

Harnessing the strength of biotechnology to meet challenges of plant disease management

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Abstract

Management of plant diseases is a continuous challenge faced by plant pathologists worldwide. Meeting the challenges posed by a wide array of plant pathogens, timely and efficiently is a must to mitigate agricultural losses, both tangible and intangible. Knowledge on biotechnology can be used to initiate more focused and safer management measures. It allows the understanding of plant pathosystems at molecular level and facilitates the control of plant diseases through options such as the use of indigenous microbial biocontrol agents. By doing so, environmental, health, economic and social issues associated with the existing management measures that are greatly dependent on synthetic chemicals can be minimized. Out of the many avenues in plant biotechnology, I focus my presentation on the use of biological control, molecular diagnostics, genome analysis of host-pathogens-biological control agents to develop a more precise and safer management strategy in plant disease control.

Harnessing the wealth of the plant microbiome (i.e. phyllosphere, rhizosphere and endosphere) as potential biological control agents of plant pathogens and inducers of host plant resistance is an environmentally-benign disease management approach. Accurate identification of plant pathogens by molecular diagnostics and tagging their genomic variations to pathogenicity are important in designing precise management practices. With the rapidly-developing technologies related to integrative 'omics' (genomics, transcriptomics, and proteomics), a large amount of information, gathered from genome-wide surveys and expression analyses of pathogen resistant host plants is available on public databases. Such information is useful to develop resistant varieties, a durable way of combating plant pathogenic infections. The publicly available information can be exploited to express or repress genes and thereby targeted to interfere with host-pathogen compatibility. This is a 'greener' plant protection approach, as the regulation of gene expression can be achieved by treating crops with non-pesticidal compounds. Our experiences in utilizing the above novel approaches in relation to the management of rice sheath blight and anthracnose of banana using indigenous plant microbial antagonists will be described. Moreover, the potential of managing banana anthracnose through exploration of resistance-governing genes with a special emphasis on defense related mechanisms in a banana cultivar will also be discussed.

Introduction

By the year 2050, the world human population has been estimated to reach nine billion, which means an additional one third of mouths need to be fed. A population boom is occurring mainly in the developing countries, with the fastest growth rate reported in Sub-Saharan Africa and the slowest rate in East Asia. To keep up with the increasing world's food production needs and per capita food consumption, the food production needs to be increased by 70% by year 2050. Parallel to the increasing trend of population growth, scarcity in

the extent of available arable land, and water are major issues faced by today's agriculture. Additionally, each year 10-16% of the global harvest of agricultural commodities is lost due to plant diseases and additional crop losses are experienced at the postharvest stage. Climate change is an emerging threat worldwide, and it further accelerates the development of some plant diseases, increasing the risk of crop losses due to plant diseases.

Globally, crop health is continuously challenged by the emerging, re-emerging and endemic plant

pathogens. Across the globe, the use of synthetic chemicals is the main control strategy against plant diseases. However, economic, health and environmental impacts associated with the misuse, overuse and/or abuse of the chemical pesticides is well documented. Despite spending millions of dollars for the management of diseases each year, crops experience heavy losses, creating alarms on the upcoming threat of failing to reach food production goals. Therefore, development of environmentally-benign alternative approaches for the management of plant diseases is indispensable for a sustainable agricultural production. Agricultural biotechnology offers several options to tackle these pressing issues while minimizing the usage of synthetic chemicals. Novel and advanced biotechnological techniques targeting molecular-level genetics, makes precise advancements compared to the existing traditional plant disease management alternatives.

Exploring the strength of the wealth of plant microbiome

One of the most promising alternative options of managing a plant disease is the use of biological control. It is mainly based on the principles of natural antagonistic interactions among microorganisms. Plant microbiome dwelling in phyllosphere, rhizosphere and endosphere and/or on phylloplane and rhizoplane could be explored as potential candidates for the control of plant pathogens. Both externally and internally, plants house microbial communities in greater numbers. The phyllosphere, the external area of the aerial plant tissues, is an enormous microbial habitat. Global phyllosphere has been estimated to reach an area of 6.4×10^6 km and it is heavily colonized by microorganisms. Microorganisms belong to all three domains, out of which bacteria are the predominant, occupying a density of 10^8 and 10^9 cells per cm. In the rhizosphere, the root-surrounding soil attracts microorganisms as a result of root exudates. In general, rhizosphere and root endosphere contain bacterial densities of 10^8 - 10^9 cfu/g and 10^8 - 10^9 /g, respectively. The microbial inhabitants of phyllosphere, rhizosphere, endosphere, phylloplane and rhizoplane present in plenty, play a significant role in the improvement of growth, productivity and protection of host plants. For example, Actinomycetes is a well-known biological control agent against plant pathogens. Its mode of action owes to the inherent

ability to produce antibacterial, antifungal, antiviral, nematicidal and insecticidal properties.

To explore potential indigenous microbial antagonists, epiphytic microorganisms on the phyllosphere of traditional and high-yielding rice varieties were isolated from different agro-ecological zones of Sri Lanka and screened for their *in vitro* and *in vivo* antagonism against the pathogen of rice sheath blight, *Rhizoctonia solani*. Of a total of 196 bacterial and 91 fungal isolates, 12 bacterial and two fungal isolates which showed more than 50% growth inhibition of *R. solani* were tested for their *in vivo* antagonism. An effective formulated biopesticide for controlling sheath blight in rice using three microbial antagonists (*Bacillus megaterium*, *Bacillus subtilis* and *Aspergillus niger*) was developed from the above isolations from the rice sheath. Application of talc based formulations of individual antagonists and mixtures of the three antagonists as spray treatments or soil applications were effective in reducing the incidence and severity of rice sheath blight. A mixture of the three biological control agents, when sprayed on rice sheath, reduced the disease incidence by up to 45% 27 days after inoculation of the sheath blight pathogen and increased rice yield. We have reported a potential biological control agent of *Colletotrichum musae*, the pathogen of banana anthracnose. The biological control agent has been identified as *Burkholderia spinosa* and has been isolated from a fruit peel of an anthracnose resistant cultivar of banana.

Majority of microorganisms associated with plants are unculturable. Therefore, a holistic view on the density and diversity of the plant microbiome cannot be figured out by the conventional methods used for isolation, identification and quantification. With the advent of novel biotechnological tools which are culture-independent, it has been possible to get a complete picture on both culturable and unculturable microorganisms that live in different niches. Metagenomics based on next-generation sequencing (NGS) is one of the most updated techniques available to scientists who assess microbial communities. It has paved the way to disclose characteristics of unculturable microorganisms and obtain a complete community signature of microorganisms living in different niche environments. Culture-independent approaches such as NGS have shown that population statistics derived from culture-

dependent approaches has underestimated the microbial populations by an alarming 95%.

In addition, molecular biological techniques, such as proteomics and transcriptomics, provide information at molecular level regarding the overall responses of microbial antagonists. For example, inside information related to desirable genes in promising biological control agents, their modes of antagonism, and the antagonistic factors produced by these agents can be understood based on these 'omics' approaches. In depth knowledge on these aspects will be helpful to fine-tune the capabilities of the biological control agents to the requirements of the plant protectionists.

Catching the culprits

Accurate identification of the causal agents is of paramount importance when it comes to effective plant disease management. Conventional methods have some limitations in accurately identifying plant disease causing agents, particularly those that are unculturable and those that cause latent infections. Morphology based identification methods are heavily influenced by environmental factors/growth conditions. Hence, effective recommendations cannot be made for management of pathogens solely based on them. Identification of causal agents, targeting their unique attributes at the molecular level (i.e. DNA, RNA, protein) provides accurate, rapid and repeatable results. A variety of molecular methods based on polymerase chain reactions (e.g. PCR, qPCR, RT-PCR, multiplex PCR, and nested PCR) and nucleic acid hybridization techniques (e.g. RFLPs, dot blots, Northern blots) have been used by plant protectionists for accurate investigation of plant pathogens. Protein based identification methods (i.e. based on antigen-antibody reactions) are also available to identify disease causing agents. The above molecular methods save time and eliminate the need for taxonomic expertise and hence, can be practiced by farmers or extension workers at field level for rapid diagnosis and quick decision making, when produced as pre-designed diagnostic kits. In addition, for accurate diagnosis of diseases and correct identification of the pathogen, molecular based techniques are helpful. Further, they could reveal genetic variations within a pathogen population, which may explain their virulence (degree of disease causing ability), sensitivity to fungicides and other problematic characteristics that create groups who does not

respond favourably to recommended management measures.

Disclosing the hidden talents

Mining the details of host plants, pathogens and beneficial microorganisms useful for plant protection at molecular level will broaden the horizons and will facilitate a precise and strategic plant disease management program. Such information can be used to develop effective and environmentally-safer disease management measures through, biological control, breeding for resistance and induction of host plant resistance. Molecular techniques, such as genomics, transcriptomics and proteomics, provide insight of overall interactive responses among plants, pathogens and biological control agents, in a given pathosystem. With respect to pathogens, genome and transcriptome analysis disclose the presence of virulence genes and reveals details about the expression of such genes and further, allow the screening of virulence suppressive factors. In addition, genomics and transcriptomics of host plants provide useful information on genes responsible for delivering resistance and defense responses against invading pathogens.

With due credit to the ever expanding era of 'omics', today the wealth of genome information is being exploited to seek solutions for current day disease management issues. PhytoPath, an integrative genomics resource of plant pathogens, contains records of complete genome sequences of 135 plant pathogens. Cumulative total of plant pathogen genes in the public domains has reached a five-fold increase compared with the known human pathogenic genes recorded in year 2010. Complete genome sequence information of biocontrol agents of plant pathogens such as *Pseudomonas fluorescens* LBUM 223, *Pseudomonas chlororaphis* UFB2 and *Pantoea vagans* C9-1 are available in public databases. Transcriptomics and proteomic analyses have been used, to identify up- and/or down-regulating genes and their gene products in situations where plants are infected with different types of pathogens such as *Ustilago scitaminea* in sugarcane (You-Xiong et al., 2011) and *Sclerotinia* in *Brassica napus* (Wei et al., 2015) and *Colletotrichum musae* infection in banana. The expression profiles of genes in banana cultivar (Seenikesel) due to infection of *C. musae* was analyzed by differential hybridization of a genome-wide cDNA library and functions of differentially-regulated expressed sequence tags

(ESTs) were deduced. Among the up-regulated ESTs, 51%, 16% and 11% were related to defence response-, stress response- and energy and metabolism-related genes, respectively. Moreover, 22% and 11% of down-regulated genes respectively account for defense- and stress response-related functions and 11% for protein synthesis, folding and stabilization. In addition, similarly-regulated gene transcripts were identified and 30%, 30% and 20% of them were respectively related to defense, energy and metabolism and protein synthesis folding and stabilization functions.

Waking up the sleeping worriers

Exposing the plants to non-pathogenic bacteria and fungi can induce the host plant resistance against a broad spectrum of pathogenic microorganisms infecting below and above-ground plant parts. This kind of resistance, is not considered as genetically controlled resistance, but is an induced systemic resistance (ISR). Through ISR, plants are primed to react rapidly and strongly to pathogen attacks. *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp. and *Trichoderma* spp. have been well-documented as promising ISR inducers. As a response to ISR, plant tissues get enriched with defense-related enzymes and are reinforced with structural barriers. Due to induced formation of such defense mechanisms, plants get protection from incoming pathogen infections. Potential of inducing the expression of defense related genes to reduce anthracnose development due to infection of *C. musae* was explored through exogenous application of jasmonic acid and a bacterial antagonist (*B. spinosa*) (Aruna Kumara and De Costa, unpublished).

Future of 'greener agriculture'

Growing concerns of consumers on environment and health hazards due to synthetic chemical inputs used in agriculture has increased the demand for cleaner and safer food commodities. Global market for biopesticides is predicted to grow at a 16% compound annual growth rate from 2014 to 2020. Finer details related to beneficial microflora are being disclosed by novel biotechnological approaches. The diversity of desirable attributes possessed by plant microbiome make them favourable candidates to be integrated into management strategies targeting a greener and safer agriculture. In addition, the availability of full and partial genome sequences of pathogens and

resistant host plants will be a valuable resource to tackle host-pathogen interactions and design tailor-made management measures to suit the needs of the future.

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