CHAPTER 6

SUMMARY

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The fact that local 'chitri' (wilt) disease is caused by Fusarium oxysporum f. sp. nicotianae was confirmed when artificially inoculated tobacco plants produced the characteristic wilt symptoms. Further anatomical studies showed that the fungus gains entry into the host vascular system after forming a colony in the outer cortical zone. Presence of the fungus (spores as well as mycelial fragments) and formation of gels in the xylem vessels were recorded. Fungus was also responsible for the destruction of parenchymatous tissue in the pith.

Inoculum potential plays a significant role in disease development. Fungal inoculum levels between 4 and 40% showed a high degree of host destruction. The fungal mycelium when inoculated with water alone produced wilt symptoms quicker (20 days) than when it was mixed with soil (more than 30 days).

However, compared to the fungal inoculation, diluted culture filtrate (CF) of the fungus showed a profound effect since it caused wilting of tobacco seedlings within 7 days. This also suggested its toxic nature. Bidi tobacco var. Anand 2 was the most susceptible among all the six varieties screened, and hence, it is used in the cellular selection method to develop wilt resistant lines.

The CF was used as a selecting agent, since it was observed that it could mimic all the wilt symptoms that a fungus can bring about.

To apply selection pressure in a reproducible manner, the quantitative determination of phytotoxic activity of the CF was done. It was found that CF was stimulatory up to 20% level and inhibited cell growth progressively at higher concentrations. Complete inhibition was noticed above 50% of CF. Similar results were obtained when CF was added in the growth study of leaf disks and whole plants.

However, CF inhibited the anthers to produce haploids, right from its lower concentration. Since, the dose sensitivity of CF is more or less similar from the whole plant upto cellular level, it is considered that CF of Fusarium causes some fundamental damage at the host cellular level.

The destruction of chloroplasts was found linearly correlated with the decrease in growth of the leaf disks, pH change of the culture medium and the increase of specific conductivity of the ambient solution. Hence, a direct proportion was seen between the toxin concentration of CF and the various metabolic activities.

To get additional information about the phytotoxin its isolation and purification was done, structure was studied and physical and chemical properties were recorded. Effect of the toxic substance(s) from the CF was also attempted.

When differentially treated CF was tested on tobacco plants, it was found that the toxin present in CF is a heat labile, low molecular weight and water soluble compound. These were confirmed by the wilt index bioassay. While fusaric acid (5 - n-butyl picolinic acid; FA) obtained in the ethyl acetate extract from CF, could cause vein-clearing and wilting, it failed to show other symptoms such as

containing respective selection pressure. Only LD50 could survive and regenerate shoots and roots. The LD50 regenerated plants were transplanted to pot and critically examined by leaf-disk-assay (Fresh weight increase) for the expression of selected resistant trait. These plants showed lot of variation in wilt resistancy from -5 to 86.5% fresh weight increase as per cent control. This indicated that genetic mosaic of genetical as well as physiological variation occurred during the selection cycles. The regenerants that acquired physiological resistancy ~~behaved~~ became susceptible after the selection pressure was removed (after transplantation).

The control regenerated plants did not show any change in the level of resistancy to the pathogen. Hence, origin of resistant variants, could be due to the selection pressure that acted as a mutagen and not due to somaclonal variation. Three lines of evidences indicate that a heritable resistance was obtained through cellular selection approach: LD50 resistant cultures were twice subcultured in the presence of the selection pressure; regeneration was also achieved in the selective medium; leaf disk screening on the field transferred plants correlated positively with the whole plant wilt index study.

The haploids obtained through anther culture, without selection pressure, were also screened for wilt resistancy

by the leaf-disk-assay. Maximum resistancy was noticed as 58.7%. Majority of the plants showed the resistancy below 35%.

Anthers were also directly inoculated on media containing selecting stress. CF beyond 25% showed 100% lethal to the androgenesis. When activated charcoal was removed from Nitsch's medium, even 25% showed complete inhibition. Moreover, the plants produced at the sublethal level in these experiments were not healthy. If this could be rectified, may be by adjusting the cultural conditions/parameters, directly resistant haploids can be generated and diplodized.

The possibility of using tissue culture techniques to produce new disease resistant plants was realized only after the research with tobacco for wild fire, disease (Pseudomonas tabaci) by Carlson, 1973a. Since then there is a consistent build-up of research which gathered momentum with developments in the general field of plant biotechnology and is now poised to make remarkable advances. Further, in vitro method has much to offer to plant pathologists to study biochemistry of host-pathogen interaction. In addition, as techniques of cellular and molecular genetics continue to develop, the possibility exists to introduce useful genes into existing crop varieties and to alter the nature of further crop species.