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The present study deals with "Induction of resistance in tea (*Camellia sinensis* (L.) Kuntze) by biotic and abiotic inducers against *Lasiodiplodia theobromae* (Pat.) Griffon & Mauble for management of diplodia disease". The study consists of: (1) Pathogenicity of *Lasiodiplodia theobromae*, the causal agent of diplodia disease, (2) Studies on physiological characteristics of the fungus, (3) Studies on serological aspects for determination of cross-reactive antigens, (4) Colorimetric assay of different defense related enzymes, (5) Studies on different defense related enzymes following application of different inducers-biotic, abiotic and plant extracts, (6) Studies on isozyme patterns of different enzymes related to induction of resistance in tea plants and (7) Assessment of disease following application of potential inducers in susceptible tea plants.

At the onset of the work, brief reviews of literature in the present lines of investigations have been presented. The main areas of the review are diseases of tea and allied crops, growth and physiology of the pathogens, pathogenicity, serology, systemic acquired resistance, induced systemic resistance and defense related enzymes.

Different experimental procedures and techniques used in the present work have been described in details in the materials and methods section.

The present work was carried out after thorough survey of different fungal diseases present in the different tea plantations including nurseries of the study area. During field survey two fungal pathogens were found to cause diseases in young tea plants. Those were isolated and identified as *Lasiodiplodia theobromae* and *Rhizoctonia solani* following Koch's postulates. *Lasiodiplodia theobromae* was found more consistently to cause disease in young tea plants.

Pathogenicity of *Lasiodiplodia theobromae* was performed following leaf inoculation and root inoculation techniques in seven different elite tea seed varieties. Tea seed variety, TS-449 was found most susceptible and TS-491 was found most resistant among the seven tea seed varieties tested.

Maintenance of fungal culture and production of fungal spores, in artificial media is very much important for different laboratory and field studies. Hence, growth and sporulation of *Lasiodiplodia theobromae* have been studied on a

variety of media with or without supplements. Important physiological parameters have also been studied. From the results it was found that PDB-RE (potato dextrose broth supplemented with tea root extract) was best for both growth and sporulation of *L. theobromae* after 25 days. Light had no significant effect on mycelial growth, but light induced sporulation of *L. theobromae*. The results also indicated that slightly acidic to neutral pH was optimum for the growth of *L. theobromae*.

To detect cross reactive antigens (CRA) in susceptible and resistant tea seed varieties against *L. theobromae*, serological techniques viz. immunodiffusion and immunoelectrophoresis were performed. Polyclonal antisera were raised against antigens of the susceptible (TS-449) and resistant (TS-491) tea seed varieties as well as of *L. theobromae*. In agar gel double diffusion (immunodiffusion) test, antisera, were subjected to react with proteins of susceptible and resistant tea varieties and also with mycelial protein of *L. theobromae*. The results showed the presence of CRA in homologous reactions as well as in cross reactions between susceptible tea variety and *L. theobromae*. No CRA was found in cross reaction between resistant tea variety (TS-491) and *L. theobromae* and *vice-versa*. Immunoelectrophoretic study revealed that susceptible tea varieties (TS-449 and TS-464) shared three precipitin bands with antisera of *L. theobromae* (LtA). Antisera of susceptible variety (TS-449) shared two precipitin bands against antigen of *L. theobromae*. No precipitin band was found between antisera of *L. theobromae* and antigen of resistant tea variety (TS-491) and *vice-versa*.

The root, stem and leaf antigens of seven different tea varieties, mycelial antigen of *L. theobromae,* and as well as non-pathogen proteins (*Trichoderma harzianum*) and antisera of two tea varieties (susceptible and resistant) and of pathogen *L. theobromae* were used to perform indirect-ELISA. Indirect ELISA was performed to detect the level of cross reactive antigens present in different tea varieties and *L. theobromae*. Higher ELISA values in heterologous reactions indicated the presence of CRA in higher level that lead to compatible reactions or more susceptible. In the present study, higher ELISA values were found in cross reactions between antisera of *L. theobromae* and antigen of susceptible tea variety (TS-449). Low ELISA values (0.038, 0.031 and 0.031) were found between antisera of *L. theobromae* and antigen of resistant tea variety (TS-491). The results of conventional pathogenicity tests were compared with the results of

indirect ELISA to establish a guideline for the degree of susceptibility of different tea varieties against *L. theobromae*.

To find out the cellular location of CRA in host tissues (tea plants) immunofluorescence and 'immunogold-silver enhancement' studies were performed using antisera of pathogen (*L. theobromae*). In indirect immunofluorescence, when the root sections were treated with antisera of *L. theobromae* and then reacted with FITC showed comparatively more fluorescence in susceptible tea varieties than the resistant one. Fluorescence was observed in the epidermal regions and xylem elements of the roots. Similarly, in case of immunogold labelling when stem and root sections of susceptible tea variety (TS-449) was treated with antisera of *L. theobromae* and labelled with immunogold-particles followed by silver enhancement, CRA was observed mainly in the epidermal regions and xylem elements as strong precipitations. In leaves of susceptible tea variety, mesophyll tissue and vascular bundle elements also showed marginal darkening which indicated the presence of CRA in those areas. When leaf, stem and root sections of resistant variety (TS-491) was treated with the antisera of pathogen, no such strong precipitations were observed.

After thorough observation of virulence studies of the pathogen, *L. theobromae* on seven different tea varieties, further works were carried out to devise environment friendly disease control measures. In this regard, susceptible (TS-449) tea plants were induced by abiotic inducers (salicylic acid, jasmonic acid and nickel chloride), biotic inducers (*Trichoderma harzianum* and *T.virens*) and leaf extracts (*Azadirachta indica, Acalypha indica, Jasminum jasminoides* and *Catharanthus roseus*) separately for induction of defense related enzymes (chitinase, β-1,3-glucanase, polyphenol oxidase, phenylalanine ammonia-lyase and peroxidise) and disease management.

From the results it was found that susceptible tea plants pre-treated separately with four different inducers (viz. 10^{-2}M Jasmonic acid,10^{-2}M salicylic acid, *Trichoderma virens* and leaf extract of *Azadirachta indica*) showed maximum phenylalanine ammonia-lyase (PAL) activity after 4 days following challenge-inoculation by *L. theobromae*.

Under similar condition, polyphenol oxidase (PPO) activity was found maximum in tea plants after pre-treated separately with five different inducers (nickel chloride, salicylic acid and jasmonic acid, *Trichoderma harzianum* and leaf

extract of *Acalypha indica*) following challenge-inoculation, in comparison to untreated-uninoculated and treated-inoculated controls.

PPO isozyme assay in tea showed differential expression with R_f values of 0.09, 0.30, 0.40 and 0.50. Two isoforms of R_f 0.09 and 0.05 were expressed constitutively in all treatments including control. PPO isozyme of R_f 0.09 was expressed with higher intensity in aqueous leaf extract (of *Acalypha indica)* and salicylic acid treated-inoculated tea plants in comparison to untreated-uninoculated and treated-uninoculated controls. PPO isozymes of R_f 0.40 and 0.30 were also expressed in higher level in treated-inoculated tea plants.

The enzyme, β-1,3-glucanase activity was found maximum in pre-treated (separately with salicylic acid, *Trichoderma harzianum, T. virens* and *Acalypha indica*) and challenge-inoculated (by *L. theobromae*) susceptible tea variety, TS-449. Isozyme patterns of β-1,3-glucanase revealed three different isozyme bands with different R_f values of 0.1, 0.35 and 0.45. Two isoforms of R_f 0.1 and 0.35 were found as constitutive. But they were expressed at higher levels in treated-inoculated plants. An unique β-1,3-glucanase isozyme of R_f 0.45 was found to express in treated-uninoculated and treated-inoculated plants.

Chitinase activity was also found to increase in pre-treated (separately with nickel chloride, *Trichoderma harzianum, Acalypha indica* and *Azadirachta indica*) and inoculated (by *L. theobromae*) tea plants. Chitinase activity in treated-inoculated plants was also studied by chitinase supplemented plate method. It was evident that pre-treated (separately with *Acalypha indica* leaf extract and nickel chloride) and challenge-inoculated susceptible tea variety showed significantly higher degree of 'chitinase activity' (expressed as intensity grade ++++). Under similar condition of pre-treatment and inoculation resistant tea variety showed comparatively lower chitinase activity (intensity grade +++).

Peroxidase activity was found higher in pre-treated (separately with nickel chloride, *Trichoderma harzianum, Acalypha indica* or *Azadirachta indica*) and challenge-inoculated (by *L. theobromae*) susceptible tea variety in comparison to untreated-uninoculated and treated-uninoculated controls. Peroxidase isoforms with two bands of molecular mass 38KDa and 33KDa were expressed prominently in pre-treated (separately with leaf extract of *Azdirachta indica* and *Acalypha indica*) and challenge-inoculated tea plants. Expression of the 33KDa peroxidase isozyme did not increase much following treatment with leaf extract

but increased dramatically when treated plants were challenge-inoculated with *L. theobromae*.

Degree of susceptibility or resistance of a particular variety to a pathogenic fungus is determined through its pathogenicity. Pathogenicity is determined, ordinarily, by disease incidence. Disease incidence was assessed and compared in the differentially induced susceptible variety, TS-449. Three different abiotic inducers, two biotic inducers and four leaf extracts were used for induction of resistance in the susceptible variety TS-449. Assessment of diplodia disease was performed from 4th day up to 16th day at 4-days intervals. Salicylic acid (abiotic inducer) and *T. virens* (biotic inducer) effectively reduced disease incidence (mean foliar disease index/plant) in tested tea plants. Disease incidence was also found to reduce in tea plants induced by three different leaf extracts (viz. *Azadirachta indica, Acalypha indica* and *Catharanthus roseus*) as evidenced by the results.

Implications of the results have also been discussed in the discussion section. The results were encouraging since several inducers showed significant resistance inducing capacity. Further these may be integrated with other bio-control agents and may be used in fields as part of integrated disease management system.

All the investigations presented in the study have confirmed and also extended some of the findings of the earlier workers. During this study, certain new facts of fundamental importance have also been revealed. Pathogenicity of *L. theobromae* has been tested in several tea seed varieties in different ways. The significance of antigenic relationship with regard to compatible interaction between *L. theobromae* and tea seed varieties has been demonstrated by various serological techniques. Correlation between pathogenicity test and different serological experiments was observed and was confirmed with indirect ELISA. Major cross-reactive antigens between the tea plants and the pathogen were detected in the cells of tea and the pathogen through immunofluorescence and 'immunogold-silver enhancement' studies. Resistance was induced in susceptible tea varieties using some chemicals and plant extracts. Hence, this study has provided an insight to formulate a definite defense inducer against diplodia disease. Present study designs the suitable control measures of the disease using resistance inducers of different nature.