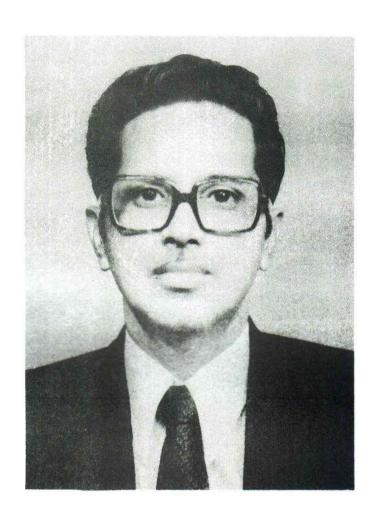Vol. 78 (I-II) 1-11

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DISEASE MANAGEMENT OF HIGHER PLANTS - CHANGING STRATEGIES AND NEW CHALLENGES

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It gives me great pleasure to take this opportunity to express my deep sense of gratitude to the distinguished members of the Executive Council of the Indian Botanical Society for unanimously electing me as the President of the Society for the year 1998. I am most grateful to the senior botanists of the country for their kindness, encouragement and whole-hearted support. We extend our sincere thanks to Prof. R.K.S. Chauhan, Vice-Chancellor, Vikram University, Ujjain for inviting the Indian Botanical Society to hold its 21st Botanical Conference at the Institute of Environment Management & Plant Sciences, Vikram University. I am well aware that the botanists assembled here are specialists in different branches of botany. Nevertheless I feel that it would be most appropriate for me to talk about my own field of specialization. The topic that I have chosen for my lecture is "Disease management of higher plants-changing strategies and new challenges". Protecting plants against diseases caused by detrimental microbes is a major challenge to plant protection research. About 33% of the world crop production is lost every year and one-third of this loss is due to plant diseases. In 1997, food grain production in India was 198 million tons and the total population was approximately 932 millions. About 20% of the crop production including post harvest products was lost due to diseases. Therefore, effective disease management strategies are pre-requisites for promoting food security of the country.

The search for novel and improved strategies for plant disease management will continue unabated since none of the existing ones can meet the new challenges. Emerging strategies based on novel concepts may prove to be more efficient and dependable than the older ones but there is no guarantee that it will not create any new problem whatsoever in future which is more serious and hazardous to ecosystem because each strategy has its own advantages and disadvantages or drawbacks. These drawbacks underlie the need for new discoveries. The purpose of this lecture is to highlight the developments in plant protection research that have changed the perceptions of researchers, planners in industry and users and also the way in which the scientific discoveries have moved forward and gradually reached the present status.

Changing strategies in fungicidal research-chemical fungicides to biofungicides (microbial and herbal) for disease management

Sulphur was one of the first to be used both as a fungicide as well as an insecticide. Initially the Greeks used sulphur without knowing the cause of disease. During the 18th and 19th Centrury, the most important and widely used fungicides were sulphur, copper and mercury. Later, the evolutionary path has shifted from inorganic salts to organo-metallic and organic chemicals since these compounds are more fungitoxic than the inorganic metals. The leading thought was to improve the well known fungicidal action of the compounds. During the 1940s organic fungicides began to displace inorganic protectants because organic protectants were easier to use, effective at lower doses and less phytotoxic. But most organic protectants remain primarily on plant surfaces and are not absorbed by plants and consequently these compounds have little effect on pathogens growing internally. These disadvantages influenced the subsequent search for new fungicides and led to the discovery of chemotherapeutants which were directly toxic to pathogens. Besides, these chemotherapeutants may also inactivate vivo toxins or may increase the resistance of plants. Sometimes these compounds are transformed within the plants to those substances which are capable of having greater or different activities from the original products or in other words the transformed compounds may be more potent than the untransformed ones. In the late 1960s,

a systemic fungicide benomyl was discovered and later several new systemically acting chemicals were also introduced for plant disease management. But the selective action of various systemic fungicides on different groups of fungi created some problems. For instance, theazole group of fungicides has been found to be very active against a broad spectrum of fungal diseases but the major problem with the azole group is the increasing population of strains resistant to azole group especially among powdery mildews of cereals. The discovery of morpholine group of fungicides is very important since these compounds act by inhibiting ergosterol biosynthesis in fungi (target site specific fungicides). It is evident from the discoveries of various groups of chemical fungicides so far that there is an increasing tendency to kill the target pathogens by inhibiting the synthesis of their vital components instead of killing both target and non-target organisms by non-specific fungicides. Recent reports also reveal that some fungicides are able to induce systemic acquired resistance (SAR) by producing antifungal PR-proteins (pathogenesis related proteins) in host plants, activating phenol oxidising enzymes or enzymes involved in phytoalexin biosynthesis. This may be regarded as host mediated effects of fungicides. However, even target specific fungicides cannot always satisfy the quality of an ideal fungicide. The deleterious effects of fungicides on non-target soil microbes, deterioration of ecosystem and development of fungicide-resistant strains necessitate us to develop biological methodologies for controlling diseases and pests as a substitute for chemical fungicides. In this case, success depends upon the correct selection of suitable organisms with antagonistic properties.

Several fungi, rhizobacteria and herbal products are now being exploited in the management of fungal diseases. Bacterization of seeds may provide protection against selected soil borne fungal pathogens. For example, *Bacillus subtilis, Arthrobacter* and *Streptomyces* antagonise *Fusarium udum, Pythium debaryanum* and *Rhizoctonia* sp., respectively. *B. megaterium* (str. B23) was used against *Colletotrichum corchori* causing anthracnose of jute (Bhattacharyya and Purkayastha, 1982). Bacterization of soybean seeds or roots with *Rhizobium japonicum* significantly reduced charcoal rot of soybean (Chakraborty and Purkayastha, 1984). Infestation of substrates with *Streptomyces parvullus* also suppressed *Macrophomina* infection of

soybean roots (Purkayastha and Chakraborty, 1984). Leeman *et al.* (1996) demonstrated that *Fusarium* wilt of radish could be controlled by co-inoculation of *Pseudomonas* spp. and other root colonizing fungi. Fluorescent pseudomonads such as *P. fluorescens* and *P. putida* can inhibit the growth of several plant pathogens. There is evidence that *P. fluorescens* was used for controlling *Gaeumannomyces graminis* var. *tritici* (causing "Take all" disease), *Thielaviopsis basicola* (tobacco black root rot), *Sarocladium oryzae* (Sheath rot of rice), *Rhizoctonia solani* and *Sclerotium rolfsii* (causing stem rot of groundnut). Besides, *Enterobacter, Rhizobium* and *Serratia* were also found to be effective in suppressing the growth of *Phytophthora cactorum, P. megasperma* and *Fusarium oxysporum* respectively (Dube, 1998).

Among fungal antagonists, a few species of *Trichoderma* (viz. *T. harzianum, T. viride, T. koningii, T. hematum, T. polysporum*) and *Gliocladium (G. roseum, G. virens, G. catenulatum)* are well known. some strains of *T. harzianum* have been reported to be highly antongonistic to many crop pathogens namely, *S.rolfsii M. phaseolina, Fusarium* spp., *Pythium aphanidermatum, Sclerotinia sclerotiorum, S.sapivorm* and *P. ultimum.* Apart from *Trichoderma* and *Gliocladium, Penicillium oxalicum, Pythium oligandrum, Verticillium biguttatum* and *Taloromyces flavus* were also antagonistic to *Pythium* spp., *Aphanomyces cochlioides, R. solani* and *V. dahliae* respectively.

Herbal products are also being used to control various fungal, bacterial and viral diseases. Several plant oils and crude extracts have been screened for antifungal activity. Oils of neem, chalmoogra and *Cymopogon* were applied to control *S. rolfsii, M. phaseolina* and *H. oryzae* causing groundnut foot rot, jute stem rot and brown spot of rice respectively (Bhattacharyya *et al.,* 1991).

Oils obtained from seeds of sunflower, olive, corn and soybean protected apple against powdery mildew (Agrios, 1997). Aqueous neem leaf extract protected barley leaves against leaf stripe disease (Paul and Sharma 1996). Search for reliable herbal fungicides is gradually increasing but it should be borne in mind that all antifungal plant products may not be harmless, stable and effective under field conditions.

Conventional plant breeding to molecular cloning of resistance genes and production of transgenic plants for disease management

In the first half of the 20th Century disease resistance has been fully established as a major means of disease management. The development of plant resistant to specific diseases through breeding and selection has been outstandingly the result of extensive research during this period. Several disease resistant crop plants belonging to cereals, pulses, oil seeds, fruits and vegetables have been developed through conventional breeding which are still popular, least expensive, safest, easiest and effective. But the reliance on major gene resistance becomes a problem when the selection pressures exerted on the pathogen population increase with the extensive use of any one variety. Possibilities of epidemics are also enhanced. Hence, search for alternative approaches continued with a view to obtain more effective and durable resistance, to make use of available genetic resources and to develop techniques for transfer of resistance genes between species or genus where hybridization by traditional breeding method is not possible at all. Gradually, several other methods such as meristem tip propagation, callus and single cell culture, production of haploid plant and protoplast fusion have been introduced for the improvement of plant resistance and to avoid sexual barrier. Recently, genetic engineering has been found to be an excellent tool for plant disease management. A number of transgenic plants resistant to fungal/ bacterial/viral and insect diseases have been developed. Some of the recent findings are discussed here.

Production of transgenic plants resistant against fungal diseases

Some Regenes have been successfully isolated from plants which are resistant to fungal diseases. For example, HM-1 gene which has been isolated from maize confers resistance to *Cochliobolus carbonum* race1, a causal organism of leaf spot disease of maize. This gene encodes a NADPH reductase that inactivates the race specific toxin produced by the pathogen (Johal and Briggs, 1992). Jones *et al.* (1994) have isolated Cf-9 gene from tomato which provides resistance specifically to *Cladosporium fulvum* (leaf mould) races that express the avirulence gene avr9. The L^6 gene from flax offers resistance to *Melampsora lini* (Lawrence *et al.*, 1994). A total of 31 loci have been identified that provide race specific resistance to the rust fungus.

Out of 31, one locus contains multiple alleles or closely linked genes.

A gene derived from grape vine *(Vitis vinifera)* coding for trihydroxystilbene synthase was transferred into protoplasts of japonica rice (cv. Nippon base) using polyethylene glycol-mediated direct gene transfer. Transgenic plants were regenerated from calli selected on Kanamycin. Results of pathogenicity tests indicated enhanced resistance of transgenic rice to *Pyricularia oryzae* (Stark *et al.,* 1997).

It is well known that Cercosporin is a non-host specific polyketide toxin required by many *Cercospora* spp. to cause plant disease. A gene Cfp. cloned from *C.kikuchi* was found to be resistant to *Cercosporin.* This was confirmed when expression of Cfp in the Cercosporin-sensitive fungus *Cochliobolus heterostrophus* significantly increased its resistance to Cercosporin. The Cfp gene was subsequently introduced into the tobacco cell line "NT-1" and "Xanthi" and their toxin resistance was assayed. Results suggest that Cercosporin resistant plants should be resistant to disease caused by Cercosporin producing *Cercospora* species (Upchurch *et al.,* 1997).

Woo *et al.* (1997) reported that expression in plant of genes coding for cell wall degrading enzymes from a mycoparasitic fungus improved plant resistance against fungal diseases. Transgenic tobacco, potato and petunia plants expressed a genomic and a cDNA copy of the chitinase Th En-42 gene from *Trichoderma harzianum,* a biological control agent. These transgenic lines were tested against foliar and soil borne pathogens. In all cases significant differences in disease symptoms between transformed and untransformed (control) plants were noted.

Transgenic plants resistant against bacterial diseases

Two R genes RPS 2 and Pto were successfully isolated from *Arabidopsis* and tomato, respectively (Martin *et al.,* 1993; Bent *et al.,* 1994). The Pto gene from tomato confers resistance to *Pseudomonas syringae* pv. tomato expressing the avirulence gene avr Pto. Originally, Pto gene was identified in a wild species of tomato but subsequent backcrossing programmes introgressed Pto gene into a variety of cultivated tomato. On the other hand, the RPS2 gene from *Arabidopsis* offers resistance to *P. syringae* pv. *maculicola* expressing the avr. Rpt 2 gene. The RPS2 gene potentially encodes a 909 amino acid, 105 KD

protein (Mindrinos *et al.*, 1994). Analysis of amino acid sequence reveals a region of leucine-rich repeats, six potential glycosylation sites and a potential leucine zipper domain.

Transformation of M.26 apple root stock with lytic proteins enhanced resistance to fire blight caused by *Erwinia amylovora*. The identity of the M.26 apple (*Malus* sp.) rootstock was confirmed by random amplified polymorphic DNA analysis. The leaf tissue was transformed with genes encoding the lytic proteins attacin E (att E) and phage T4 lysozyme by *Agrobacterium*-mediated transformation. The transformed plants were tested for resistance to *E. amylovora* (Borejsza-Wysocka *et al.*, 1997).

Transgenic plants resistant against viral diseases

Although a large number of transgenic virus resistant plants have been raised using viral coat protein genes, only the recent findings are cited here. Most of them are pathogen-derived resistance. Tabacco plants (*Nicotiana tabacum* "Xanthi") were transformed with a binary vector containing the coat protein gene of tomato mottle-virus (To MoV) modified in the N-terminus region. The progeny of the transformed tobacco plants were screened for resistance to To Mov and cabbage-leaf curl-virus (Cab LCV) by challenge with viruliferous white flies. A range in response to To MoV from immunity to tolerance to susceptibility was observed. But no resistance to Cab LCV was noticed (Sinisterra *et al.*, 1997) in inoculated plants.

Transgenic barley plants (T_2 lines) resistant to mild mosaic virus were obtained by particle bombardment using the virus coat protein gene. Initially, immature embryos from T_0 plants (cv. New Golden) were bombarded with DNA coated gold particles. After selection on the medium containing hygromycin, 83 plants were propagated. Out of these 83, only 30 plants contained coat protein. No plants of the T_2 line were infected when inoculated with the virus (Hagio *et al.*, 1997).

Mirkov *et al.* (1997) collected seven strains of sugarcane-mosaic virus (SC MV) from different regions of the world. These included SC MV strains (A, B, D and E) and Sorghum mosaic virus (Sr MV) strains (H, I and M). Cloned cDNAs containing the coat protein coding region of the genome were obtained and sequenced for each of these strains. Chimeric constructs were made and these constructs were used in biolistic co-transformation experiments on sugarcane using the npt II gene as a selectable marker. More than 500 plants into which the coat protein gene had been introduced were screened for resistance by repeated inoculations. Several lines inoculated up to 6 times with strain H remained virus free. These lines also offered resistance to the closely related Sr MV strains I and M but not to the distantly related SC MV strains A, D and E.

Transgenic plants resistant against insect diseases

The Cry genes of *Bacillus thuringiensis* encode a diverse group of Crystal-forming proteins that exhibit insecticidal activity particularly against the larvae of lepidopteran, coleopteran and dipteran insects. The efficacy of *B. thuringiensis* based biopesticides may be improved through the genetic manipulation of these genes. A gene transfer system has been developed for the introduction and maintenance of cloned insecticidal Cry genes on small plasmids in *B. thuringiensis*. This vector system combines a foreign DNA from the recombinant bacterium after introduction of Cry-encoding plasmid (Baum *et al.*, 1996). The crystal proteins, δ-endotoxins of *Bacillus thuringiensis* are specifically lethal to *Lepidopteran* insects were shown by Chau *et al.* (1996). A truncated B. t-toxin gene, Cry IA(a) encoding an insecticidal crystal protein (ICP) directed by the Cauliflower mosaic virus 35S promoter was transferred to potato plants by an *Agrobacterium*-mediated transformation system. Seven out of 30 transgenic plants expressed the Cry IA(a) gene. Those transgenic plants containing multiple transgenic copies did not express the Cry IA(a) gene.

An insect resistant transgenic plant has been developed by Sane *et al.* (1997). The cowpea *(Vigna sinensis)* trypsin-inhibitor (CpTI) gene was transferred to tobacco plants. The transgenic tobacco plants offered resistance against a wide range of insects. The pressure and expression of the CpTI gene in the primary transformants R_0 and R_1 progeny was also confirmed by the authors. The efficacy of the expressed CpTI protein against *Spodoptera litura* was tested by feeding trial larvae. Reduction to the extent of 50% was observed in the biomass of *S.litura* larvae fed on transgenic leaves, expressing 3-5 µg CpTI/g fresh tissue.

Induced resistance/immunization of plants-an approach to plant disease management (Cross protection to chemical/herbal induction)

The importance of induced resistance in plant

disease management has been pointed out earlier by several workers (Chester, 1933; Sequeira, 1983; Kuc, 1987 and Sticher *et al.,* 1997). Extensive work has been done on biological and chemical induction of resistance in plants against various diseases, but suitable application technology is not yet available for complete immunization of plants in the field. However, in this part, attempt has been made to discuss how resistance could be induced and utilized in plant disease management.

Low virulent strains of TMV have been employed in Japan and in the Netherlands to cross protect tomatoes against virulent strains of the pathogen. Similarly, mild strains of "potato virus X" were used to protect plants against the more virulent strains of the same virus. There is evidence that TMV-infected tobacco plants conferred resistance against diseases caused by TMV, *Peronospora tabacina* and *Pseudomonas tabaci* in the field. It was reported that the roots of tomato seedlings dipped in avirulent spore suspension of *Verticillium albo-atrum* at the time of transplantation offered resistance of *Verticillium* wilt caused by virulent strains. But the results were encouraging only in artificially inoculated soil and not in the naturally infected ones (Sequeira, 1983).

An interesting observation was made by Hiramoto *et al.* (1995). They first detected two types of activities of exudates collected from cut end of barley seedlings. Exudates induced systemic resistance in barley seedlings when collected 3-6h after pruning but induced susceptibility when collected after 9-12h of pruning. A correlation was established between the accumulation of antifungal substances in barley leaves and the timing of induced resistance. It was concluded that signals for both systemic resistance and susceptibility might exist in the exudate obtained from the cut end of barley seedlings.

A non-phytotoxic, systemic resistance inducing agent was isolated from the leaves of *Clerodendrum aculeatum* and purified. Treatment of susceptible tobacco plants with this purified basic protein (34 kDa) preparation induced a very high level of systemic resistance against virus infection. Gel electrophoretic analysis also indicated a relationship between induced resistance and accumulation of the 34 kDa protein in the leaves (Verma *et al.,* 1996). In 1997, Kumar *et al.* isolated and characterised cDNA encoding the a foresaid systemic resistance inducing protein (34 kDa). The

leaves of *C. aculeatum* usually contain this endogenous virus inhibitor protein. When susceptible plants are treated with this inhibitor, it confers resistance to a number of plant viruses. It was also reported that coconut *(Cocos nucifera* L.) and Sorghum *(Sorghum bicolor* L.) leaf extracts containing the antiviral principles were used for the management of bud necrosis virus disease of groundnut under field conditions. Two sprays of either coconut or sorghum leaf extracts at an interval of 15 days effectively reduced disease and significantly increased yield (64%-100%) compared to control (Kulkarni *et al.,* 1997). Presence of antiviral proteins in higher plants has been reviewed extensively by Verma *et al.* (1995).

Systemic acquired resistance (SAR) in plants could be compared with immunization in animals although the mechanisms are quite different. SAR can be induced by microbes, natural products and inorganic and organic compounds (Table 1). It is not unreasonable to speculate that the induction of SAR may provide new solutions to plant disease problems in future. But a thorough understanding of the components involved in pathogen recognition and expression of resistance in the host is imperative for designing new strategies. It is necessary to mention here that there is no difference in induction of resistance by biological and chemical agents. In tobacco, INA (Dichloro-isonicotinic acid) induced the same 9 families of SAR genes as TMV or salicylic acid (Kessmann *et al.,* 1994). It was conclusively demonstrated that transgenic tobacco plant containing nah G gene (transferred to tobacco from *Pseudomonas putida)* encoded an enzyme salicylate hydroxylase which converted salicylic acid (SA) to catechol and as a result plant became susceptible to TMV. Because there was no accumulation of SA. But when the same transgenic plant was treated with INA (Dichloro-isonicotinic acid), the plant restored its resistance to TMV although INA-treated plants were unable to accumulate SA. It strongly suggests that like SA some other chemical compound(s) may also induce SAR. However, the levels of endogenous SA have been correlated with the induction of the PR-proteins. Although it has been demonstrated that SA is involved in the development of SAR in tobacco it is not known whether SA is involved in all cases of SAR induction. Several *Arabidopsis* mutants that fail to establish SAR have been isolated and it has been shown that npr 1 (non-expressor of PR genes) and nim 1 mutants (non-inducible immunity) are insensitive to chemical inducers

Table 1: Microbes and chemicals induce systemic acquired resistance (SAR) in plants against diseases

Inducer	Host	Protection against	Reference
Microbes			
Bean yellow mosaic virus	Red Clover	*Erysiphe polygoni*	King *et al.* (1964)
Colletotrichum laginarium	Soybean	*C. truncatum*	Wrather and Elrod (1990)
C. lindemuthianum	Alfalfa	*C. lindemuthianum*	O'Neill and Baker (1989)
Erysiphe graminis f. sp. *hordei*	Barley	*E. graminis*	Hwang and Heitefuss (1982)
Turnip crinkle virus	*Arabidopsis*	*Pseudomonas syringae*	Summermatter *et al.* (1995)
Leptospheria maculans	Oilseed rape	*Leptosphaeria maculans*	Mahuku *et al.* (1996)
Pseudomonas syringae	Rice	*Magnaporthe grisea*	Hofmann and Babuin (1993)
Tobacco mosaic virus	Tobacco	*Thelaviopsis basicola*	Madamanchi and Kuc' (1991)
Sclerospora graminicola	Pearl millet	*S. graminicola*	Kumar *et al.* (1993)
Chemicals			
Arachidonic acid	Potato	*Phytophthora infestans*	Coquoz *et al.* (1995)
Benzo-(1,2,3)-Thiadiazole-7-Carbothioic acid S-methyl ester (BTH)	Wheat	*	Gorlach *et al.* (1996)
DL-3amino butyric acid (BABA)	Tomato	*P. infestans*	Cohen and Gisi (1994)
	Tobacco	*Peronospora tabacina*	Cohen (1994)
INA	Barley	*Erysiphe graminis*	Kogel *et al.* (1994)
Kitazin	Rice	*Rhizoctonia solani*	Bera and Purkayastha (1997)
Methyl jasmonate	Barley	*Erysiphe graminis hordei*	Mitchell and Walters (1995)
Probenazole	Rice	*Magnaporthe grisea*	Sekizawa and Mase (1980)
Phosphate salts	Cucumber	*Colletotrichum laginarium*	Gottstein and Kuc' (1989)
Acetyl salicylic acid (aspirin)	Tobacco xanthine	TMV	White (1979)

* No antimicrobial activity but mimic the biological induction of SAR against some pathogens.

of SAR such as SA and INA (Cao *et al.*, 1994; Delaney *et al.*, 1995; Shirasu *et al.*, 1996). Unlike nah G plants, these mutants are not able to respond to SA or INA. Though nim 1 mutant accumulates SA after inoculation with *Peronospora parasitica* it fails to inhibit the growth of the pathogen. Similarly, npr1 mutants fail to express PR-genes. But mutants that have high constitutive SAR gene expression and resistance are known as cims (constitutive immunity). All cim *Arabidopsis*

mutants are characterised by increased disease resistance. A major advance is the isolation of a gene controlling the onset of SAR. The ankyrin repeats encoding npr1 gene may function as a regulator of transcription of SAR genes or as a salicylic acid receptor (Cao *et al.*, 1997). The significance of PR-proteins is that they show strong antifungal or antimicrobial activities.

Like PR-proteins, phytoalexins (PA) are also antimicrobial and are produced in plants in response to physical, chemical or biological stress (Purkayastha, 1986, 1995, 1996). Resistance occurs only when one or more phytoalexins accumulate in appreciable amount to inhibit pathogen development. More than 350 phytoalexins have been chemically characterised from approximatly 30 plant families (Kuc', 1995). Since PA is not a translocatable compound it is primarily involved in localised acquired resistance (LAR). Induction of resistance by eliciting PA production in plants depends upon several factors such as signal transduction, elicitors, suppressors and detoxification of PA. It is not improbable that production of PA in a susceptible host after infection is prevented by suppressor molecules (may be glucans or glycoproteins) produced by the pathogens.

Apart from PR-proteins and phytoalexins, resistance could also be induced in plants by activation of oxidative burst. The cell membrane is an activating site for induction of defence mechanisms. This membrane contains the R-gene coded proteins that recognise the elicitors released by the pathogen and later trigger hypersensitive response (HR). Doke (1983) reported the involvement of superoxide (O_2-) and H_2O_2 generation in HR of potato tuber tissue to infection with an incompatible race of *Phytophthora infestans*. Rapid generation of oxidants has been observed in several incompatible host-parasite interactions. It indicates that oxidative burst has a role in the host's defence response although it is different from classical transcription depended defences as in case of phytoalexins and PR-proteins. Highly reactive oxygen radicals are believed to be released by NADPH oxidase enzyme complex of the host cell plasma membrane immediately after the fungus of its elicitors come in contact with the cell. Oxygen radicals act through the oxidation of phenolic compounds into quinones which inhibit the growth of the pathogen. Besides, oxygenation of membrane lipids also appears to involve various lipooxygenases.

It has been reported that soybean cells inoculated with either virulent or avirulent races of *Pseudomonas syringae* pv. *glycinea* caused a rapid but weak transient accumulaion of oxidants (Phase I) (Orlandi *et al.,* 1992; Baker and Orlandi, 1995). But when the cells were inoculated with an avirulent race there was a second massive and prolonged oxidative burst between 3 to 6h after inoculation (Phase II). Similar two-phase kinetics were also observed in tobacco cells after inoculation with *P. syringae* pv *syringae* which is a non-pathogen of tobacco. Induction of O_2 and/or H_2O_2 accumulation is possible with specific bacterial or fungal avirulence factors (Baker *et al.,* 1993; Mehdy, 1994). For instance, a race specific peptide elicitor derived from *Cladosporium fulvum* avr 9 gene rapidly stimulates an oxidative burst after infection of leaves of the tomato genotype of 9. A number of non-race specific fungal elicitors and endogenous oligogalacturonide elicitors derived from the plant cell wall were also able to induce oxidative burst within 2 min. of exposure to plant cells (Levine *et al.,* 1994). However, molecular mechanism underlying the activation of oxidative burst after interaction with an avirulent pathogen remains yet to be clearly understood. Manipulation of oxidative burst may be expolited in plant disease management.

Various traditional control measures to modern integrated disease management (IDM) strategies

Most traditional disease control measures have been found to be inadequate and hence attempts are being made to identify desirable combinations of methods for management of various plant diseases. The main objectives are (a) to eliminate or reduce the initial inoculum; (b) to reduce its effectiveness; (c) to increase the resistance of host and (d) to delay onset of disease (Agrios, 1997). Several methods including regulatory inspection of healthy seeds, cultural practices, biological, physical, chemical controls and bioenvironmental regulations are considered to be effective. But the integration of several control strategies appears to be the best approach so far to plant disease management. A few examples are cited here.

Manibhushanrao and Baby (1997) reported that an integration of fungal antagonists such as *Gliocladium virens* and *Trichoderma longibrachiatum* and organic soil amendments (*Gliricidia* leaf and neem cake) provided excellent control of sheath blight of rice in the field. Survival of pathogen (*Rhizoctonia solani*)

propagules in the soil was also considerably decreased in combined treatments than individual ones. In a separate investigation Gupta (1997) has demonstrated that usage of broad spectrum fungicides is the main management practice for apple diseases though other methods are also being tested. It is noted that disease forecasting is an important component to reduce the number of fungicide sprays. Protectant fungicides are used initially on disease appearance followed by broad spectrum fungicide which reduce powdery mildew and canker fungi. Pre-leaf fall sprays with urea minimise inoculum of *Venturia inaequalis,* a causal organism of apple scab. Besides, scab-infected leaves are burnt and scab resistant varieties are used for large scale cultivation. Attempts are being made to produce virus free apple plants.

Integrated disease management of Pearl Millet has also been reported. Pearl Millet is a host of four major diseases, namely, downy mildew, ergot, smut and rust of which downy mildew caused by *Sclerospora graminicola* is of more wide occurrence and more destructive. Use of seed dressing fungicide metalaxyl can protect plants up to 40 days but combination of fungicide with host plant resistance is more effective. But this is not suitable for other diseases. It is suggested that resistant lines of crop with seed dressing fungicides and cultural practices for specific areas may help to develop more effective IDM strategy for Pearl Millet (Thakur, 1997).

It appears from the available literature that host plant resistance is the key factor in integrated disease management followed by either fungicidal treatment of cultural practices or both. It is now possible to identify genetic diversity in pathogen population by DNA finger printing and hence a better understanding of the genetics of virulence diversity may help to develop the strategy of resistance gene transfer to a desired host.

Disease management, allied problems and new challenges

Disease management is an urgent need and urgent need impels one to devise appropriate means. Several strategies and technologies have been devised to control plant diseases but the problem remains unsolved. Although spectacular advances have been made in plant protection research during the last few years with the help of advanced biotechnology and genetic

engineering, little attention has been paid to newly created problems. For example (a) a novel recommended disease resistant cultivar (s) may be more susceptible to another disease, (b) changes due to artifical or natural mutations in strains of pathogenic fungi, bacteria and viruses may cause more serious disease to their respective host plants, (c) introduction of new crops and intensive agricultural practices may create new disease problems, (d) "ecologically sustainable plant disese management" is still a challenge to plant protectors, (e) it is not yet clearly understood as to why single gene transfer from a non-host wild plant to a cultivated one confers resistance to a specific race or a few races while the non-host is immune to all races of the same pathogen, (f) what is the factor(s) responsible for total immunity of a non-host plant to a pathogen of another plant? (g) "Replant disease" on "soil sickness" is a problem for fruit tree growers in many countries who use old nurseries or orchards for growing their plants, (h) use of chemical pesticides is economic and socially acceptable but it causes environmental hazards, (i) target specific fungicides or pesticides may increase population of fungicide/pesticide resistant strains in nature which are more harmful to plants, (j) immunization of plants is now a reality but characterization of the translocatable "immunity signal" that sensitizes the plant to react rapidly following challenged inoculation remains yet to be elucidated, (k) to develop suitable application technology for immunization of plants is a major challenge to plant pathologists, (1) several R genes have been isolated from plants but how R-gene products function following perception of pathogen avirulence signals is not clearly defined. In other words, the molecular mechanism whereby pathogen recognition is transmitted to the cell and activates a variety of plant defense responses is still unclear, (m) currently, major genes are being extensively used in agriculture for plant disease control but evry little is known about their structure, origin and mode of action, (n) several pathogenesis related proteins (PRs) have been characterised but how and in which combination they act against different diseases remain enigmatic. All detected PR-proteins are not yet properly identified and characterised.

Concluding remarks

Regardless of advances in all disease management disciplines, it is expected that traditional practices will continue as usual along with modern technology in the 21st Century. Gradual progress in fungicidal research during the last several decades clearly shows the evolution of concepts for chemical management of plant disease. Currently, research on fungicides is being carried out on new formulations, new molecule synthesis, analysis of residuals, toxicity, screening and field testing. Although attempts are being made to modernise fungicides by developing non-toxic, non-hazardous chemical compounds, biofungicides of herbal or microbial origin are gaining popularity despite their limited success. It should be borne in mind that all biofungicides are not harmless to all non-target organisms. Conventional plant breeding method has some limitations and therefore production of transgenic plants through genetic engineering may be accepted as a better alternative. Several disease resistant transgenic plants have already been raised. Now their values should be proved under field conditions and economic benefit to growers should also be ensured.

Systemic acquired resistance (SAR)/immunity could be induced in plants by a variety of biological and chemical agencies but the nature of molecular events required for resistance/immunity is still largely unknown. The signal transduction events in resistant plant that follow after recognition of an avr-dependent signal are also not known. Plant immunization technology appears to be a promising area in plant sciences and needs more attention of researchers. Integrated management of plant diseases will be most successful provided the effective methods are carefully selected for integration purpose. Innovative approaches to disease management are always thrilling and beneficial but new developments/discoveries sometimes create new problems to the plant protectors. However, disease management of higher plants remains to be an exciting field of research in future also.

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