GM Crops developed conferring enhanced resistance to plant pathogens, special reference to Rice and Jute

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Plant- Pathogen Interaction

- **Non-host interaction** - the pathogen is incapable of replication - no infection, no disease symptom.

- **Compatible interaction** - the pathogen establishes an initial infection where it can multiply and spread systemically through the plant - causing disease symptom.
  
  Interaction between **virulent pathogen** & **susceptible plant**.

- **Incompatible interaction** – Invading pathogen is capable of establishing an initial infection in a region or near the site of entry, but **lacks the capacity to** systemically spread through the plant resulting **hypersensitivity**.
  
  Interaction between **avirulent pathogen** & a **resistant plant**.

Compatibility of host pathogen relation depends on both partners and is characterized by a highly efficient genetically determined specificity.

In the first of three *Cell* papers (Mackey et al., 2002, *Cell* 108:743), Dangl’s lab demonstrated that sequence-unrelated type III effectors, AvrB and AvrRpm1, target the same plant protein RIN4. Biochemical modification of RIN4 by these effectors is the signal through which the plant immune receptor RPM1 senses bacterial invasion.
Jonathan Jones investigates mechanisms of disease resistance in plants, and how plant pathogens overcome host resistance using defence-suppressing ‘effectors’.

The *Arabidopsis* (thale cress) RPS4/RRS1 NLR receptor pair recognises bacterial effectors, and mechanisms of *Arabidopsis* resistance to the oomycete pathogens that cause downy mildew and white rust (*Albugo*). *Arabidopsis* resists *Brassica*-infecting races of *Albugo* (so-called "non-host resistance"). The potato relative *Solanum americanum* resists all races of the late blight pathogen *Phytophthora infestans*.

R genes from wild relatives introduced via genetic modification and/or gene editing. He conducts field trials to evaluate the use of such R genes to confer late blight resistance in GM potato.
INDUCED DEFENCE

- Hypersensitive response (HR)
- Small RNAs
- Programmed cell death (PCD)
- Induced structural barriers
- Phytoalexins
- Pathogenesis related proteins (PR-proteins)
- Post transcriptional gene silencing (PTGS)
- Systemic acquired resistance (SAR)
- Antimicrobial peptides
The general features of plant AMPs are small molecular size, net positive charge, amphipathic properties, and rich in cysteine residues conferring a high thermostability.

Recent analyses suggest that plant genomes are rich in genes encoding cysteine-rich peptides resembling AMPs, which might account for up to 2-3% of the predicted genes, suggesting that plant possess a formidable defense arsenal (Silverstein et al. 2007).

**Major plant antimicrobial peptide families**

- Thionins
- Defensins
- Lipid transfer proteins
- Puroindolines
**Thionins**

- Thionins are a family of low molecular weight antimicrobial peptides (approximately 5kDa), with conserved cysteine residues.

- They are mainly positively charged at physiological pH due to the presence of basic amino acids like arginine and lysine.

- Thionins interact with the negatively charged phospholipid membrane leading to formation of ion pore channels in the cell membranes (Hughes et al. 2000) and causing membrane depolarization and cell death.

**Defensins**

- Defensins are 45-54 aa long disulphide cationic peptides omnipresent in both monocots and dicots.

- These acts against fungi seems by binding with membrane bound sphingolipids (Thevissen et al. 2003) and penetrate the fungal membrane leading to disruption and destabilization of membranes coupled with leakage of ions.

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Fig 1: Transgenic tomato plants expressing the Arabidopsis thionin (Thi2.1) exhibited enhanced resistance to *Ralstonia solanacearum* (Bacterial wilt). WT-wild type, RV-resistant variety, T1,T2,T3- Transgenic plants (Chan et al. 2005).

Fig 2: Transgenic tobacco plants expressing the TvD1 defensin exhibited enhanced resistance to *Rhizoctonia solani* (Target spot disease). WT-wild type, RV-resistant variety, T1,T13,T26- Transgenic plants (Vijayan et al. 2013).
Lipid transfer proteins

- Lipid transfer proteins are cationic peptides that derive their name from their ability to transfer lipid molecules between different membranes.

- They are involved in a wide variety of processes like biosynthesis of membranes, cutin synthesis, maintenance of the fatty acid pool, somatic embryogenesis and also a part of the defense response against phytopathogens.

- LTPs penetrates and forms pore in the fungal membranes leading to ion efflux and ultimately cellular death.

Puroindolines

- The puroindolines are small 13kDa proteins with five conserved disulfide bonds which are cationic at physiological pH and exclusively contain a tryptophan-rich domain.

- Puroindolines additionally have both antibacterial and antifungal action via interactions with the membranes.

- They were able to cause loss of membrane organization causing cellular lysis via pore formation.
Pathogenesis related protein (PR)

- Proteins coded by the host plant but induced in pathological or related stress situation
- These proteins are mostly low molecular weight, extracted at low pH, resistant to proteolysis and localized predominantly in the intracellular spaces of leaves.

Classes

- PR-1 - Unknown
- PR-2 - β-1,3-glucanase
- PR-3 - Chitinase
- PR-4 - Chitinase
- PR-5 - Thaumatin like protein
- PR-9 - Peroxidase
- PR-10 - Ribonuclease
Target gene I \( \rightarrow \) PR-15-Oxalate oxidase (Osoxo4) gene

- Oxalate oxidase (encoded by oxo gene) belongs to germin superfamily of protein.
- Protease and SDS-resistant apoplastic glycoprotein.
- Play a role in development, osmotic regulation, and defense response.

How it works?

Rhizoctonia solani

Necrotrophic pathogen \( \rightarrow \) OA (oxalic acid) \( \rightarrow \) Oxalate oxidase

\[ \text{Osoxo4 gene} \]

\[ \text{2CO}_2 \] \( \rightarrow \) Lipid peroxidation

\[ \text{H}_2\text{O}_2 \] \( \rightarrow \) Phytoalexins synthesis

Activation of PR genes
TRANSGENIC RICE WITH PR GENES FOR SHEATH BLIGHT RESISTANCE

*Rhizoctonia solani* Kuhn

- Widest in distribution
- Yield loss 3-50%
- Transgenics with ~40-70% protection

Deployment of pathogenesis related protein

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<td>Datta <em>et al</em> (2000)</td>
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Tissue-specific expression of *Arabidopsis NPR1* gene in rice for sheath blight resistance without compromising phenotypic cost

Kutubuddin Ali Molla\textsuperscript{a,b}, Subhasis Karmakar\textsuperscript{a}, Palas Kumar Chanda\textsuperscript{a,c}, Sailendra Nath Sarkar\textsuperscript{a}, Swapan Kumar Datta\textsuperscript{a,d}, Karabi Datta\textsuperscript{a,\*}

Green tissue specific (Pd540) overexpression of *AtNPR1* gene in rice showed enhanced protection against sheath blight (53\% protection)
Overexpression of Rice oxalate oxidase 4 (OsOXO4) enhances resistance against Rhizoctonia solani (50% resistance)
Case studies: 3

Green tissue-specific co-expression of chitinase and oxalate oxidase 4 genes in rice for enhanced resistance against sheath blight

Subhasis Karmakar · Kutubuddin Ali Molla · Palas K. Chanda · Sailendra Nath Sarkar · Swapan K. Datta · Karabi Datta

Transgenic rice plants showing significant and enhanced resistance against sheath blight (63% resistance)
Case studies: 4

**Dual gene expression cassette is superior than single gene cassette for enhancing sheath blight tolerance in transgenic rice**

Subhasis Karmakar¹, Kutubuddin A. Molla¹-², Kaushik Das³, Sailendra Nath Sarkar³, Swapan K. Datta¹-³ & Karabi Datta¹

Whole plant bioassay of T₂ transgenic and wild type (WT) plants with sheath blight fungus, *R. solani* using two different green tissue specific promoter 66% resistance against sheath blight disease
Pathogen inoculation assay and morphological alternations of two sets of three representative plants [3 plants from WT (Wild type) and 3 progeny plants from homozygous transgenic 105-5 rice line].

Representative 2-DE gel images of leaf proteins of WT and transgenic rice plants in three different conditions.

Functional classification of identified proteins and relative protein abundance. (a) Pie chart showing functional categorization of identified proteins based on biological process. (b) Network of different high and low abundant proteins downstream to AtNPR1 found from 2D-proteomics data analysis after 24 and 48 hpi.
M. phaseolina infects more than 500 different plant species of more than 100 plant families.

Jute fibre losses 35–40% in India and 30% in Bangladesh by this fungus.
X. oryzae enters rice via hydathode water pores
TRANSGENIC RICE WITH Xa 21
RESISTANT TO BACTERIAL BLIGHT

- BB\(^r\)  IR24A  IR24B
- BB\(^s\)  O. longistaminata
- IRBR21  (IRRI variety)
- (wild rice)
- conventional breeding (by crossing)
- genetic transformation

- IR72 (BB\(^s\) Xa-21)
- IR72 (IRRI)
- IR72 (transformed)
- BB\(^r\)  BB\(^r\)  BB\(^r\)
- Available
- for field testing
- improved variety (homozygous)

Genetic locus:
- Chr. 11
- RG 03
- RZ537
- Transgene insertion: 3.8 kb

Genetic markers:
- T\(_3\)
- IR24
- IRBB21
- IR72-C
- IR72-T\(_0\)
TAL effectors are virulence/avirulence effectors

- In susceptible plants, some TAL activate the expression of susceptibility genes to support bacterial virulence (divert nutritional resources)
  - Activate transcription of OsSWEET genes (encode sugar transporters)

- In resistant plants, some TAL “interact” with resistance genes products, leading to the induction of specific defense reactions.

Chen et al., 2012; Moscou, Bogdanove, 2009; Boch et al., 2009; Streubel et al., 2013, etc. JL
TALEN-Gene Editing: Knowledge of how TAL effectors regulate gene activation now used for genome editing in plants, animals, etc.

**TALENS enable efficient plant genome engineering**

Plant Physiology November, 2012 (Epub ahead of print)

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF.

High-efficiency TALEN-based gene editing produces disease-resistant rice

Nature Biotechnology 2012 30: 390-392

TALEN(ted) rice is resistant to disease! JL
Genomes to gene editing in rice

3K Rice Genome

EBE-SWEET Promoter variation in 3K

Genome Editing

Wang et al. 2018; Nature

Zaka et al. 2018; PLoS One

Oliva et al. 2019; Nature Biotech
How does the phytobiome (the microbial community associated with rice) influence the biology of BB and rice?

- **The Phytobiome**: microbes in and associated with the plant
  - Seeks to understand how the phytobiome influences the plant or is influenced by the plant
  - An opportunity to partner?

**Outcomes**
- **New strategies** for: reduced plant disease, improved yields, reduced environmental degradation, safer food, healthier soils, improved response to weather extremes, managing microbial communities, improved human health, reduced resistance to antimicrobials, reduced non-renewable inputs
- Increased and more robust human, genetic and technological infrastructure

**Impact**
- Increase in safe and healthy food, feed, and fiber

www.apsnet.org/ppb
Climate change and preparedness

The Planetary Panopticon

Technology will soon allow the world to be mapped in near-real time and at high resolution. Declan Butler investigates the potential for operational monitoring of the planet.
Present level of CO₂ is 380 ppm; it would be 550 levels by 2050.

Expts. have been conducted with soybean at 550 ppm shows malfunctioning of SA and ethylene resulting more susceptible to diseases.
Salicylic Acid Suppresses Jasmonic Acid Signaling

Dieuwertje Van der Does, Antonio Leon-Reyes, Annemart Koornneef, Marcel C. Van Verk, Nicole Rodenburg, Laurens Pauwels, Alain Goossens, Ana P. Körbes, Johan Memelink, Tita Ritsema, Saskia C.M. Van Wees, and Comé M.J. Pieterse

Rice Bacterial Blight: Disease pressure increases with increasing temperature

- High BB disease in hot season (particularly if accompanied by high humidity); little disease in cool season (Yamada et al., 1979; Horino et al., 1982; Ou, 1985; Ezuka & Kaku, 2000; Balidion et al., unpubl.)

- Longer BB lesions at high than low temperature regimes in controlled environments (Webb et al., 2010; Corral et al., unpubl.; Balidion et al., unpubl.)

What is the influence of high temperatures on resistance to BB disease?
Conclusion

Co-evolution of H/P
Host specific recognition
Defense signal transduction
Exchange of molecular signal through
Signalling cascade and modified gene expr.
Genome editing, transgenesis, MAS, gene pyramiding
Sustainable, Durable Plant protec, Conserve.
Ecosystem

Thank you