distilled water.

Leaf tissue (500 mg) was homogenized with 5 ml of 80 per cent ethanol. The macerate was transferred to centrifuge tubes and centrifuged at 5000 rpm for 15 min. The pellet was washed thrice with 80 per cent ethanol. The supernatant were pooled and made upto know volume with 80 per cent ethanol. The supernatant were pooled and used for sugar estimation. The pellet was subsequently used for extraction of starch.

The pellet which was collected from the above process was solubilised in 5 ml of 52% PCA boiled at 80°C for 10 min. The solution was filtered through glasswool. The filtrate was measured and made upto 10 ml with PCA, 20 μl filtrates of different acidlime clones were taken separately 2.95 ml of distilled water and 5 ml of anthrone reagent were added to the above samples and incubated for 10 min in icebath. The

absorbance of the samples was read out 625 nm in a Spectrophotometer. The amount of starch was calculated by using glucose standard curve.

3.24 TITRABLE ACIDITY

The method described by Ranganna (1977) was adopted for estimation of titrable acidity. A known amount of juice from healthy fruit of different acidlime clones were transferred separately to volumetric flasks and the volume was made up with distilled water to a known amount. After standing for 30 minutes, the suspension was filtered through Whatman No.1 filter paper and the filtrate was used subsequently. An aliquot was taken and titrated against standard Sodium hydroxide using phenolphthalein as an indicator. Titrable acidity was expressed as percentage malic acid equivalents using the following formula.

$$\text{Acidity (\%)} = \frac{\text{Titre of alkali} \times \text{Normality} \times \text{Volume madeup} \times \text{Equivalent of malic acid}}{\text{Volume of sample taken for estimation} \times \text{Weight of the sample}} \times 100$$

3.25 PER CENT DISEASE INDEX

Per cent disease index was calculated on leaves, fruit of different acidlime clonal selections.

$$\text{Per cent diseases index} : \frac{\text{Sum of all ratings}}{\text{No. of leaves scored}} \times \frac{100}{\text{Maximum scoring}}$$

For leaf canker estimation, forty leaves were randomly selected from four sides of each clonal selection and disease incidence was scored using 0-4 scale (0 = no infection, 1 = traces to 10%, 2 = 10-25%, 3 = 25-50% 4 = > 50% leaf area affected). (Plate-2).

For fruit canker estimation fifty fruits were randomly selected from four sides of each clonal selection and disease incidence was scored using 0-4 scale. (Plate-3).

For twig canker fifty twigs were randomly selected from four sides of each tree and disease incidence was scored and per cent disease index were calculated. (Plate-4).

Bark eruptions were graded as area and circumference of the disease eruption on the trunk. (Plate-5 and 6).

3.26 YIELD

Yield data were recorded from each tree of different clonal selections; number of fruits per tree, weight of the fruits were also recorded and cumulative yield from 1995-98 was computed. Juice per cent, rind per cent was also calculated.

3.26.1 Juice per cent

For estimating juice per cent, juice weight and fruit weight was taken separately and juice per cent was calculated for 100 fruits. Juice per cent was calculated in Tenali clone compared to other acidlime clones.

$$\text{Juice per cent} = \frac{100}{\text{Fruit Weight}} \times \text{Juice Weight}$$

3.26.2 Rind per cent

For estimating rind per cent, rind weight and fruit weight was taken separately and rind per cent was calculated for 100 fruits in Tenali compared to other acidlime clones.

$$\text{Rind per cent} : \frac{100}{\text{Fruit Weight}} \times \text{Rind Weight}$$

RESULTS

CHAPTER IV

RESULTS

4.1 ISOLATION OF THE PATHOGEN AND CULTURAL CHARACTERS

Raised, lemon yellow coloured colonies were observed on the streaked plates of Nutrient dextrose agar (NDA) after 72 hours of incubation which were well isolated on last streaked petridishes (Plate-7). These colonies were transferred on NDA slants under refrigerated conditions. The bacterium produced copious slime on NDA medium. It looked red with Gram negative staining with rod shaped cells.

4.2 PATHOGENICITY TESTS

Symptoms appeared about fifteen days after inoculation as small water soaked spots on inoculated leaves, which gradually increased in size becoming corky on both surfaces of the inoculated leaves. Characteristic chlorotic halo developed around the spots (Plate-8).

Reisolations from such lesions consistantly yielded the same bacterium. Based on the above Gram negative reaction and pathogenicity test, the bacterium was identified as Xanthomonas axonopodis pv. citri.

No symptoms appeared in controls and in Tenali clone the symptoms were negligible.

Plate 7. Xanthomonas axonopodis pv. citri culture colonies on NDA medium

Plate 8. Pathogenicity test with Xanthomonas axonopodis pv. citri on acid lime no symptoms in control

4.3 NATURE OF RESISTANCE

In view of the relation of stomatal frequency and stomatal size, cuticle and epidermis thickness and the role of phenols, sugars, amino acids, proteins, nitrogen, ascorbic acid and chlorophylls with canker disease resistance. These were estimated in resistant and susceptible clones of acidlime and the results are presented below.

4.3.1 Stomatal Frequency in Test Acidlime Clones

The results of experiments on stomatal frequency in the lower leaf surface are presented in Table 1, (Plate 9 and 10), (Figure-1).

The results presented in Table 1 indicated that there was a positive correlation to stomatal frequency with susceptibility to canker disease. The resistant Tenali clone had the lowest number of stomata 374.26 mm^{-2}, while in other susceptible clones it ranged from 416.53 to 538.01 mm^{-2}. There was a significant difference in stomatal frequency between resistant Tenali and other susceptible acidlime clones.

4.3.2 Size of Stomatal Aperture in Test Acidlime Clones

The results of experiments on size of stomatal aperture in lower leaf surface are presented in Table 2 (Fig.2).

Table 1. Stomatal frequency.in test acidlime clones

S.NO.	Test acidlime clones	No. of stomata per mm^2
1.	Tenali	374.26
2.	Pramalini	416.53
3.	Vikram	538.01
4.	Chakradhar	421.05
5.	Kasipentla	444.44
6.	Mungilpattu	467.83
7.	Local	479.50
	CD at 5%	34.61

Table 2. Size of stomatal aperture in test acidlime clones

S.NO.	Test acidlime clones	Size of stomatal aperture μ
1.	Tenali	2.34
2.	Pramalini	4.21
3.	Vikram	3.91
4.	Chakradhar	4.59
5.	Kasipentla	3.25
6.	Mungilpattu	3.45
7.	Local	3.79
	CD at 5%	0.34

ANGRAU
Central Library
Hyderabad
D05741

Fig.No.1 Stomatal frequency in test acidlime clones

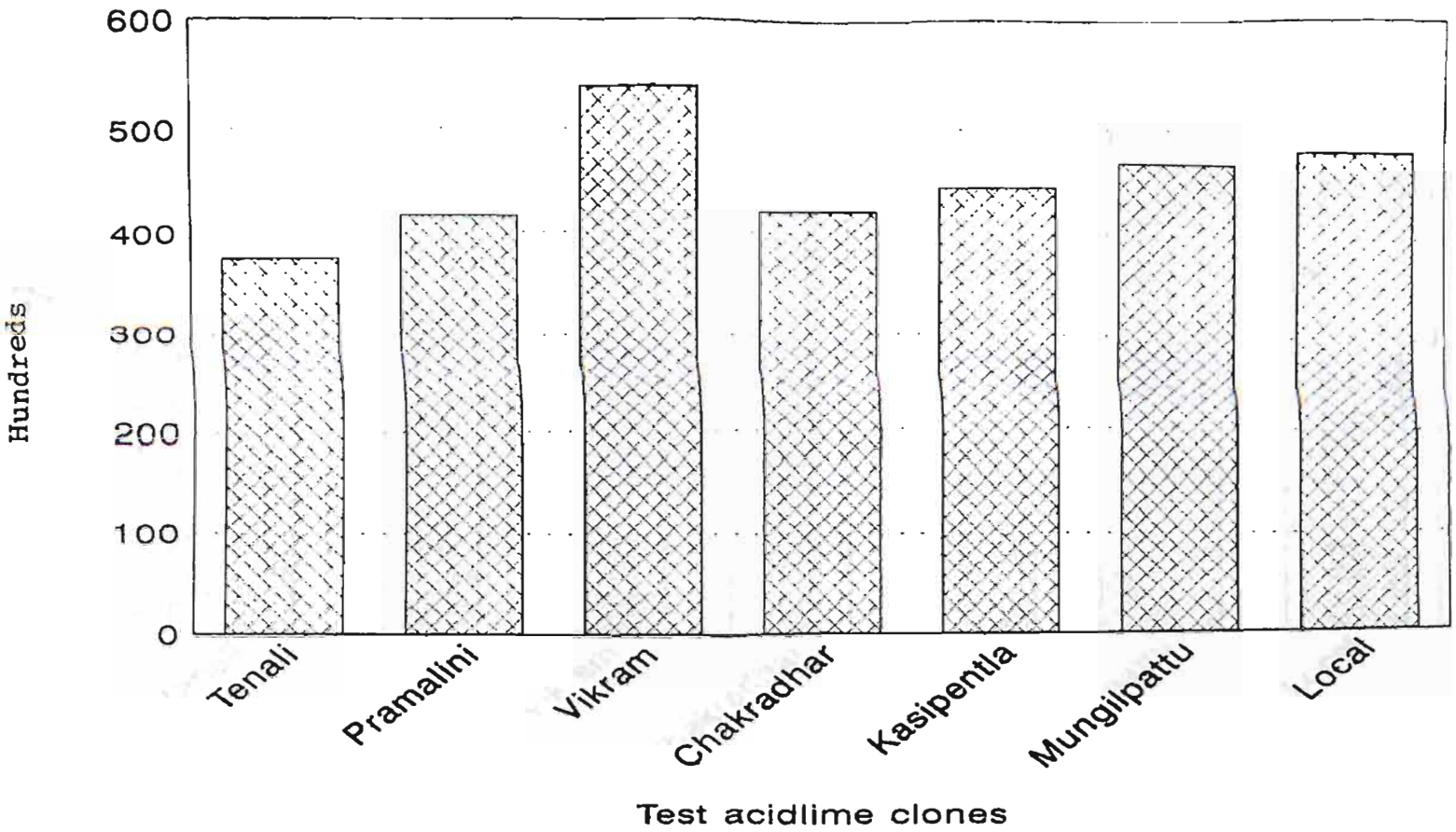
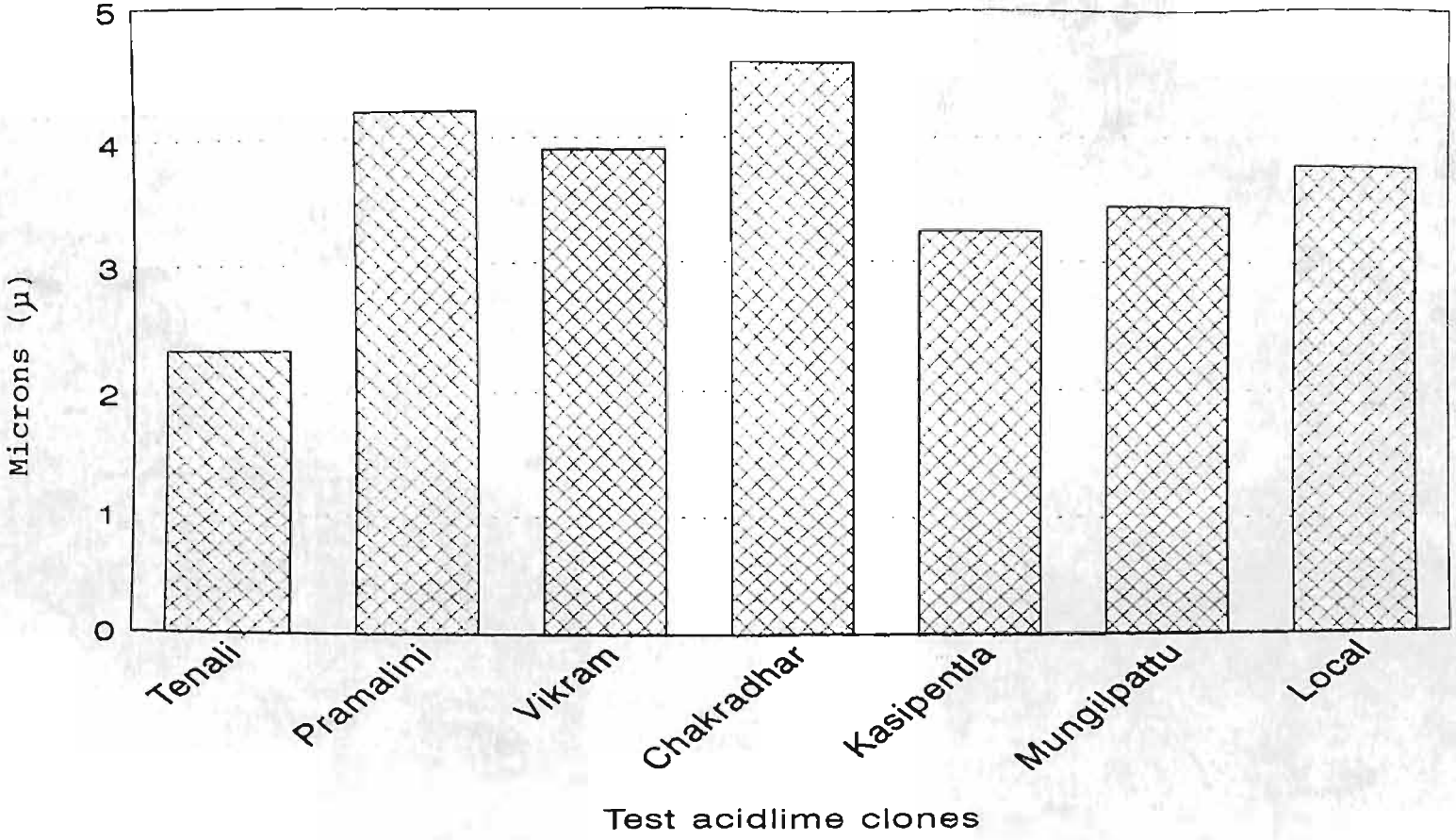


Fig.2 Size of stomatal aperture in test acid lime clones



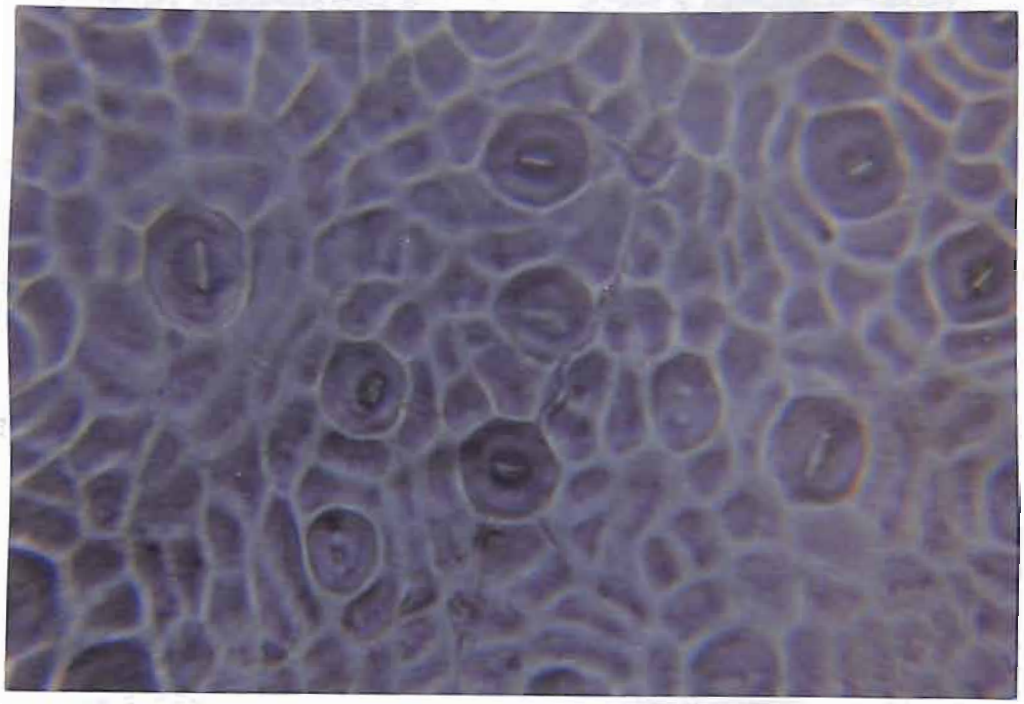


Plate 9. Less stomatal frequency in the resistant Tenali clone

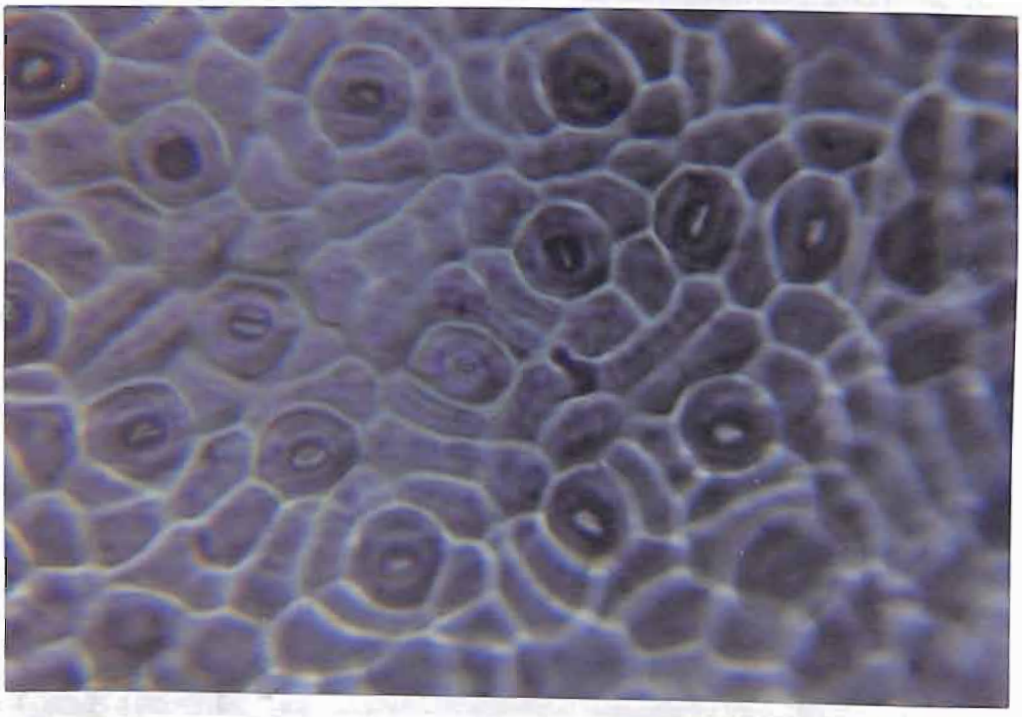


Plate 10. More stomatal frequency in the susceptible clone

From Table 2, it is evident that there was a positive correlation between stomatal aperture with susceptibility to canker disease. The stomatal aperture in resistant Tenali acidlime clone was 2.34 µ, while in susceptible acidlime clones it ranged from 3.25 to 4.59 µ. The resistant Tenali clone had narrow stomatal aperture compared to other clones. There was significant difference in size of stomatal aperture between resistant and susceptible acidlime clones.

4.3.3 Cuticle Thickness in Test Acidlime Clones

The results of experiments on cuticle thickness of leaf were presented in Table 3, (Fig.3).

From Table 3, it is evident that cuticle thickness had a positive correlation with resistance to canker disease in acidlime. Cuticle thickness was more in resistant Tenali clone 2.0 µ, while in other susceptible clones it ranged from 1.0 to 1.5 µ. There was a significant difference in cuticle thickness between resistant Tenali clone and other susceptible acidlime clones.

4.3.4 Epidermal Thickness in Test Acidlime Clones

The results of experiments on epidermal thickness in the upper leaf surface of leaf are presented in Table 4.

Table 3. Cuticle thickness in test acidlime clones

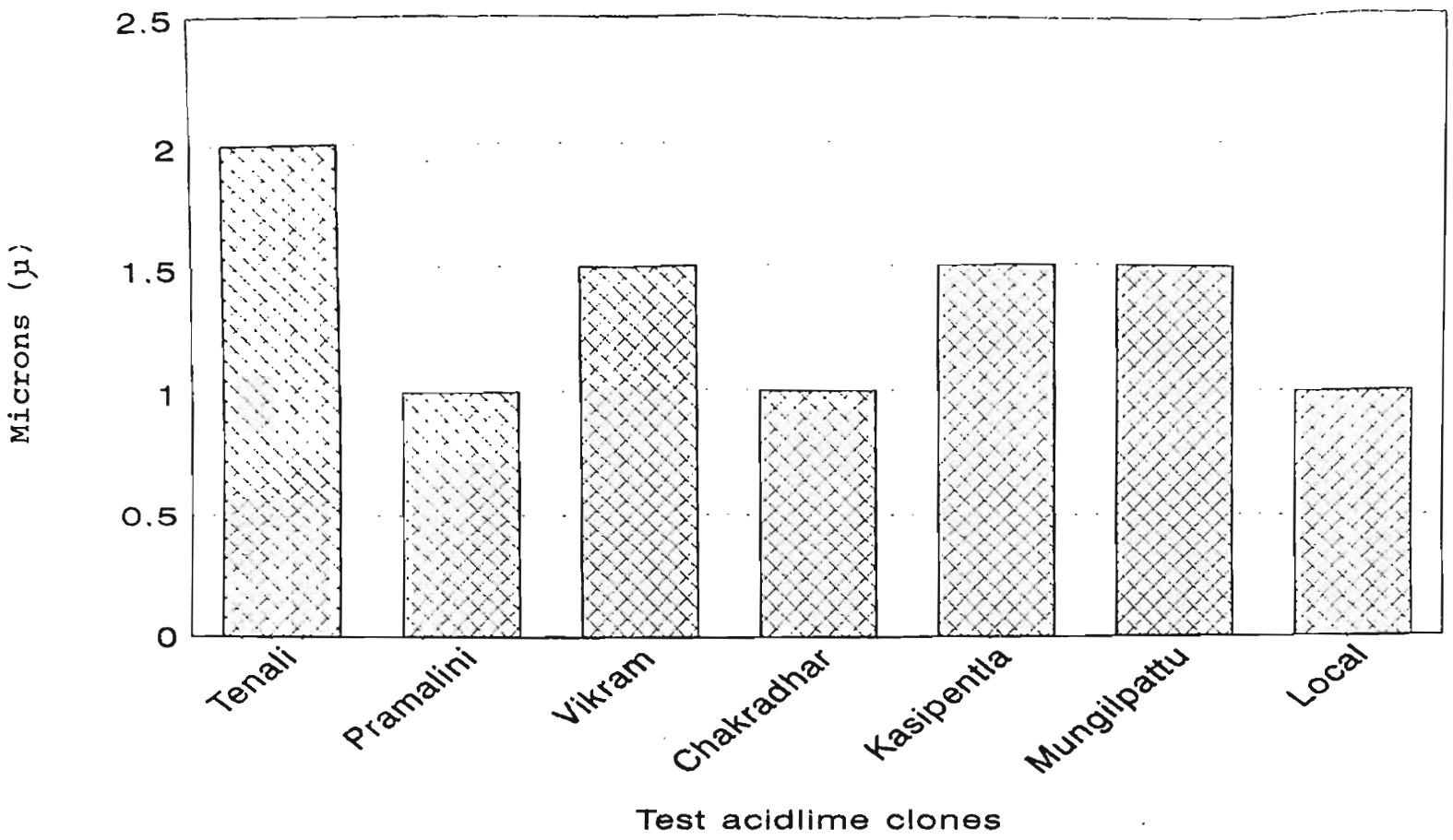
S.NO	Test acidlime clones	Cuticle thickness μ
1.	Tenali	2.0
2.	Pramalini	1.0
3.	Vikram	1.5
4.	Chakradhar	1.0
5.	Kasipentla	1.5
6.	Mungilpattu	1.5
7.	Local	1.0
	CD at 5%	0.12

Table 4. Epidermal thickness in test acidlime clones

S.NO.	Test acidlime clones	Epidermal thickness μ
1.	Tenali	2.0
2.	Pramalini	1.9
3.	Vikram	2.0
4.	Chakradhar	2.0
5.	Kasipentla	2.0
6.	Mungilpattu	2.0
7.	Local	1.9
		NS

APAU CENTRAL LIBRARY
Acc: No: D5741
Date: 4.9.99

Fig.3 Cuticle thickness in test acid lime clones



The results indicated that epidermal thickness in Tenali clone was 2.0 μ, while in susceptible clones it ranged from 1.9 to 2.0 μ.

It is evident that there was no significant difference in epidermal thickness between resistant Tenali kand other susceptible kacidlime clones.

4.3.5 Total Phenols in Test Acidlime Clones

The results of experiments on total phenol content in leaves of test acidlime clones were presented in Table 5, (Fig.4).

The results indicated that phenol content was higher in resistant Tenali clone 9.25 mg g^{-1}, while in susceptible clones it ranged from 5.75 to 7.75 mg. g^{-1}. Phenols were positively correlated with resistance. There was a significant difference in total phenols between resistant Tenali clone and the susceptible acidlime clones.

4.3.6 Ascorbic Acid Content in Test Acidlime Clones

The results of experiments on ascorbic acid content of leaves of test acidlime clones were presented in Table 6, (Fig.5).

Table 5. Total phenols in test acidlime clones

S.NO.	Test acidlime clones	Total phenols ($mg.g^{-1}$ dry weight)
1.	Tenali	9.25
2.	Pramalini	7.25
3.	Vikram	6.75
4.	Chakradhar	5.75
5.	Kasipentla	7.75
6.	Mungilpattu	7.50
7.	Local	6.00
	CD at 5%	0.86

Table 6. Ascorbic acid content in test acidlime clones

S.NO.	Test acidlime clones	Ascorbic acid content ($mg.g^{-1}$ fresh weight)
1.	Tenali	1.1
2.	Pramalini	2.1
3.	Vikram	1.8
4.	Chakradhar	3.6
5.	Kasipentla	3.9
6.	Mungilpattu	1.6
7.	Local	3.1
	CD at 5%	0.17

Fig.4 Total phenols in test acid lime clones

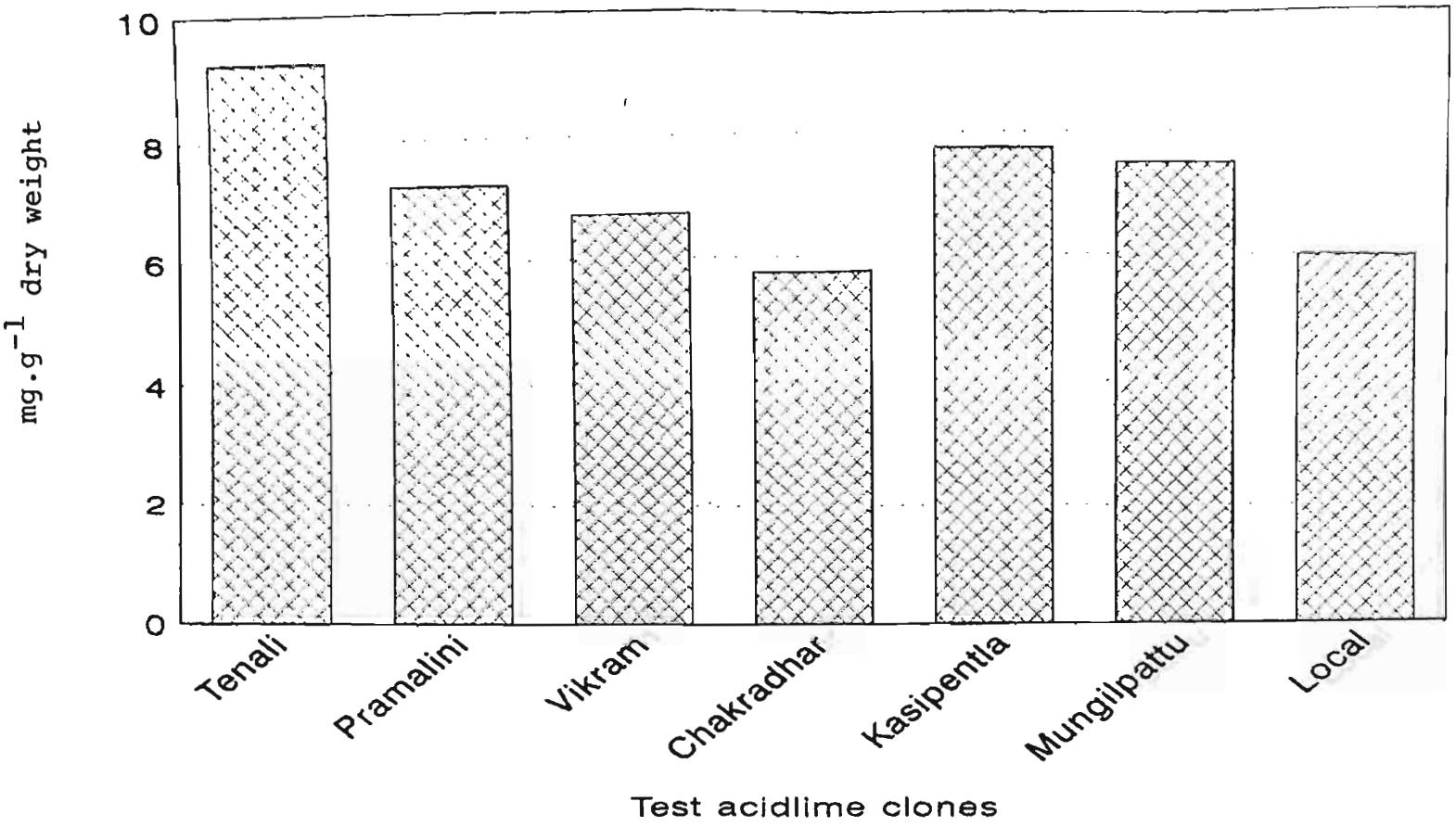
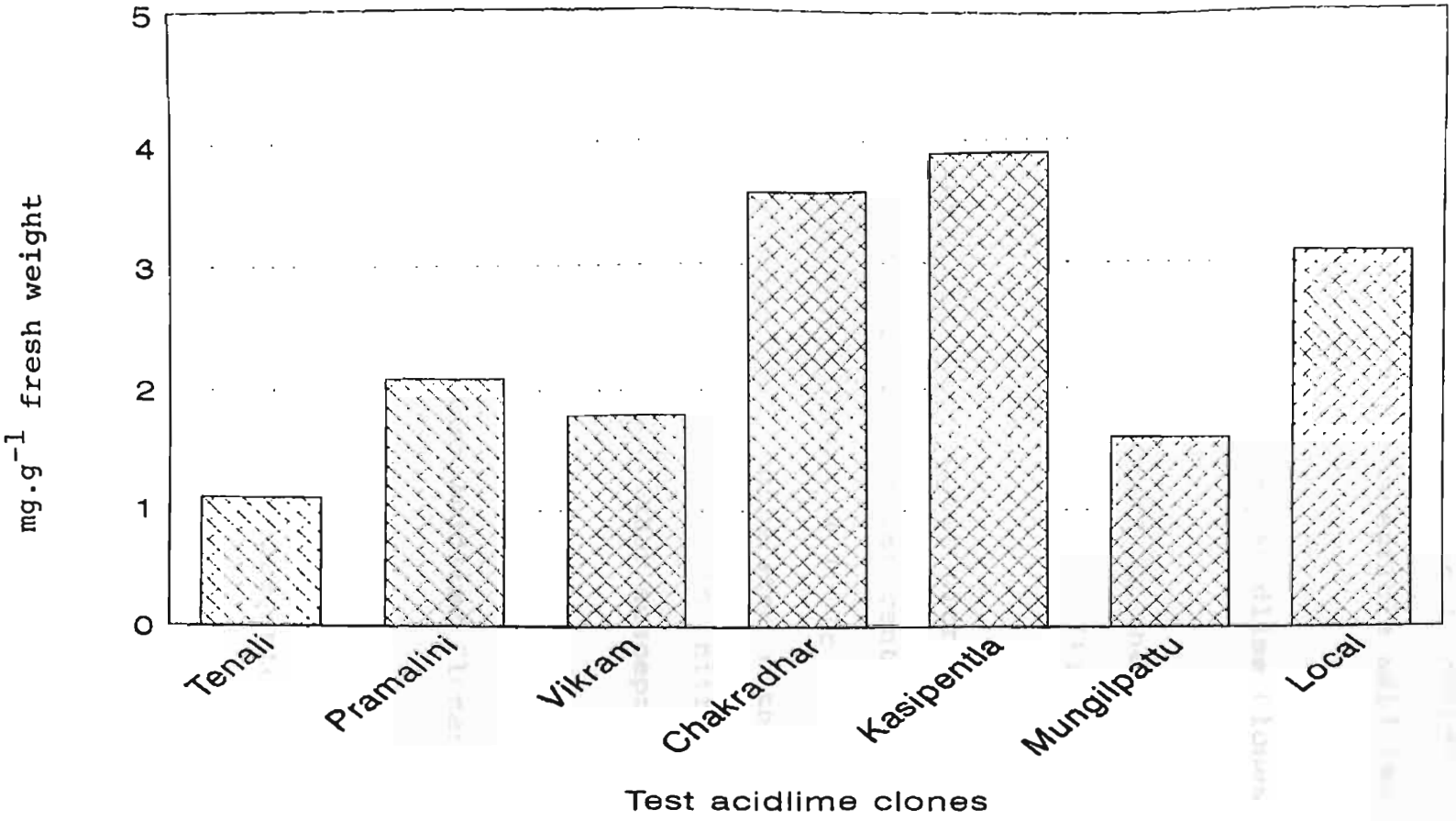


Fig.5 Ascorbic acid content in test acid lime clones



The results showed that ascorbic acid content was less in resistant Tenali 1.1 mg g^{-1}, while in susceptible clones it ranged from 1.6 to 3.9 mg.g^{-1} . Ascorbic acid was negatively correlated with resistance. There was a significant difference in ascorbic acid content between resistant Tenali clone and other susceptible acidlime clones.

4.3.7 Total Nitrogen Content in Test Acidlime Clones

The data on total nitrogen content in the leaves of test acidlime clones were presented in Table 7, (Fig.6)

The results indicated that total nitrogen content was less in resistant Tenali clone 3.28 per cent while in other susceptible clones it ranged from 4.31 to 6.02 per cent. Nitrogen content was negatively correlated with resistance. There was a significant difference in nitrogen content between resistant Tenali clone and other susceptible acidlime clones.

4.3.8 Total Sugar Content in Test Acidlime Clones

The results of experiments on total sugar content in leaves of test acidlime clones were presented in Table 8, (Fig.7).

The results indicated that the total sugars was more in resistant Tenali clone 31.70 mg.g^{-1}, while in other clones it

Table 7. Total nitrogen content in test acidlime clones

S.NO.	Test acidlime clones	Total nitrogen (%)
1.	Tenali	3.28
2.	Pramalini	4.34
3.	Vikram	5.04
4.	Chakradhar	4.76
5.	Kasipentla	6.16
6.	Mungilpattu	6.02
7.	Local	4.31
	CD at 5%	0.86

Table 8. Total sugars in test acidlime clones

S.NO.	Test acidlime clones	Total sugars ($mg.g^{-1}$ dry weight)
1.	Tenali	31.70
2.	Pramalini	26.00
3.	Vikram	26.00
4.	Chakradhar	26.00
5.	Kasipentla	26.00
6.	Mungilpattu	27.90
7.	Local	26.00
	CD at 5%	2.59

Fig.6 Total nitrogen content in test acid lime clones

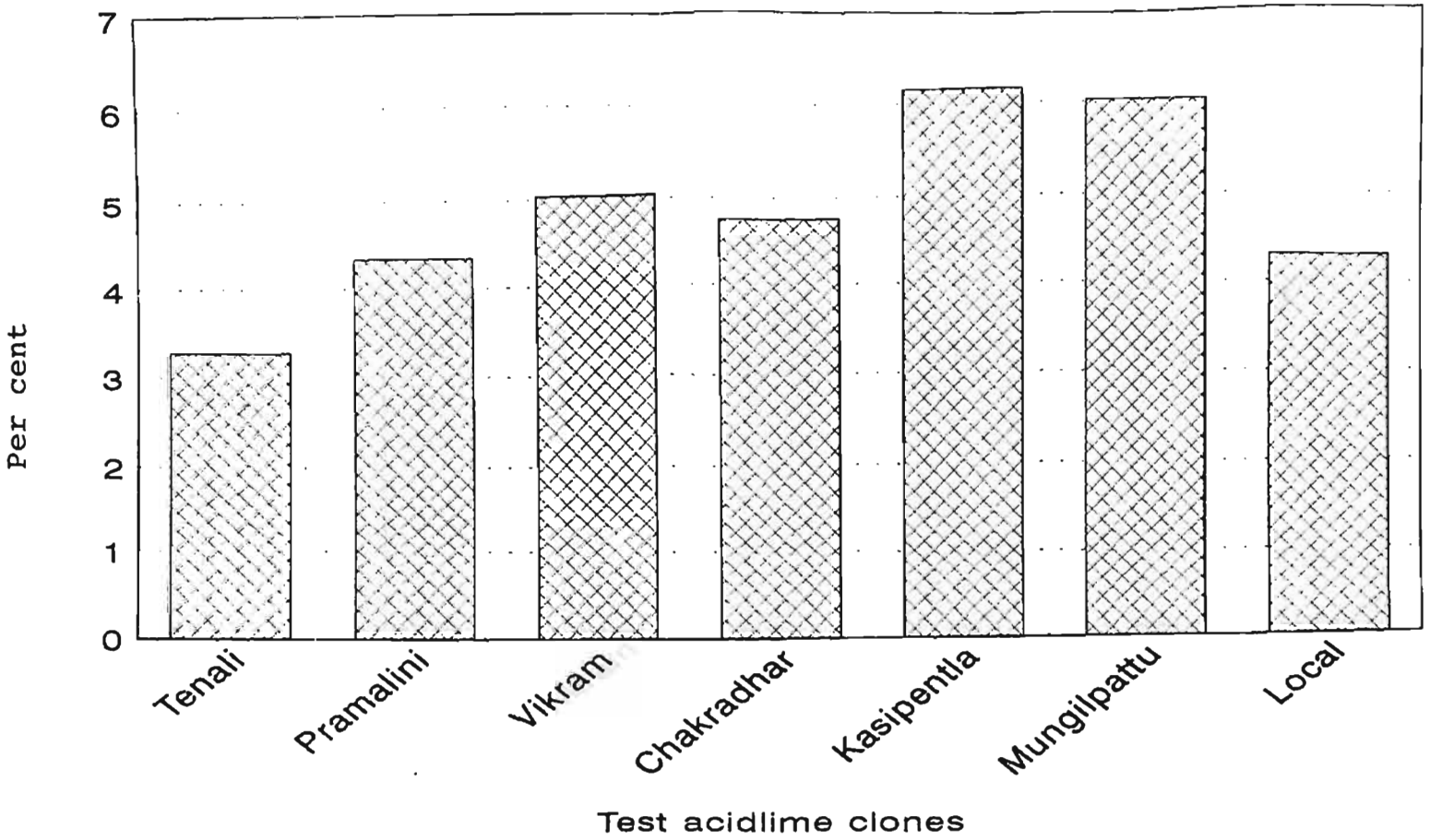
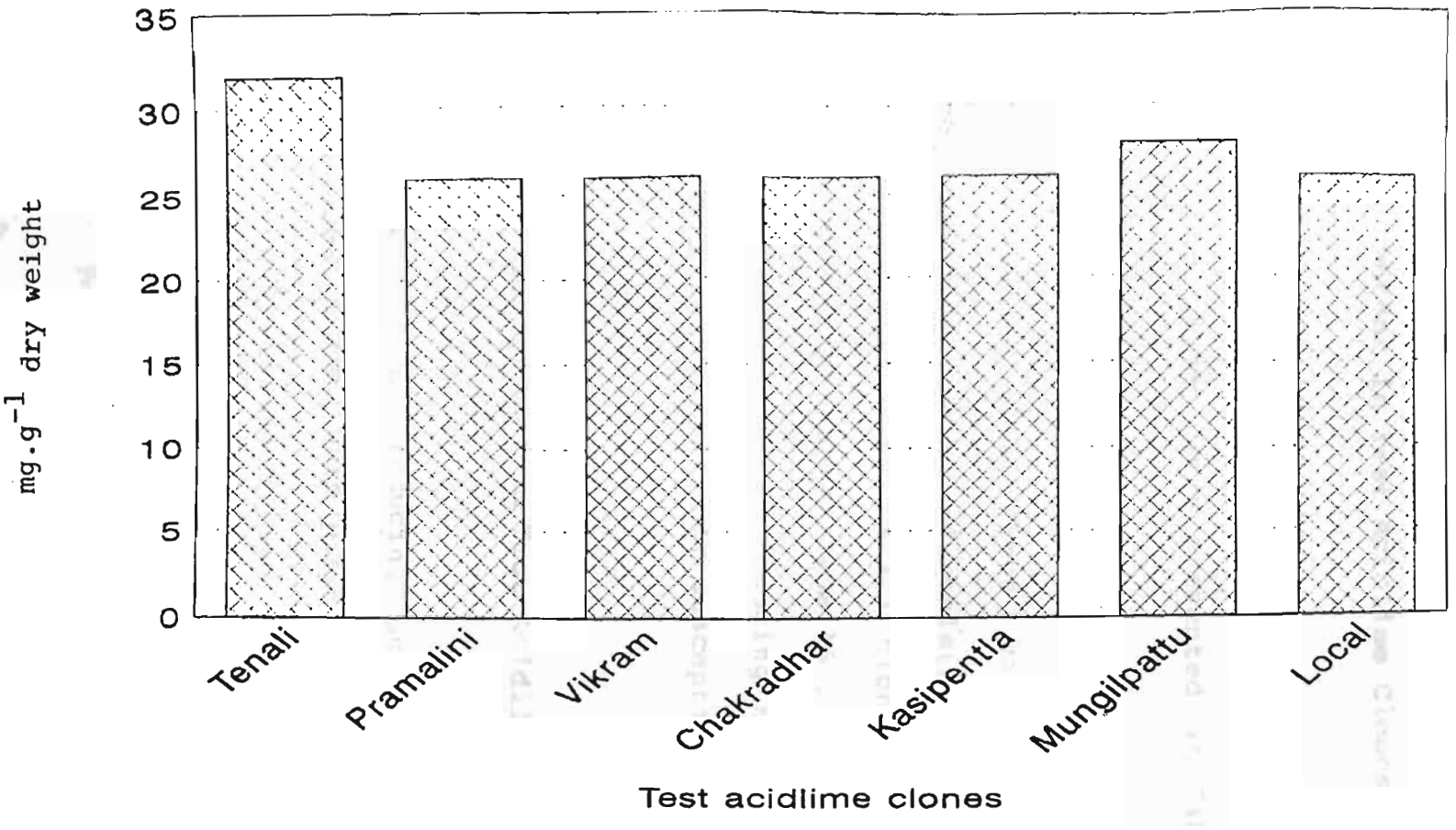


Fig.7 Total sugars in test acid lime clones



ranged from 26.00 to 27.90 $mg.g^{-1}$. Total sugars was positively correlated with resistance. There was a significant difference in total sugars between resistant Tenali clone and other susceptible acidlime clones.

4.3.9 Reducing Sugar Content in Test Acidlime Clones

The results of experiments on reducing sugar content in leaves of test acidlime clones were presented in Table 9, (Fig.8).

The results indicated that there was a positive correlation between reducing sugars and resistance. Reducing sugar content was more in resistant Tenali clone 18.8 mg. g^{-1} while in susceptible clones it ranged from 15.2 to 16.9 $mg.g^{-1}$ There was a significant difference in reducing sugar content between resistant Tenali clone and other susceptible acidlime clones.

4.3.10 Non-reducing Sugar Content in Test Acidlime Clones

The data recorded on non-reducing sugar content in leaves of test acidlime clones were presented in Table 10, (Fig.9).

The results indicated that non-reducing sugar content was more in resistant Tenali clone 12.06 $mg.g^{-1}$, while in

Table 9. Reducing sugars in test acidlime clones

S.NO.	Test acidlime clones	Reducing sugars ($mg.g^{-1}$ dry weight)
1.	Tenali	18.8
2.	Pramalini	16.2
3.	Vikram	15.9
4.	Chakradhar	15.2
5.	Kasipentla	15.7
6.	Mungilpattu	16.9
7.	Local	15.2
	CD at 5%	1.73

Table 10. Non-reducing sugars in test acidlime clones

S.NO.	Test acidlime clones	Non-reducing sugars ($mg.g^{-1}$ dry weight)
1.	Tenali	12.06
2.	Pramalini	9.11
3.	Vikram	9.39
4.	Chakradhar	10.04
5.	Kasipentla	9.57
6.	Mungilpattu	10.23
7,	Local	10.04
	CD at 5%	1.73

Fig.8 Reducing sugars in test acid lime clones

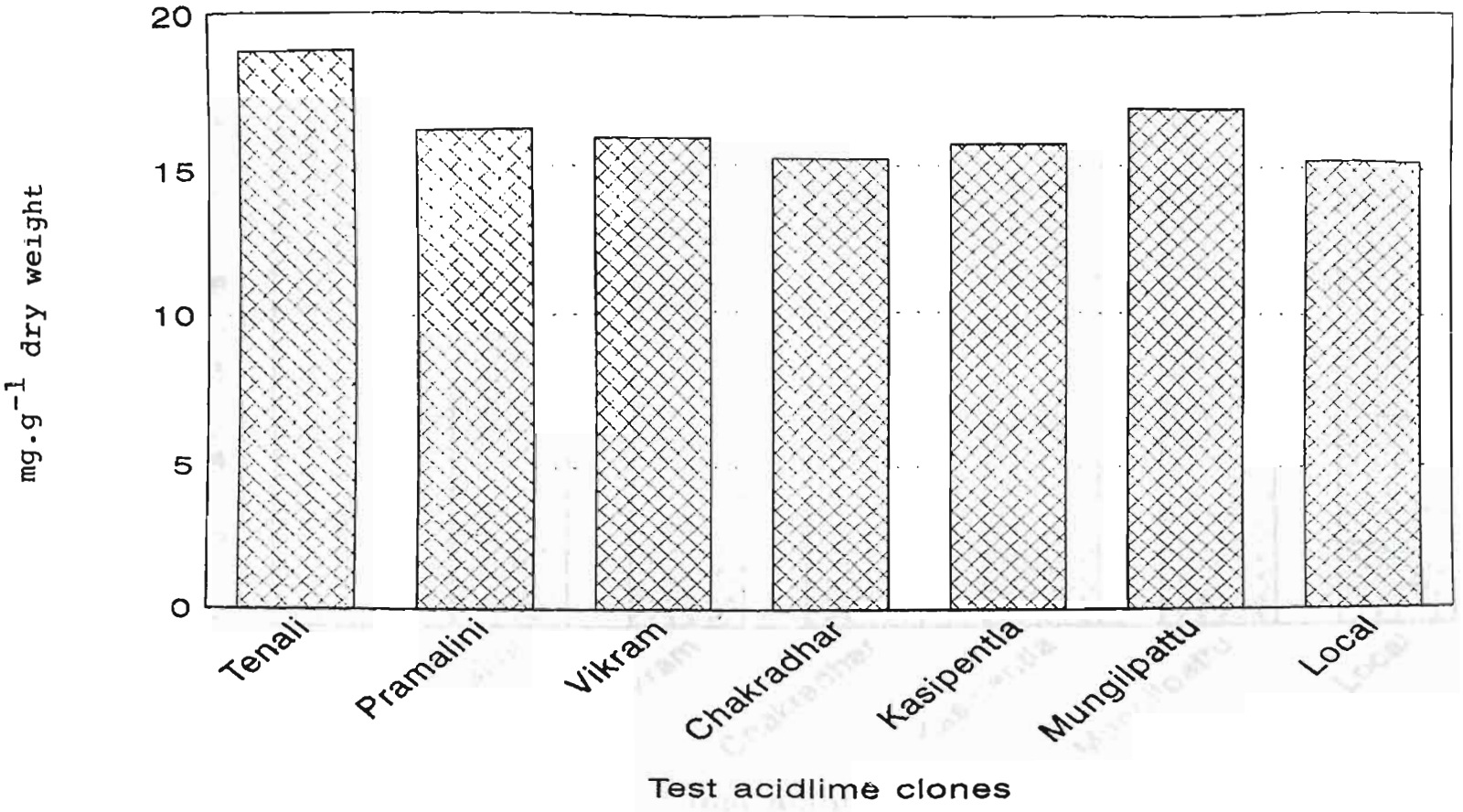
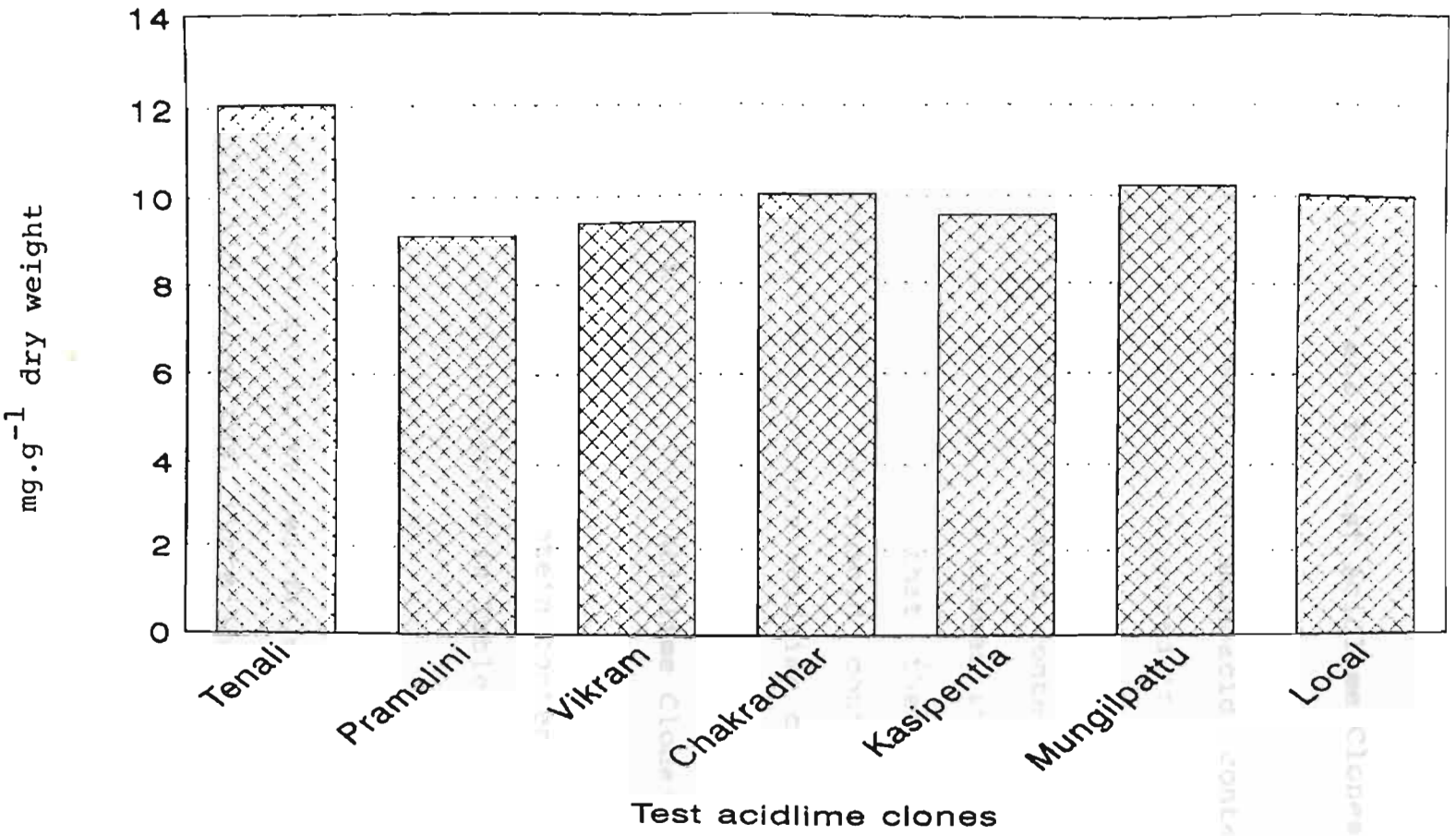


Fig.9 Non-reducing sugars in test acid lime clones



susceptible clones it ranged from 9.11 to 10.23 $mg.g^{-1}$. There was a significant difference in non-reducing sugar content between resistant Tenali clone and other susceptible acidlime clones.

4.3.11 Total Aminoacid Content in Test Acidlime Clones

The results of experiments on aminoacid content in leaves of test acidlime clones were presented in Table 11.

The results indicated that aminoacid content in Tenali was 23.95 $mg.g^{-1}$, while in susceptible clones it ranged from 19.12 to 24.11 $mg.g^{-1}$. It is evident that there was no significant differencein total aminoacid content between resistant Tenali and other susceptible acidlime clones.

4.3.12 Total Protein Content in Test Acidlime Clones

The results of experiments on protein content in leaves of test acidlime clones were presented in Table 12.

The protein content in Tenali 17.42 $mg.g^{-1}$ fresh weight, while in susceptible clones it ranged from 16.35 to 18.60 $mg.g^{-1}$. The results indicated that there was no significant difference in protein content between resistant Tenali and other susceptible acidlime clones. The results showed that the proteins are not correlated with canker resistance in acidlime clones.

Table 11. Total aminoacid content in test acidlime clones

S.NO.	Test acidlime clones	Total aminoacid content $mg.g^{-1}$ dry weight
1.	Tenali	23.95
2.	Pramalini	21.75
3.	Vikram	24.11
4.	Chakradhar	23.25
5.	Kasipentla	23.80
6.	Mungilpattu	24.00
7.	Local	19.12
		NS

Table 12. Total protein content in test acidlime clones

S.NO.	Test acidlime clones	Total proteins $mg.g^{-1}$ fresh weight
1.	Tenali	17.42
2.	Pramalini	17.25
3.	Vikram	18.60
4.	Chakradhar	17.25
5.	Kasipentla	16.35
6.	Mungilpattu	17.25
7.	Local	16.81
		NS

4.3.13 Total Starch Content in Test Acidlime Clones

The results of experiments on starch content of leaves of test acidlime clones were presented in Table 13.

The results indicated that starch content in Tenali was 23.14 $mg.g^{-1}$, while in susceptible clones it ranged from 21.85 to 23.52 $mg.g^{-1}$. There was no significant difference in starch content between resistant Tenali and other susceptible acidlime clones. The results indicated that the starch had no correlation with host reaction.

4.3.14 Chlorophyll a, Chlorophyll b, Total Chlorophylls in Test Acidlime Clones

The data on chlorophyll a, chlorophyll b and total chlorophyll content in test acidlime clones were presented in Table 14, (Fig.10).

It is evident that the total chlorophyll content was more 32.56 $mg.g^{-1}$, in resistant Tenali clone while in other clones it ranged from 22.13 to 28.56 $mg.g^{-1}$, Chlorophyll a content was more 13.78 $mg.g^{-1}$, in resistant Tenali clone while in other clones it ranged from 9.31 to 11.93 $mg.g^{-1}$. Chlorophyll b content was more 18.6 $mg.g^{-1}$, in resistant Tenali clone while in other clones it ranged from 10.32 to 15.92 $mg.g^{-1}$. There was a significant difference in

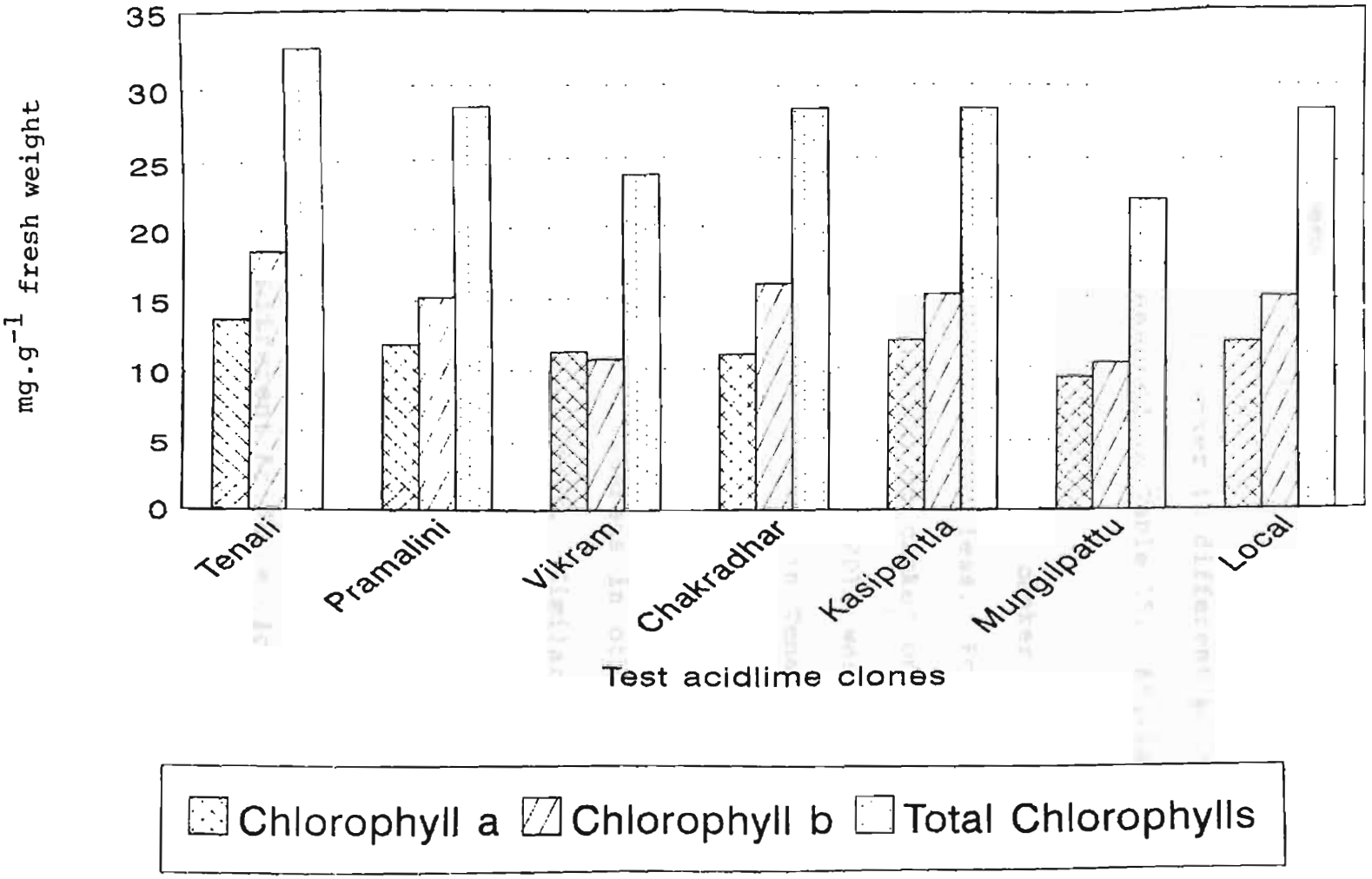
Table 13. Total starch content in test acidlime clones

S.NO.	Test acidlime clones	Total starch $mg.g^{-1}$ dry weight
1.	Tenali	23.14
2.	Pramalini	21.85
3.	Vikram	22.62
4.	Chakradhar	21.91
5.	Kasipentla	23.52
6.	Mungilpattu	22.87
7.	Local	23.02
		NS

Table 14. Chlorophyll a, chlorophyll b and total Chlorophyll content in test acidlime clones

S.NO.	Test acidlime clones	Chlorophyll a	Chlorophyll b	Total chlorophylls
1.	Tenali	13.78	18.60	32.56
2.	Pramalini	11.93	15.23	28.56
3.	Vikram	11.25	10.67	23.82
4.	Chakradhar	11.02	15.92	28.45
5.	Kasipentla	11.90	15.18	28.40
6.	Mungilpattu	9.31	10.32	22.13
7.	Local	11.90	15.80	28.40
	CD at 5%	1.03	1.73	3.46

Fig.10 Chlorophyll a, Chlorophyll b and total chlorophyll content in test acid lime clones



chlorophyll content between resistant Tenali and other susceptible acidlime clones.

4.3.15 Incidence of Canker in Different Plant Parts of Acidlime Clones

The data on incidence of canker in different plant parts of acidlime clones were presented in Table 15, (Fig.11).

It is evident that the incidence of canker in different plant parts of Tenali clone was very less. For this, data were collected on per cent disease of canker on fruit, leaf and twig and per cent disease index (PDI) was calculated. Per cent disease index of fruit canker in Tenali clone was 8.08 per cent, where as in other clones it ranged from 40.88 to 50.85 per cent. Per cent disease index of leaf canker in Tenali clone was 2.20 per cent, whereas in other clones it ranged from 36.66 to 49.02 per cent. Similarly per cent disease index of twig canker in resistant Tenali clone was 6.0 per cent, whereas in other susceptible clones it ranged from 52.0 to 64.0 per cent.

4.3.16 Bark Eruption in Different Acidline Clones

The incidence and intensity of bark eruption in different acidlime clones were expressed in Table 16.

Table 15. Incidence of canker in different plant parts of test acidlime clones

S.NO.	Test acid lime clones	Fruit canker (%)	Leaf canker (%)	Twig canker (%)
1.	Tenali	8.08	2.20	6.0
2.	Pramalini	46.66	41.77	60.0
3.	Vikram	44.53	44.83	52.0
4.	Chakradhar	40.88	36.66	60.0
5.	Kasipentla	48.50	44.25	58.0
6.	Mungilpattu	50.85	47.23	58.0
7.	Local	47.12	49.12	64.0

Table 16. Bark eruption in test acidlime clones

S.NO.	Test acidlime clones	Bark eruption
1.	Tenali	-
2.	Pramalini	Moderate
3.	Vikram	Mild
4.	Chakradhar	Moderate
5.	Kasipentla	Moderate
6.	Mungilpattu	Mild
7.	Local	Severe

Plate 2. Canker intensity on acid lime leaves (0-4 scale)

Plate 3. Canker intensity on acid lime fruits (0-4 scale)

Plate 4. Acid lime twigs with canker symptoms

Plate 5. Bark eruption on the stem of young acid lime seedling

Plate 6. Bark eruption on the trunk of acid lime tree

The results have shown that the incidence of bark eruption in Tenali clone was not observed, whereas in other susceptible clones it was observed as mild in Mungilpattu, Vikram and moderate in Chakradhar, Pramalini, Kasipentla and severe in Local clone.

4.3.17 Rind Per cent, Rind Thickness, Juice Per cent and Titrable Acidity in Fruit of Different Acidlime Clones

The data on rind per cent, rind thickness, juice per cent and titrable acidity in fruit of different acidlime clones were presented in Table 17.

The results indicated that the rind per cent, juice per cent and titrable acidity in Tenali clone was on par with other acidlime clones. Rind thickness in Tenali clone was also similar to that of all other acidlime clones.

4.3.18 Cumulative Yield of Acidlime Clones from 1995-98

The data on cumulative yield of acidlime clones from 1995-98 were presented in Table 18, (Fig.12).

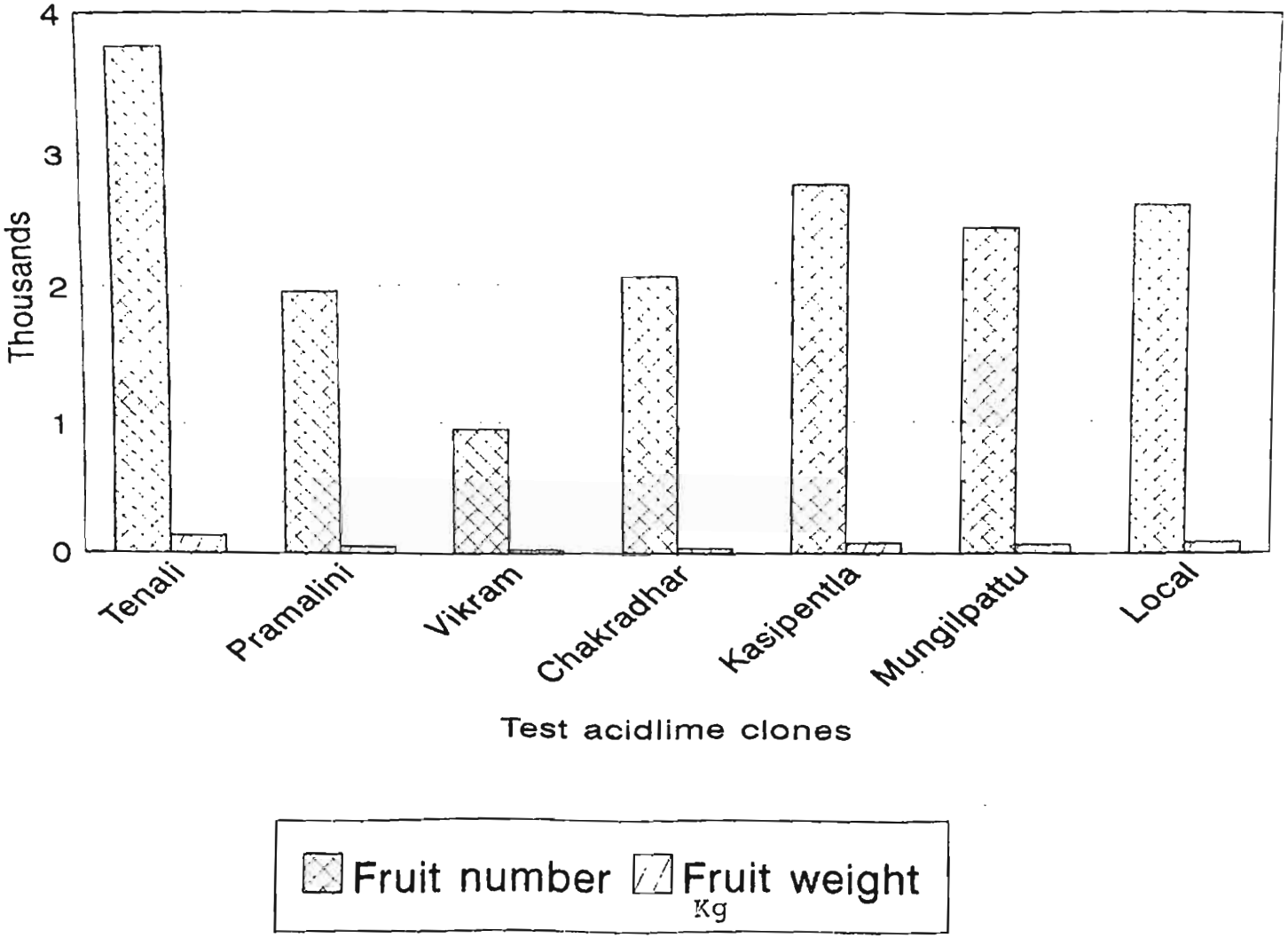
Table 17. Rind per cent, rind thickness, juice per cent and titrable acidity of different acidlime clones

S.NO.	Test acidlime clones	Rind per cent	Rind thick-ness cm	Juice per cent	Titrable acidity mg/100 g of juice
1.	Tenali	16.89	0.10	44.38	6.38
2.	Pramalini	16.16	0.10	36.12	7.23
3.	Vikram	18.05	0.10	41.43	6.03
4.	Chakradhar	21.26	0.10	46.39	6.20
5.	Kasipentla	17.86	0.10	43.50	6.23
6.	Mungilpattu	18.31	0.10	44.02	6.45
7.	Local	18.56	0.10	44.40	6.74

Table 18. Cumulative yield in test acidlime clones from 1995 to 1998

S.NO.	Test acid lime clones	Cumulative yield	
		Fruit number	Fruit weight (kg)
1.	Tenali	3768	128.00
2.	Pramalini	1961	50.20
3.	Vikram	953	25.50
4.	Chakradhar	2049	36.70
5.	Kasipentla	2753	79.40
6.	Mungilpattu	2434	67.02
7.	Local	2612	81.40

Fig.12 Cumulative yield in test acid lime clones from 1995 to 1998



The cumulative yield in fruit number was more in resistant Tenali clone (3768), whereas in other clones it ranged from 953 to 2753. Similarly cumulative yield in terms of fruit weight was also more in resistant Tenali clone (128.00 Kg), whereas in other clones it ranged from 25.50 to 81.40 Kg. It shows that cumulative yield in terms of fruit number and fruit weight was more in Tenali clone compared to other clones.

DISCUSSION

CHAPTER V

DISCUSSION

Acidlime and sathgudi sweet orange are the two commercial cultivars grown in Andhra Pradesh. Out of several diseases, canker incited by Xanthomonas axonopodis pv. citri causes much more economic losses in these cultivars. Though effective management practices are available, they are not economical and cause polution. The economic and eco-friendly way to manage this disease is only through host resistance. Among these acidlime and sathgudi sweet orange cultivars, acidlime is more susceptible to canker disease. Of the several acidlime clonal selections available at AICRP on citrus, Tirupati. Tenali selection was found to be the least affected by canker disease and free from bark eruption.

The defence mechanisms operating in plants are morphological and biochemical. They are either pre-existing or post-infectional. An attempt was made in the present studies to understand the nature of pre-existing morphological and biochemical resistant factors involved in cankerless Tenali selection compared to other canker susceptible clones. As such the relative nature of stomatal characters like their frequency and stomatal aperture in leaf along with cuticle thickness,

epidermal thickness in the morphological resistance and the biochemical constituents like total phenols, total nitrogen, ascorbic acid, total sugars, total amino acids, total proteins, total starch, total chlorophylls in the biochemical resistance and other fruit characters like rind per cent, rind thickness, juice per cent, titratable acidity were studied in resistant Tenali selection compared with other susceptible acidlime clones and also studied per cent disease index of canker on fruit, leaf, twig; incidence of bark eruption on trunk and also recorded cumulative yield from 1995 to 1998 in resistant Tenali clone compared with other susceptible clones.

More stomatal frequency determines susceptibility as shown by Mc Lean (1921) with citrus and Pseudomonas citri, Tang (1958), Kishore and Chand (1972) with citrus and Xanthomonas citri, Pullaiah et al. (1994) with citrus spp. and X. compestris pv. citri. These systems have established that resistance is due to less number of stomata.

In the present investigation, it was found that a significantly less number of stomata (374.26 mm^{-2}) were found in the resistant Tenali clone and more in other susceptible acidlime clones with stomatal number ranging from 416.53 to 538.01 mm^{-2}.

Wider stomatal opening was associated with susceptibility as observed by Mc Lean and Lee (1921) with citrus variety grapefruit and Pseudomonas citri, Tang (1958) with different citrus species viz., Citrus sinensis, Citrus limon, Citrus aurantium and Poncirus trifoliata with Xanthomonas citri, Kishore and Chand (1972) with Citrus aurantifolia and X. citri, Pullaiah et al. (1994) with Citrus spp. and X. campestris pv. citri systems.

In the present investigation also the resistant Tenali clone had narrow stomatal opening (2.34 µ), while in other susceptible acidlime clones had wider stomatal opening ranging from 3.25 to 4.59 µ.

Thick cuticle was associated with resistance as observed by Bassi et al. (1984) with rose and Sphaerotheca pannosa, Xie and Leng (1990) with apple and Marssonia coronaria, Chowdhury A M et al. (1997) with Lentil and Stemphylium botryosum systems.

In the present investigation the resistant Tenali acidlime clone had thick cuticle of 2.0 µ while in other susceptible acidlime clones had thin cuticle which ranged from 1.0 to 1.5 µ.

Thick epidermis was associated with resistance as observed by Kheladze *et al.* (1984) with pear and *Gymnosporangium sabinae*, Tomy-philip *et al.* (1991) with Mulberry and *Cerotelium fici*, Chowdhury A M *et al.* (1997) with lentil and *Stemphylium botryosum* systems.

In the present investigation there was no significant difference in the epidermis thickness between resistant Tenali and other susceptible acidlime clones. This shows that epidermis has no role in host reaction to canker in acidlime.

Several types of chemical constituents in the plants like phenols, ascorbic acid, nitrogen, sugars, aminoacids, proteins and starch are known to paly an important role in offering resistance against pathogens.

Phenols are antimicrobial compounds which restricts the advancement of the pathogen in the host giving resistant reaction as shown by Kishore and Chand (1975) with citrus spp. and *Xanthomonas citri*, Raghuchander *et al.* (1988) with triticale and *Bipolaris sorokiniana*, Pullaiah *et al.* (1993) with Citrus spp. and *X. axonopodis* pv. *citri* systems.

In the present investigation there were significantly greater amounts of phenols in resistant Tenali clone (9.25 $mg.g^{-1}$ dry wt.), while in other susceptible acidlime clones it ranged from 5.75 to 7.75 $mg.g^{-1}$ dry weight of the leaf.

Less quantity of ascorbic acid was found in the resistant plants when compared to the susceptible ones as shown by Ragab *et al*. (1987) with barley and *pyrenophora teres*, Reddy N and Khare (1988) with groundnut and *Puccinia arachidis*, Chen-lifeng *et al*. (1997) with wheat and *Fusarium graminearum* systems.

In the present investigation there was significantly less ascorbic acid in the resistant Tenali clone (1.1 $mg.g^{-1}$) and in other susceptible clones it ranged from 1.6 to 3.9 $mg.g^{-1}$ on fresh weight basis of the leaf.

Nitrogen content is linked with resistance and susceptibility in plants. More quantities being found in susceptible as shown by Padmanaban *et al*. (1974) with citrus and *X. c.* pv. *citri*, Gupta *et al*. (1987) with sesamum and *Alternaria* spp., Pullaiah *et al*. (1993) with citrus spp. and *X. axonopodis* pv. *citri* systems.

In the present investigation there was significantly less quantity of total nitrogen found in the resistant Tenali clone (3.28%), while in other susceptible clones it ranged from 4.31 to 6.16 per cent on dry weight basis of the leaf.

Total sugars, reducing and non-reducing sugar content is associated with resistance and susceptibility; more quantities being found in resistant plants as shown by Mahto *et al*. (1987) with rice and *x.c.* pv. *oryzae*, Siddiqua and Kashem (1993) with rice and *x.c.* pv. *oryzae*, Satyanarayana *et al*. (1995) with maize and *Macrophomina phaseolina*.

In the present investigation there were significantly more quantities of total sugars, reducing and non-reducing sugars in the resistant Tenali clone (31.70 $mg.g^{-1}$, 18.8 $mg.g^{-1}$ and 12.06 $mg.g^{-1}$) respectively, whereas in other susceptible clones the total sugars ranged from 26.00 to 27.90 $mg.g^{-1}$, the reducing sugars ranged from 15.2 to 16.9 $mg.g^{-1}$ and non-reducing sugars ranged from 9.11 to 10.23 $mg.g^{-1}$ dry weight of the leaf.

Amino acids either quantitatively or qualitatively associated with resistance and susceptibility. More quantities of amino acids were found in the resistant plants when compared to the susceptible ones as shown by

Shree and Reddy (1986) with sorghum and Helminthosporium, Chourasia *et al*. (1987) with betelvine and *X. c.* pv. *beticola*, Agrawal (1990) with pigeonpea and *P. drechsleri* f.sp. *cajani*. Pullaiah *et al*. (1973) with Citrus spp. and *X. axonopodis* pv. *citri* systems.

In the present investigation there was no significant difference in amino acid content between resistant Tenali clone and other susceptible acidlime clones which shows that amino acids have no role in host reaction.

Proteins have also got bearing on the resistance mechanism in plants. More quantities of proteins were found in resistant plants when compared to susceptible plants as shown by Samiran Gangopadhyay *et al*. (1986) with maize and *Rhizoctonia solani*, Raghuchander (1988) with triticale and *Bipolaris sorokiniana*, Angra-Sharma and Sharma (1994) with maize and *Helminthosporium maydis* systems.

In the present investigation there was no significant difference in protein content between resistant Tenali clone and other susceptible clones and indicated that there was no role of protein content in host reaction.

Starch content is also associated with resistance and susceptibility. More quantities of total starch being found in resistant plants as shown by Temirbekova (1981) with wheat and Alternaria, Helminthosporium, Fusarium, Cladosporium spp. and bacteria, Pall (1994) with Eleusine coracana and blast, Marik (1997) with barley variety Luxor and Erysiphe graminis systems.

In the present investigation there was no significant difference in starch content between resistant Tenali clone and other susceptible acidlime clones indicating that there was no role of starch content in host reaction.

Chlorophyll content had directly related to yield. High yielding varieties posses more amounts of chlorophylls per unit leaf weight as shown by Agaev et al. (1988) with red clover, Eid et al. (1991) with garlic plants, Babu et al. (1993) with Vigna radiata systems. The net blotch resistant barley variety (Giza 117) contained higher levels of chlorophyll a and chlorophyll b was shown by Ragab et al. (1987) in barley.

In the present investigation there were significantly more quantities of chlorophyll a and chlorophyll b and total chlorophylls in the resistant Tenali clone compared to other susceptible acidlime

clones. There was a positive correlation between chlorophyll content and yield potential of resistant Tenali clone. Yields were more in resistant Tenali clone compared to other susceptible acidlime clones.

Per cent incidence of canker was studied in different plant parts of acidlime clones. The per cent incidence of fruit canker, leaf canker, twig canker was very less in resistant Tenali clone and in other susceptible clones it was more. Tenali clone is free from bark eruption whereas in other clones mild to severe bark eruption was noticed on trunk.

Cumulative yield in terms of fruit weight and fruit number was more in resistant Tenali clone compared to other clones.

Rind per cent, rind thickness, juice per cent and titrable acidity was also studied in resistant Tenali clone compared to other acidlime clones. There was no significant difference in rind per cent, juice per cent and titrable acidity in different acidlime clonal selections. They were on par with one another; rind thickness is similar in all acidlime clones.

SUMMARY

CHAPTER VI

SUMMARY

Citrus canker is a major disease causing severe economic losses. Though effective management practices are available they are not economical. The only economic and eco-friendly way is through host resistance. Screening was done with few acidlime clonal selections available at AICRP on citrus, Tirupati viz., Tenali, Pramalini, Vikram, Chakradhar, Kasipentla, Mungilpattu and Local selections.

In the present investigation the nature of resistance in the above resistant and susceptible acidlime clones was studied with particular reference to the pre-existing morphological and biochemical nature.

Since the bacterium (Xanthomonas axonopodis pv. citri) causing citrus canker is known to enter through stomata, the frequency and size of stomatal aperture were studied. Thick cuticle and thick epidermis are also known to abstruct the penetration of the pathogen into the host was also studied in resistant Tenali clone of acidlime in comparison with other susceptible acidlime clones, to understand the nature of morphological resistance.

There were significantly less number of stomata (374.26 mm^{-2}) and narrow stomatal aperture (2.34 µ) in the resistant Tenali clone compared to other susceptible clones, where the stomatal number ranged from 416.53 to 538.01 mm^{-2} and the size of aperture ranged from 3.25 to 4.59 u. The cuticle thickness was more in the resistant Tenali clone (2.0 µ) while in other susceptible clones it ranged from 1.0 to 1.5 µ. There was no significant difference with epidermal thickness between resistant and susceptible acidlime clones. These results indicated that there is morphological resistance in the resistant Tenali acidlime clone with reference to stomatal frequency, stomatal aperture and cuticle thickness.

The involvement of biochemical factors particularly the total phenols, ascorbic acid, total nitrogen, total sugars, reducing and non-reducing sugars, total amino acids, total proteins, total starch, chlorophyll a, chlorophyll b and total chlorophylls for disease resistance was also investigated in the leaves of resistant Tenali clone compared to other susceptible acidlime clones.

There were higher quantities of total phenols, total sugars, reducing and non-reducing sugars, total chlorophylls, chlorophyll a and chlorophyll b and observed

lower quantities of ascorbic acid, total nitrogen in the leaves of resistant Tenali clone compared to other susceptible acidlime clones. There was no significant difference with total amino acids, total proteins, total starch in resistant Tenali clone compared to other susceptible acidlime clones. The per cent incidence of canker on fruit, leaf, twig and bark eruption on the trunk was also studied. The per cent incidence of canker on fruit, leaf, twig was less in resistant Tenali clone whereas in other clones the was more. Further Tenali clone was found free from bark eruption.

Cumulative yield interms of weight of fruit and fruit number was also studied, cumulative yield both interms of fruit weight and in fruit number was more in resistant Tenali clone whereas in other clones it was far less.

Some of the fruit qualities like rind per cent, rind thickness, juice per cent and titrable acidity was also studied in resistant Tenali clone compared to other clones. There was no significant difference in rind per cent, juice per cent and titrable acidity in different acidlime clonal selections. They are on par with one another. Rind thickness is similar in all acidlime clones.

Further studies on certain aspects such as post infectional morphological and biochemical resistance mechanism and histopathological aspects, relative humidity in the sub-stomatal chamber, the components present in the cuticle, the role of oxidizing enzymes like polyphenol oxidase, ascorbic acid oxidase enzymes and qualitative differences in phenols etc. in the resistant Tenali clone in comparison with other susceptible acidlime clones may be carried out to understand fully the mechanism of resistance in Tenali acidlime clone to canker disease.

LITERATURE CITED

LITERATURE CITED

*Agaev F N, Samorodova and Bianki G B 1988 Interrelation between content of nutrients, plastid pigments in the green matter of red clover. Sbornik - Nauchnykh - Trudov - po - prikladnoi - Botanike - Genetike - i -Selektsii 121 : 45-54.

Agrawal S C 1990 Effect of phytophthora stem blight on aminoacids and protein contents of pigeonpea plants. Indian Journal of Pulses Research 3 : 171-174.

Angrasharma R and Sharma D K 1994 Biochemical and histological studies on susceptible and resistant maize leaves infected by Helminthosporium maydis. Plant Pathology 43 : 972-978.

Arnon D T 1949 Copper enzymes in isolated chloroplasts, polyphenol - oxidase in Beta vulgaris. Plant Pathology 24 : 1-25.

Babu R C, Muralidharan V, Rathnaswamy R and Rangaswamy S R S 1993 Photosynthesis, chlorophylls, leaf areas and seed yield in mungbean hybrids and their parents. Indian Journal of Pulses Research 6 : 57-59.

*Bartholomow J W 1962 Variables influencing results and the precise definition of the steps in gram staining as a means of standardizing the results obtained. Stain Technology 37 : 139-155.

*Bassi M, Conti G G, Maffi D and Bocci A M 1984 Preliminary observations on Sphaerotheca pannosa development in a susceptible and a resistant rose cultivar Rivista-di-patologia-vegetale, 20 : 134-137.

Bhatia J N and Thakur D P 1994 Biochemical components of pearl millet in relation to downy mildew disease. Indian Journal of Mycology and Plant Pathology 24 : 216-219.

Bisen P S 1975 Changes in amino acid content of healthy and Aspergillus niger affected apple tissue. Indian phytopathology 28 : 131-133.

Chattopadhyay A K 1989 Relationship of phenols and sugars in Alternaria blight resistance of rapeseed mustard. Indian Journal of Mycological Research 27 195-199.

Chauhan M S 1987 Amino acids carbohydrates and phenolics in tobacco varieties susceptible and moderately resistant to Helminthosporium spiciferum. Indian Journal of Mycology and Plant Pathology 17 : 17-23.

Chen-LiFeng, Ye-Moobing, Chen-yong xing, xu-langlai, xu-yong Gao, Chen-LF, Ye-MB, Chen-yx, xu-LL and xu-YG 1997 The relationship between ascorbic acid and resistance of wheat to scab. Acta-phytopathologica-sinica 27 : 113-118.

Chourasia R K, Nayak M L and Khare M N 1987 Some biochemical and structural indicators of resistance to bacterial leaf spot of betelvine (Piper betel L.). Indian Journal of Mycology and Plant Pathology 17 : 175.

*Chowdhury AM, Asheque-Ahmed Zaman M, Chowdhury AM and Ahmed A 1997 Studies on the defence structural factors of some susceptible and resistant varieties of lentil plants. Journal of Mycopathological Research 35 : 35-39.

*Danilova A N and Kotukhov Yu A 1991 Susceptibility to rust in Allium nutans plants from natural populations. Byulleten - Glavnogo - Botanicheskogo - Sada. No. 159 : 76-81.

* Dubois M, Gilles K A, Hamliton J K, Rebers P A and Smith F 1956 Calorimetric method for determination of sugars and related substances. Analytical Chemistry 28 : 350-356.

* Easwaran K S S 1973 Physiology of resistance to a bacterial disease in sorghum. Nature of nitrogen synthesis in sorghum tissues. Phytopathologische Zeitschrift 76 : 117-122.

* EI-Khateeb M A, Badway-E M and Boselah N A E 1991 Effect of some growth regulators on growth, chemical composition and essential oil of Ruta graveolens L. Plants. Bulletin of Faculty of Agriculture, University of Cario 42 : 829-848.

* Eid S M, Shafshak N S and Abo Sedera F A 1991 Effect of potassium fertilization and foliar application of certain micro-nutrients combinations on growth, yield and chemical composition of garlic plants. Annals of Agricultural Science, Moshtohor 19 : 981-993.

Farkas G L and Kirally Z 1961 Amide metabolism in wheat leaves infected with stem rust. Physiologia plantarum 14 : 344-353.

* Gaponenko V I, Baleva E F, Nikolaeva G N, Trufanova N I, Zhebrakova I V, Kashevshaya O V, Dovnar V S and Babich N S 1984 Activity of ribulose bisphosphate carboxylase in barley plants of different yield at the headling stage. Doklady Akademii Nauk Belorusskoi S S R 28 : 664-667.

Gupta S R, Gupta P P, Kaushik C D and Sharma C S 1987 Biochemical changes in leaf surface extract and total chlorophyll content of sesame in relation to Alternaria leaf spot disease. Indian Journal of Mycology and Plant Pathology 17 : 165.

Have H and Kauffman H E 1972 Effect of nitrogen and spacing on bacterial leaf blight of rice. Indian Farming 21 : 8-13.

* Jindal S, Arora Y K and Bajaj K L 1979 Studies on the phenols of resistant and susceptible varieties of muskmelon in relation to powdery mildew disease. Plant Biochemical Journal 6 : 48-53.

Khatri H L and Chenulu V V 1969 Metabolism of resistance and susceptible cowpea varieties infected with cowpea mosaic virus changes in Carbohydrates contents. Indian Phytopathology 22 : 453-457.

* Kheladze V S, Dvurechenskaya and Tskhvendazde L P 1984 Resistance factors in varieties of pear against rust Gymnosporangium sabinae (Dicks) Wint. Mikologiya-i-Fitopatologiya 18 : 143-144.

Kiraly Z 1974 Methods in Plant Pathology, Published by Els Vier Scientific Publishing Co., New York, 138.

* Kishore V and Chand J N 1972 Citrus canker in Haryana. Haryana Agricultural University Journal of Research 2 : 124-127.

Kishore V and Chand J N 1975 Resistance of citrus to citrus canker caused by Xanthomonas citri analysis of phenols and sugars. Indian Phytopathology 28 : 46-47.

* Legenchenko B I, Raskin V I, Rakovskii V D and Berestov I I 1988 Characterisation of potential yield in cereals from parameters of low temperature fluorescence of the chlorophyll. Sel Skokhozyistvennaya Biologiya. No.6 : 9-12.

Lowry H, Rosebrough N T, Far A L and Randall A J 1951 Protein measurement with the Folinphenol reagent. Journal of Biological Chemistry 193 : 256-275.

* Luthra Y P, Joshi U N, Gandhi S K and Arora S K 1996 Biochemical constituents in seeds of sorghum varieties resistant and susceptible to leaf spot disease. Plant Disease Research 11 : 54-56.

Mahadevan A and Sridhar R 1982 Methods in Physiological Plant Pathology Published by Sivakami Publications, Madras pp.115-118.

* Mahto B N, Singh R N, Awasthi C P and Abidi A B 1987 Sugars and Phenolic compounds in rice leaves in relation to varietal resistance to bacterial blight pathogen. International Rice Research News letter 12 : 12-13.

* Marik P 1997 Winter barley Luxor Genetika-a-Slechteni 33 : 75-76.

Marimuthu T and Kandaswamy T K 1980 Changes in the amino nitrogen and aminoacids in the mung lines moderately resistant and susceptible to bacterial leaf blight organism. Indian Phytopathology 36 : 345-348.

Mc Cready R, Goggle M J, Silviera V and Owens H S 1950 Determination of starch analysis in vegetables application to peas. Analytical Chemistry 29 : 1156-1158.

* Mc Lean F T 1921 A study of the structure of the stomata of two species of citrus in relation to citrus canker. Bulletin of Torrey Botanical Club 48 : 101-106.

* Mishra A, Siradhana B S and Shirpuri A 1980 Phenols in relation to resistance of sorghum to anthranose. Philippine Agriculturist 63 : 71-73.

Mogle T R and Mayee C D 1979 Comparative changes in reducing sugars, starch and free amino acids accompanying downy mildew infection in resistant moderately resistant and susceptible lines of pearl millet. Indian Journal of Mycology and Plant Pathology 11 : 61-65.

Mohammed M A, Hassan A A, Okshi I and Hilal R 1981 Nature of resistance to Fusarium wilt in watermelon. Egyption Journal of Horticulture 8 : 1-12.

Pall B S 1994 Biochemical studies or pathogenesis of fingermillet blast. Research and Development Reporter 11 : 43-47.

Padmanaban D, Vidyasekaran P and Rajagopalan C K S 1974 Changes in photosynthesis and carbohydrates content in canker and regions in Xanthomonas citri infected citrus leaves. Indian Phytopathology 27 : 215-217.

Patel RS, Desai MV and Feltier GL 1970 Control of Citrus Canker. Indian Journal of Horticulture 27 : 93-98.

* Pavlenko E P 1993 features of the leaf epidermal tissue in interspecfic hybrids of Allium in relation to resistance to Peronospora Byulleten-Glavnogo-Botanicheskogo-sada 168 : 65-71.

Pavlenko E P 1993 Histometric analysis of elements of the periderm in leaves of interspecific onion hybrids in relation to resistance to Peranosporay destructor. Byulleten-Glavnogo-Botanicheskogo-Sada 167 : 88-95.

Philip T and Govindaiah 1996 Studies on the factors contributing for disease resistance in mulberry against Cercospora moricola. Indian Journa of Sericulture 35 : 39-42.

* Popova E V and Temirbekova S K 1981 Physiological and biochemical bases of increasing the resistance in wheat to enzymomycotic exhaustion. Trudy vsesoyuznogo Nauchno Issledovatel skogo Instituta Zashchity Rastenii. Biological bases and practical use of induced immunity of plants to diseases and pests. 40-49.

Pullaiah N, Moses G J and Kumar P S 1994 Morphological nature of resistance in citrus to canker disease Xanthomonas campestris pv citri (Hasse) Dye.) Journal of Research APAU 22 : 139-140.

Pullaiah N, Moses G J and Kumar P S 1993 Biochemical nature of resistance in citrus to canker disease (Xanthomonas campestris pv citri (Hasee) Dye.) Journal of Research APAU 21 : 172-173.

* Ragab M M, Osman A R, Ghobrial E, Barakat F M and El-Nashar F K 1987 Relation between chemical composition of barley and net blotch. Agricultural Research Review 65 : 225-232.

* Raguchander T, Srikant Kulkarni Hedge R K and Kulkarni S 1988 Studies on leaf blight of triticale caused by Bipolaris sorokiniana (Sacc.) Shoem. Plant Pathology News letter 6 : 45.

Rai J N, Singh R P and Saxena V L 1980 Phenolic and cell wall degrading enzymes in Sclerotinia infection of resistant and susceptible plants of Brassica juncea Indian Journal of Mycology and Plant Pathology 9 : 160-165.

Ramos L J, Narayanan K R and Mc Millan R T Jr 1992 Association of stomatal frequency and morphology in Lycopersicon species with resistance to Xanthomonas campestris pv. vesicatoria. Plant Pathology 41 : 157-164.

Ranganna S 1977 Manual of analysis of fruit and vegetable products. Hill Publishing Company Ltd., New Delhi : 7-8.

Rangaswami G and Natarajan 1966 Changes in the free amino acid and C:N ratios of banana leaves infected with fungal pathogens. Indian Phytopathology 29 : 59-63.

Reddy P N and Khare M N 1988 Physiology of ground nut rust disease : Changes in total sugars, phenols, ascorbic acid, Peroxidase and phenol oxidase. Journal of oil seeds Research 5 : 102-106.

*Reddy M R S, Murthy V D, Rao G N and Reddy G S 1981 Bark eruption on acidlime (Citrus aurantifolia Christm.) Swingle.) an additional symptom of citrus canker. Current Research 10 : 21-22.

Reddy M R S and Naidu P H 1986 Bacterial canker on roots of acidlime (Citrus aurantifolia Christm.) Swing). Indian Phytopathology 39 : 588-589.

Riker A J and Riker R S 1936 Introduction to research on plant disease. John S. Swift Co Inc. St. Louis, Chicago, New York, Cincinnati, Cleveland pp 117.

Sadasivm S and Manickam A 1996 Biochemical methods. New age international (P) Limited, Publishers, II (ed), New Delhi : 193-194.

Samiran Gangopadhyay, Sangam Lal, Gangopadhyay S and Lal S 1986 Changes in certain biochemical constituents in maize (Zea mays L) leaf sheath infected with Rhizoctonia solani Kuhn. Indian Journal of Plant Pathology 4 : 9-16.

Saradadevi C 1982 Physiological studies in safflower (Carthamus tinctorius L.) under NaCl salinity. M.Phyll. Thesis Sri Venkateswara University, Tiruapti.

Satyanarayana E, Saikumar R and Reddy M S S 1995 Relationship of some prime biochemical compounds with charcoal rot resistance in corn (Zea mays L.). Mysore Journal of Agricultural Sciences 29 : 120-124.

* Shree M P and Reddy C N 1986 Effect of Helminthosporiose infection on certain biochemical constituents in the resistant and susceptible varieties of Sorghum. Indian Journal of Plant Pathology 4 : 46-52.

Siddiqua A and Kashem M A 1993 Total phenol and soluble sugar in rice leaf sheaths in relation to resistance to sheath blight pathogen. Indian Journal of Agricultural Research 27 : 57-63.

Singh R S and Prashar M 1984 Studies on metabolic changes in aminoacids, sugars, total acidity and vitamin C in pea fruit infected with Rhizopus stolonifer. Indian Phytopathology 37 : 334-335.

* Stein W H and Moore S 1948 Chromatography of amino acids on Starch columns separation of phenyl alanine leucine, methionine, tyrosine and valine. Journal of Biological Chemistry 176 : 337-365.

* Tang C Y 1958 The epidermal structure of citrus in relation to its susceptibility to canker infection. Acta. Phytopath. Sin 4 : 56-70.

Thind T S, Saksena S B and Agrawal S C 1977 Post infectional changes in aminoacids, sugars, phenols, substances and organic acids of apple fruits incited by Clathridium corticola. Indian Phytopathology 30 : 323-325.

Thind B S, Sharma R R and Singh N 1979 Changes in sugars amino acids and phenols in chilli leaves (Capsicum annum) in response to Xanthomonas vesicatoria infection. Indian Journal of Mycology and Plant Pathology 10 : 131-135.

Tomy Philip, Govindaiah, Sangupta K, Naik V N and Philip T 1991 Anatomical nature of resistance in mulberry genotypes against Cerotelium fici causing leaf rust. Indian Phytopathology 44 : 249-251.

Verma J P and Singh R P 1974· Studies on the nutrition of Xanthomonas malvacearum and its relationship to free amino acids, organic acids and sugars of resistant and susceptible cotton cultivars. Acta. Phyto. Acad. Sci. Hungarica 9 : 53-63.

Xie W L and Leng H Q 1990 Studies on apple blotch 2. Relation between the resistance of apple cultivars and their physical and physiological characters. Journal of Sichuan Agricultural University 8 : 51-54.

Yang J, Verma P R and Lees G L 1992 The role of cuticle and epidermal cell wall in resistance of rape seed and mustard to Rhizoctonia solani. Plant and Soil 142 : 325-321.

* Original not seen

APAU
Acc: No: D5741
Date: 4.999