academicJournals

Vol. 9(15), pp. 1207-1217, 10 April, 2014
DOI: 10.5897/AJAR2014.7311
Article Number: 7677EFA43830
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Review

Plant disease management in India: Advances and challenges

Sanjeev Kumar

Department of Plant Pathology, Jawahar Lal Nehru Krishi Vishwa Vidyalaya Jabalpur- 482004 (M.P), India.

Received 22 May 2013; Accepted 13 January, 2014

The responsibility of protecting food crops from diseases and pests in the challenging environment is rising with increase in human population and its needs. The crop losses due to pests are assessed to be ranging approximately between 10 to 30% of crop productions. Status and importance of various diseases have changed over the years in India. Awareness is needed to know the status of these problems and to develop management modules to protect these in eco-friendly manner. IDM is a multidisciplinary approach that seems promising to manage diseases effectively by integration of cultural, physical, biological and chemical strategies. Of the diverse components in IDM, biocontrol is important, but notwithstanding their known efficacy, biocontrol formulations have only a inadequate share in the national pesticide scenario. How to make biocontrol more effective, feasible and popular needs to be reviewed thoroughly. Development and use of molecular techniques for pathogen detection, resistance identification and cloning of genes for resistance seems very promising to realize the goal. So, further research thrust is needed in India to develop and utilize new novel technologies and strategies like gene cloning, recombinant DNA technology and other biotechnological and molecular modules to minimize the crop losses due to existing and new emerging diseases in the light of climate changes. Plant pathologists have a crucial role to play in this scenario. We have to be more proactive in our approach. Some of the current advances and emerging challenges in crop disease management in India are briefly discussed in this review.

Key word: Integrated disease management, biocontrol, India.

INTRODUCTION

India is known as growing economic giant but the benefits of this progress are mostly confined to urban or semi-urban areas. More than 65% of the population in the country lives in rural areas and depends on agriculture and related avenues for their sustenance. Hunger and poverty persists because of lack of work opportunities, thus inadequate income for farming communities. Indian agriculture, basically characterized as a means of subsistence, is changing fast as per market demands both domestically and international. Modern high input mono cropping based intensive agriculture has resulted in loss of biodiversity, outbreaks of pests and diseases, degradation of soil and water, which has ultimately led to stagnating agricultural production and productivity. Climatic changes are becoming a major factor in the present scenario (Kumar, 2013e).

E-mail: sanjeevcoa@gmail.com.

The crop yield losses, on field and during post harvest period, caused by pests, diseases and weeds are of paramount importance. The crop losses due to pests, diseases and weeds are approximately assessed to be ranging between 10 to 30% of crop productions. If we consider, on a average, crop loss of 20%, and the present gross value of our agriculture produce as Rs, 7 lakh crore, the loss comes to Rs. 1,40,000 crore, which is colossal (Kumar and Gupta, 2012). Even if we could save 50% by using plant protection, it will add Rs. 70,000 core additional income to our farmers. At a same time, when all of us are concerned about National Food Security, can the country afford these losses? Various type of direct and indirect losses caused by plant diseases include, reduced quality and quantity of crop produce, increased cost of production, threat to animal health and environment, limiting the type of crops/varieties grown, loss of natural resources and less remunerative alternatives adopted (Kumar and Saxena, 2009). In order to combat the losses caused by the plant diseases, it is mandatory to define the problem and seek solutions, At the biological level, the requirements are for fast and accurate identification of the causal organism, accurate estimates of the severity of diseases and its effect on yield, and identification of its virulence mechanisms. Disease may then be minimized by the reduction of the pathogen's inoculums, inhibition of the virulence mechanisms, and promotion of genetic diversity in the crop.

Success in disease management, as in most walks of life, depends on having right tools and the confidence to apply them. The key tool for disease management is knowledge and having knowledge gives the confidence. Diagnostic and advisory support systems are facing huge challenges in making relevant and effective knowledge and support available to farmers and market chains and ensuring that upstream researchers are informed of the real priority problems and issues requiring resolution.

Chemical pesticides have reduced crop losses in many situations, but even with a substantial increase in pesticide use, the overall proportion of crop losses and the absolute value of these losses from diseases appear to have increased over time (Kumar and Gupta, 2012). Nonetheless, an increase in pesticide use still appears to be profitable. Increased monoculture, reduced crop diversity and rotation, and use of herbicide have all increased vulnerability to diseases as well. Pathogens tend to develop resistance to pesticides, requiring higher use to sustain production. Inappropriate and excessive pesticide use led to increased and unnecessary disease out breaks and additional disease loss because of the inadvertent destruction of natural enemies of diseases, disease resistance and resurgence of secondary diseases. Ultimately, overuse of pesticides can reduce food production. Proponents with varying perspective on chemicals agree threat integrated disease management (IDM) must be science based and economically viable for farmers. The emphasis in on disease problems and preventing them form reaching economically damaging levels.

Host–plant resistance, natural plant products, biopesticides, natural enemies and agronomic practices offer a potentially viable option for IDM. They are relatively safe for the non target organisms and humans. Biotechnological tools such as marker assisted selection, genetic engineering, and wide hybridization to develop resistant crop cultivars will have a great bearing on future disease management programs. Disease modeling, decision support systems, and remote sensing would contribute to scaling up and dissemination of IDM strategies.

Plant pathology is challenging and an important science that deals with science of disease development and ability of managing diseases. Society, consumers and growers will only be able to continue to benefit from plant pathology if the discipline can evolve appropriate disease management schemes that can respond to the significant changes in agricultural practices in India; the ultimate goal being to produce more and safer food in sustainable agricultural systems that conserve natural resources and the environment. Information technology, communication and the integration of conventional and new technologies are essential and must be integrated by the modern practitioners of plant pathology into effective disease management schemes that can be implemented at the farm level. In view of this, a review article on current advances and emerging challenges in crop disease management in India should be viewed as very timely and appropriate.

Changing disease scenario

The status and importance of various diseases have changed over the years. Numerous diseases like papaya ring spot (papaya ring spot virus) and *phytophthora* diseases (*Phytophthora parasitica, Phytophthora palmivora, Phytophthora citrophthora* etc) have taken serious proportions. In 2008, there was flare up of neck blast and brown plant hopper of rice in Haryana causing losses up to 40% due to persistent and unprecedented rains at the flag end of monsoon season (Bambawale et al., 2008). Likewise, diseases with unfamiliar or complex etiology such as mango malformation and para wilt and grey mould of cotton and other diseases such as sheath blight of rice/maize, bract mosaic of banana, downy mildews in maize and several other crops have become devastating by virtue of evolution of virulent races or resurgence capabilities (Kumar and Gupta, 2012).

The nematode problems have taken their toll and with each year passing some of them are gaining momentum to decimate the entire cropping systems- the root knots, reniform and burrowing nematodes are important in different states; *Meloidogyne indica* is devastating the kagzi lime in Gujarat, the root knot complex has similarly created serious situation with *Ceratocystis fimbriata* on pomegranate in Maharashtra, Karnataka and North Gujarat; the root knot is severely infesting mulberry plants which is adversely affecting the silk industry in Karnatka. Mostly, the reactions to outbreaks of diseases are in fire fighting mode and once the fire is doused, there is no follow up or recording the causes which had resulted in outbreaks. Even where the causes are investigated by eminent teams, their recommendations remain on paper.

Climate change

Climate change and the response of pathogens to changing conditions are matters for utmost priority for plant pathologists.

Research on impacts of climate change on plant diseases has been limited in India, although some striking progress has been made lately.

The rise in temperature at Kanpur may have been beyond the tolerance limit of *Aceria cajani*, the mite vectoring Sterility Mosaic Virus of pigeonpea, which could have influenced decline in the disease there. On the other hand, the weather factors might have shifted in favour of the vector at Bangalore that may have resulted in rise of the disease on the crop there (Kumar et al., 2013e). Climate change may have also influenced Phytophthora blight incidence at Kanpur and Pantnagar is mutually opposite directions (Kumar et al., 2013f).

While *Alternaria* blight is increasing on pigeonpea in Andhra Pradesh, Cercospora leaf spot is on the rise on the crop in Karnataka and Stemphylium blight is growing on lentil, chickpea, in some parts of India which could be due to the effect of climate change.

Root rot of oilseeds Brassica is an emerging threat for Rape seed – mustard production system, recently reported from the farmers' field in some pockets of the country. Some isolates of *Alternaria brassicae* sporulated at 35°C and several isolates had increased fecundity under higher RH, it seems that as per recent changes towards warmer and humid winters, being in line with current projections for future climate change (Waugh et al., 2003).

Global warming resulting in elevated carbon dioxide and temperature in the atmosphere could influence plant parasitic nematodes directly by interfering with their developmental rate, survival strategies and indirectly by altering host physiology. Studies have also demonstrated that the geographical distribution range of plant parasitic nematodes may expand with global warming spreading nematode problems in newer areas (Somasekhar et al., 2012).

There are also reports of upsurge in infestation by *Rotylenchulus* and *Pratylenchus* on several crops viz., chickpea, vegetables, etc (Somasekhar et al., 2012).

Seed pathology

Current emphasis for increased crop yields is to increase seed replacement rate. This requires seed health and rigorous seed health testing. Seed pathology involves the study and management of diseases affecting seed production and utilization, as well as management practices applied to seeds. Research innovations in detection of seed borne pathogens and elucidation of their epidemiology; advances in development and use of seed treatments; and progress towards standardization of phytosanitory regulations are to be strengthened (Chahal, 2012). With the globalization of agriculture, seed health testing is going to be mandatory for seed quality control. This needs highly sensitive, foolproof and quick methods for indexing seed borne pathogens. Immunodiagnostic and molecular technologies which are highly specific and sensitive test methods are yet to be simplified, standardized and commercialized in India for indexing seed borne pathogens of concern. There is a need to develop simple and efficient diagnostic test kits which can be defined as a commercially packaged system of the principal or key components of a seed health testing method (Vishunavat, 2009).

Tackling threat of Ug 99

Wheat rusts have been very imperative diseases of wheat worldwide. The large scale cultivation of wheat carrying a single gen Sr31 for protection against stem rust, over a large area proved to be a primer for the development of a new race virulent on this gene. Initially, this gene, used in breeding at CIMMYT, was selected as it was found allied with resistance to other two rusts like yellow (Yr 9) and brown rust (Lr 26). Achieving triple resistance with one gene was very striking for wheat breeders and this gene was used in breeding programmes all over the world. This gene was further found to be also linked with other positive traits like enhanced yield and adaptability over a array of environments. With the adoption of Sr 31 bearing cultivars, grown all over the world, the incidence and occurrence of stem rust became meager and sporadic. Thus, breeders stared to view stem rust of wheat as less of a threat. In such circumstances, it was anticipated that sooner or later the pathogen may heat back and this gene may lose its effectiveness. This was what precisely happened when a new race, popularly termed as Ug 99, emerged in highlands of Kenya and threatened wheat cultivation in the world (Sharma , 2012). The projection of its spread out of the continent, as happened in the past for Yr-9 virulence's, was a serious concern for the whole wheat community. Taking cognizance of this menace, global community was warned by Dr. Borlaug to take up suitable measure to embark upon the threat as stem rust has the potential to wipe out the crop. India, too, was at risk country as it has been growing a cultivar carrying a single gene (Sr31) for protection, over a huge area. In addition to threat of Ug99, however same cultivar was also susceptible to yellow rust in the main wheat bowl of the country of the North West India. So, Indian wheat researchers need to address the threat of both yellow rust and Ug 99. Realizing the significance of wheat crop for the country, India adopted a range of activities that helped not only in assessing the damage to wheat crop but also helped in urging the wheat workers to replace the susceptible wheat with resistant one. The activities included screening against Ug99, extensive survey and surveillance, raising awareness of field workers, and developing new resistant stocks and identification of fungicides against Ug99. The first consignment for screening Indian wheat against Ug99 was sent to Kenya in 2002 and the majority of these varieties were found susceptible to Ug99. However, two genetic stocks were identified resistant. Subsequent screening of Indian wheat at Kenya revealed that some of the presently cultivated wheat's were resistant to Ug 99 (Prashar, 2012). So, it was a huge challenge for the country to replace varieties susceptible to both yellow and stem rusts. Second activity encompassed imparting training field workers to identify stem rust and distinguish susceptible response from resistant one. Thirdly, developing resistant genetic stocks with major genes and energized efforts to integrate adult plant resistance of polygenic kind were adopted. However, progress has been made with screening and developing resistant stocks, the challenge still remains on many vital areas *viz*, the role of stem rust in north hills, exploring new area of survival of wheat rust and reviewing epidemiology of wheat. For India, it remains a big challenge to replace susceptible cultivars with the resistant ones and exploring role of weather factors and grasses in survival of wheat rusts.

Plant parasitic nematodes

Plant parasitic nematodes are gaining significant importance worldwide due to their devastating effects on crops leading to major economic and social impacts. It is estimated that the 20 most important life sustaining crops undergo 10.7% yield losses due to nematodes, and other 20 economically important crops suffer 14% losses, the average amounts to 12.3%. While the average losses in developing nations including India is 14.6% (Ganguly and Dutta, 2012). According to trials in the 1960s, in Rajasthan alone the molya disease due to *Heterodera avenae* caused crop losses worth Rs. 400 million at current prices. Even ear cockle nematode, *Anguina tritici* caused less than 1% average loss of wheat amounts to Rs, 450 million at current prices. Similarly, root lesion nematode, *Pratylenchus* coffee cause damage worth of Rs. 200 million on coffee. At the global level root knot nematodes, *Meloidogyne incognita* is the major impediment in crop production as rarely any crop is free from its damage. In India, *M. incognita* cause colossal loss in vegetables like tomato, brinjal, okra, cucurbits etc. Rice root knot nematode, *M. graminicola* has emerged as a foremost predicament in rice wheat agro – ecosystem particularly in south –east Asia. Disease caused by nematodes are divided into subgroups according to their habitat and parasitic habit (sedentary, migratory, endo and ectoparasites).

Majority of the plant parasitic nematodes are ectoparasites include Xiphinema, Longidorus, Trichodorus Tylenchorhychus, Helicotylenchus, Hemicriconemoides, Paratylenchus etc. Some of the main migratory nematodes of above ground plant parts are *Ditylenchus dipsaci, D. angustus Aphelenchoides besseyi, Rhynchophorus palmarum, Bursaphelenchsu xylophilus* etc. Parasitic stages of some genera like *Tylenchulus* and *Rotylenchulus* partially enter roots of citrus and castor known as sedentary semi – endoparasites. While sedentary endo parasites like root knot and cysts complete their life cycle almost within the root itself followed by production of medium to large size galls in case of *Meloidogyne* incurring heavy damage. In addition to the direct damage, nematode also predispose plants to a variety of other pathogens particularly bacteria and fungi forming disease complexes. Some of them are tundu, vascular wilt, damping off, cortical rot, black shank etc. Other than that species of *Xiphinema, Longidorus* and *Trichodorus* are known to transmit soil borne viruses in plants leading to disease like grapevine fan leaf, raspberry ring spot, tobacco black ring etc. Under the changing climate and agricultural scenario, a sustainable management approach need to be adopted with an objective to uphold nematode population densities at the levels below the economic injury level or to lessen their levels such as to derive utmost profits out of the management cost is incurred (Ganguly and Dutta, 2012).

Emerging viral menace

Ever since the mosaic disease of tobacco was associated with a virus that is, Tobacco mosaic virus by the turn of 19^{th} century, more and more plant diseases were found to be caused by viruses. In India, several viral diseases of agriculturally important crops such as citrus, sugarcane and tobacco were identified namely tristiza in citrus, sugarcane mosaic in sugarcane and leaf curl in tobacco in 1920s and 1930s. Subsequently viral diseases of vegetables and ornamental, horticultural and field crops were studied. Many of these viral diseases have caused significant economic losses in several crops. A few emerging viral diseases which are of huge concern to our farmers are rice tungro, groundnut bud necrosis, sunflower necrosis, yellow mosaic of legumes, pigeonpea sterility mosaic, soybean bud blight, cotton leaf curl, cassava mosaic, potato apical leaf curl, banana bunchy top, banana bract mosaic, papaya leaf curl, papaya ring spot, chlorotic leaf spot in peach, piper yellow mottle (Prabha and Baranwal, 2009). These new viral diseases are emerging because of intensive agriculture coupled with change in cropping system. This has allowed the prevalence of important virus vectors such as white fly and aphids throughout the year. It is important to develop short term and long term strategies for the management of viral diseases. There is an urgent need to develop diagnostics for detection of different viruses in field crops and vegetables. Emphasis is given on integrated disease management involving use of disease free planting material, agronomic practices to control vectors, development, and use of resistant varieties and timely diagnosis. Development and utilization of detection and diagnostic methods based on enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and Microarray are gaining importance. Biotechnological tools have augmented conventional approaches in identification and isolation for resistant sources/genes and development of crops resistant to specific viruses through transgenic approaches. Viral resistant transgenic plants are obtained by inserting segments of viral nucleic acid into plant genomes that leads to silencing of genes of the virus that have homologous sequences, thereby making plants resistant (Singh and Malhotra, 2010).

Biotechnology in plant pathology

Diverse approaches in plant disease management have very much been influenced by the recent advances in molecular biology. Many biotechnological tools and techniques have been developed by using different plant pathogens as experimental materials. It has provided way to understand host pathogen correlation under diverse environment to give a novel look to this branch of science paradoxically viewed as 'cut and burn' technology. Different aspects are as follows:

(i) Molecular diagnosis of plant pathogens

Conventionally, cultural methods have been employed to isolate and identify potential pathogens. This is relatively slow process, often requiring skilled taxonomists to reliably identify the pathogen. However, over the last 30 years, several techniques have been developed which have found application in plant pathogen diagnosis; these include the use of monoclonal antibodies (Kohler and Milstein, 1975) and enzyme linked immunosorbant assay (ELISA) (Clark and Adams, 1977) and DNA based technologies, such as the polymerase chain reaction (PCR), which enable regions of the pathogen's genome to be amplified by several million fold, thus increasing the sensitivity of pathogen detection. Furthermore, diagnostic PCR has been significantly improved by the introduction of second generation PCR, know as the real time PCR. It is now possible not only to detect the presence or absence of the target pathogen, but also to quantify the amount present in the sample. Enumerating the pathogen upon detection is crucial to estimate the potential risks with respect to disease development and provides a useful basis for diseases management decisions. The DNA micro array technology, originally designed to study gene expression and generate single nucleotide polymorphism (SNP) profiles is currently a new and emerging pathogen diagnostic technology and offers a platform for unlimited multiplexing capability. The fast growing databases generated by genomics and biosystematics research provide unique opportunities for the design of more versatile, high throughput, sensitive and specific molecular assays that will address the major limitation of the current technologies and benefit plant pathology. Finally, the so far restricted use of robotics to DNA technology will become economically feasible and thus accessible to farmers and will offer the possibility of using single DNA chip as practical tool for the diagnosis of hundreds of plant pathogens (Kumar, 2013d).

(ii) Analysis of molecular variability in plant pathogens

Characterization of genetic diversity in plant pathogens have been made feasible, beyond use of differential hosts, through diverse molecular techniques. Different molecular markers have been used in characterization of genetic diversity of plant pathogens. In most of the cases, these are RAPD (Williams et al., 1991), RFLP (Botstein et al., 1980), AFLP (Vos et al., 1995), SSR/ISSR (Guleria et al., 2007), ITS (Powell et al., 1996). The RAPD markers have been mostly used for characterization of fungal pathogens, followed by AFLP and ITS markers. This might be because of their ease and simplicity in use. Guleria et al. (2007) collected 19 *Rhizoctonia solani* isolates from rice growing regions of India, used two marker systems that is, RAPD and ISSR for molecular characterization of genetic variability. Of these two types of DNA markers, RAPD markers were able to detect more genetic variability when compared to ISSR markers.

(iii) Mapping of disease resistance genes using DNA markers

Molecular mapping can be used for direct selection of disease resistance genes for the use in plant breeding programmes. Commonly used markers are restricted fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSRs), single nucleotide polymorphism (SNP) with predilection of PCR based markers. There is very few reports available form India which is required to be strengthened to support marker assisted selection (MAS) in plant breeding for disease resistance.

(iv) Marker assisted pyramiding of disease resistance genes

Marker assisted pyramiding of disease resistance genes termed as 'Breeding by Design' can help to control the pathogen which recurrently and rapidly develop their new virulence. Efforts are made in India under Asian Rice Biotechnology network (ARBN) to pyramid resistance gene against bacterial blight of rice. Rice is among the first crops where marker assisted pyramiding of disease resistance genes was initiated. Rice varieties developed by using MAS have now been released for commercial cultivation for the first time in India. The variety amend as Improved Pusa Basmati-1 was developed by using conventional plant breeding approach integrated with MAS and two bacterial blight resistance genes Xa13 and Xa21 incorporated in Pusa Basmati-1(Gopalakrishnan et al., 2008). Another variety of rice resistant to bacterial blight was developed in non basmati type rice in India by using MAS. PCR based molecular markers were used in a backcross –breeding program to introgress three major bacterial blight resistance genes (Xa21, Xa13 and Xa5) into Samba Mashuri from a donor line (SS1113) in which all the three genes are present in a homozygous condition (Sundaram et al., 2008). These two reports successfully demonstrate the application of marker assisted selection for targeted introgression of BLB resistance genes into Basmati type and non-Basmati types varieties of rice in India. So, there is call for to take up such initiatives for other host pathogen system for control of serious loss causing diseases. Molecular markers can also help in assaying the germplasm for presence or absence of a particular disease resistance gene. Cloning of disease resistance genes by tagging approaches can identify the function of a specific genes by uncovering a specific pathotype. Initiation of his work will help to understand complex pathotype system of *Pyricularia* grisea (Jalali, 2008).

(v) Transgenics

Development of disease resistance through transgenic research is yet at primitive stage in India. Non availability of resistance to plant pathogens can be overcome by search and transfer of resistance genes from other sexually incompatible species which is possible using genetic engineering approach. It requires to first search for new genes which have broad spectrum resistance to pathogen population present in the region and then following transgenic approach, transferring of resistance to commercial varieties to achieve resistance, working at filled level. Disease resistance transgenic have been developed in banana and tobacco by transferring a synthetic substitution analogue of a short peptide, Maganin (Chakarbarti et al., 2003). Magainin is one of the earliest reported antimicrobial peptides from skin secretions of the African clawed frog. The peptide is not stable in its native form and, therefore, researchers modified it to express in foreign plant systems. Tobacco plants transformed with the peptide showed enhanced reisistance against *Sclerotinia sclerotium*, *Alternaria alternata* and *Botrytis cinerea*. Transgenic banana plants showed resistance to *Fusarium oxysporum f. sp. cubense* and *Mycosphaerella musicola* (Kumar and Gupta, 2012). However, it remains to be seen how these plants perform under natural disease conditions.

(vi) Application of genomics

Genomic has emerged as one of the frontier technologies during this century. Its application in different areas of plant pathology can be enormous in structural, functional or comparative genomics. Using high throughput genome sequencing technologies many plant pathogens are being sequenced world over. A list of pathogens which are at different stages of the genomic sequencing has been given by Jalali (2008).The massive genome sequence data being generated on different microorganisms can be used for simultaneous detection of multiple plant pathogens. The unique sequence from a wide range of pathogens could be used to develop micro-arrays for the simultaneous detection of large number of different strains. The probes and primers could be designed for differential detection of pathogens and their characterization at molecular level by using the unique sequence data of the pathogen's DNA.

(vii) Application of RNA interference

RNA interference (RNAi) has emerged as a powerful tool for battling some of the most notoriously challenging diseases caused by viruses, bacteria and fungi (Wani et al., 2013). RNAi is a mechanism for RNA guided regulation of gene expression in which double stranded ribonucleic acid (ds RNA) inhibits the expression of genes with complementary nucleotide sequences. The application of tissue specific inducible gene silencing in combination with the use of appropriate promoters to silence several genes simultaneously will result in protection of crops against destructive pathogens. RNAi application has resulted in successful control of many economically important diseases and pests in plants. Baum (2007) used RNAi to develop transgenic corn expressing ds RNA to silence genes of the corn root worm. Similarly, cytochrome p450 cy6AE14 genes of the cotton bollworm were silenced to disable the bollworm from feeding on gossypol in plants (Mao et al., 2007). In addition, RNAi approaches have also been used effectively to knockout the expressions of genes and to understand their biological functions (Anandalakshmi, 2013). The RNAi based technologies have tremendous potential in significantly reducing our reliance on chemical pesticides and thus pave way for the efficient, cost effective, eco –friendly alternative which will ensure a paradigm shift in pest management in India.

(viii) Post transcriptional gene silencing

The RNA silencing mechanism is also a powerful tool to develop crop species resistant to viruses. The expression of virus derived sense or antisense RNA in transgenic plants conferring RNA mediated virus resistance appears to induce a form of post transcriptional gene silencing (PGTS). It's a nucleotide sequence specific process that includes mRNA degradation, RNA silencing, an evolutionary mechanism protecting cells from pathogenic RNA and DNA, is viewed as an adaptive immune system of plants against viruses (Krishnaraj, 2013). Several lines of research indicate that RNA silencing can be induced locally and then spread throughout the organisms, and this aspect of the process likely reflects its role in viral defense.

Role of nanotechnology

Nanotechnology offers an imperative role in improving the existing crop management techniques. Generally only a very low concentration of agrochemicals have reached the target site of crops due to leaching of chemicals, degradation by photolysis, hydrolysis and microbial degradation. Hence, repetitive application is needed for effective control causing unfavorable effects such as rapid occurrence of resistance and soil and water pollution. Nano-formulated agrochemicals should be designed in such a way that they hold all necessary properties like effective concentration (with high solubility, stability and effectiveness), time controlled release in response to certain stimuli, improved target activity and less eco-toxicity with safe and easy mode of delivery. Therefore, an urgent need is to evaluate and develop natural, biodegradable, and environment safe nano-formulated compounds.

Biological control of plant diseases

The increased reflection on environmental concern over pesticide use has been instrumented in a large upsurge of biological disease control. Among the various antagonists used for the management of plant diseases, *Trichoderma* and *Pseudomonas* play a vital role. Among the various isolates of *Trichoderma viride, Trichoderma harzianum, Trichoderma virens* and *Trichoderma hamatum* are used against the management of various diseases of crop plants especially with dreaded soil-borne pathogens in India (Kumar et al., 2009). It has many advantages as a bio-control agent due to its high rhizosphere competence, ability to synthesize polysaccharide–degrading enzymes, amenability for mass multiplication, broad spectrum action against various pathogens and environmental friendliness (Kumar, 2013c). Fluorescent pseudomonads suppress the pathogens either directly through the production of various secondary metabolites or indirectly by inducing plant-mediated defense reactions. The crucial factor in the success of biological control by fluorescent pseudomonads is their ability to colonize the rhizosphere and their persistence throughout the growing season. Fluorescent pseudomonads are root colonizers because they occur in the natural habitat of rhizosphere and thus, when they are reintroduced to roots through seed or seed-piece inoculation, they colonize root surface profusely. Fluorescent pseudomonads suppress the pathogens by antibiosis through the production of various antibiotic substances such as 2,4-diacetyl phloroglucinol, phenazine-1-carboxylic acid, oomycin A, oxychlororaphine, pyoluteorin, pyrrolnitrin and pyocyanine. Siderophores are extracellular, low molecular weight substances which selectively complex iron with high affinity. Fluorescent pseudomonads produce siderophores such as pseudobactin and pyoverdine which chelate the iron available in the soil and make it unavailable to pathogen thus the pathogen dies for want of iron. In rice, seed treatment followed by root dipping and foliar spray with *Pseudomonas fluorescens* showed a higher induction of ISR against sheath blight pathogen, *R. solani* (Singh and Singha, 2004).While dealing with biocontrol we have to have a critical look at the following aspects which have been hindering its successful applications as bioprotectants. Greatest limiting factor is very short shelf life (4-6 months) under tropical and subtropical conditions. There is a strong need to increase the shelf life to make biological control application practically feasible. Sensitiveness of biocontrol agents (BCAs) to pH, temperature, moisture, substrates, etc are very important factors restricting their application. Modern biotechnological tools could be used to reduce these limitations (Mukhopadhyay, 2012). To date, 26 microbes have been included in the schedule to the insecticide act 1968 for production of microbial biopesticides. *T. viride*, *T. harzainum*, *Pseudomonas* sp., *Beauvaria bassiana, Metarrhizium anisopliae* and *Bacillus subtilis* are important biocontrol agents for management of various pest and diseases in India (Singh, 2012). However, their use is still limited to some selected states in our country. The major region for this phenomenon is the mushrooming of some fly –by-night spurious companies. This not only sow the seeds of doubt in farmers mind about the profitability of microbial pesticides but also the ill effect of these biopesticides.

The research on BCAs can only be fruitful when we commercialize and register the product based on superior strains with the Central Insecticide Board (CBD) (Singh, 2012). Efforts must be on to work on the above issues by interdisciplinary approaches involving biotechnologists, microbiologists, plant pathologists, biochemists and also through strict control by government agencies.

Disease forecasting and monitoring

Plant disease forecasting and monitoring provides early information about the probable occurrence of a disease to facilitate chemical prophylaxis at appropriate time either to stop pathogen multiplication or further spread of the disease. Early information is essential to determine number of sprays and schedules to make economically sound disease control, and limit the chance of development of pathogen resistance to the pesticides. In developed countries pesticide use is warranted by well established monitoring, surveillance and forecasting system. It is generally done by established relationships between pathogen population and physical weather parameters like air temperature, rainfall, relative humidity, cloudiness, dry wetness or leaf wetness duration. Field monitoring for pre disease symptoms at susceptible stage and monitoring of favorable weather conditions make the basis of disease monitoring. Forecasting and monitoring of major air borne diseases have been relying on the knowledge on biology and ecology of the pathogen, quantitative seasonal studies over several years, season range, and variation in the population pattern, geographical distribution and weather records. Forecasting systems for potato late blight, apple scab, powdery mildew of mango, beer and rice blast are now available (Sinha and Banik, 2009) However, forecasting systems developed elsewhere could be adopted with local situation after expert judgments and field trials. But for practical utility in growers' field there is still a lacuna. In this connection, training programme for the plant pathologists, extension persons as well as for the grower is necessary.

Plant quarantine measures

Exchange of plants/planting material is a potential source of introducing exotic pests. Quarantine measures act as filters against entry of such pests by restricting their introduction and in case introduced, preventing their establishment and further spread. The International Plant Protection Convention (IPPC) of FAO encourages cooperation among various countries and ensures that each country establish a National Plant Protection Organization to discharge such function. In India, the Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture implements the plant quarantine regulations for bulk consignments and National Bureau of Plant Genetic Resources under Indian council of Agricultural Research (ICAR) is the nodal agency for safe movement of germplasm including transgenics. This work under the Plant Quarantine Regulation of Import in India order 2003 which came into force from 1st January 2004, under the destructive Insect and Pests Act of 1914. Although, the regulations are now in place, there are number of issues related to quarantine of germplasm as it has been drafted more for facilitating bulk imports than for exchange of germplasm. Under this order a pest risk analysis (PRA) has been made mandatory and the various schedule of the order give lists of crops for which a generic PRA is given. A number of cultivated crops (and their germplasm including wild relatives/land races) do not find mention in any of the schemes. Hence, a detailed PRA becomes obligatory for them prior to import. The size of sample/consignment, technique to be used for detection of minute amounts of pest, availability of diagnostic reagents and reference collection of exotic pests, and post entry quarantine testing are important technical issues in quarantine. Over the years, during quarantine processing, the pests intercepted include many like late blight of potato, banana bunchy top, bacterial blight and streak disease of paddy etc that are not known to occur in India, have different races/ biotypes/strains not known to occur in India; are present on new host, are from a country from where they were never reported before or are an entirely new pest species hitherto unreported in science or are reported to be present widely in India. These interceptions, especially of pests not yet reported from India signify the importance of quarantine in preventing the introduction of destructive exotic pests. The need of the hour is to strengthen the agricultural biosecurity system of the country.

New generation fungicides

The process of fungicides discovery has undergone a noteworthy change over the years. After the era of broad spectrum multisite and site specific systemic fungicides, several novel action fungicides of different chemical classes have been developed in the past two decades. These are more eco- friendly and used at a much lower dose rates as compared to the earlier compounds. Most noted among these are the strobilurins (QoIs), derived from *Strobilurus tenacellus*, a wild mushroom. These are analogues to strobilurins –A and have broad range of disease control. Azoxystrobin was the first strobilurin introduced in 1996 and currently nine strobilurins compounds are available. Other important fungicides introduced for the control of diverse diseases in the last decade are Oxazolidinediones (faoxadone), Phenoxyquinolines, (quinoxyfen), Anilinopyrimidines (cyprodinil, pyrimethanil), Valinamides (iprovalicarb, benthiocarb) Mandelamides, (mandipropamid), phenylpyrroles (fenpicloil, fludioxonil), MBIs (carpropamid) Spiroketalamines (spiroxamine), Benzamides (mandipropamid), Cyanoimidazoles (cyazofamid), Thiocarbamates (ethaboxam) and Amdoximes (cyflufenamid), Phenoxyquinolines (quinoxyfen), Imidazoles (fanmidone), Benzamides (fluopicolide, zoxamide) representing different chemistries and mode of action (Thind, 2012). The majority of these have been developed for use against oomycete pathogens. The main advantages of new generation compounds are ecologically safer and are required to be used at much lower rates than their earlier counterparts. A few of the lately developed fungicides have been registered for use in India and a good number of novel action fungicides are currently under evaluation. Azoxystrobins and fenamidone have been registered for use against grape downy mildew and potato blight. Prominent among those being tested against different diseases are mandipropamid. iprovalicarb, benthivalicarb,, fluopicolide, famoxadone, cyazofamid, pyraclostrobin and kresoxim methyl (Kumar, 2013a).

These recently introduced fungicides to the market represent major advances in technology, potency against target diseases, selectivity, safety and rate reduction. However, they tend to have single site modes of action which makes them potentially affected by target site resistance (Leadbeater, 2012). Thus it is very important to proactively design and implement resistance management strategies and recommendations for new fungicide classes, as well as maintaining existing products. Swift development of resistance to strobilurins is now well documented and these are now categorized under high risk fungicides. Their use has to be regulated and FRAC guidelines adopted so as to sustain their efficacy levels (Kumar and Gupta, 2012).

Spurious pesticides

One of the evils faced by poor farmers of India is the spurious pesticides. It is estimated that spurious pesticides account for about Rs. 1000-1200 Cr. of sales. This results in a net loss to the farmers of crops worth about Rs 6,000 crore (Kumar, 2013a and b). The producers of spurious products manage to escape from the clutches of law, often because of the local authorities either turning a blind eye or being a partner in the crime. Because of this collusion between the criminals and the law enforcing agencies, genuine manufacturing companies have to seek the help of private agencies, to identify and nab the culprits and seek legal course to punish the offenders. This is a very difficult and challenging task, as the local officials from the agriculture and police departments have to be involved to make the entire operation fool proof and to ensure that the offenders do not escape. The manufacturers of spurious pesticides most often target popular and expensive brands from MNCs or leading Indian companies which have better acceptance amongst the farmers. While some of the spurious pesticides contain the active ingredients, mentioned on the label but at a much less percentage than declared on the label, some do not contain any active ingredients. It could be talcum/chalk powder or simply a solvent or kerosene. There are two types of spurious pesticides- those who have 9(4) registration and manufacturing licenses. For them producing products is relatively easier to escape the attention of law makers. The second types are those hard core criminals, often with strong political and official support. If at all, they are caught, they shift their operations to another location in the neighboring state. It is impossible to get them nabbed and punished.

Farmers not only lose their investment on pesticides, but because of spurious fungicides do not control the diseases, the farmers lose their crops also. Here again, the Agricultural department should play their role effectively, by being vigilant, nab the culprits and punish the producers and sellers of spurious pesticides so that this menace is totally eliminated to save farmers.

Adoption / Implementation of IDM

IDM, is multidisciplinary approach seems promising to manage diseases effectively by inclusive amalgamation of cultural, physical, biological and chemical strategies. However, the boon in the knowledge centric array of IDM ironically proves a bane on reaching the knowledge – poor and resource deficient farmers at the implementation stage. Socio economic and educational status of farmers have had a lot of bearing on the adoption/ success of the IDM programme. More worrisome are environmental insensitivities of a majority of farmers, either due to lack of knowledge or desperate economic situations, mainly the rural indebtedness. Unprofessional and unstructured farm mechanism of small land holding makes the socio economic situation of most of the Indian farmers unenviable. Under such situations, the expectation that knowledge intensive IDM should succeed appears far fetched. Under such compelling situations, most of the IDM implementation efforts in the country have been rather sporadic in terms of temporal and spatial. As per current estimates only 5% of the cropped area in the country is under IDM (Mayee, 2006).

Role of plant clinics

Plant health clinics counsel farmers on sick plants the way a health clinic advises humans on their ailments. Diagnosis of the problem is often made on the spot with a prescription given to the farmer. Plant clinics give underprivileged farmers national and international diagnostic expertise. They have enabled scientists to reach more farmers in a timely low –cost way.Preliminary results have shown that the plant health clinics increase incomes and crop harvests and minimize pesticides abuse. They also lay the foundation for plant health systems. Plant clinics help farmers avoid the futile costs of self-medication, dosing fields with the erroneous pesticides and make sure they use the right one instead or none at all if that works best (Paul, 2012).

Human resource development

The science of plant pathology has a key role in escalating the crop productivity through management of disease. Therefore, disease diagnosis as well as management requires the services of trained plant pathologist who are well acquainted both with basic concept as well as allied fields that is, molecular biology and plant breeding. Of late, new course curricula for PG students have been framed keeping in view the need of the hour. The essential key courses are mycology, plant bacteriology, plant virology, molecular aspects of plant pathogenesis etc. There is dire need to have specialists in these areas. The course on molecular diagnostics, molecular basis of host pathogen interaction, population biology of pathogens using molecular tools and genetic engineering for disease resistance have been added to the course curricula in universities but may not have been much impact since most of the agricultural universities, there is scarcity of scientists to teach basic fundamental courses as well as advance courses. In most of the agricultural universities, mycology and basic bacteriology have taken a back seat. Similarly, knowledge of biotechnological technique is the need of the hour to develop disease resistance genotype and bulk of the teachers do not have adequate exposure to this field. There must be meticulous trainings to teachers at entry level. Most teachers and student of the same university without out side exposure and unaware of many national and international perspectives of agriculture. It would be in the interest of the improvement of agricultural teaching if out of the three degrees, two are from different universities. International exposure of teachers is also indispensable and adequate opportunities to participate in international trainings, professional conferences and other events should be provided. In agricultural universities, many seats in master and doctorate degree are lying vacant due to non availability of students. Steps need to be taken up in promoting agriculture education from the level high school, organizing education fair, encouraging high school student's trip to university and colleges and other means to create general awareness.

Number of fellowships at degree level should be augmented. These steps will help in bridging gap between demand and supply of quality graduates. Further to draw students towards masters and doctoral level education, adequate and large number of fellowships must be available as to lessen the students and parents from financial load. Specialized diploma in plant protection can cater to the need of the farmers at block level. Farmers can also be saved from the clutches of greedy business men in pesticide sale through trained persons. These steps if taken up in right earnest will go in long way in generating the human resource for quality teaching as well as mitigating the sufferings of the farmers.

Perspective

Requirement of disease management in food crops pressed plant pathologist to focus attention on practical plant pathological aspects. It led to lay more emphasis on field oriented research amalgamation of efforts for development of disease resistant varieties. Since 1990s, molecular plant pathology has received attention. Notably, molecular markers have been developed for identification and diagnosis of a number of plant pathogens.

It requires to be strengthened further to garner the benefit of technological advancements. It can be highly useful in understanding the disease of unknown etiology. Molecular plant pathology should be integral part of course curricula for master and doctoral teaching in SAUs. There is a very limited information on impact of climate change on plant diseases. Some focused studies in relation to climate changes needs to be taken up in India. Most effective disease surveillance and expert systems for farmer's advisory are required to be developed.

Forecasting systems for potato late blight, apple scab, powdery mildew of mango, beer and rice blast are now available. But for practical utility in growers' field there is still a lacuna. In this connection, training programme for the plant pathologists, extension persons as well as for the grower is necessary.

Other specific areas of research like disease management using RNA interferences or Post transcriptional gene silencing, nanotechnology and integration of various technologies should draw attention. Plant pathologist is definitely expected to play a grater role in the twenty first century because there is need to meet the international commitment of an open trade under the WTO. There is need to emphasize research on trans boundary movement of plant pathogens since plant pathogens are not only disease causing agents now but also entities of trade significance. National standards of for survey, surveillance and pest free areas (PRA) need to be developed. Hence plant pathologist have a special role for developing PRA, improved and effective quarantine measures developed of national standards is very much required to enhance export of Indian commodities. It needs competent human resources with special trainings. Indian economy is based on agriculture and plant pathology has a special role to meet new challenges for sustainability and advancements of the Indian agriculture.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Anandalakshmi R (2013). Application of RNAi for engineering disease tolerance in crops with special reference to horticultural crops. J. Mycol. Plant Pathol. 43(1):111.

Bambawale OM, Saradana HR, Arora S (2008). Expanding dimensions of plant protections as per current needs. Crop Care 34(2):15-21.

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007). Control of coleopteran insects pests through RNA interference. Nature Biotechnol. 25:1322-1326. http://dx.doi.org/10.1038/nbt1359

Botstein B, Ehite RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragement length polymorphism. Am. J. Hum. Genet. 32:314-331. PMid:6247908 PMCid:PMC1686077

Chahal SS (2012). Indian Agriculture: Challenges and Opportunities in post Borlaug era. Souvenir, 3rd Global. Conference on Plant. Pathol. Food Sec. pp. 48-55.

Chakarbarti A, Ganapathi TR, Mukherjee PK, Bapat VA (2003). MSI-99, a magainin analogue, imparts enhanced disease resistance in transgenic tobacco and banana. Planta 216:587-596.

Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme – linked immunosorbent assay (ELASA) for the detection of plant viruses. J. Gen. Virol. 34:475-483. http://dx.doi.org/10.1099/0022-1317-34-3-475

Ganguly AK, Dutta TK (2012) Plant parasitic nematodes: An emerging problem under changing climate and agricultural practices. J. Mycol. Plant Pathol. 43(1):541.

Gopalakrishnan S, Sharma RK, Rajkumar KA, Joseph M, Singh MP, Singh AK, Bhat KV, Singh NK, Mohapatra T (2008). Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breed. 127:131-139.http://dx.doi.org/10.1111/j.1439-0523.2007.01458.x

Guleria S, Aggarwal R, Thind TS, Sharma TR (2007). Morphological and pathological variability in rice isolates of Rhizoctonia solani and molecular analysis of their genetic variability. J. Phytopathol. 155:654-661. http://dx.doi.org/10.1111/j.1439-0434.2007.01291.x

Jalali BL (2008) Molecular plant pathology: Where do we stand. J. Mycol. Plant Pathol. 38(3):419-429.

Kohler G, Milstein C (1975). Continuous culture of fused cells secreting antibody of predefined specificity. Nature pp. 495-497. http://dx.doi.org/10.1038/256495a0

Krishnaraj PU (2013). Post transcriptional gene silencing: A tool to develop virus disease resistance J. Mycol. Plant Pathol. 43(1):133.

Kumar A (2013f). Forewarning models for *Alternaria* blight in mustards (*Brassica juncea*) crops Ind. J. Agric. Sci. 81:116-119.

Kumar S (2013a). Role of fungicides in food and crop health security for better tomorrow. Res. Rev. J. Agric. Sci. Tech. 2(1):1-11.

Kumar S (2013e). Plant disease management under changing climatic scenario. J. Mycol Plant Pathol. 42(2):149-154.

Kumar A, Kumar V, Bhattacharya BK, Singh Niranjan, Chattopadhyay A (2013). Integrated disease management: Need for climate resilient technologies. J. Mycol Plant Pathol. 43(1):28-36.

Kumar J, Saxena SC (2009) Proceedings of the 21st training on Recent Advances in Plant Disease Management, GBPUA&T Pantnagar pp. 1-3.

Kumar S (2013b). Selection of suitable medium and organic substrate for maximum growth and sporulation of *Trichoderma viride* Proceedings of IX World Soybean. Res. Conf. Durban South Africa, pp. 2-3.

Kumar S (2013c). Trichoderma; A biological weapon for managing plant diseases and promoting sustainability. Int. J. Agric. Sci. Vet. Med. 1(3):1-16.

Kumar S, Gupta O (2012). Expanding dimensions of plant pathology. JNKVV Res. J. 46 (3):286-293.

Kumar S, Upadhyay JP, Rani A (2009). Evaluation of Trichoderma species against *Fusarium udum* Butler causing wilt of Pigeon pea. J. Bio. Cont. 23(3):329-332.

Kumar S (2013d). Molecular diagnostics in plant pathogens: Recent advances. J. Mycol Plant Pathol. 43(1):135.

Leadbeater A (2012). The role of FRAC in resistant management. J. Mycol Plant Pathol. 42(1):25.

Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnol. 25:1307-1313.http://dx.doi.org/10.1038/nbt1352

Mayee CD (2006). Plant pathology in growth of Indian Agriculture. J. Mycol Plant Pathol. 36(3):355-359.http://dx.doi.org/10.1111/j.1365-3059.2006.01447.x

Mukhopadhyay AN (2012). Trichoderma for plant disease management- A gift of god to humankind. J. Mycol Plant Pathol. 42(1):23.

Paul.YS (2012) Role of Plant clinics in global food security. Souvenir, 3rd Glo. Con. Plant Pathol Food Security, Udaipur, Rajasthan, P. 34.

Prabha K, Baranwal VK (2009). Emerging viral menace in plants: An overview. Indian Farming pp. 51-56.

Prashar M (2012). Tackling threat of Ug99 or wheat rust variants. J. Mycol. Plant Pathol. 42(1):28.

Powell W, Machray GC, Provan J (1996.) Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1:215-222. http://dx.doi.org/10.1016/1360-1385(96)86898-1 http://dx.doi.org/10.1016/S1360-1385(96)86898-0

Singh HB (2012). Commercialization of biocontrol agents. J. Mycol Plant Pathol. 42(1):25.

Singh HP, Malhotra SK (2010). Research and development in vegetables- issues and strategies. Indian Horticulture. pp. 3-10.

Singh R, Singha AP, (2004). Comparative efficacy of local bioagents, commercial bioformulations and fungicide for the management of sheat blight of rice, under glass house condition. Ind. Phytopath. 57:494-496.

Sinha P, Banik S (2009). Plant disease forecasting and monitoring: An imperative in precision Agriculture. Indian Farm. 59(8):46-50.

Sharma YP (2012). Wheat stem rust Ug 99- A threat to food security. Proceedings of the 21st training on Recent Advances in Plant Disease Management, Pantnagar, pp. 171-173.

Somasekhar N, Praseas JS, Ganguly AK (2012). Impact of climate change on soil nematodes- implications for sustainable agriculture. Ind. J. Nemat. 40:125-134.

Sundaram RM, Manne R, Vishnupriya R, Biradar SK, Laha GS, Reddy GA, Rani NS, Sharma NP, Sonti RV (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, and elite indica rice variety. Euphytica. 160:411-422. http://dx.doi.org/10.1007/s10681-007-9564-6

Thind TS (2012). New generation fungicides for managing plant diseases: advantages and limitation. J. Mycol Plant Pathol. 42(1):26.

Vishunavat K (2009). Advancement of seed health testing techniques for better disease management. Proceedings of the 21st training on Recent Advances in Plant Disease Management, Pantnagar. pp. 87-91.

Vos P, Hoggers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zebean M (1995). AFLP-A new technique for DNA fingerprinting. Nucl. Acids Res 23:4407-4414. http://dx.doi.org/10.1093/nar/23.21.4407 PMid:7501463 PMCid:PMC307397

Waugh MM, Kim DH, Ferrin DM and Stanghellini ME. (2003) Reproductive potential of Monosporascus cannonballus. Plant Dis. 87:45-50. http://dx.doi.org/10.1094/PDIS.2003.87.1.45

Wani SH, Sanghera GS, Singh NB (2013). Biotechnology and plant disease control-Role of RNA interference. Amer. J. Plant Sci.1:55-68.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1991). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535. http://dx.doi.org/10.1093/nar/18.22.6531