# Interactions among rice-*Xanthomonas-Rhizoctonia* and biostimulants

Design of a framework to test the effect of a multiple species control of *Xanthomonas* and *Rhizoctonia* in rice

Andre van der Wurff, Marta Streminska and Anne Elings

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**Referaat**

Het Indonesische Ministerie van Landbouw heeft de ambitie om de duurzame productie van rijst te stimuleren om aan de toenemende vraag te voldoen. De 'resource use efficiency' kan worden verbeterd als productiederving als gevolg van ziekten wordt aangepakt. Kennis van de rol van biodiversiteit op de stabiliteit van het systeem moet worden vertaald in landbouwkundige handelingen. Soorten met overlappende functies kunnen vormgeven aan biologische beheersmaatregelen om een weerbaar systeem tegen pathogenen te creëren. Aangezien soorten elkaar ook kunnen tegenwerken, moet de aanpak ook rekening houden met de diverse effecten van verschillende boven- en ondergrondse pathogenen. Zowel bovengrondse als ondergrondse biologische gewasbescherming kunnen profiteren van een aanpak die is gebaseerd op meerdere soorten. Plaag-predator interacties zijn vaak onderdeel van gemeenschappen van meerzijdig interacterende pathogenen en natuurlijke vijanden. Het ontwerpen van geïntegreerde gewasbescherming vereist begrip van alle interacties tussen soorten, zowel plagen als natuurlijke vijanden. Het doel van het onderzoek is om te bepalen welke momenteel beschikbare biologische bestrijders compatibel of zelfs synergistisch zijn, om een weerbaar en duurzaam systeem van gewasbescherming tegen *Xanthomonas* en *Rhizoctonia* in rijst in Indonesië te bouwen.

**Abstract**

The Indonesian Ministry of Agriculture has the ambition to boast the sustainable production of rice in order to meet the increasing demand. Resource use efficiency can be improved if production loss owing to plant pathogens is resolved. Knowledge on the role of biodiversity on system stability must be translated agricultural practices. Species with overlap in function may constitute biological control measures to create a resilient system against pathogens. As species may counteract, the approach needs to take into account the diverse effects caused by different pathogens, both above-ground and below-ground. Above-ground as well as below-ground biological control may benefit from a multiple species approach. Pest-enemy interactions are often embedded in rich communities of multiple interacting pathogens and natural enemies. Designing IPM for multiple pests requires understanding of all interactions among species, both pests and natural enemies. The goal of the research is to investigate which currently available biological control agents are compatible or even act synergistically in order to build a resilient and sustainable crop protection system against *Xanthomonas* and *Rhizoctonia* in rice in Indonesia.

**Address**

**Wageningen UR Greenhouse Horticulture**

Violierenweg 1, 2665 MV Bleiswijk
P.O. Box 20, 2665 ZG Bleiswijk
The Netherlands
+31 (0) 317 - 48 56 06
+31 (0) 10 - 522 51 93
glastuinbouw@wur.nl
www.glastuinbouwur.nl/glastuinbouw

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# Summary

The Indonesian Ministry of Agriculture has the ambition to boast the sustainable production of rice in order to meet the increasing demand. Resource use efficiency can be improved if production loss owing to plant pathogens is resolved. Knowledge on the role of biodiversity on system stability must be translated to agricultural practices. Species with overlap in function may constitute biological control measures to create a resilient system against pathogens. As species may counteract, the approach needs to take into account the diverse effects caused by different pathogens, both above-ground and below-ground. Above-ground as well as below-ground biological control may benefit from a multiple species approach. Pest-enemy interactions are often embedded in rich communities of multiple interacting pathogens and natural enemies. Designing IPM for multiple pests requires understanding of all interactions among species, both pests and natural enemies.

The goal of the research is to investigate which currently available biological control agents are compatible or even act synergistically in order to build a resilient and sustainable crop protection system against *Xanthomonas* and *Rhizoctonia* in rice in Indonesia.

# 1     Introduction of framework

The Indonesian Ministry of Agriculture has the ambition to boast the sustainable production of rice in order to meet the increasing demand. Resource use efficiency can be improved if production loss owing to plant pathogens is resolved. Traditional crop protection uses chemicals that can be hazardous for the environment, life stock, humans and beneficials and even increase system vulnerability towards pests and diseases. A sustainable control of plant pathogens needs to be preventive and resilient against a wide array of pests and diseases. Therefore, we propose to design a multi-species system approach including currently available above and below-ground biological control agents. In order to investigate the system, a hypothetical framework needs to be designed to overview interactions and to decide for tools to monitor.

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## 1.1     Problem definition

Indonesia is a rapidly developing country with a relatively young and fast growing population of almost 250 million, making it the most populous country in South East Asia. Although the country's average economic growth has exceeded 6% per annum during the past five years, over 100 million people still live of less than $2 a day. Rice is by far the dominant staple crop in Indonesia, as it is in many other Asian countries, particularly for the poorer segments of society. Next to importance for consumption, millions of Indonesians are engaged in rice production, rice processing and other post-harvest activities related to rice. Smallholder farmers account for around 90% of Indonesia's rice production, each holding an average land area of less than 0.8 hectares. Although Indonesia is the third-largest country regarding global rice production, it is still a net rice importer as demand is increasing and production is levelling. According to the FAO, 2013 rice production in Indonesia is projected at around 36.55 million tons (milled basis) against a consumption of around 39.5 million tons.

The Indonesian Ministry of Agriculture has the ambition to boast rice production in order to meet the increasing demand and trying not too much dependent on imported rice. The national strategy is to increase rice production (National Rice Production Increase P2BN) to target an annual production increase of around 6% to reach a national rice surplus of 10 million tons by 2014. This ambition is in line with the goals of the Dutch Embassy in Jakarta, which underlines in its Multi Annual Strategic Plan 2012-2015 the importance of innovation in the agriculture sector. The Embassy promotes initiatives that are focused on applying Dutch knowledge & expertise in Indonesia that could add value to food security. Horticulture is among the 4 priority subsectors.

The average rice production level in Indonesia is 4.9 ton ha$^{-1}$ y$^{-1}$ which is low compared to 10.1 ton ha$^{-1}$ y$^{-1}$ in neighbouring Australia. Sustainability in terms of resource use efficiency can be improved if production limiting factors are resolved. Of particular interest are the economic loss caused by plant pathogens. Important pathogens are bacterial leaf blight, fungal rice blast disease as well as above-ground leaf folders, plant hoppers and below-ground pathogens such as root knot nematodes and the Fusarium fungus. Blast disease alone already affects about 12% of the total area of rice fields in Indonesia (Badan Pusat Statistika 2008). In the current situation local public and private stakeholders are unable to tackle these problems. The opportunity lies in the introduction of a crop protection system that strengthens natural defence mechanisms of the crop.

The consortium of private and public parties will integrate their practical and scientific knowledge in a 3-year applied research with the objective to generate new knowledge and insights and add value to rice crop protection products & technologies, tested for their applicability under local conditions in Indonesia. The results will answer local business needs and enable (small-scale) farmers to increase sustainable rice production contributing to food security.

Indonesian farmers and consumers will benefit directly from the results of this project as reduced crop diseases and the use of biological crop protection systems (instead of current chemical products) will improve the quality and quantity of production output, resulting in higher incomes and diminish negative environmental effects (benefiting both nature and men).

Entrepreneurs & employees along the extensive Indonesian rice value chain and other relevant stakeholders such as governments, industry associations, NGO's and local research organisations will indirectly benefit by higher incomes, more environmental sustainable production and less dependency of imported rice.

Traditional crop protection uses chemicals that can be hazardous for the environment, life stock, humans and beneficials. Chemicals can increase system vulnerability, as happened with insecticide application against rice hopper (Gallagher 1988). Integrated Pest Management (IPM) was introduced in Indonesia around 1988 (Kenmore 1997). Significant progress has been made during the last decade (Thorburn 2014), using crop rotation, resistant cultivars, and natural enemies or antagonists. However, this strategy remains under-developed and counteracted by broad-spectrum chemicals, mono-cultivation, use of new hybrids and fertilizer use (Kuyek *et al.* 2000). IPM needs to be preventive and resilient against a wide array of pests and diseases. Knowledge on the role of biodiversity on system stability (Van der Wurff *et al.* 2006) must be translated agricultural practices. Species with overlap in function (functional redundancy) may constitute biological control measures to create a resilient system against pathogens. As species may counteract, the approach needs to take into account the diverse effects caused by different pathogens, both above-ground and below-ground (Pieterse *et al.* 2009).

Below-ground species such as *Trichoderma harzianum* T22 and *Bacillus subtilis* act synergistically and are good means to control plant pathogens. *B. subtilis* produces antibiotics (e.g. He *et al.* 1994; Stabb *et al.* 1994, Huang *et al.* 1993). *Trichoderma* T22 enhances plant nutrient uptake, elicits plant defence responses, physically protects the plant and can neutralize various toxicants (Ruocco *et al.* 2009). Evaluations with mixed cultures have shown promising mechanisms and results (Dong *et al.* 2002; Rukayadi and Hwang, 2009; Surayadi *et al.* 2013; Zhang and Dong, 2004).

Above-ground biological control may also befit from a multiple species approach (Holt & Lawton, 1994; Letourneau *et al.* 2009; Messelink *et al.* 2012). Pest-enemy interactions are often embedded in rich communities of multiple interacting pests and natural enemies. Designing IPM for multiple pests requires understanding of all interactions among species, both pests and natural enemies. Generalist predators and multiple prey communities are important for pest control in rice (Settle *et al.* 1996). An important generalist predator of the rice brown plant hopper is mirid *Cyrtorhinus lividipennis* (Lou & Cheng, 2003). Understanding the role of prey diversity, alternative food, and banker plants needs is required to develop practical IPM solutions (Huang *et al.* 2011; Messelink *et al.* 2012).

Below-ground organisms induce systemic changes in the plant that influence for example water and nutrient uptake and above-ground biomass, and above-ground pests and diseases (Soler *et al.* 2012; Bezemer & Van Dam 2005). Also, below-above-ground interactions can influence non-pathogenic soil micro-organisms (Bezemer *et al.* 2005; Kostenko *et al.* 2012). Above-ground species can have various effects on the rice crop, such as leaf area reduction and reduced photosynthesis rate (Elings *et al.* 1999).

A new and sustainable IPM approach to control pathogens of rice would include multiple species and means that show functional redundancy, act synergistically, confer resistance against multiple pests and diseases, and take into account plant interactions, below- and above-ground. Fundamental and applied science reveals a wealth of information in order to design and test such system. A pragmatic first step is to use a model crop species rice and model pathogens that represent different life styles below, as well as above-ground.

## 1.2 Goal

Which currently available biological control agents are compatible or even act synergistically in order to build a resilient and sustainable crop protection system against *Xanthomonas* and *Rhizoctonia* in rice in Indonesia.

## 1.3     Why framework needed?

State–of-the-art scientific knowledge shows that biocontrol agents are subjected to a complex of interactions that determine their success in battling a plant pathogen. Interactions include those between agents and plants, agents and growth substrate (soil, substrates such as perlite, coco or potting soils), agents and local microbiology, and above-, below-ground pathogens and biocontrol agents. Therefore, it is of importance to make an inventory of possible interactions. This is referred to as a framework. The framework which includes all possible interactions is used to determine the tools that are necessary to monitor important aspects and validate the framework (system).

# 2    *Xanthomonas oryzae* pv. *oryzae*

## 2.1    Epidemiology

Bacterial blight of rice a vascular disease resulting in a systemic infection of susceptible rice plant. The disease manifests itself tannish-grey to white lesions along the veins. Symptoms are most often observed at the tillering stage (Gnanamanickam *et al.* 1999). The most developed manifestation of the disease is so called Kresek. Then the leaves of the entire plant turn pale yellow and wilt. Research shows that young plants (3 weeks old) are more susceptible. Moreover, environmental conditions such as humidity and high temperature (>28°C) are conducive to kresek symptoms development (Gnanamanickam *et al.* 1999).

Bacteria causing the disease are yellow, slime-producing, motile, gram negative rods with a polar flagellum. They belong to the species *Xanthomonas oryzae*. This species has two pathovars:
1.  *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice, also called *Xoo*.
2.  *Xanthomonas oryzae* pv. oryzicola causing bacterial leaf streak of rice. The second pathovar is of less significance when it comes to problems during rice cultivation.

In most cases the infection begins bacteria enter the plant via wounds or natural openings. Bacteria spread through plant tissues via xylem vessels. The pathogen moves vertically through the leaf through primary veins but also progresses laterally through commissural veins. The important fact to note is that bacteria switch between two lifestyles upon entering the plant. First the bacteria are mobile. They poses flagellum for the movement purposes. Upon entering the plant they become sessile (lose their ability to swim). It has been hypothesized that at this stage Xanthomas *oryzae* pv. *oryzae* may function in the form of a biofilm. Bacteria cells produce EPS (Extracellular polymeric substances) which fill up the xylem vessels (Nino-Liu *et al.* 2006).

During infection process bacteria produce a wide range of metabolites apart from EPS, which help in spread of the infection. These metabolites include: lipopolysaccharides, adhesins, cell wall degrading enzymes, and type III effectors (Xu *et al.* 2015).

Recently, experimental research has shown that many of pathogenic traits of this bacteria are being controlled by quorum sensing (QS). QS is an intracellular communication system which allows bacteria to sense changes in cell density and adjust gene expression accordingly. Quorum sensing is possible thanks to the production of extracellular signal molecules. In recent year there is increasing evidence that there are two quorum sensing circuits in Xanthomas *oryzae* pv. *oryzae* (Xu *et al.* 2015). The first circuit depends on production of Diffusible Signal Factor (DSF) molecule, cis-11-methyl-2-dodecenoic acid. DSF signalling playing a vital role in *Xoo* virulence, by regulating xylanase activity, swimming ability and EPS synthesis. The second circuit is most likely governed by production of Diffusible Factor (DF) characterized as 3-hydroxybenzoic acid (3-HBA), This system regulates production of yellow pigments (xanthomonadins). Xanthomonadins play essential roles in protecting bacteria from photobiological and peroxidative damage and are involved in epiphytic survival and systemic plant infection (Xu *et al.* 2015).

Recent scientific research has given a significant boost to the amount of information available on *Xanthomonas* plant host interaction, which may form a promising route for manipulation of plant susceptibility to bacterial diseases.

## 2.2    Cultural control measures of bacterial blight of rice

Control measures for bacterial leaf blight of rice include cultural practices, chemical and biological control, disease forecasting, and, most importantly, host genetic resistance, typically major gene resistance. Cultural practices are useful for the disease control, but their effects may vary significantly depending on the location and disease incidence records.

Common methods used at the nursery stage include seed disinfection, proper nursery drainage, and removal of diseased plants, weeds and debris.

In practice the method of field disinfection by burning the rice straw left from the previous season is also being used. At the paddy field stage, judicious fertilization and proper plant spacing are the most recommended cultural methods of control. It is also very important to take a good care of proper fertilisation schedule. The most important factor being avoiding the nitrogen excess, which may favour the disease in rapidly growing crop. Application of fertilizers rich in potassium and phosphorus, as well as application of agrochemicals at the maximum tilling to booting stages or after a typhoon or a severe flood are common practices.

Chemical control bacterial leaf consists of preventative application of Bordeaux mixture (hydrated lime and copper sulphate).

## 2.3      Biological control of bacterial leaf blight of rice

Biological control is perceived as an environmentally friendly and cost effective alternative to chemical control. There are studies on application of bacteriophages specific to *Xanthomonas oryzae* (Chae *et al.* 2014). Bacterial antagonists of *Xoo* also have received particular attention as biocontrol candidates, largely because of their rapid growth, easy handling and effective colonization of the rhizosphere. There are numerous scientific articles detailing isolation and characterisation of bacterial antagonistic to *Xoo* (Chithrashree *et al.* 2011; Velusamy *et al.* 2006). Many of these bacteria re native to the rice rhizosphere. The most widespread examples are rhizobacterial fluorescent pseudomonads, which are also found to be antagonistic to many other bacterial and fungal diseases (Shivalingaiah1 and Umesha, 2013; Velusamy *et al.* 2006). There are also reports of importance of endophytic bacteria in control of of *Xoo* (Chung *et al.* 2015). The ability of the antagonistic organism to survive as endophyte of rice influences significantly its ability to counteract *Xoo*. It is not surprising due to the fact that primary infection begins within the plant tissue and by applying endophytic microorganisms, the pathogen can be stopped from spreading within plant. There are also reports available about different isolates of *Bacillus* which have been successfully employed to inhibit *Xoo* in planta (Chung *et al.* 2015).

Biological control agents can be employed as seed treatment before sowing, root dips prior to transplanting and foliar sprays in the fields. In several studies application of biological control not only resulted in lower disease intensity (or occurrence), but also resulted in higher grain yields. This had been attribute to probable plant growth promotion by used microorganisms

## 2.4      How can rice plant protect itself from infection by *Xanthomonas oryzae* pv. *oryzae*?

Bacterial leaf blight is just one of over 70 diseases of rice, which are caused by bacterial, fungi or nematodes, Plants are confronted with potential attackers all the time. Plants are equipped with a diversity of self-protection systems. First of all they have constitutive defences, such as cell wall barriers. During the initial contact between plant and a pathogen, plants exert innate immunity. In case of bacterial leaf blight of rice it means that flagellin present in bacterium's flagellum is recognised by receptors located outside the plant cell. This leads to activation of defence signalling events.

The first pillar of the plant protection system against disease is disease resistance encoded in plant's own DNA. The most well know example of such resistance is protection of rice against *Xoo* by Xa21 gene. It is the first of the defence genes well documented in scientific literature. Xa21 encodes the receptor which recognises the 194-amino acid protein Ax21 from *Xoo*. Xa21 induces downstream defence mechanisms: expression of PR (pathogen related) genes and development of hypersensitive reaction (programmed cell death).

Xa21 is not the only one defence genes described. Major genes from various resistant donors have been identified and to date more than 25 loci conditioning resistance against bacterial leaf blight have been found. Some of these resistance genes or loci have been widely used in rice breeding programs, and some of them have been mapped to their chromosomal locations and are subject to cloning efforts.

Genes conferring resistance to the major classes of plant pathogens have been isolated from a variety of plant species and are termed 'R genes'. An overview of cloned R genes against *Xoo* in rice are given in White and Yang (2009).

Specific resistance genes are not the only possible way for a plant to protect itself. Plant hormones may play a very important role in plant-bacteria interactions, modulating plant immune responses and/or acting as antagonists of bacterial quorum sensing (QS) systems on the other.

One of the phytohormones is salicylic acid (SA). In dicotyledonous plants, SA is the main chemical regulator of the plant defence response called systemic acquired resistance (SAR). In short it means that when plant is being locally challenged by leaf pathogens (such as *Xanthomonas oryzae* pv. *oryzae*), signals are being send to distal leaves to induce the systemic response. This response in turn will protect plant from a range of subsequent attack by different pathogens. In dicotyledonous plants SAR is mostly effected against biotrophic pathogens. The precise role of SA in monocots, such as rice, is yet to be elucidated. Monocotyledonous plants, are characterised by high endogenous SA concentration in their tissues compared to dicotyledonous plants extensively studied to this date.
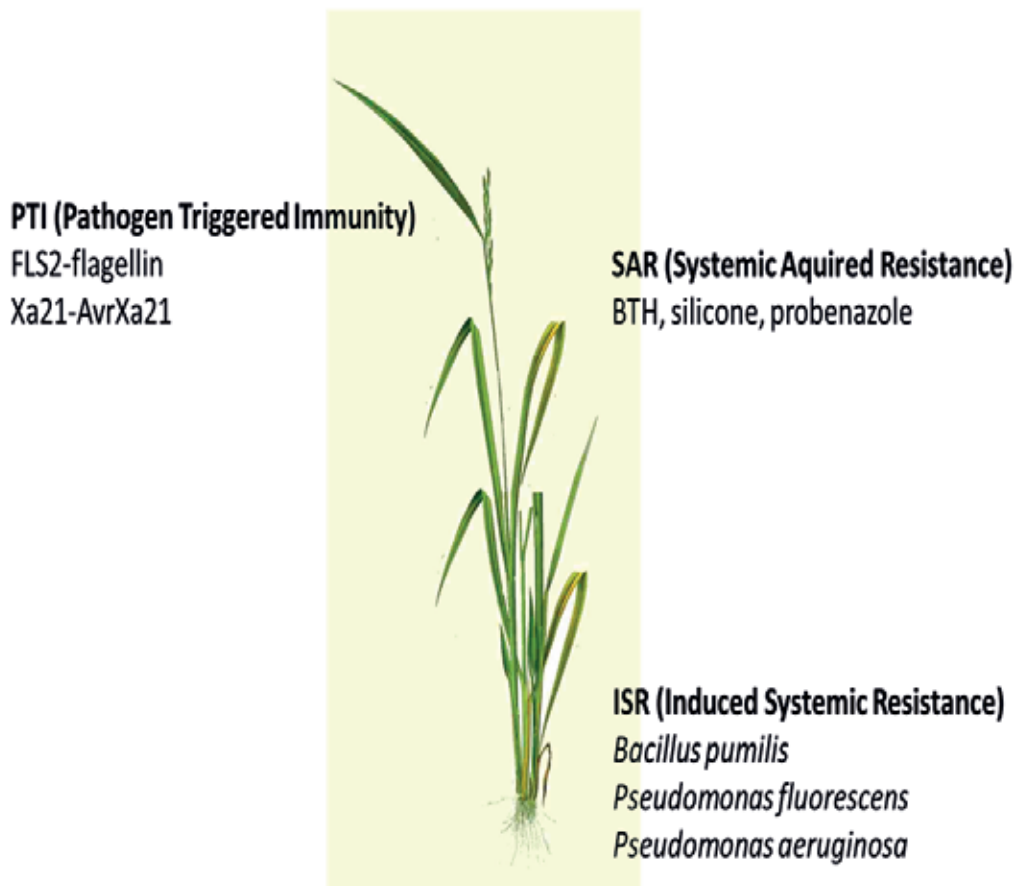


**PTI (Pathogen Triggered Immunity)**
FLS2-flagellin
Xa21-AvrXa21

**SAR (Systemic Aquired Resistance)**
BTH, silicone, probenazole

**ISR (Induced Systemic Resistance)**
*Bacillus pumilis*
*Pseudomonas fluorescens*
*Pseudomonas aeruginosa*

**Figure 1** *Overview of plant defence response and genes and species that are involved in rice.*

There are however some implications that salicylic acid (SA) has an important role to play also in SAR response in rice. Research on the subject has shown that salicylic acid is one of the key players in rice's machinery against attack by *Xanthomonas oryzae* pv. *oryzae*. For example, rice plants overexpressing genes, which production in the cells is governed by salicylic acid, are less susceptible to *Xanthomonas oryzae* pv. *oryzae*. Additionally rice plants, which were subjected to treatment with exogenous SA, where subsequently less susceptible to bacterial leaf blight.

Systemic acquired resistance (SAR) in rice can be also induced by a number of synthetic compounds, such as INA (2,6-dichloroisonicotinic acid), BTH (S-methyl benzo-1,2,3-thiadiazole-7-carbothioate), probenazole (3-allyloxy-1,2-benziso-thiazole-1,1-dioxide), BIT (1,2-benzisothiazole-1,1-dioxide) or NCI (N-cyanomethyl-2-chloroisonicotinamide;) or tiadinil (3'-chloro-4,4'-dimethyl-1,2,3-thiadiazole-5-carboxanilide). This is actually a very positive development. These compounds do switch on the rice's defence responses, but are not effective directly against pathogens themselves. It means that there is lower likelihood that the pathogens will eventually develop resistance to them, which is often the case with bactericides.

Rice plant can also benefit from colonisation of their roots by plant growth promoting rhizobacteria (PGPR) or endophytic bacteria and fungi. These beneficial organisms not only improve nutrient uptake by plants. They can directly inhibit plant pathogens by production of antibiotic substances or via quorum sensing quenching (in the case of bacterial pathogen *Xoo*).They are also able to indirectly reduce the plant disease incidence by induction of systemic resistance. This defence reaction is called Induced systemic resistance (ISR). ISR does not involve the accumulation of pathogenesis-related proteins or salicylic acid, but instead, relies on pathways regulated by jasmonate and ethylene.

Some of the rhizobacteria can also produce plant hormones themselves, such as *Pseudomonas aeruginosa* or *Bacillus amyloliquefaciens*. In rice, pyocyanin (antibiotic produced by Pseudomonas) is also shown to be a trigger of ISR. In dicotyledonous plants ISR is thought to be effective mostly against necrotrophic plant pathogens. In rice however this clear distinction is not possible. There are reports of PGPR that were able to induced the ISR response towards *Xoo* and other pathogens. Examples of such bacteria are: *Bacillus pumilis*, *Bacillus subtilis* GBO3, *B. pasteurii*, *B. cereus*, *B. mycoides*, and *B. sphaericus* and *Pseudomonas fluorescens* Pf1.

# 3    Rhizoctonia

## 3.1    Epidemiology

Rice sheath blight was first reported in Japan in 1910. The causal agents was unclear and was referred to as *Corticium sasakii*, *C. vagum*, *Sclerotium irregulare*, *Hypochnus sassaki*, *Pellaculare sassaki* and more recent *Thanatephorus cucumeris* (A.B. Frank) Donk (anamorph: *Rhizoctonia solani* Kuhn). Initial symptoms are usually lesions on lower sheets of leaves. As a lesion develops the centre becomes grey - white or light green with a purple-brown border. Favourable conditions are low sunlight and high humidity of over 95% and high temperature between 28 - 32$^o$C. Mycelium spreads through the plant and via other neighbouring plants into the canopy. There the increased sunlight dries the lesions and under these unfavourable conditions, sclerotia survival spores are formed.

Soil born sclerotia, and to a lesser extend mycelium in plant debris are generally considered as the main factor of transmittance. Sclerotia become buoyant after approx. 30 days and float on water where they accumulate with infested plant debris around submerged rice plants. Thus initial infection occurs near the waterline. Sclerotia can survive multiple years in soil.

Factors that contribute to the increasing importance of sheath blight include the increased acreage planted to susceptible long-grain varieties, the increase in the acreage of rice grown in rotation with soybeans, the increased use of broadcast seeding and the higher rates of nitrogen fertilizers used with the modern commercial rice varieties. Sheath blight also affects many grasses and weeds other than rice, causing similar symptoms and representing a source of new infestations.

## 3.2    Indirect interaction via the plant

Different forms of resistance in rice exist. A thick wax cuticle on leaves is known to inhibit infection. Japonica types of rice seem more resistant than Indica types. Young plants are more resistant than older plant. Also nitrogen fertilization influences outbreaks of diseased plants.

Breeding programs have so far been not promising. Species such as Fusarium and *Rhizoctonia* have a complicated lifestyle (Bouarab *et al.* 2009). Therefore, in rice, the defence response to pathogens is co-activation/ suppression of both SA- and JA-mediated defence signalling pathways (Zhao *et al.* 2008).

Table 1 List of genes involved in plant defense against *Rhizoctonia*

*List of genes are involved in Rhizoctonia and Xanthomonas plant defence, gene function and scientific reference. Adapted from Sayarai et al. 2014.*

| gene | role |
| --- | --- |
| Ubiquitin | |
| PR-3 | |
| PR-5 | |
| LOX | First enzyme in production of JA |
| PR-10 | |
| Defensin | |
| Thionin | |
| PAL | Catalyzes broad range phenylpropanoid, like lignin, pigments, flavonoids, phytoalexins. |
| POX | |
| NH1 | Increase resistance to *Xanthomonas* |

# 4    Multispecies and primers

## 4.1    *Trichoderma harzianum*

In agriculture, *Trichoderma* may improve plant growth and development, has biological control activity against other fungi and nematodes depending on the species. *Trichoderma* survives in environments with remnants of fungicide molecules such as Captan (Chaparro *et al.* 2011). *Trichoderma* has the capability of degrading xenobiotic compounds and can survive field concentrations of chemical fungicides. As such, they are suited for integrated management as species may not sensitive to chemical based crop protection agents.

## 4.2    *Bacillus spp.*

*Bacillus* species have been widely studied for their opportunity to control *Rhizoctonia* sheath blight because of a. their broad production of active compounds and b. endospore formation to allow a stabile product formulation. In addition they are suited for integrated management as species may not sensitive to chemical based crop protection agents (Peng *et al.* 2012). *Bacillus* species are known to display a variety of control mechanisms, such as induced plant systemic resistance, production of antimicrobial compounds and competition for nutrients and colonisation sites with pathogens (Kloepper *et al.* 2004).

### 4.2.1    *B. subtilis*

*Bacillus subtilis* NJ-18 is known to inhibit many pathogens of rice including *Xanthomonas* and *Rhizoctonia*. It was studied in efficacy trials in rice in combination with the fungicide jinggangmycin (a glucosaminidase glycoside antibiotic produced by Streptomyces var. jinggangensis which resembles the action of validamycin). The result was reported as a synergism, when *Rhizoctonia* was suppressed to a greater extend when both means were applied than when either of them were applied alone (Peng *et al.* 2012).

### 4.2.2    *B. megaterium*

*Bacillus megaterium* is a rhizobacterium the plant growth promoting properties of which have been widely documented in scientific literature. It is hypnotised that this bacterium alters the phytohormonal balance of the plant by producing cytokinins.

### 4.2.3    *B. mycoides*

*Bacillus mycoides* is reported to play a significant role in Induced systemic resistance to foliar diseases in sugar beets.

## 4.3    Paenibacillus azotofixans

Strains belonging to the species *Paenibacillus azotofixans* were shown to be efficient nitrogen fixers prevalent in the rhizospheres of maize, sorghum, sugarcane, wheat, banana, and forage grasses. Some strains are able to produce antimicrobial substances and solubilize organic phosphates (Seldin *et al.* 1998).

## 4.4     Glomus spp.

### 4.4.1     G. mosseae

This mycorrhizal fungus was studied in relation to rice seedlings growth. Inoculated rice seedlings grew significant better compared to non-mycorrhizal controlled seedlings, and had increased height (34%), shoot biomass (122%), root biomass (590%) at maximum tillering stage, and grain yield (9.7%) at harvesting stage.

### 4.4.2     G. fasciculatum

This fungus was indicated a possible inoculant for rice seedlings to partially mitigate the effect of drought on upland rice. In screens of vesicular-arbuscular mycorrhizal for wetland rice it was shown to be improve plant height, tiller number, total biomass, panicle number, grain weight and plant P and Zn concentrations. Inoculation with G. fasciculatum resulted in 59% increase in grain yield.

### 4.4.3     G. intraradices

In greenhouse experiments with rice this fungi was shown to influence the plant – herbivore interactions. Specifically, oviposition of rice water weevil (*Lissorhoptrus oryzophilus*) was influenced. When rice was inoculated with AMF fungi, oviposition (i.e., the numbers of eggs laid by weevil females in leaf sheaths) was enhanced. This is an indirect indication of the increased nutritional status of the rice leaves, as insect choose to lay eggs on leaves, where the offspring growth will be optimal.
This fungus was also shown to play a role in phosphorus solubilisation in flooded rice fields.

### 4.4.4     *G. aggregatum*

Combination of *G. aggregatum* and neem cake significantly increased plant growth characters viz., shoot and root length, plant biomass and leaf yield of tobacco followed by application of *G. aggregatum* plus peanut cake. Similarly, reduction in root-knot nematode population, egg mass production, root galling and root-knot index was recorded in *G. aggregatum* plus neem cake.

### 4.4.5     *G. etunicatum*

The *G. etunicatum*-colonized rice plants were more active in acquiring Zn from either added or native sources than non-colonized plants, and consequently the available-Zn content in soil was lower after the harvest of rice.

## Table 2

*List of Species in Multispecies product and proposed primers.*

| Species | Primers | F/R | Sequence | SA/JA | Reference |
|---|---|---|---|---|---|
| *Trichoderma harzianum* | ITS S | F | TACAACTCCCAAACCCAATGTGA | | Rubén López-Mondéjar *et al.* 2010 |
| | ITS R | R | CCGTTGTTGAAAGTTTTGATTCATTT | | Rubén López-Mondéjar *et al.* 2010 |
| *Bacillus subtilis* | | F | GCG GCG TGC CTA ATA CAT GC | | Lahlali *et al.* 2013 |
| | | R | CTC AGG TCG GCT ACG CAT CG | | Lahlali *et al.* 2013 |
| *Bacillus megaterium* | RAPD 1 | F | | | Reddy *et al.* 2010 |
| | RAPD 2 | R | | | Reddy *et al.* 2010 |
| | BmphaC015F | F | CGTGCAAGAGTGGGAAAAAT | | Nayak *et al.* 2013 |
| | BmphaC931R | R | TCGCAATATGATCACGGCTA | | Nayak *et al.* 2013 |
| *Bacillus mycoides* | BMSH-F | F | TTT TAA GAC TGC TCT AAC ACG TGT AAT | | Park *et al.* 2007 |
| | BMSH-R | R | TTC AAT AGC AAA ATC CCC ACC AAT | | Park *et al.* 2007 |
| *Paenibacillus azotofixans* | BAZOl | F | GAG TTG TGA TGG AGC | | Rosado *et al.* 1996 |
| | BAZO2 | R | AGG TTG AGC CCA TGGTT | | Rosado *et al.* 1996 |
| *Glomus mosseae* | Mos-F | F | GAAGTCAGTCATACCAACGGGAA | | |
| | Mos-R | R | CTCGCGAATCCGAAGGC | | |
| *Glomus fasciculatum* | | F | | | |
| | | R | | | |
| *Glomus intraradices* | VC-F | F | GAGACCATGATCAGAGGTCAGGT | | Alkan *et al.* 2006 |
| | VC-R | R | GGTCATTTAGAGGAAGTAAAAGTCGTAAC | | Alkan *et al.* 2006 |
| *Glomus aggregatum* | GA-F | F | GGTATATTTCAAAGAGTAAGGTTCG | | Kiers *et al.* 2011 |
| | GA-R | R | TGTCTCTACGCCTTAGTATGC | | Kiers *et al.* 2011 |
| *Glomus etunicatum* | ITS1Kpn | F | see article for sets | | Millner *et al.* 1998; Millner *et al.* 2001 |
| | ITS4Pst | R | see article for sets | | Millner *et al.* 1998; Millner *et al.* 2001 |

# 5 Yield loss due to *Xanthomonas* and *Rhizoctonia*

## 5.1 Crop growth and production

Loss of yield can be caused by different effects of the disease on the crop. Figure 2 summarizes the yield formation process. The amount of leaf area determines with the radiation level the amount of intercepted radiation. The amount of intercepted radiation and air $CO_2$ concentration are the major determinants of crop photosynthesis rate that, integrated over for example a day, determine the gross amount of assimilates. Temperature and vapour pressure deficit (jointly they determine relative air humidity) are of minor influence on the rate of photosynthesis. Various maintenance components have priority in the use of assimilates ($CH_2O$). The net amount of assimilates is partitioned over the plant organs, and the daily growth rates integrate over time to organ weight. Seed weight is ultimately of greatest importance. Leaf weight in combination with the specific leaf weight (the weight of a leaf per unit area) determines leaf area, which closes the circle.

Sufficient leaf area must be maintained to ensure high amounts of intercepted radiation, and the photosynthetic processes must not be hindered to ensure high rates of $CH_2O$ formation. Healthy tissue requires less maintenance than diseased tissue, and healthy transport bundles will enable good transport of assimilates to, and therefore growth of, the organs.
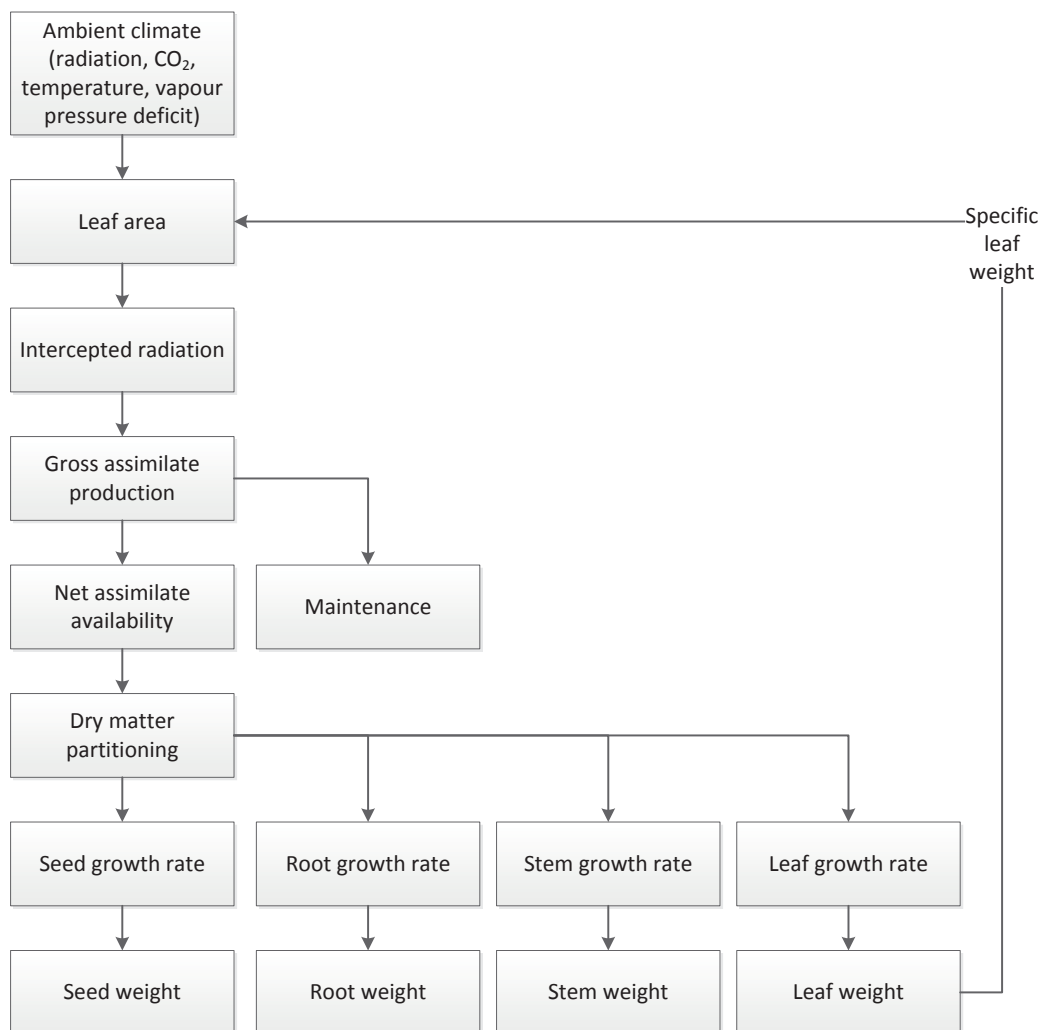


**Figure 2** *Crop growth analysis. The maintenance component has various components at various places, but is presented in a simplified manner.*

## 5.2       Mechanisms of yield loss

### 5.2.1       *Xanthomonas*

*Xanthomonas* lesion formation on a leaf leads reduction of green leaf area and therefore to reduced interception of solar radiation (Pinnschmidt *et al.* 1995). In addition, Elings *et al.* (1997, 1999) found a reduced photosynthesis rate in the green leaf tissue of an infected leaf. The presence of bacteria in the xylem vessels blocks water transport, affecting transpiration (Mew *et al.* 1993), which also plays a role in reduced photosynthesis rates.

### 5.2.2       *Rhizoctonia*

Sheath blight influences crop growth and production through leaf senescence and reduction of green leaf area and therefore the photosynthesis rate; phloem blockage and reduction of assimilate translocation; xylem blockage and reduced of water uptake by the roots and transpiration; and the plant's biochemistry that leads to increased respiration (Pinnschmidt *et al.* 1995; Willocquet *et al.* 2000). Savary *et al.* (1997) distinguished primary infection that results from the mobilisation of primary soil borne inoculum and occurs in the lower part of the canopy, and secondary infection that takes place in the upper part of the canopy and that results from contact between healthy and diseased tillers. Senescence, mortality and recovery rates of tillers result in number of healthy, fully productive tillers.

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# Annex 1 Pathogens of Rice

Table 1

*List of pathogens in rice and type of plant defence response (JA/SA).*

| Scientific name | Popular name |
| --- | --- |
| *Rhizoctonia solani* | Sheat blight |
| *Xanthomonas oryzae* pv. *oryzae* | Leaf blight |
| *Xanthomonas oryzae* pv. *oryzicola* | Bacterial leaf streak |
| *Magnaporthe oryzae* | Rice blast fungus |
| *Cochliobolus miyabeanus* | Brown spot |
| *Pseudomonas fuscovaginae* | Bacterial sheath brown rot |
| *Sarocladium oryzae* | Sheath rot |
| *Pyricularia oryzae* | Blast disease |
| *Meloidogyne* | Root knot nematode |
| *Sphaerulina oryzina* | Narrow brown spot |
| *Microdochium oryzae* | Scald |

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of nature to
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