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Preliminary Investigations on *Corynespora* Leaf Fall Disease of *Hevea brasiliensis* through *il vitro* Screening and Biochemical Assay

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Corynespora cassiicola is a devastating fungal pathogen which causes *Corynespora* Leaf Fall disease (CLFD) in *Hevea brasiliensis*, a perennial tree crop widely cultivated throughout the Asian countries as the sole commercial source of Natural Rubber. The severity of infection varies among different clones of *Hevea*. Cassiicolin, the toxin secreted by the fungus, is the primary determinant of *Corynespora* pathogenicity. Objectives of the present study are *in vitro* confirmation of susceptibility/tolerance of various clones of *Hevea* towards CLFD and a preliminary investigation towards the correlation between chitinase activity and incidence of CLFD. Eight clones of *Hevea*, four each from susceptible and tolerant groups were selected for this study. *In vitro* experiments were carried out using two sources viz. detached leaves and calli for determining the degree of tolerance of these clones to the disease. Detached leaves were subjected to vacuum infiltration with the crude toxin for specific time intervals and the extent of damage caused was determined from the necrotic lesions induced in each case. The necrotic browning effect was predominantly observed in leaf pieces of susceptible clones after 12 hours of toxin treatment whereas the treated leaves of tolerant clones remained green and unaffected even after 24 - 36 hrs. In the second experiment, newly developed calli were exposed to cassiicolin by transferring to media enriched with different concentrations of the crude toxin (0.5, 1, 2 & 3 ml/100 ml of medium). Calli of susceptible clones turned brown and became necrotic in the presence of lower levels (0.5 & 1.0 ml) of toxin whereas calli from tolerant clones survived and remained intact even in the presence of 2.0 - 3.0 ml of toxin. Pathogenic infection also causes the coordinate induction of chitinase, a PR protein which is capable of inhibiting fungal growth *in vitro*. Assay of chitinase activity was carried out in the field grown *Hevea* clones belonging to both susceptible and tolerant groups. Leaves of these plants were exposed to cassiicolin toxin for 12 hrs and the induced chitinase activity was measured. Untreated leaves were taken as the control. Comparison of control plants of different clones showed difference in chitinase activity, tolerant ones having more chitinase activity than the susceptible clones. It was observed that upon exposure to cassiicolin there was significant increase in chitinase activity in tolerant clones as compared to susceptible ones.

Inference from the initial screening experiments using detached leaves and callus cultures are clearly in conformity with the earlier observations and field reports. Results from these preliminary studies open up the scope of *in vitro* screening of pipeline clones of *Hevea* towards CLFD thus enabling early selection of tolerant clones. Moreover the positive correlation between high chitinase activity and increased tolerance to CLFD throws light into the possibility of imparting CLF tolerance to elite clones of *Hevea* by overexpression of chitinase gene through genetic manipulation techniques.