14th Triennial Symposium of ISTRC, CTCRI, Kerala, India

Pre- and Post - infection Resistant factors in Taro[*Colocasia esculenta*] in response to *Phytophthora* leaf blight

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Phytophthora- the 'Killer Pathogen' has devastating disease effects on numerous crop and ornamental plants. *Phytophthora colocasiae* causes leaf blight of taro and remains the most serious constraint to taro production. The disease is notoriously difficult to manage.

Durable genetic resistance provides option for effective control of this disease Durable genetic resistance provides the best option for effective management of this disease. Taro plants show diverse response to *Phytophthora* leaf blight

- **The resistance might occur intra-species or varietals level i.e. Cultivar specific or race specific resistance**
- **Inter-species or genus level resistance i.e. non-host resistance**

Factors related to resistance reactions are associated with quantitative phenotype, hypersensitive response subjected to cytological and biochemical changes which is triggered up by signal transduction system in host-pathogen interaction.

Objectives of the works to investigate the factors related to :

- **Symptoms expression pattern of blight in different Taro cultivars under natural conditions**
- **Cultivars phenotypes and blight intensity**
- **The cytological characteristics of the pathogen invaded tissue of taro leaves evidence of disease – The HR response**
- **Changes in isoenzymes and its impact on defense reaction**
- **Involvement of signal molecule (elicitor) in *Phytophthora colocasiae***

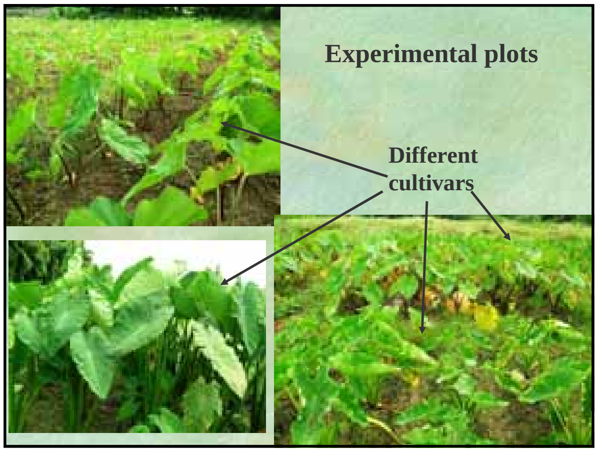
We used nine cultivars/varieties of Taro

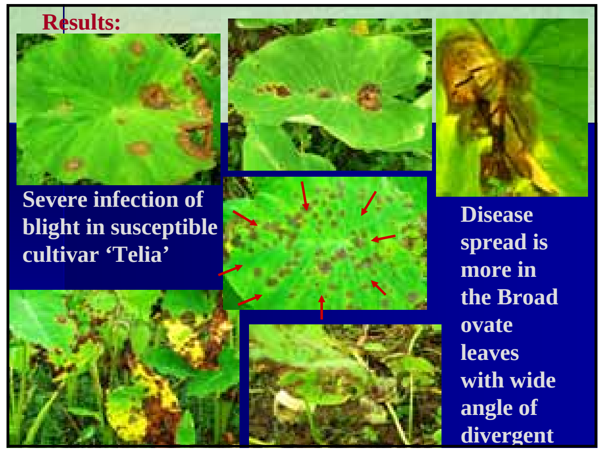
Telia (Susceptible)

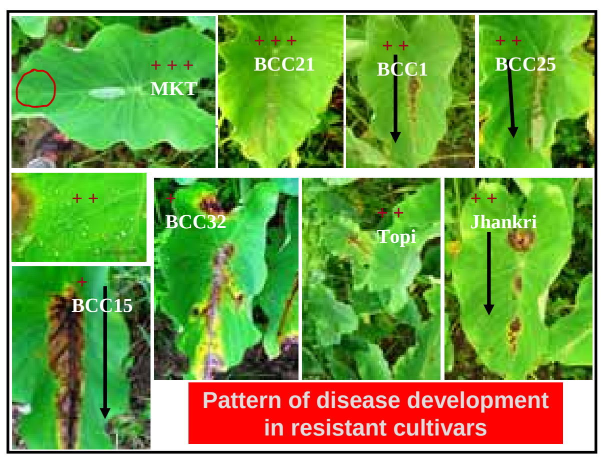
Muktakeshi (Resistance)

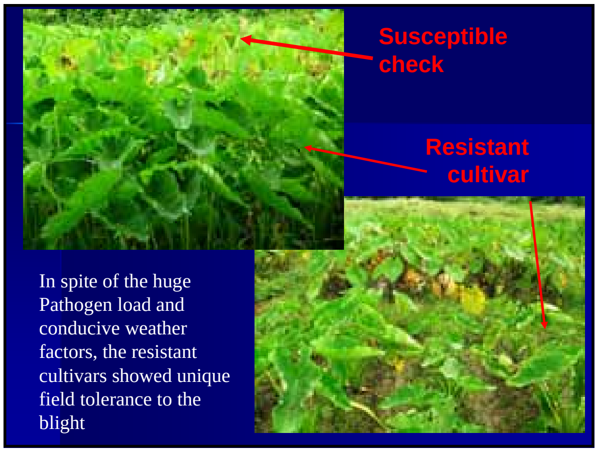
Topi, BCC-1 (Bidhan Chaitanya), BCC-15, BCC-21, BCC-25, BCC-32

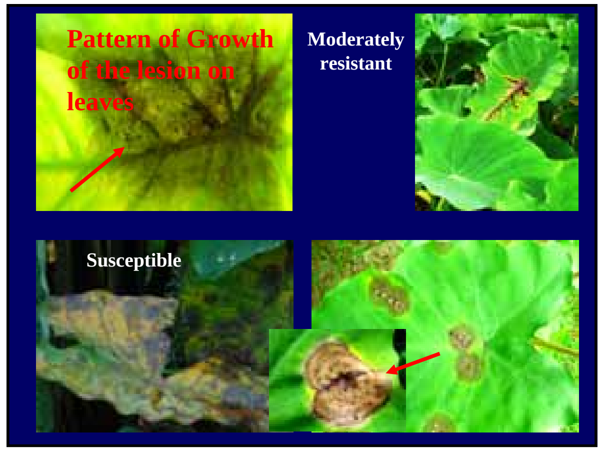
Target Disease : **Leaf blight of Taro**
Pathogen : ***Phytophthora colocasiae***

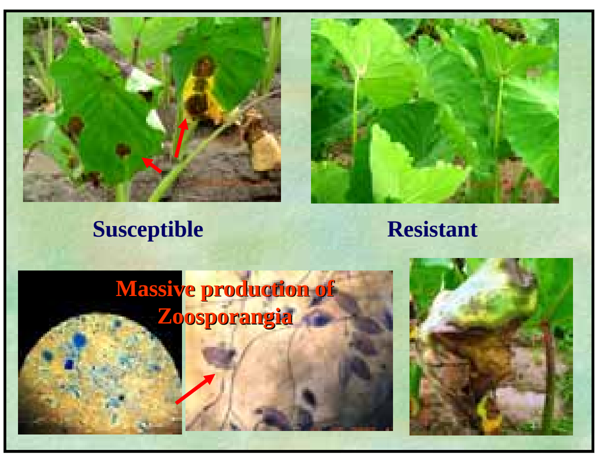

Experimental plots
Different cultivars


Results:
Severe infection of blight in susceptible cultivar 'Telia'
Disease spread is more in the Broad ovate leaves with wide angle of divergent


+ + + MKT
+ + + BCC21
+ + BCC1
+ + BCC25
+ +
+ BCC32
+ Topi
+ + Jhankri
+ BCC15
Pattern of disease development in resistant cultivars







Incidence of *Phytophthora* blight in nine promising *Colocasia* entries during 2004-05 and 2005-06 seasons

Cultivar	Average blight lesion (cm)	Ssporangium in leaf exudates (No./200µl)	Severity of leaf blight		Mean PDI	Days of 1st appearance of disease		Severity index of the blight	AUD PC
			2004-05	2005-06		2004-05	2005-06		
Mukta-keshi	0.53	12.5	17.79 (24.88)	17.10 (24.35)	17.44	130	120	1	644.91
BCC –21	5.6	7.5	22.63 (28.38)	24.14 (30.06)	23.38	110	120	1	840.32
BCC –1	6.0	9.8	34.47 (36.15)	28.16 (32.11)	31.31	115	105	2	889.84
Telia	7.33	43.21	91.83 (73.36)	81.03 (64.51)	86.43	95	95	4	2260.05
Topi	4.27	3.00	31.04 (33.83)	34.70 (36.00)	21.91	110	125	2	955.82
BCC –32	5.60	16.6	38.16 (38.06)	43.83 (41.38)	40.99	110	115	1	928.06
BCC –15	0.70	21.5	41.01 (39.82)	45.14 (41.82)	43.07	105	115	2	874.43
Jhankri	3.17	7.0	28.56 (32.33)	26.67 (31.09)	27.61	120	110	1	757.41
BCC - 25	3.23	7.5	29.86 (33.09)	29.34 (32.72)	29.60	105	105	1	752.92
SEM±	0.71	--	1.92	1.63	--	--	--	--	

Contd.

Name of the cultuvar	Leaf colour	Stem colour and type	Petiole colour	Vein colour	Leaf shape	Petiole girth	Leaf margin type	Leaf margin colour
Muktakeshi	Deep green	Light green, Erect	Light green	Green	Cup (Ovate)	Flat	Wavy	Whitish green
BCC-1	Deep green	Green	Light green	Whitish green	Lanceolate	Round	Entire	Purple
Topi	Green	Green, Erect	Light green	Whitish green	Lanceolate	Round	Entire	Whitish green
BCC-21	Deep green	Green	Light green	Whitish green	Lanceolate	Round	Entire	Purple
Jhankri	Deep green	Deep green, Erect	Deep green	Green	Cordate	Round	Wavy	Greenish white
Telia	Purplish green	Purple, Drooping	Purple green	Purple green	Oval	Round	Plain	Purple
BCC-15	Deep green	Deep green, Erect	Light green	Green	Cup	Round	Plain	Whitish green
BCC-25	Deep green	Deep green, Erect	Deep green	Green	Cordate	Round	Wavy	Greenish white
BCC-32	Bass green	Deep green, Drooping	Deep green	Purple green	Cup	Flat	Wavy	Whitish green
SEM± C.D. (P= 0.05)	--	--	--	--	--	--	--	--

Morphological characterization of nine *Colocasia* cultivars

Name of the cultuvar	Plant Height (cm)	Petiole length (cm)	Leaf length x breadth (cm)	Leaf thickness (mm)
Muktakeshi	91	67.5	35.7x20.5	0.38
BCC-1	91.3	69.4	35.2x21.9	0.39
Topi	86.3	62.55	26.1x21.6	0.39
BCC-21	89	71.1	31.8x20.8	0.36
Jhankri	90.2	67.2	32.6x19.6	0.36
Telia	75.5	53.5	36.25x33.8	0.44
BCC-15	82.9	68.4	31.3x19.5	0.44
BCC-25	90.6	71	33.8x20.7	0.42
BCC-32	70.7	52.8	30.2x29.01	0.38
SEM± C.D.(P=0.05)	1.25 3.70	1.18 3.49	--	N.S.

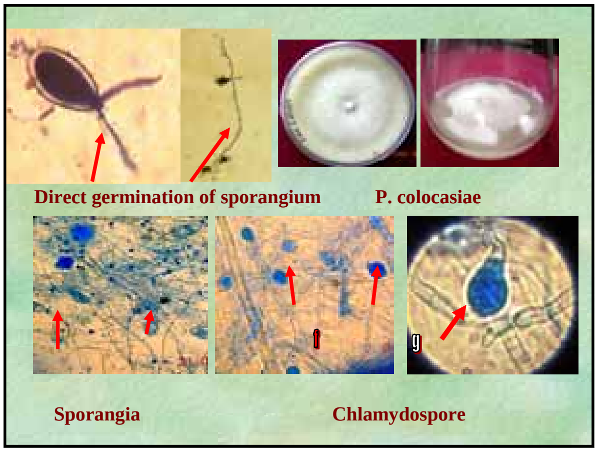
The blight pathogen, *Phytophthora colocasiae*, has a complex life cycle and can attack Taro (Other Aroids) in several ways. The assault usually begins when airborne spores land on the foliage and either germinate directly or release swimming zoospores. In either case, the pathogen penetrates the leaf or stem and grows into a dark lesion that releases thousands of new spores. Carried by wind or rain-splash, the spores can infect the same or other plants, sometimes miles away.

Zoospores can be washed from the foliage to the taro corms in the soil, causing them to rot immediately or in storage. Infected seed tubers can start a new cycle of infection in a following season.

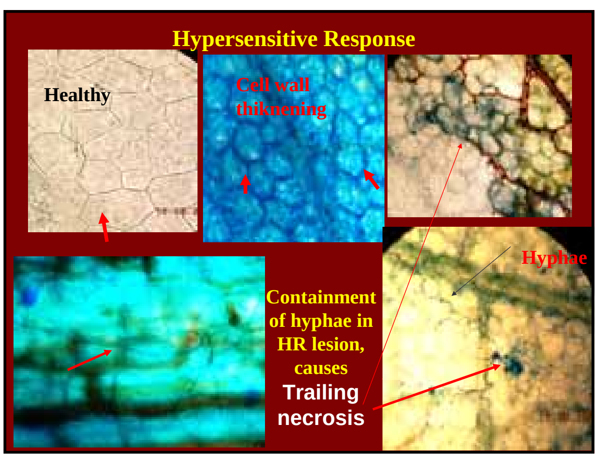
Post infection histological Factors in susceptible and resistant taro plants

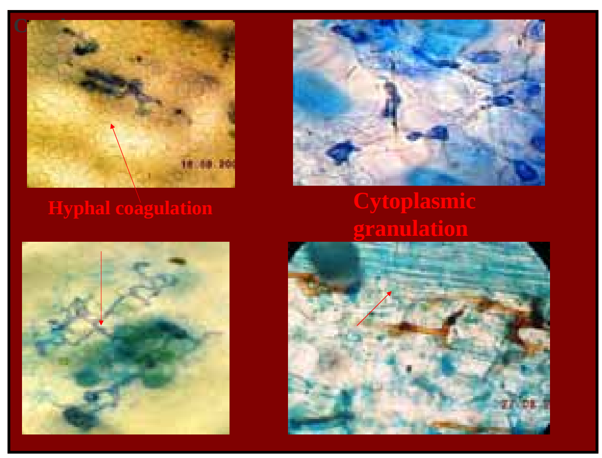
The Hypersensitive (HR) occurs in taro cultivars inoculated with Phytophthora colocasiae

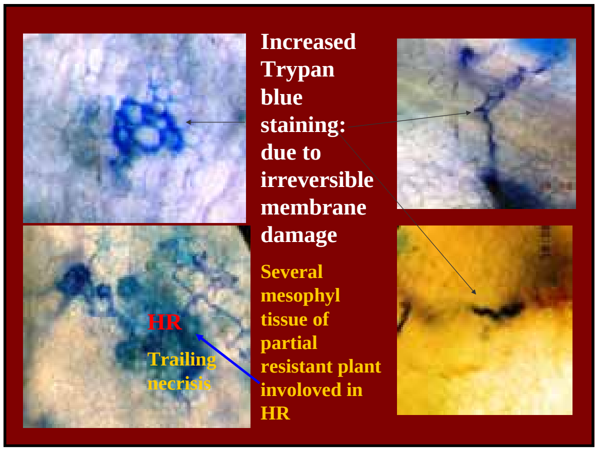
External symptoms manifestation: Massive gum deposition

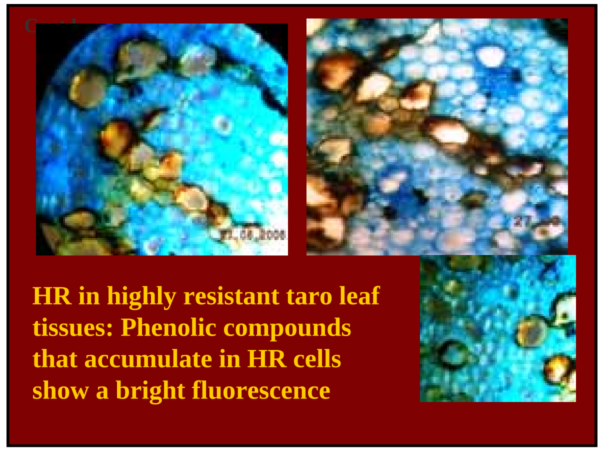

Direct germination of sporangium
P. colocasiae
Sporangia
Chlamydospore

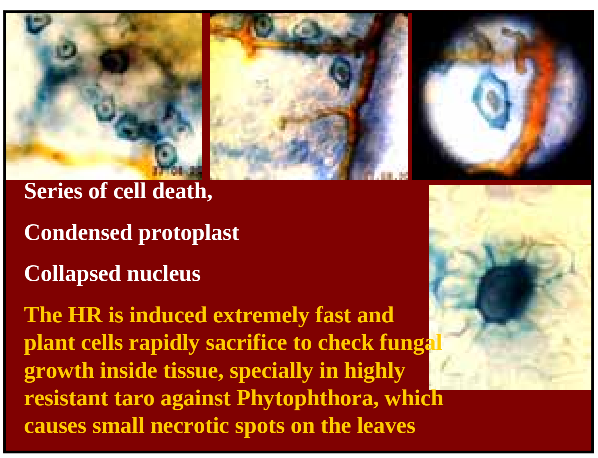

Healthy cells of
Taro Leaves

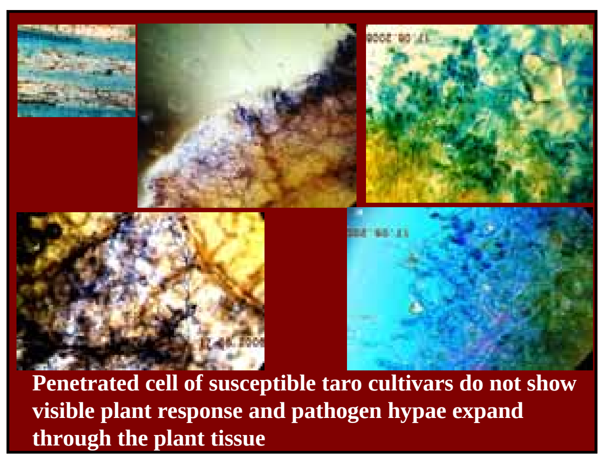

Hypersensitive Response
Healthy
Cell wall
thiknening
Hyphae
Containment
of hyphae in
HR lesion,
causes
Trailing
necrosis


Hyphal coagulation
Cytoplasmic
granulation


Increased Trypan blue staining: due to irreversible membrane damage
Several mesophyl tissue of partial resistant plant involoved in HR
HR
Trailing necrisis


HR in highly resistant taro leaf tissues: Phenolic compounds that accumulate in HR cells show a bright fluorescence


Series of cell death,
Condensed protoplast
Collapsed nucleus
The HR is induced extremely fast and plant cells rapidly sacrifice to check fungal growth inside tissue, specially in highly resistant taro against Phytophthora, which causes small necrotic spots on the leaves


Penetrated cell of susceptible taro cultivars do not show visible plant response and pathogen hypae expand through the plant tissue

Biochemical factors associated with resistance / susceptibility to blight in different Taro cultivars.

The activity and migration of some isoenzymes of taro

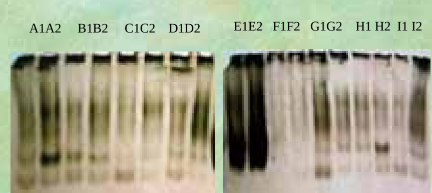
Following enzymes were studied:-

Esterase (Est.), Peroxidase (PO), Superoxide dismutase (SOD), Nitrate reductase (NR), Glucose 6-phosphate dehydrogenase (G6-PD)

Variation of total phenol and bound phenol (%) in healthy and disease cultivars

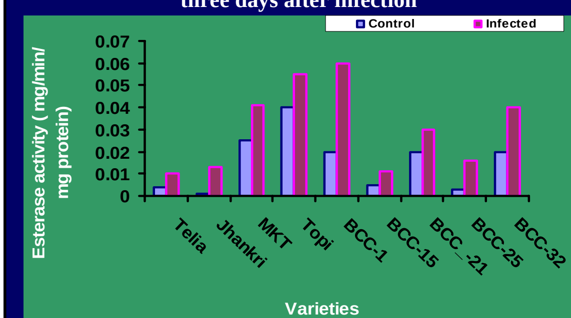
Cultivars	Total phenol (%)		Bound phenol (%)	
	Healthy	Disease	Healthy	Disease
Telia	5.75(13.81)	7.27(15.68)	2.12(8.33)	3.24(10.30)
Jhankri	8.21(16.64)	12.19(20.44)	3.40(10.62)	5.54(13.56)
Muktakeshi	8.74(17.15)	14.23(22.14)	4.17(11.83)	6.52(14.77)
Topi	9.17(17.66)	15.27(20.03)	3.80(11.24)	6.06(14.30)
BCC-1	8.38(16.85)	12.13(20.36)	2.92(9.80)	3.27(10.47)
BCC-15	7.34(15.68)	10.20(18.63)	2.88(9.80)	3.69(11.09)
BCC-21	6.74(15.0)	10.89(19.28)	3.37(10.62)	4.40(12.11)
BCC-25	7.16(15.56)	12.82(20.96)	2.71(7.49)	3.72(11.09)
BCC-32	6.44(14.65)	10.55(18.91)	2.83(9.63)	3.75(11.09)
SEm(±)	0.14	0.05	0.27	0.09
CD (0.01)	0.57	0.22	1.10	0.37

Esterase in taro cultivars in healthy and inoculated plants



1- Healthy, 2- Infected
A=BCC-15, B= BCC-21, C= BCC-25, D=BCC-32, 1- E=Topi, F= Telia, G=Jhankri, H= Muktakeshi, I= BCC-1

Activity of esterase in healthy and infected taro cultivars three days after infection



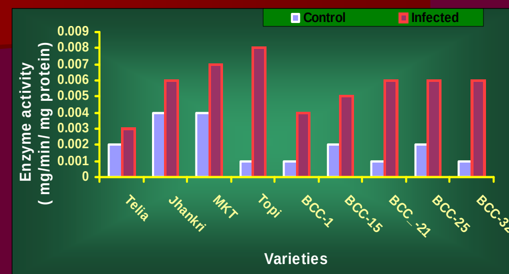
Superoxide dismutase isozyme in healthy and infectd plants of taro cultivars

A1 A2 B1 B2 C1 C2 D1 D2 E1 E2 F1 F2 G1 G2 H1 H2 I1 I2

1= Healthy, 2= Disease; A- Telia, B- Jhankri, C- Muktakeshi

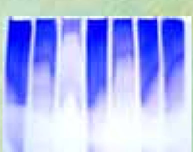
D= Topi, E= BCC-1, F= BCC-15, G= BCC-21, H= BCC-32, I= BCC-25

Enzyme activity of super oxide dismutase in healthy and infected taro cultivars (three days after infection)



Nitrate reductase in taro cultivars

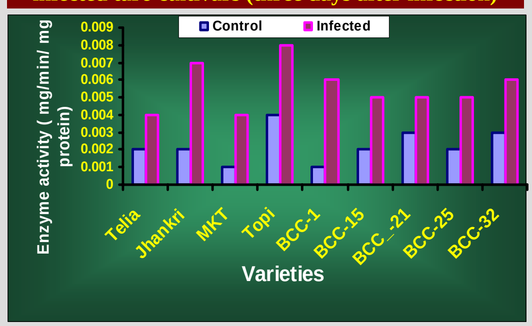
A2 A1 M B2 B1 C2 C1



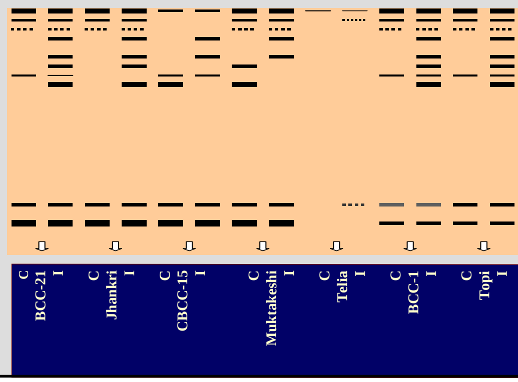
1= Healthy, 2= Disease,

M= Marker; A= Muktakeshi, B=Jhankri, C= Telia

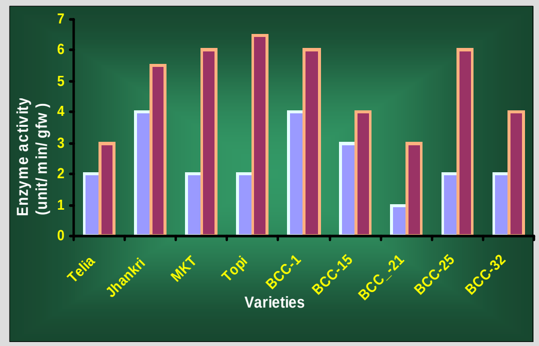
Enzyme activity of nitrate reductase in healthy and infected taro cultivars (three days after infection)



Compiled Zymogram of the Peroxidase (PO) Isozyme profile in the healthy and *Phytophthora colocasae* Inoculated plants of *Colocasia* cultivars

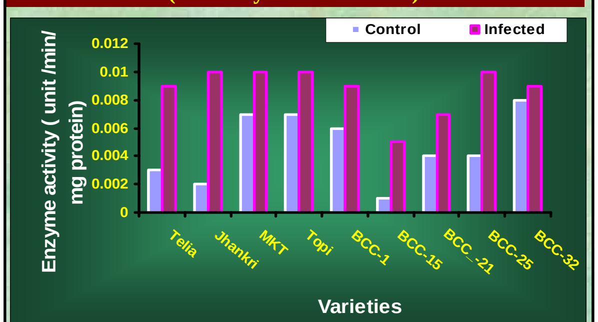


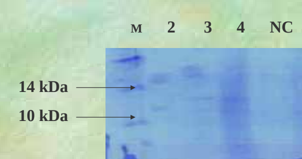
Enzyme activity of peroxidase of healthy and infected taro cultivars (three days after infection)



Enzyme activity of glucose 6 phosphate dehydrogenase in healthy and infected taro cultivars

(three days after infection)

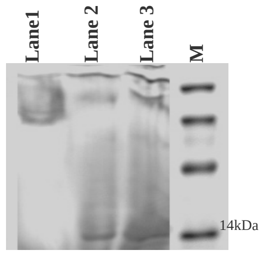




Tricin-SDS-PAGE

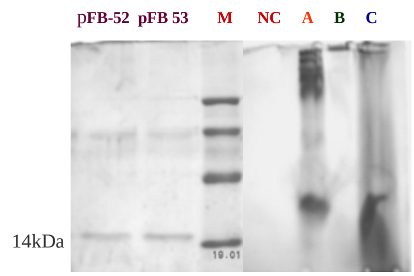
Bacterial expression of the cloned *inf1* cDNA. Recombinant proteins in Tricin-SDS-PAGE of soluble cellular fractions (lanes 3 and 4) of *Escherichia coli* strain pFB52 (FLAG-INF1 *S3*) and strain pFB53 (FLAG-INF1) cloned from *P. infestans*,. Lane 2 crude mycelia protein fraction of *P. colocasiae*. Molecular weight standards are shown in lane 1.Lane 3 & 4 Expressed protein

Analysis of INF-1 like extracellular protein (elicitin) production in the same strain of *P.colocasiae*



Lane 1-3: Fungal extracts- 1,oat meal; 2,V8juce; 3, Pea broth

INF 1 like elicitin production in *P. colocasiae* and recombinant elicitin from pFB 52 & 53 of *P.infestans*

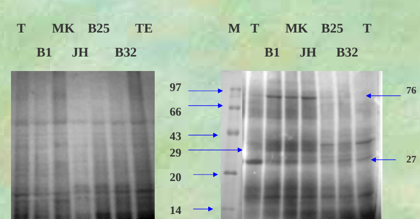


A-Partial purified mycellial extract

B-Only broth filtrate

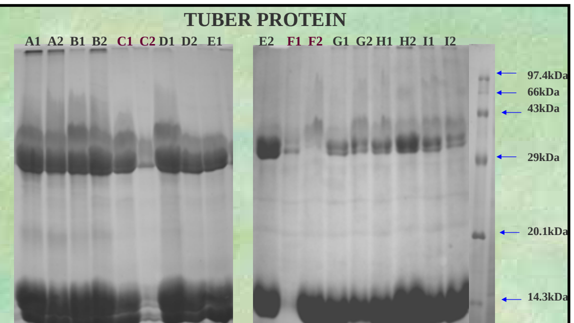
C-Filtrated Culture meium

LEAF PROTEIN PROFILE (48HRS AFTER INFECTION)



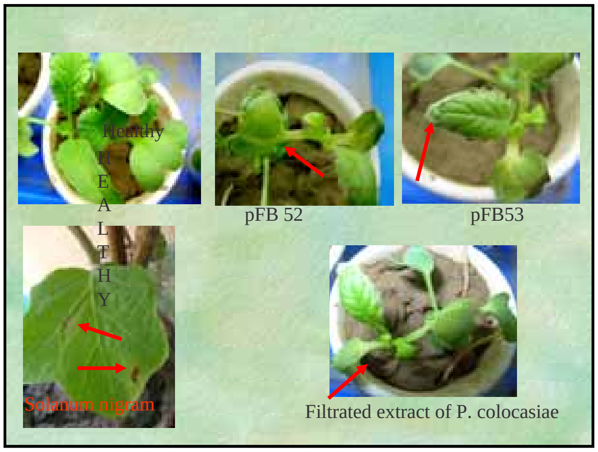
kDa

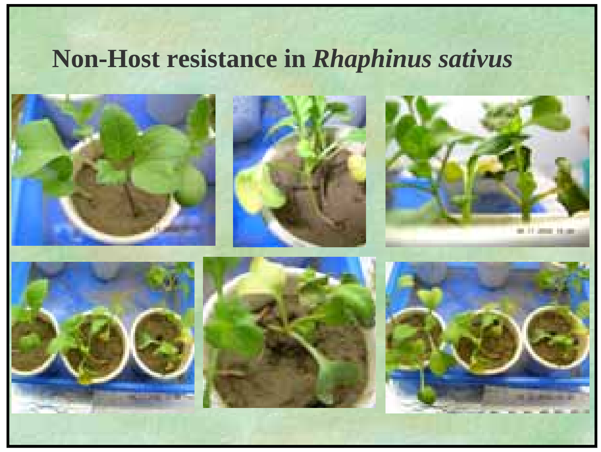
TUBER PROTEIN

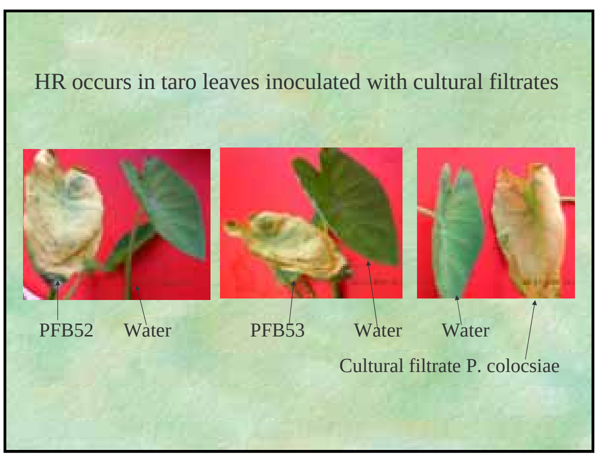


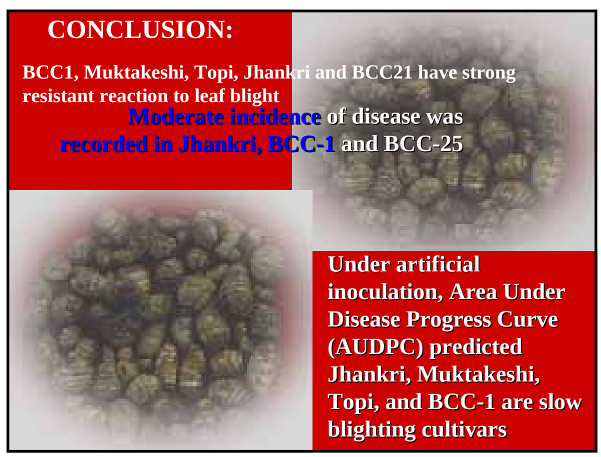
1=Healthy, 2=Infected

A= Muktakeshi, B=Jhankri, C= topi, D= BCC-1, E= BCC-15, F=Telia, G=BCC-21, F=BCC-25, G=BCC-32


Healthy
pFB 52
pFB53
Solanum nigrum
Filtrated extract of P. colocasiae


Non-Host resistance in *Rhaphinus sativus*


HR occurs in taro leaves inoculated with cultural filtrates
PFB52
Water
PFB53
Water
Water
Cultural filtrate P. colocsiae


CONCLUSION:
BCC1, Muktakeshi, Topi, Jhankri and BCC21 have strong resistant reaction to leaf blight
Moderate incidence of disease was recorded in Jhankri, BCC-1 and BCC-25
Under artificial inoculation, Area Under Disease Progress Curve (AUDPC) predicted Jhankri, Muktakeshi, Topi, and BCC-1 are slow blighting cultivars

Our results suggest that plant peroxidases have many putative functions including lignification, and likely to play indispensable roles in plant defense system agaisnt pathogen attack.
Moreover, SOD, EST, G6 PD exudates may work together as a multicomponent defense system to protect plant tissue during pathogenic assault. Isozyme steadily increased in activity during pathogenesis. The diversity of peroxidases, EST in individual cultivar expressed in leaf tissue of taro which are quick migrating isozymes, was expressed at a slightly higher level in infected plant tissue than in healthy tissues.
Nitrate reductase is also an important signaling & defense molecule, plays a key role in activating disease resistance in plants.

Hypersensitive is always associated with the resistance response of taro to Phytophthora colocasiae; the timing, severity and extent of the HR varied considerably, depending upon the examined cultivars.

As observed in taro leaves –p. colocasiae interactions, various level of pathogen ingress correlated with the different necrotic responses.

The postulate that elicitins are avirulence factors that restrict the host range of Phytophthora spp. points to a number of biotechnological applications.

THANK YOU