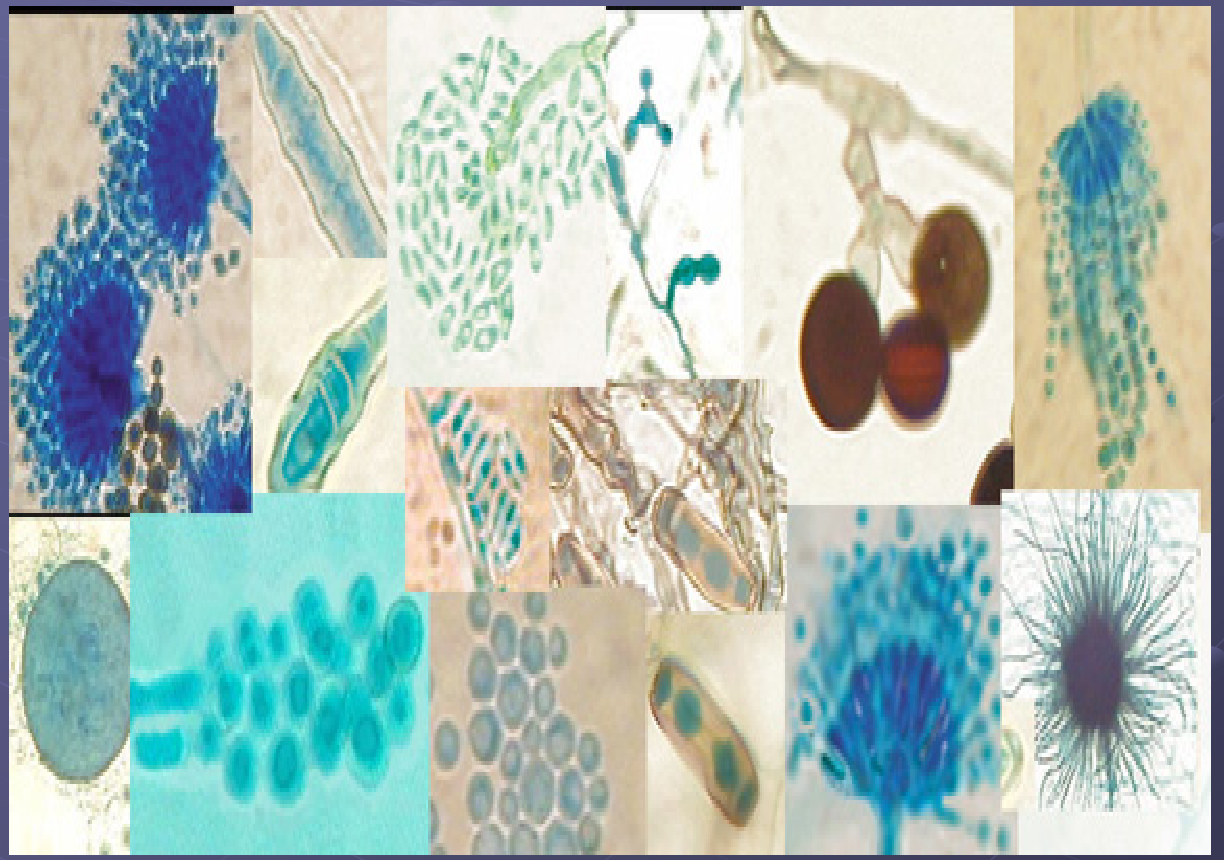
Detoxification of *fusarium* toxins in transgenic crop plants



RAKESH KHADKA
M.Sc. Agrobiotechnology

Lay out

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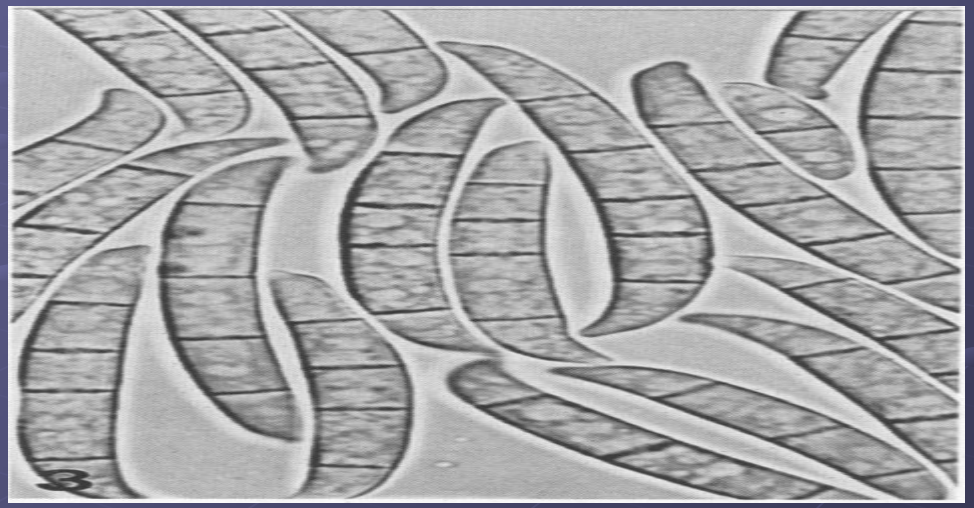
Fusarium spp.



Taxonomic Classification

Kingdom: Fungi
Phylum: Ascomycota
Order: Hypocreales
Family: Hypocreaceae
Genus: *Fusarium*

- Contains over 20 species.
- Filamentous fungus widely distributed on plants and in the soil.
- Fusarium species are toxigenic.
- Common contaminant and a well-known plant pathogen causing destructive and agriculturally important diseases of small grain, cereals and maize.
- Mycotoxins produced are often associated with animal and human diseases.

Mycotoxins in General:

Mycotoxins are the toxic chemicals produced by fungi for a variety of reasons.

1. To attack or gain access to hosts by helping to dissolve cell membranes.
2. For as protective measures against encroaching organisms.

- Mycotoxins, such as Mycotoxin T2 (Fusariotoxin) or the Amanita-toxins can be lethal to animals.

- Most-studied mycotoxins in Fusarium are toxic to both plants and animals. including hemorrhagic, estrogenic, emetic, and feed refusal syndromes, fescue foot, degnala disease etc etc.

- Disease caused by fusarium toxin do not only severely reduce yield,but also results in contamination of grain with unacceptable high amounts of mycotoxins, a problem of world wide significance.

Chemical Names of major *Fusarium* Mycotoxins; Marasas *et al.*

Some of the names are redundant, and some
the result of research in different countries

- Deoxynivalenol
- Fumonisin B1
- Moniliformin
- Neosolaniol
- Nivalenol
- Scirpentriol
- Vomitoxin
- Zearalenol
- Zearalenone
- Trichothecenes

Head blight of wheat caused by *Fusarium graminearum*

Fusarium mycotoxins may leach into the soil, causing damage to plants and animals through leaching even after the fungus is no longer active.

Importance

- Fungal diseases are common problems in crop agriculture.
- Mycotoxins, such as those produced by fusaria fungi for instance, are in fact a major food safety issue.
- Many strides have been made against plant diseases as exemplified by the use of hybrid plants, pesticides and improved agricultural practices.
- However, the problems of fungal plant disease continue to cause difficulties in plant cultivation.
- Thus, there is a continuing need for new methods and materials for solving the problems.

These problems can be met through a variety of approaches:

1. the infectious organisms can be controlled through the use of agents that are selectively biocidal for the pathogens.
2. interference with the mechanism by which the pathogen invades the host crop plant.
3. interference with the mechanism by which the pathogen causes injury to the host crop plant.
4. is interference with toxin production, storage, or activity.

Detoxification of mycotoxins

- Although reducing fungal infections is the most desirable method of eliminating mycotoxins.
- Cultural practices and genetic approaches have achieved limited success in disease control in terms of effectiveness and cost.
- Efforts to produce GM crops with enhanced disease resistance have not been successful on a practical level.
- An alternative solution, transgene-mediated detoxification of mycotoxins has been proposed.
- A prerequisite for this is the availability of genes encoding enzymes with detoxification activities in plants by the deployment of anti-mycotoxin antibodies (plantibodies) or mycotoxin-degrading enzymes.

Methods for identifying organisms capable of degrading mycotoxins.

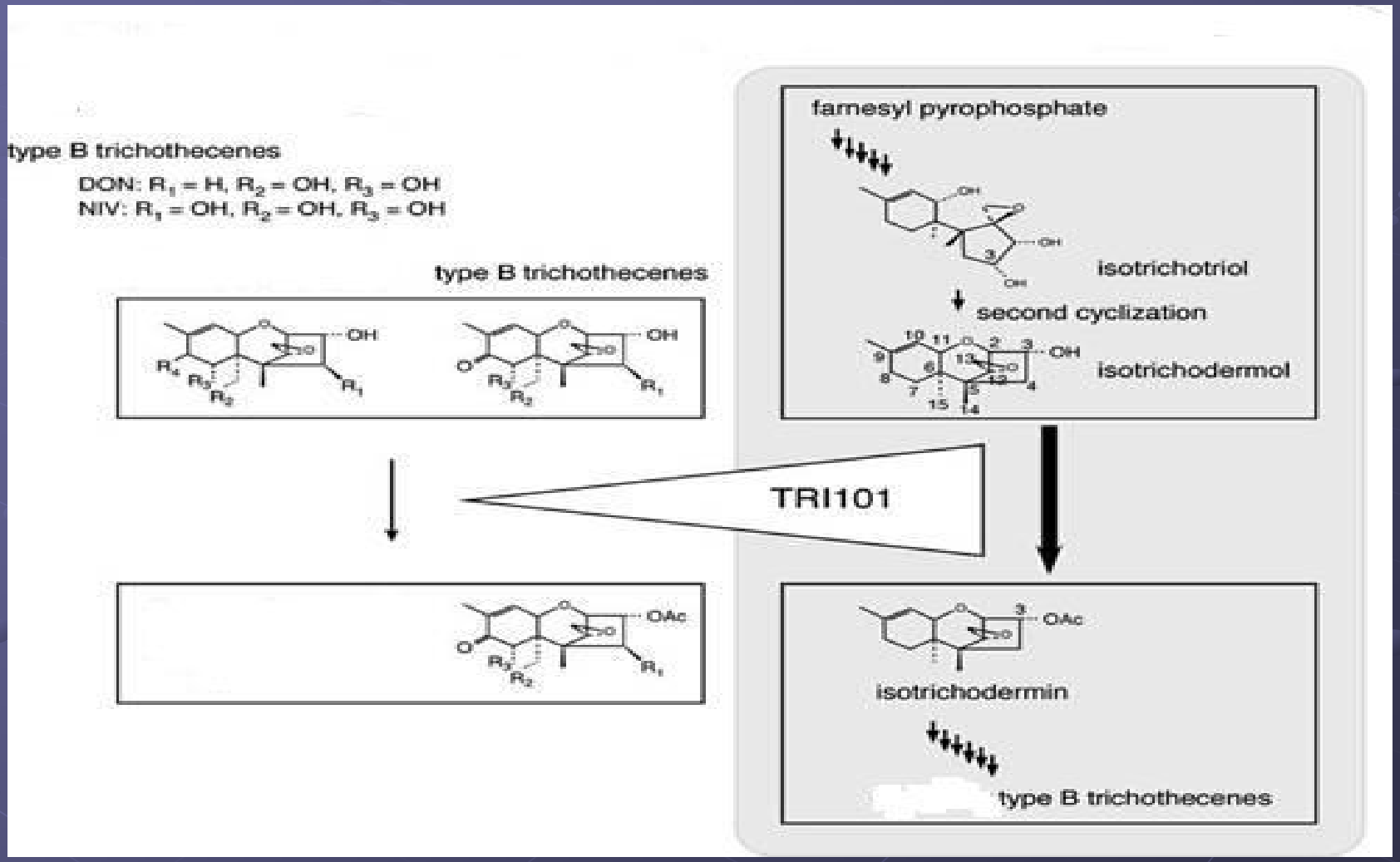
- Mycotoxin is incorporated into culture medium for selection of organisms.
- These organisms are used to isolate the enzyme and the gene responsible for conferring mycotoxin-resistance.
- The gene is cloned and inserted into a suitable expression vector for the further characterization of protein.
- The DNA encoding for mycotoxin-resistance can be used to transform plant cells normally susceptible to Fusarium or other toxin-producing fungus infection.
- Plants can be regenerated from the transformed plant cells.
- A transgenic plant can be produced with the capability of degrading mycotoxin, as well as with the capability of producing the degrading enzymes.

Case Study- on Detoxification of Fusarium Toxin

- The necrotrophic fungal pathogens *Fusarium graminearum* and *Fusarium culmorum* cause Fusarium head blight (FHB).
- A loss of yield.
- Serious threats to humans and animals by contaminating grains with the tri-chothecene mycotoxin deoxynivalenol (DON).
- DON inhibits protein synthesis in eukaryotes.
- Stimulates the development of plant diseases as a phytotoxin presumably by interfering with the expression of defense-related genes.
- *Fusarium* species have *Tri101* gene for self protection.
- *Tri101* gene encodes trichothecene 3-*O*- acetyltransferase.

Wheat spikes with symptoms of Fusarium head blight

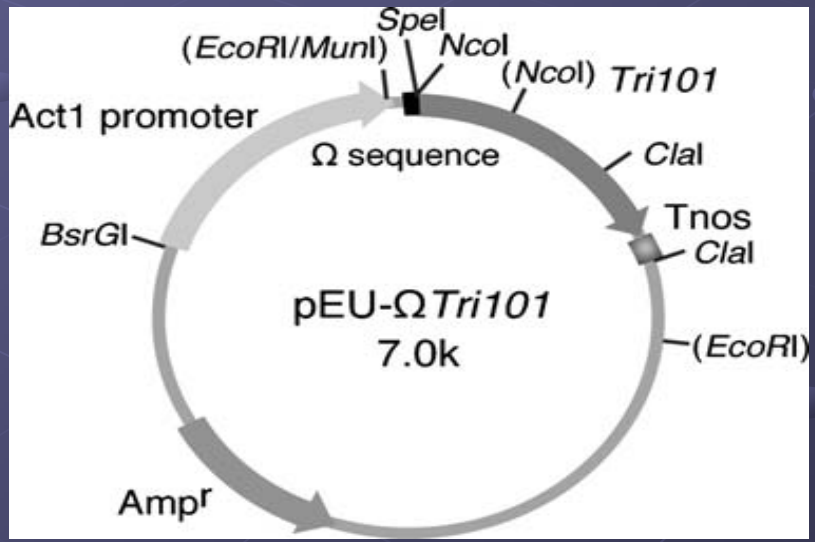
Fusarium trichothecenes biosynthesis pathway



Transformation of *Tri101* Gene in Rice.

- Total DNA and RNA were extracted from leaves using a Nu-cleon PhytoPure plant and fungal DNA extraction kit and RNeasy Plant Mini kit.
- DNA and RNA probes were prepared using a PCR DIG probe synthesis kit and a DIG RNA labeling kit.

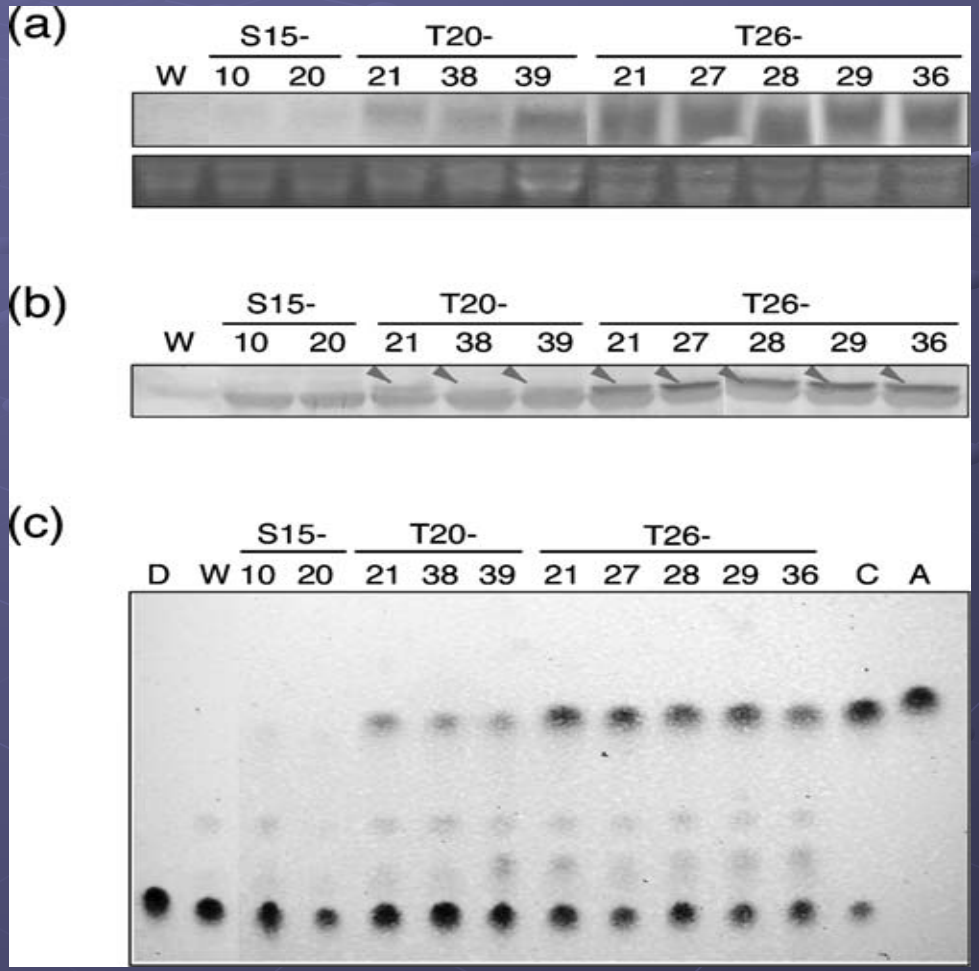
Construction of pEU-*Tri101*



Transformation of *Tri101* Gene in Rice.

- Mature seeds of japonica rice, ***Oryza sativa*** L. cv. Nip-ponbare, were used for callus induction.
- Scutellum tissues were excised from a 1-week culture on LS medium solidified by 0.25% gellan gum containing 2 mg/l of 2,4-dichlorophnoxyacetic acid (2,4-D).
- Bombarded with plasmid-coated gold particles (0.6 *μ*m).
- pAct1-*gfbsd1* containing an enhanced green fluorescence protein gene (*egfp*) fused to the blasticidin S (BS) resistance gene (*bsd*) was used as a transformation vector.
- Plantlets were regenerated from transgenic calli and grown in a greenhouse under natural light.

Detection of in vitro trichothecene 3-*O*-acetyltransferase activity in transgenic lines



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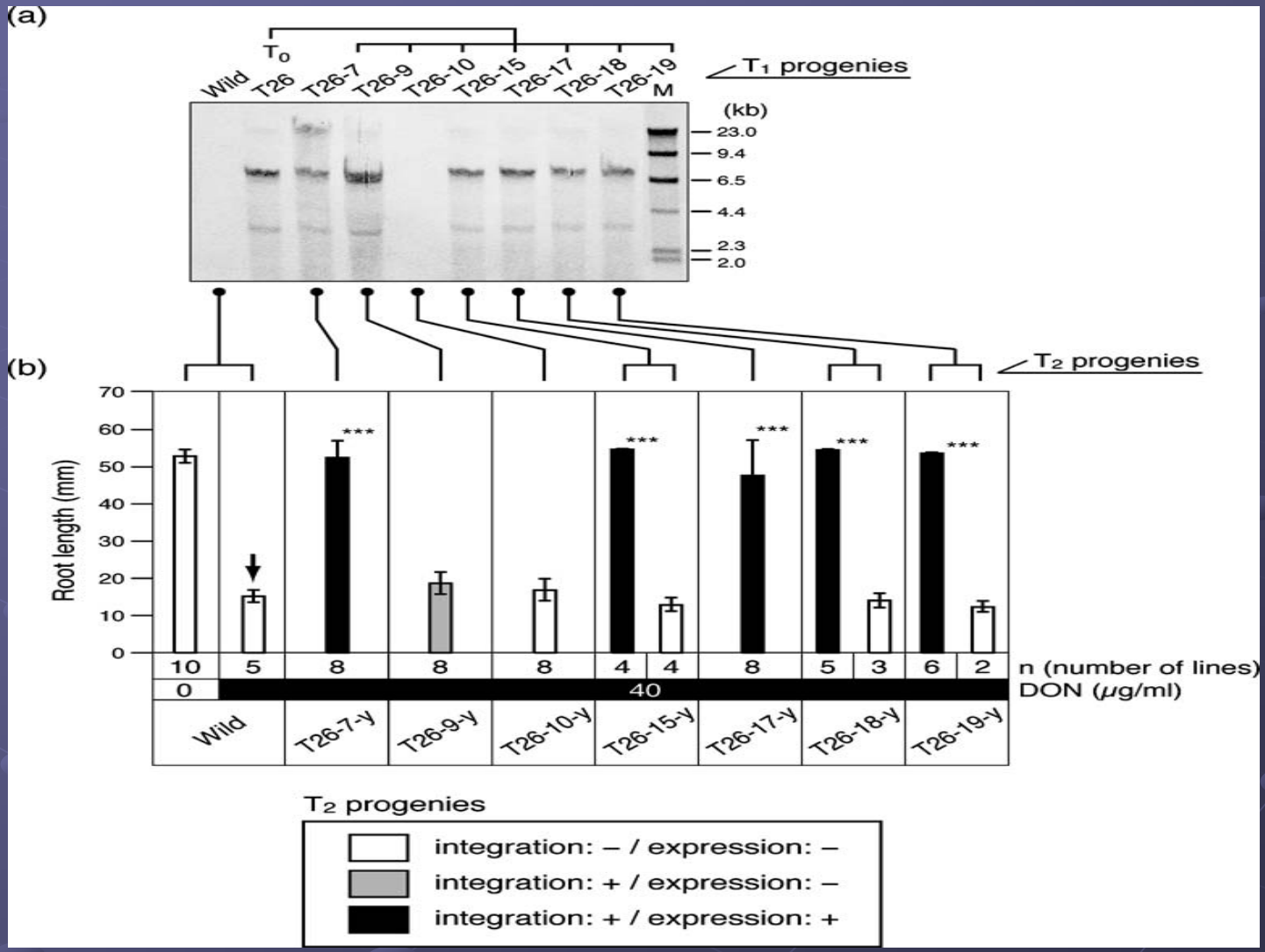

Discussion

- Fungal diseases are common problems in crop agriculture. Many strides have been made against plant diseases as exemplified by the use of hybrid plants, pesticides and improved agricultural practices.
- The problems of fungal plant disease continue to cause difficulties in plant cultivation.
- There is a continuing need for new methods and materials for solving the problems

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- Efforts to produce GM crops with enhanced disease resistance have not been successful on a practical level.
- An alternative solution, transgene-mediated detoxification of mycotoxins has been effective to control somewhat..

References

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Thank you