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Prevention of Bacterial Blight in Oryza sativa (rice) caused by Xanthomonas oryzae pv. oryzae

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ABSTRACT

Oryza sativa (rice) is a staple food crop all over the world, about more than 50% of the world's population feed on rice. A large part of the total rice yield around the world is affected by various bacterial, fungal and viral diseases, one such disease is bacterial blight. Bacterial blight of rice has high epidemic potential and is destructive to high-yielding cultivars in both temperate and tropical regions especially in Asia. Here, we tried to prevent bacterial blight in rice using genetic engineering approach. We could prevent the disease by introducing mannose binding ligand (MBL) gene into rice such that it prevents the production of xanthan gum. Xanthan is an extracellular, sticky, glue-like polysaccharide produced by bacterium *Xanthomonas oryzae pv. oryzae*.

KEYWORDS : Bacterial blight, *Oryza sativa*, *Xanthomonas oryzae*, Mannose binding ligand (MBL) gene

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I. INTRODUCTION

Oryza sativa is a member of family Poaceae. The genome size of *Oryza sativa* is 426.3 Mbp across 12 chromosomes. It is the agricultural commodity with the third- highest worldwide production. This plant variety has always been prone to be attacked by diverse and widespread potential pathogens which cause numerous diseases. One such pathogen is *Xanthomonas oryzae pv. oryzae* (Xoo). Xoo causes bacterial blight in rice.

Fig. 1: *Oryza sativa* in field

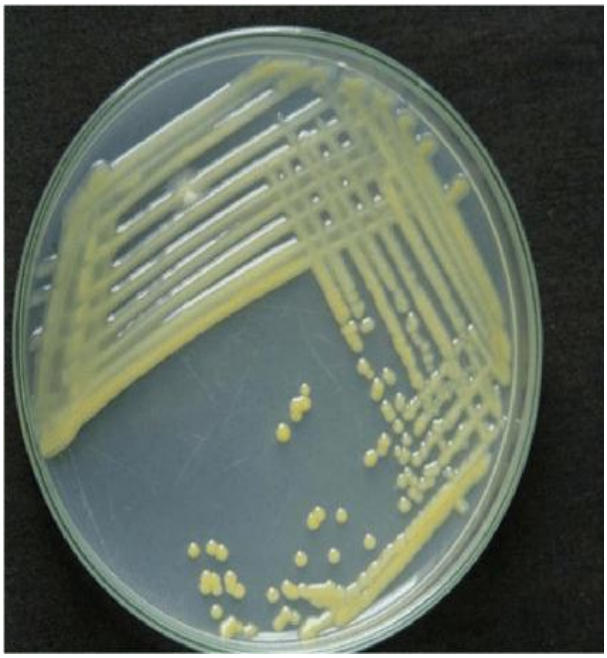

Fig.2: Colony morphology of X. oryzae on Peptone sucrose Agar plate.

Xanthomonas oryzae pv. oryzae (Xoo) is a bacteria which belongs to class Gammaproteobacteria and family Xathomonadaceae.

II. BACKGROUND OF BACTERIAL BLIGHT

Bacterial blight is a deadly bacterial disease that is among the most destructive afflictions of cultivated rice. In India, during 2009-10, rice occupied 44.62 million hectares with a total production of 89.3MT. The losses in yield varied from 6-60% in different states depending upon stage and severity of infection. In 2007, millions of hectares was severely infected with the disease causing yield loss upto 40%.

Fig. 3: Infected rice plants by *Xanthomonas oryzae pv. oryzae* (Xoo)

Currently disease control methods primarily based on chemicals are used. Some resistant varieties are developed but they may not be durable in the field leading to frequent resistance breakdown. It is required to develop some sustainable alternative strategies to control the disease.

Rice plants become infected with *Xanthomonas oryzae* through rice seeds, stems and roots that are left behind at harvest. It lives on dead plants and seeds and probably move plant - to - plant through patty water. The bacterium infiltrates the plant through hydathodes or wounds, multiplies in the intercellular spaces of the underlying epitheme, and propagate to reach the xylem vessels. The bacteria moves through the veins of the leaves and spread into the plant. Xoo produce xanthan gum causing blockage and plant wilting.

Symptoms appear on leaves of young plants as pale – green and grey-green, water – soaked streaks near the leaf-tip and margins. These lesions coalesce and become yellowish - white with wavy edges. Eventually, the whole leaf becomes yellow and then the plant die.

Xanthomonas oryzae pv. oryzae produces xanthan gum. Xanthan is a polymer of repeating pentasaccharide units consisting of two glucose units, two mannose units, and one glucuronic acid in the molar ratio of 2.8:2.0:2.0 (with structure mannose-(β-1,4)-glucuronic acid-(β-1,2)-mannose-(β-1,3)-cellibose).

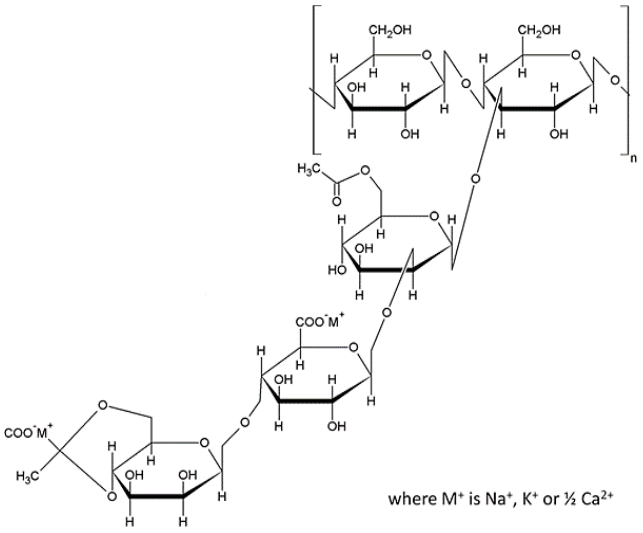

Fig. 4: Structure of Xanthan gum

The *gum* gene cluster comprises of fourteen genes which are responsible for the biosynthesis of this exopolysaccharide.

The assembly of pentasaccharide repeating unit, polymerization, and the export of xanthan are operated by *gum* operon with promoter located upstream of *gum* B, although weak promoters may exist upstream of *gum* K and *gum* D.

The twelve open reading frames (*gum* B to *gum* M) are involved in following activities:

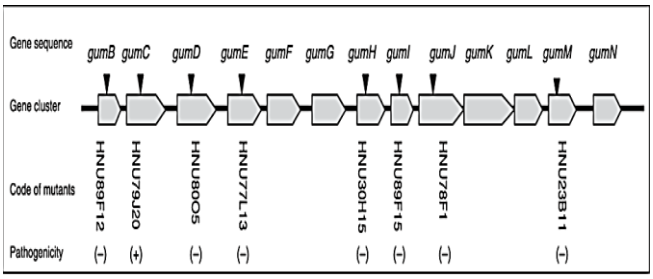

Fig. 5: Gum operon in *Xanthomonas oryzae pv. oryzae*

- There is leaky synthesis of *gum* I encoding mannosyltransferase that catalyses the entry of mannose to form the Xanthan structure. The expression of *gum* I is driven by the promoter upstream of *gum* B.
- *Gum* D is responsible for addition of glucose to lipid anchor inside the cell membrane.
- *Gum* M and *Gum* H add glucose and mannose to pentasaccharide, respectively.
- *Gum* K adds glucuronic acid.
- *Gum* F and *gum* L are responsible for modification of the polysaccharide by adding acetyl and pyruvate residues.
- *Gum* B, *gum* C and *gum* E help in polymerisation of repeating units and export of polymer.
- *Gum* J has the function of secretion of exopolysaccharide outside the bacterium due to which xylem vessels block and eventually results in the death of the plant.

III. MATERIALS AND METHODS

Here, we try to develop a hybrid rice plant variety which is resistant to bacterial blight using recombinant DNA technology.

Obtained synthetically prepared mannose binding ligand (MBL) gene and Act1 promoter.

Ti plasmid from *Agrobacterium tumefaciens* isolated and employed to develop transgenic rice plant. This plasmid is capable of transferring a piece of DNA, T-DNA (transfer-DNA) to the plant cell nucleus. The gene sequences capable of expression into the plant is marked with Nos terminator sequences.

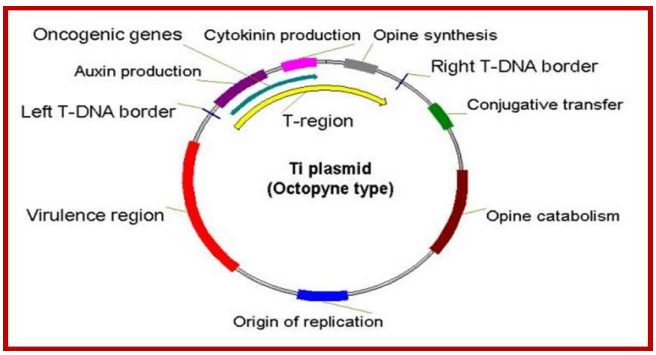

Fig. 6: *Ti* plasmid of Agrobacterium *tumefaciens*

The genes harmful to plant (tumor genes and opine synthesis genes) are removed using restriction enzymes and gene of interest (mannose binding ligand (MBL) gene) and a strong promoter (Act1, an efficient promoter for regulating the constitutive expression of a foreign gene in transgenic rice plant) are inserted with the help of ligase enzyme. This is called substitution of genes.

A selectable marker gene (antibiotic resistance gene) is also introduced to screen *Agrobacterium tumefaciens* with the gene of interest. Virulence genes remain as they are necessary to transfer the T-DNA from *Agrobacterium tumefaciens* into the plant.

The *Agrobacterium tumefaciens* is cultured with undifferentiated plant cell protoplasm on an antibiotic medium to ensure only growth of cells with antibiotic resistance.

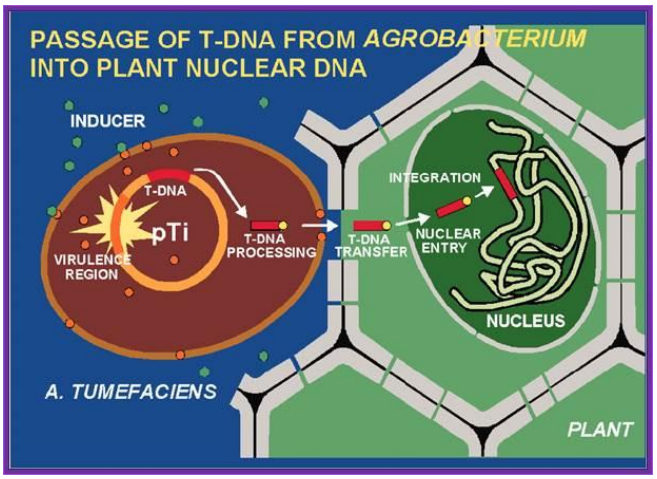

Fig. 7: Mechanism of *Agrobacterium tumefaciens* to infect plant cell

After inoculation of this system, formation of callus occurred under the influence of phytohormones (micro propagation).

Finally, obtained modified variety of rice plant variety that produces mannose binding ligand/lectin.

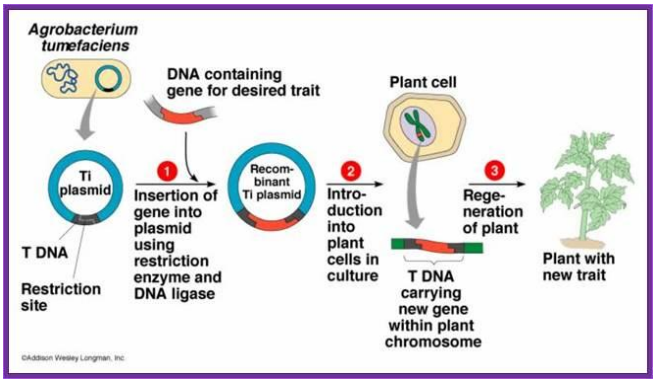

Fig. 8: Diagrammatic summary of the method used to develop hybrid variety of *Oryza sativa* (rice) resistant to bacterial blight

IV. RESULTS AND DISCUSSION

The transgenic rice plant developed produces mannose binding ligand/lectin by expressing mannose binding ligand (MBL) gene into its system. The gene regulates the production of xanthan gum. The MBL protein encoded by MBL gene captures mannose by making it unavailable for *gum* operon to act upon for the biosynthesis of xanthan gum, thus regulating the *gum* operon.

Xanthan is not produced, as mannose is not available for its synthesis and thus preventing the blockage of xylem vessels and wilting of rice plants. This ultimately prevents bacterial blight in rice.

V. ACKNOWLEDGMENT

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