Attack and defense in pathogen-plant interactions

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Wandoor, Andaman islands

How do plants stay healthy?

When disease does occur, what is the special attribute that a pathogen must have to cause disease?

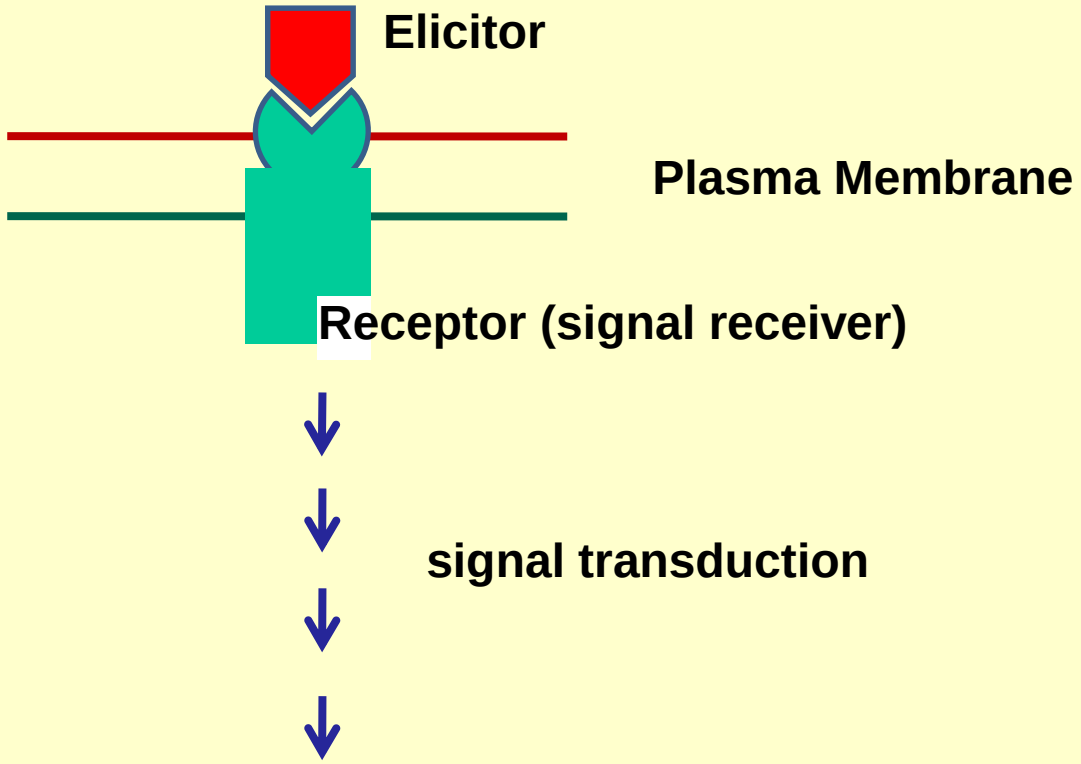
- **Plants have powerful inducible innate immune systems that protect them against the vast majority of potential pathogens**

  Pathogen associated molecular patterns (PAMPs); flagellin, lipopolysaccharide, etc

  Damage associated molecular patterns (DAMPs): cell wall degradation products

- **Pathogens can cause disease only because they can suppress these innate immune responses**

Simplified scheme for activation of innate immunity

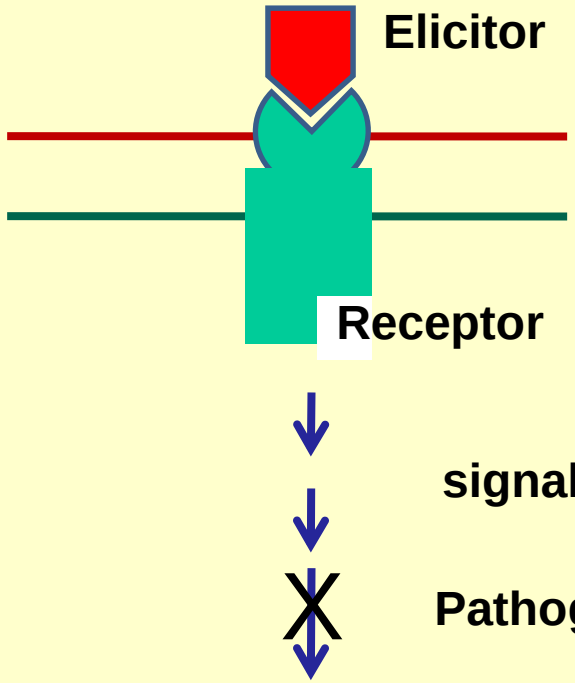


Activation of transcription factors (switches in nucleus)

↓

Defense response and resistance

Simplified scheme for inactivation of innate immunity



signal transduction

Pathogen inactivates signaling pathway

No activation of transcription factors (switches are off)

↓

No Defense response; susceptibility

Induction and suppression of innate immunity in plant-pathogen interactions

Rice

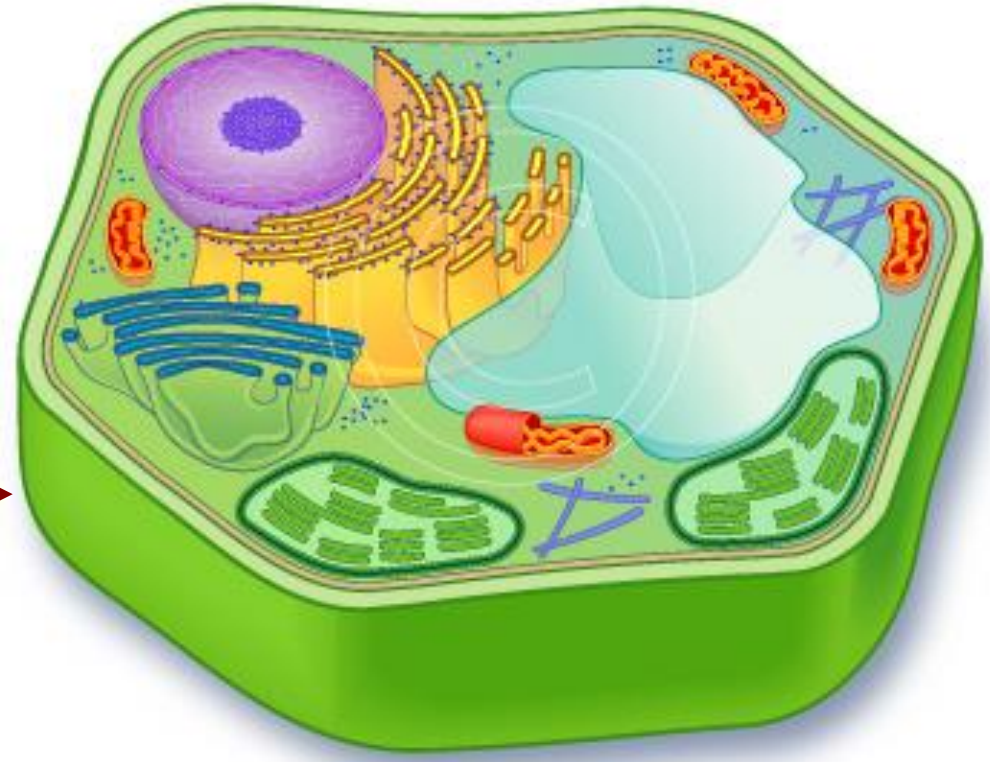
Xanthomonas oryzae pv. oryzae (Xoo)

Xoo causes Bacterial blight (BB), a serious rice disease

BB affected field of a rice variety called Samba Mahsuri in Nandhyal, Andhra Pradesh

The Plant cell wall is a formidable barrier for microbial pathogens

cell wall →



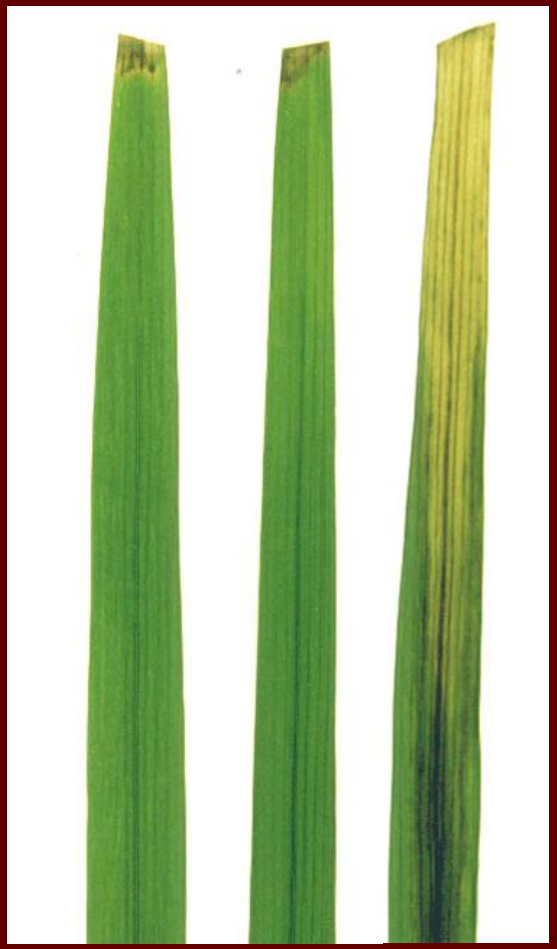
http://teamcarterlces.com/images/plantcell.jpg

- The Xoo bacterium uses a type 2 protein secretion system (T2S) to secrete enzymes such as celluases, xylanases, etc which degrade different components of the rice cell wall

- Bacterial mutants that are unable to produce either the secretion system or the secreted enzymes exhibit defects in virulence

- The capability to degrade rice cell walls is crucial for the ability of Xoo to cause disease on rice

Virulence deficient phenotype of Xoo mutant that is defective in secreting cell wall degrading enzymes



Control BXO 801 Wild type

Wild type is normal Xoo

BXO801 is T2S mutant

Control is mock inoculation

Rice

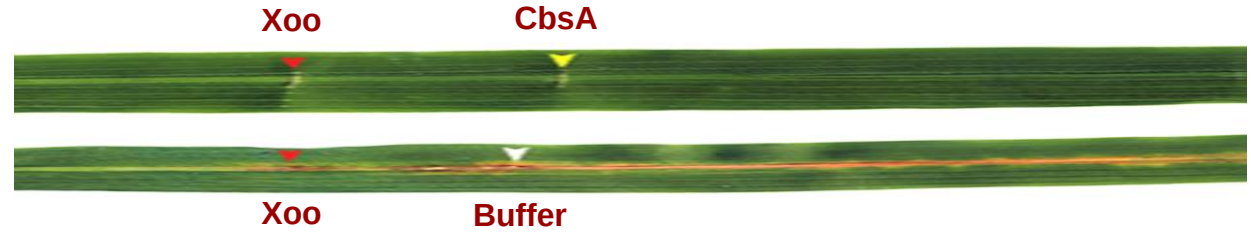
Xoo

Cell wall degrading enzymes

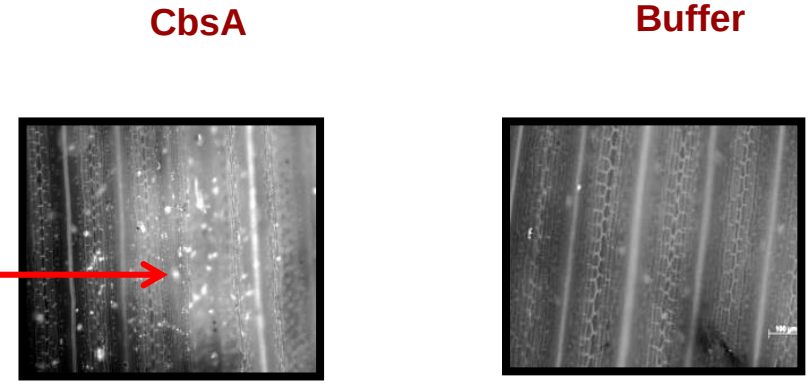
Defense responses

Innate immune responses of rice are induced by treatment with a cell wall degrading enzyme (CbsA; cellubiosidase)

Functional resistance against subsequent Xoo infection.



Callose deposits in rice leaves



Callose deposition is a defense response that strengthens the plant cell wall against infection

Rice

Xoo

Cell wall degrading enzymes

Defense responses

Suppression of
defense responses

Rice innate immune responses are suppressed by Xoo using the bacterial Type 3 protein secretion system (T3S).

The T3S secretes proteins directly into plant cells.

What are the T3S secreted proteins that suppress rice innate immune responses?

Identification of Xoo T3S effectors that suppress cell wall damage induced rice innate immune responses

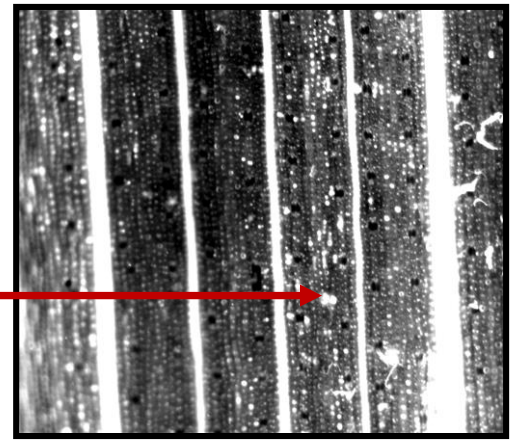
Dr. Seiji Tsuge in Japan has shown that Xoo secretes 16 different proteins into rice cells using the T3S

We have introduced each of these proteins into rice cells and assesed their individual abilities to suppress rice innate immune responses induced by cell wall damage

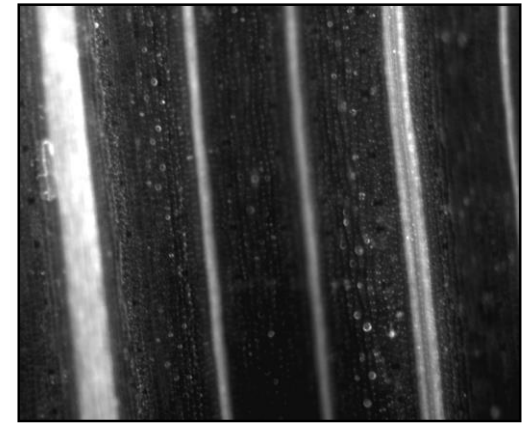
Four T3S secreted proteins are suppressors of cell wall damage induced innate immune responses. These are: Xanthomonas outer protein N (XopN), XopQ, XopX and XopZ

XopQ protein is a suppressor of cell wall degrading enzyme induced callose deposition in rice leaves

Callose deposits



Cell wall degrading enzyme



Cell wall degrading enzyme + XopQ

Xoo mutants that are defective in single, double or triple Xops are proficient at suppression of plant innate immunity

The *xopQ xopN xopX xopZ* quadruple mutant is defective in suppression of plant innate immunity

The *xopQ xopN xopX xopZ* quadruple mutant is itself an inducer of plant immune responses

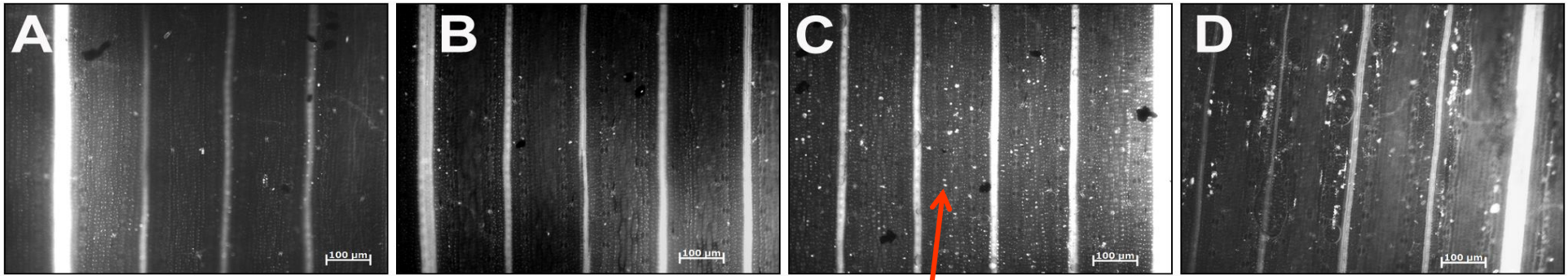
A *xopN xopQ xopX xopZ* quadruple mutant is an inducer of rice innate immune responses

Water

Wild type Xoo

xopN^{-} *xopZ*$^{-}$ *xopQ*$^{-}$ *xopX*$^{-}$

T3S^{-} mutant



callose

The XopN, XopQ, XopX and XopZ proteins function reduntantly in suppression of host innate immunity

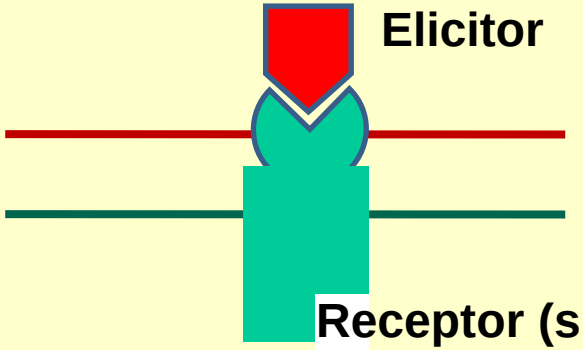
How do Xanthomonas Outer Proteins (Xops) suppress rice immune responses?

Rice has eight proteins called 14-3-3 proteins that function as key adaptors in signal transduction cascades

The XopQ, XopX and XopZ proteins interact with specific rice 14-3-3 proteins

The ability to interact with 14-3-3 proteins is crucial for the ability of Xops to suppress rice innate immunity

Simplified scheme for activation of cell wall damage induced innate immunity in rice



Plasma Membrane

Receptor (signal receiver) Identified

↓ 14-3-3

↓ 14-3-3

signal transduction

↓ 14-3-3

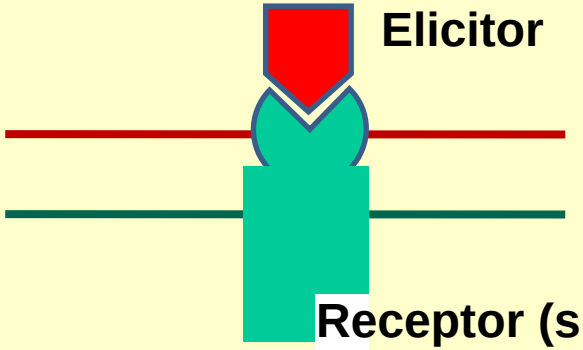
↓ 14-3-3

Activation of transcription factors (switches)

↓ one switch identified

Defense response and resistance

Simplified scheme for activation of innate immunity



14-3-3 + XopQ

14-3-3 + XopQ

14-3-3 + XopX

14-3-3 + XopZ

signal transduction
does not take place
On account of Xop
binding to 14-3-3s

No Activation of transcription factors (switch is OFF)

↓

Defense response and resistance

SUMMARY

XopN, XopQ, XopX and XopZ proteins of Xoo are involved in suppression of rice cell wall damage induced innate immunity

They appear to suppress innate immunity by interaction with distinct rice 14-3-3 proteins, each of which functions positively in induction of innate immunity

We are trying to determine the exact roles of 14-3-3 proteins in the rice innate immunity pathway

Xoo

virulence functions

innate immunity

Suppression of
innate immunity

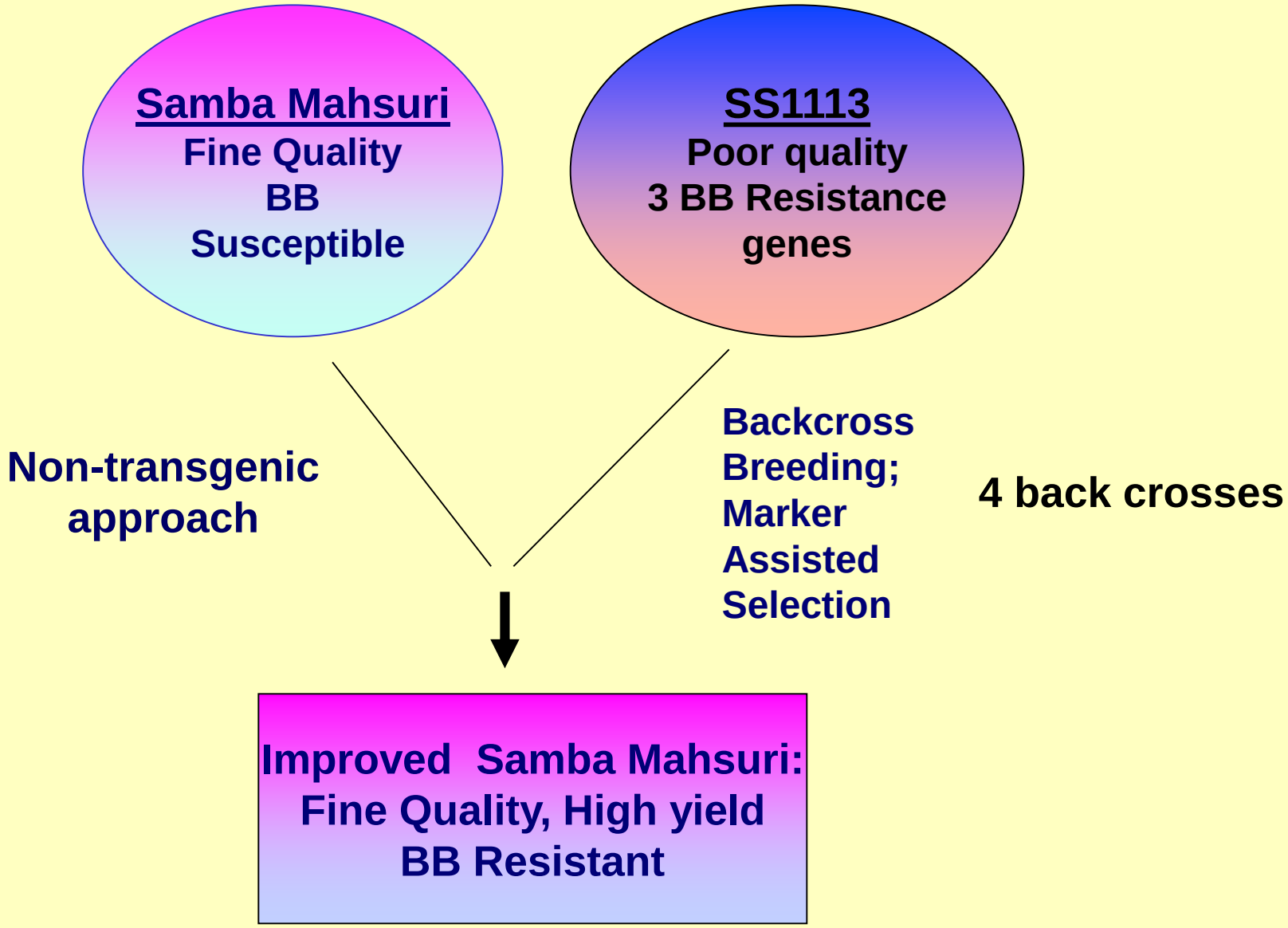
Resistance genes

rice

- **Genetic grouping of Indian strains of the BB pathogen was done using DNA fingerprinting methods and pathological testing**

- **Three rice resistance genes that are effective against Indian strains of the pathogen were identified**

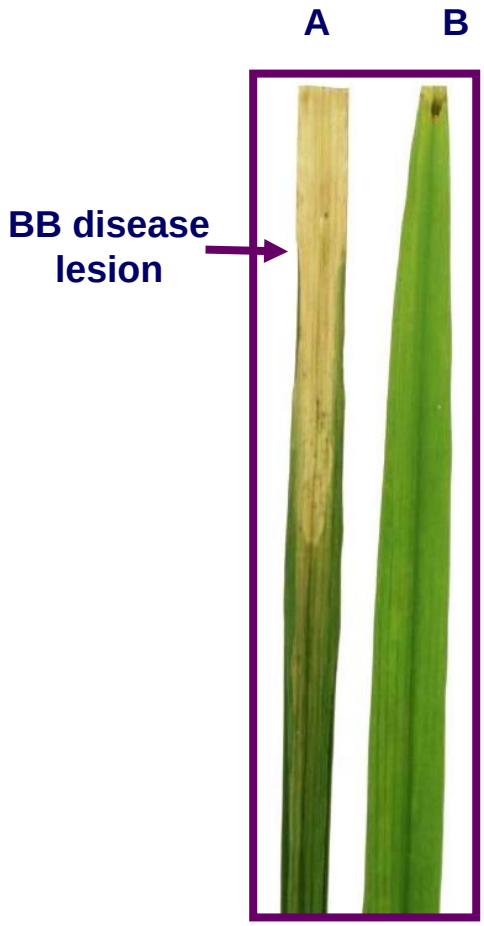
- Samba Mahsuri (also called BPT5204) is a leading rice variety in India.
- This variety was developed by scientists at the ANG Ranga Agricultural University, Andhra Pradesh
- It has exceptional quality and yield characteristics
- Samba Mahsuri is susceptible to several diseases including Bacterial Blight (BB)
- Our objective was to introduce genes for BB Resistance into Samba Mahsuri background without loss of it's unique quality and yield characteristics



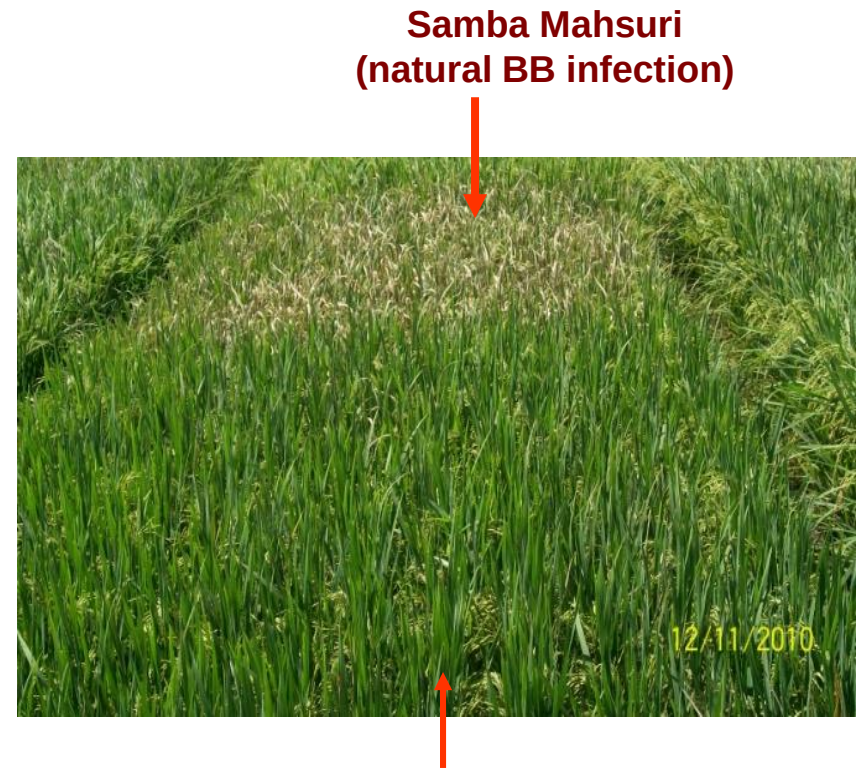
Collaboration between Indian Institute of Rice Research and CCMB

Improved Samba Mahsuri Rice Variety

Bacterial blight resistant derivative of Samba Mahsuri



A = Samba Mahsuri
B= Improved Samba Mahsuri



Research station in Gangavathi, Karnataka

Introduced into national field trials under All India Coordinated Rice Improvement Project of the Indian Council of Agricultural Research

After several years of testing, it was approved for commercial cultivation by Central Varietal Release Committee of ICAR

Released as a new variety by Ministry of Agriculture, Government of India through notification in the Gazette of India

Improved Samba Mahsuri is resistant to bacterial blight

Farmers fields near Nandhyal, Andhra Pradesh.
In collaboration with the Indian Institute of Rice Research, Hyderabad

EXTENSION

Seed distribution through CSIR-800 program

During the last five years, we have distributed 10 kg seed packets/farmer to 6000 farmers in Telangana, Andhra Pradesh, Tamil Nadu, Chhatisgarh, Karnataka and Uttar Pradesh to promote farmer to farmer spread of the variety.

IMPACT

Improved Samba Mahsuri has been cultivated in ~200,000 hectares of farmers' fields in Andhra Pradesh, Karnataka, Chhattisgarh, Tamil Nadu, Telangana, Bihar, etc.

Total production value at the level of farmers is ~Rs. 2000 crores (as per 2016 rates)

Trait specific value is ~Rs 400 crores

Licensed to Metahelix Life Sciences/Rallis India

Improved Samba Mahsuri has low glycaemic index and is diabetic friendly

CONCLUSION

Plants have powerful inducible innate immune responses

Plant pathogens can cause disease only because they can suppress these immune responses

In turn, plants have evolved resistance genes

Three genes for bacterial blight resistance have been introduced into a commercial important rice variety to develop a novel bacterial blight resistant variety using molecular breeding

Acknowledgements

Induction of rice innate immunity
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Mahesh Kumar

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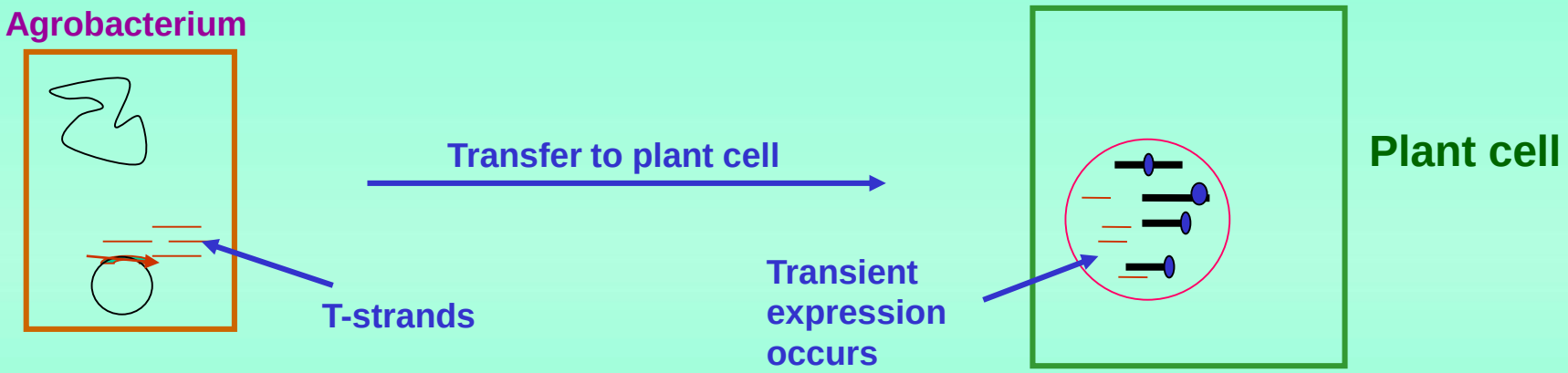
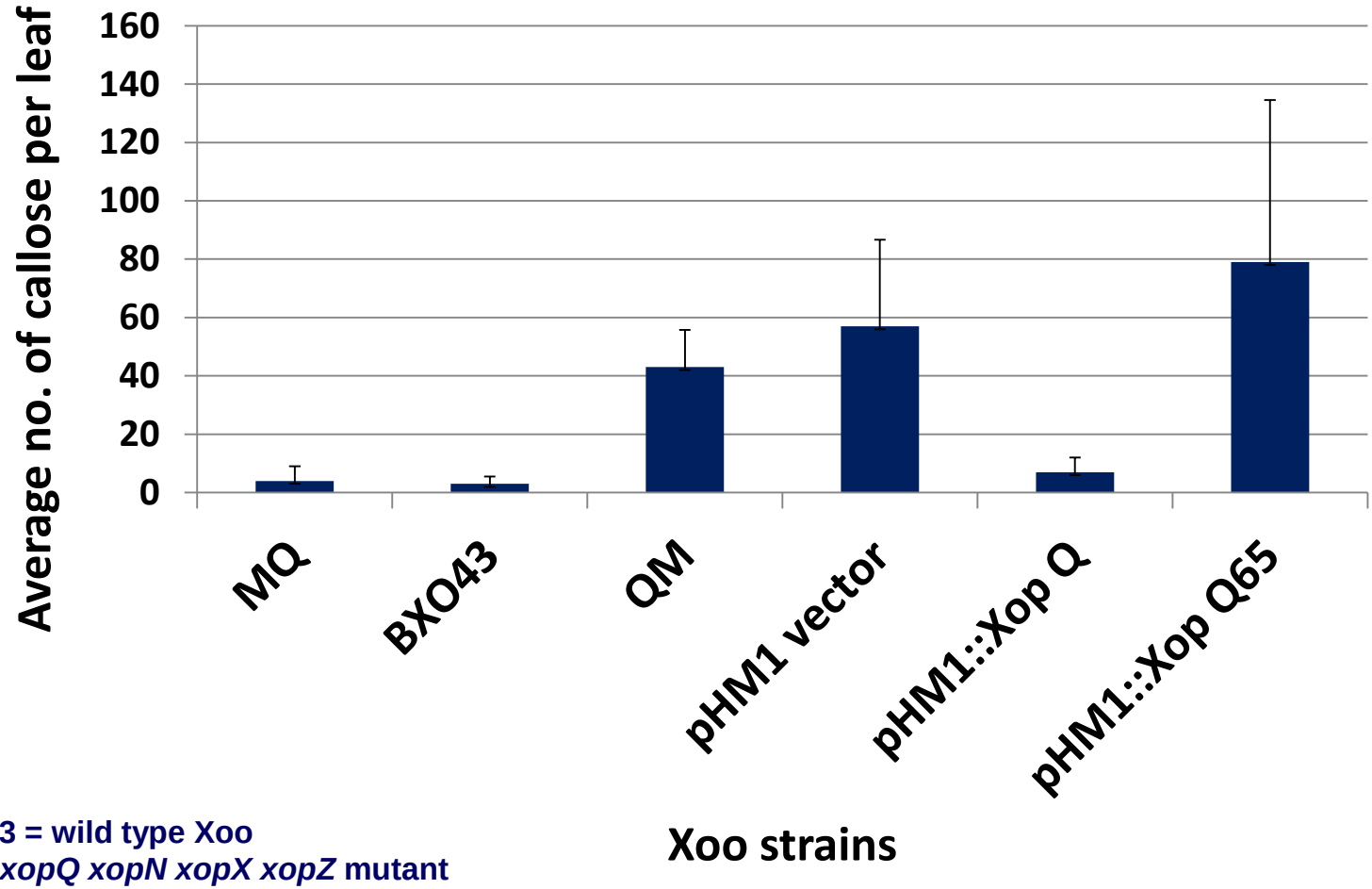
THANK YOU

Transient transfer of Xoo *xop*Q gene into rice cells



Xoo *xopQ* gene was cloned into a T-DNA vector

A mutation in the 14-3-3 binding motif affects the ability of XopQ to suppress rice innate immunity



BXO43 = wild type Xoo
QM = *xopQ xopN xopX xopZ* mutant
MQ = water