

Biotechnology and plant disease management-case studies

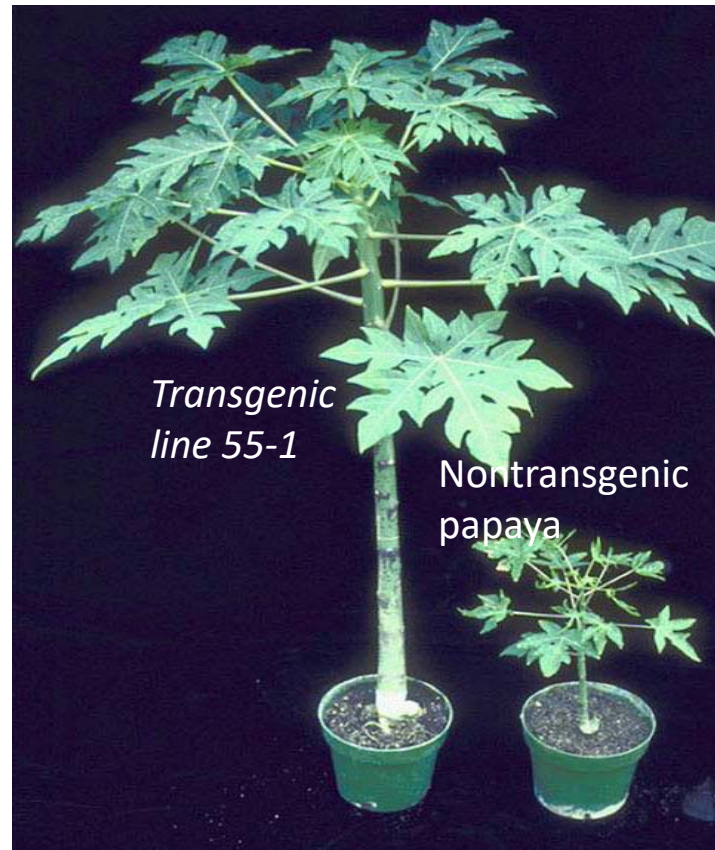
Pathogen Derived Resistance (PDR) in plant disease management

- Papaya cultivation was severely affected by Papaya Ring Spot Virus (PRSV) in Puna district of Hawaii, USA during early 1990s.
- PRSV is a potyvirus that is rapidly transmitted by a number of aphid species in a nonpersistent manner

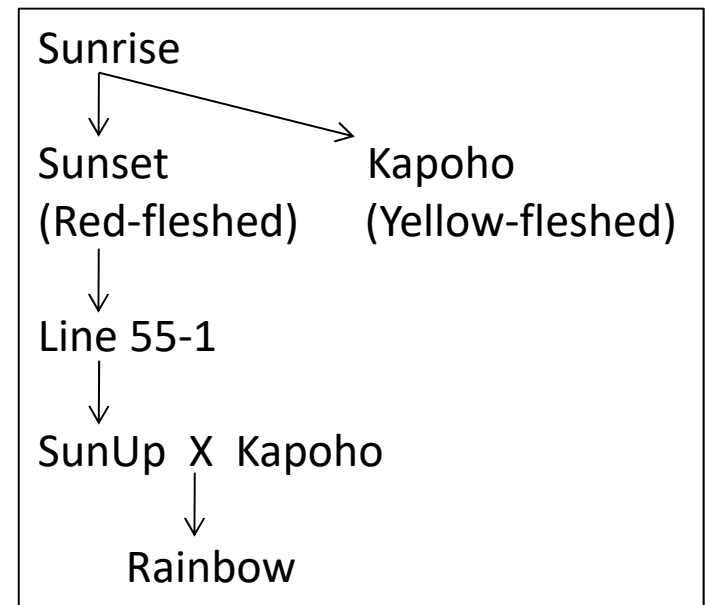


- The coat protein gene of PRSV was cloned in 1986 and transgenic lines were developed through the transformation of embryogenic cultures of papaya using the biolistic approach in 1988
- Transforming was done on commercial cultivars of 'Sunrise', its siblings 'Sunset', and 'Kapoho'.
- 'Kapoho' was the cultivar grown almost exclusively in Puna.

- 17 independently transformed plants were obtained
- In 1991, *line 55-1* was identified to be highly resistant PRSV HA, a severe strain from Hawaii
- The resistant line was the red-fleshed 'Sunset', which was much less desirable than the yellow-fleshed 'Kapoho'.



- Two new transgenic cultivars were developed—‘SunUp’ and ‘Rainbow’.
- ‘SunUp’ is a transgenic red-fleshed ‘Sunset’ that is homozygous for the coat protein gene.
- ‘Rainbow’ is a yellow-fleshed F1 hybrid developed by crossing ‘SunUp’ and nontransgenic yellow-fleshed ‘Kapoho’



- Transgenic papaya test field in Puna. Yellow plants are nontransgenic papaya; plants on right are transgenic 'Rainbow' papaya.



- Aerial view of transgenic papaya test field in Puna, showing block of healthy transgenic 'Rainbow' surrounded by severely infected nontransgenic 'Sunrise' papaya.



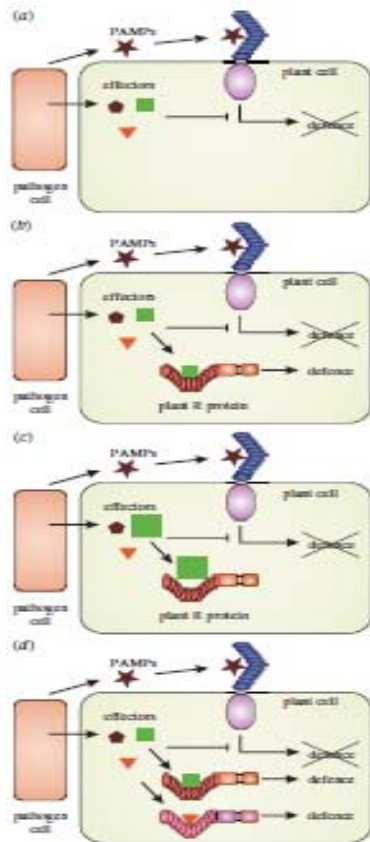
- Commercial transgenic 'Rainbow' papaya field



- Transgenic papaya field of R3 plants in Thailand in 2002.



Transgenic potato against Late Blight Disease: genes from wild potato



(a) Pattern recognition receptors (PRRs) in the plant cell membranes confer recognition of pathogen associated molecular patterns (PAMPs), resulting in PRR-triggered immunity (PTI). Despite PTI, plants are susceptible to their pathogens owing to the delivery of effector molecules that attenuate this host resistance response.

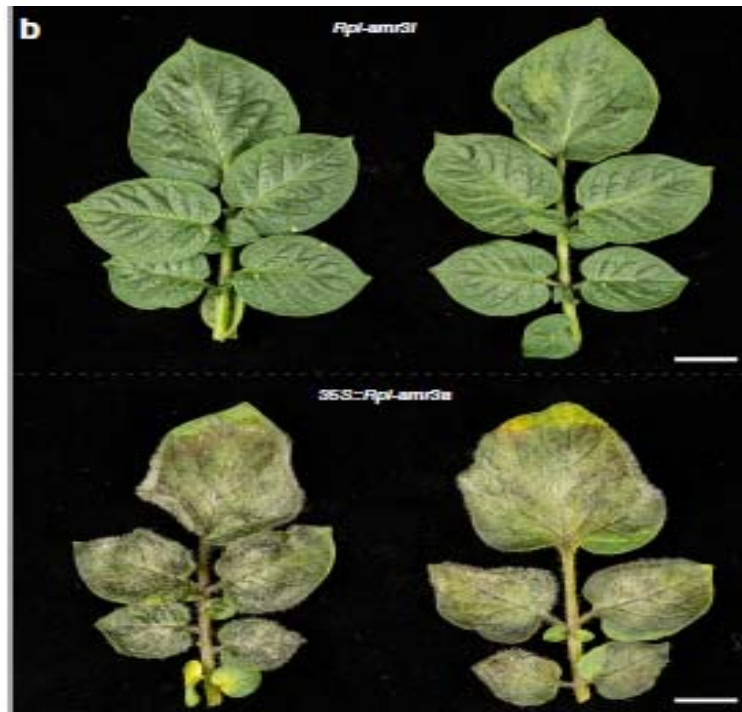
(b) Classical breeding for late blight resistance has focused on the introgression of single dominant R genes from wild sources such as *Solanum demissum*. These single genes resulted in strong selection on the pathogen effector genes, resulting

(c) in the selection of mutated effectors that evade recognition, or in complete loss of recognized effectors.

(d) It is postulated that the cloning and transgenic stacking of several R genes should be done. Stacked R genes will provide a more durable defence system, because each R gene abolishes the selection for single effector mutations that circumvent a different R gene.

Genes from tobacco

- Diploid non-tuber-bearing *Solanum americanum* harbors multiple *Rpi* genes
- Candidate gene *Rpi-amr3i* from *S. americanum* confers full resistance against *P. infestans* in stable transgenic potato plants.



Transgenic diploid potato "Line 26" (Solynta B.V.) expressing *Rpi-amr3i*

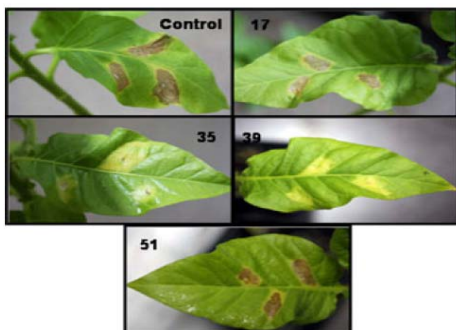
Control plants carrying the nonfunctional Candidate gene *Rpi-amr3a*



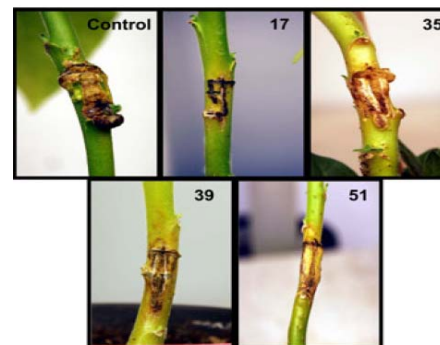
- *Rpi-vnt1.1*-transgenic and non-transgenic Desiree in field trials. Left, transgenic plants; right, non-transgenic.

Tobacco bacterial disease resistance using cytosolic ascorbate peroxidase and Cu, Zn-superoxide dismutase

- Transgenic tobacco plants were prepared by over-expressing *cytsod* from spinach and/or *cytapx* from pea
- Transgenic line 17 carried two copies of *sod*, line 51 two copies of *apx*, lines 35 and 39 carried both *sod* and *apx*



Disease resistance against the pathogenic bacteria *Pseudomonas syringae* pv. *tabaci* in transgenic tobacco lines.



Disease resistance to the pathogenic strain C58 of *Agrobacterium tumefaciens* in transgenic tobacco lines

- Bacterial pathogens stimulate superoxide (O_2^-) generation in apoplastic space by activating plasma membrane NADPH oxidase, which is subsequently dismutated to H_2O_2 by the apoplastic SOD.
- Apoplastic H_2O_2 generation takes place through peroxidases (POX), amine oxidases (AOx) and oxalate oxidases (Oox).
- H_2O_2 participate in lignification process as well as in direct killing of pathogens. ROS, such as O_2^- (perhydroxyl radical), can permeate biological membranes.
- Therefore, an important part of apoplastic ROS can also originate from an oxidative stress in cytosol, in addition to that produced by ROS originating from chloroplasts, mitochondria and peroxisomes and can contribute to induction of oxidative stress in cytosol.
- This ROS accumulation can induce HR, manifested as cell death and tissue necrosis. Transgenic lines overexpressing both *cytsod* and *cytapx* can limit ROS accumulation and avoid tissue necrosis.

