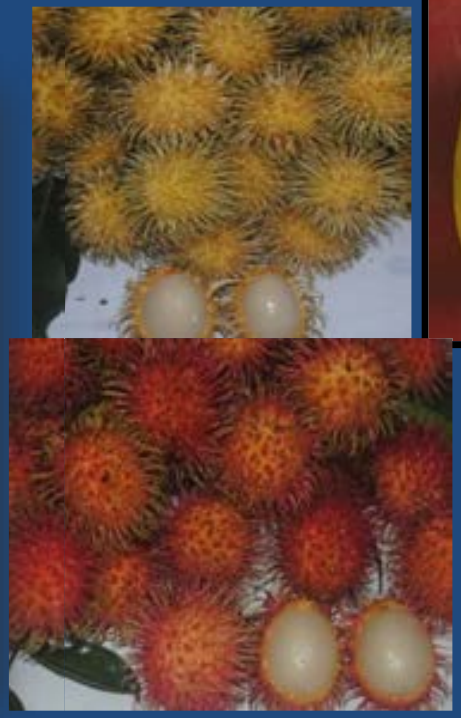
IPB, CAFS, UPLB Research Mandate

- By virtue of PD 729 (June 5, 1975), IPB under CAFS, UPLB was established and mandated…
- To undertake development of new and improved varieties of important agricultural crops like fruit crops.
- Another is to conduct investigations on allied disciplines related to plant breeding such as crop biotechnology.
- 1992 under RA 7308 of the Seed Industry Development Act, IPB was identified as the lead agency in crop biotech research.

This is line with one of UPLB's major banner programs on food security, and SEARCA on rural development, hence this lecture.

Classical Breeding and Biotechnology Approaches for Improvement of Selected Fruit Crops for Rural Development*

Dr. PABLITO M. MAGDALITA
Prof. I & UP Scientist II



*SEARCA Regional Professorial Chair Lecture delivered in the Drillon Hall, SEARCA Headquarters College, Laguna, March 26, 2018.

Rationale of the Lecture

- World's population will exceed 9 billion by the 2040s & in 2050, half of the world's population will reside in the tropics.
- The problem is already with us, with many of world's poorest people live in tropics. Changes are necessary in our priorities for development, research and education to ensure a safe, adequate & secure food supply.
- Breeders & horticulturists need to work together to exploit the potential of tropical crops to meet the needs of our growing population.
- There have been some real success stories in production of tropical horticulture crops. China and Vietnam produce large quantities of fruits and vegetables to feed their populations and export horticultural produce to other countries.
- Thailand is well known for its production and export of tropical fruits.

Rationale of the Lecture

- There have been many recent advances in tropical plant breeding in countries such as India and Brazil. More than 3,000 good varieties of fruit species grow in the Brazilian rainforest.
- In the Asia Pacific region, more than 400 species of tropical fruits and nuts are grown commercially (ISTTH, 2016, Nocker & Gardiner 2014).
- The Philippines has 3,600 identified native trees, 67% of which are endemic or found only in our archipelago.
- In terms of fruit-bearing species, there are 2,500 tropical fruits worldwide and of this, more than 300 edible fruit species have been reported.
- However, only 5 are considered major such as banana, pineapple, mango, papaya and citrus.

Rationale of the Lecture

- Recent advances in the breeding of papaya include: resistant to PRSV, blemish-free skin, gynodioecious types and those with high-yield have been done in Malaysia, India, Brazil & Thailand (Dinesh 2010, Chan 2015).
- Breeding of bananas have been focused on developing disease resistant varieties to *Fusarium* and viruses, drought tolerance and short-statured types in Taiwan & Central America (Dantas et al. 1995, Molina 2017).
- Further, the improvement of durian, guava and rambutan was focused on developing superior trees that are high-yielding, early fruiting, off-season & and disease resistant types (Magdalita et al. 2011, Ogata et al. 2016).
- This lecture aims to inform the public and discuss the utilization of conventional breeding and biotechnology for breeding selected fruits for rural development.

Classical breeding methodology

- **Acquisition of selected germplasm**
- **Evaluation, selection & purification**
- **F_1 hybrid breeding**
  - **-inbred line development (F8)**
  - **-diallel crossing**
  - **-preliminary evaluation**
  - **-replicated field trial**
  - **-on-farm trials**
  - **-seed production**
  - **-variety release**
  - **-registration of variety**

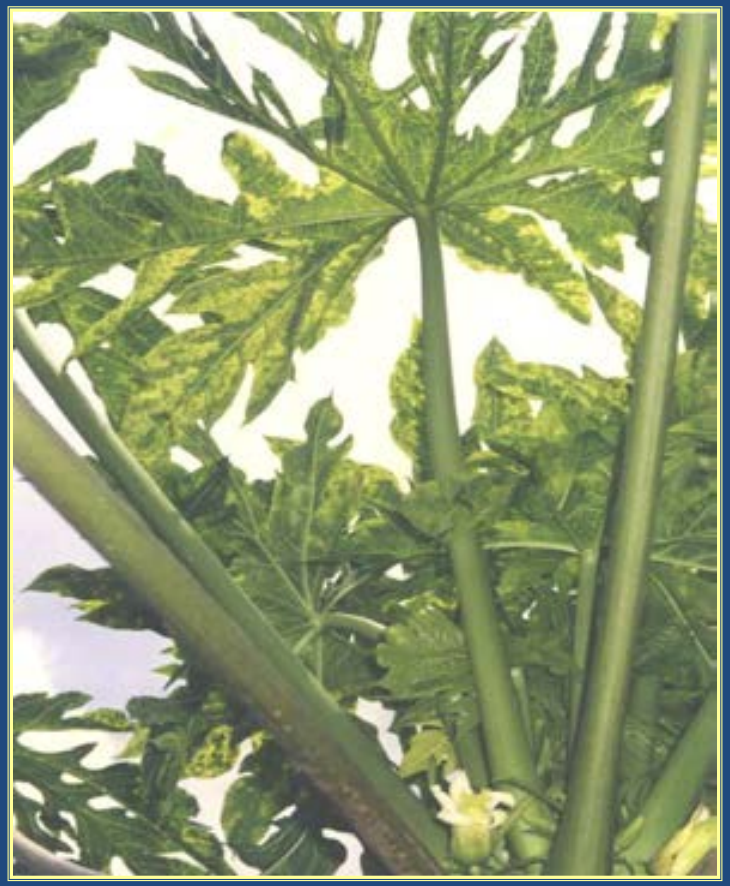
Important qualities of improved fruit varieties:

- **High-yielding or prolific bearers of fruits**
- **Non-biennial bearing**
- **Sweet and juicy**
- **Attractive flesh color**
- **Thick and firm flesh**
- **Non-fibrous**
- **Pleasant flavor and aroma**
- **High edible portion**

- **Since perennials fruit after 5-7 years, selected and proven true-to-type varieties should be grown only.**

- **These qualities will make fruit growing a profitable enterprise for commercial growers and orchardists.**

F_1 Hybrid Breeding for Tolerance to PRSV)



Chlorosis on the leaves

Concentric rings of the fruit

Symptoms of PRSV

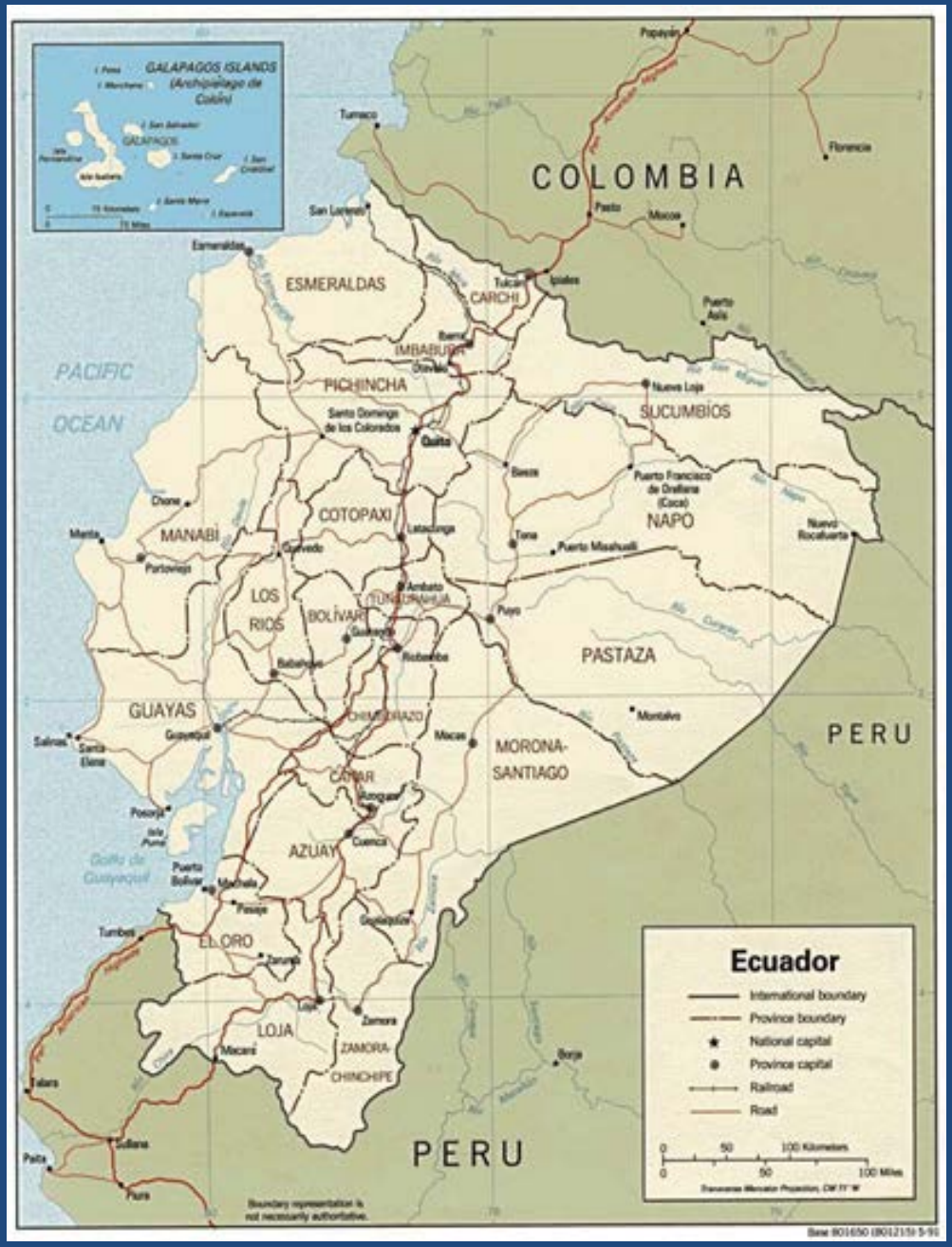
Transmission Electron Microscopy of PRSV

long flexuous rod 780 to 800 nm (potyvirus)

Cytoplasmic inclusion bodies (Pin wheel & circular structures)

Vector of PRSV (non-persistent)

Source: Opina 1986



- **Loja area in Peru: important centre for diversity of *Carica***
- ***Vasconcellea* species has 20% x higher amount of papain than *Carica papaya***

Vasconcellea x heilbornii cv. Babaco

Vasconcellea microcarpa

Vasconcellea monoica

Sources of resistance to PRSV

Vasconcellea guodotiana

- **Resistance to *Phytophthora* root rot**
- **Pickling type**

Vasconcellea parviflora

- **Ornamental type with pink flowers**

Vasconcellea pubescens

Carica cauliflora* now *Vasconcellea cauliflora

Tainung (A), Legaspi Special (B), Morado (C)

Papaya amerikano-232 (A), Pineras-232 (B), Semi-dwarf Cavite Special-243 (C)

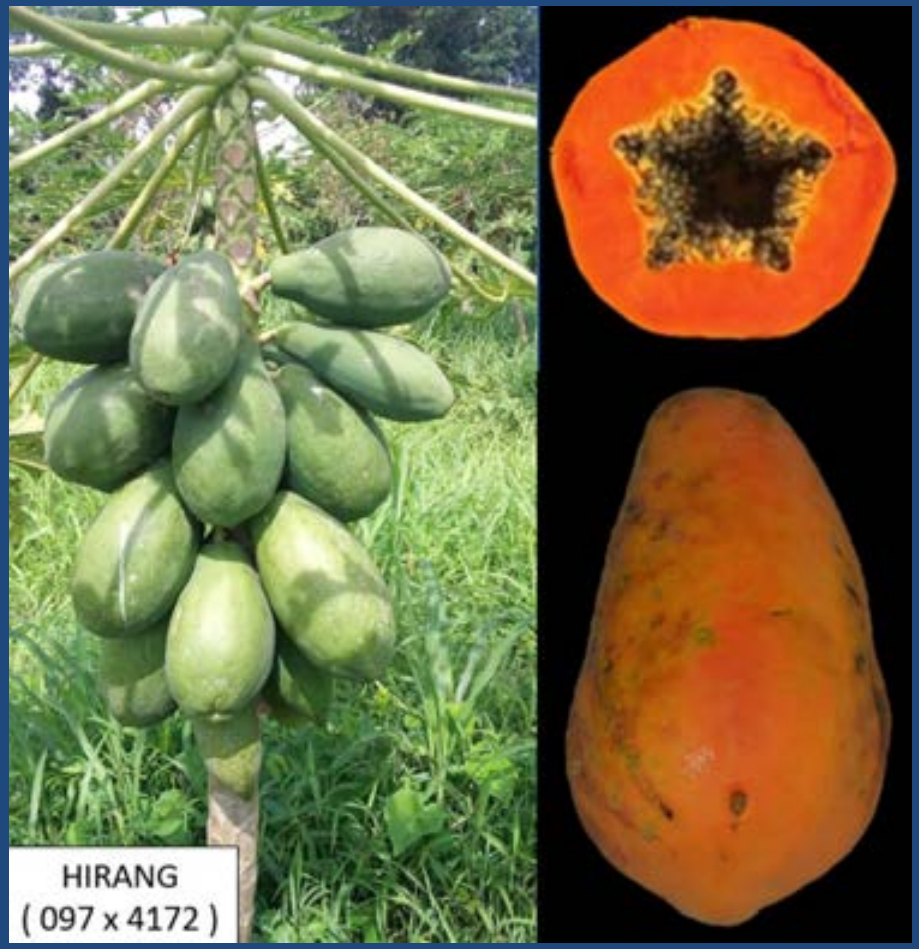
***Carica papaya* (‘Cariflora’) x *C. papaya* (‘Cavite Special’)**

'Sinta' is the first Philippine-bred F_1 hybrid papaya.

- **Tree is gynodioecious, semi-dwarf & prolific.**
- **Fruit is sweet (11.5°Brix), & firm that weigh 0.5 to 3.5 kg/fruit**
- **High edible portion (78%), juicy**
- **Marketable yield of 17-50 fruits per tree per year, or 48 t/ha**
- **Moderately tolerant to papaya ringspot virus (PRSV), the most devastating disease of papaya worldwide.**
- **Registered with the National Seed Industry Council (NSIC)**

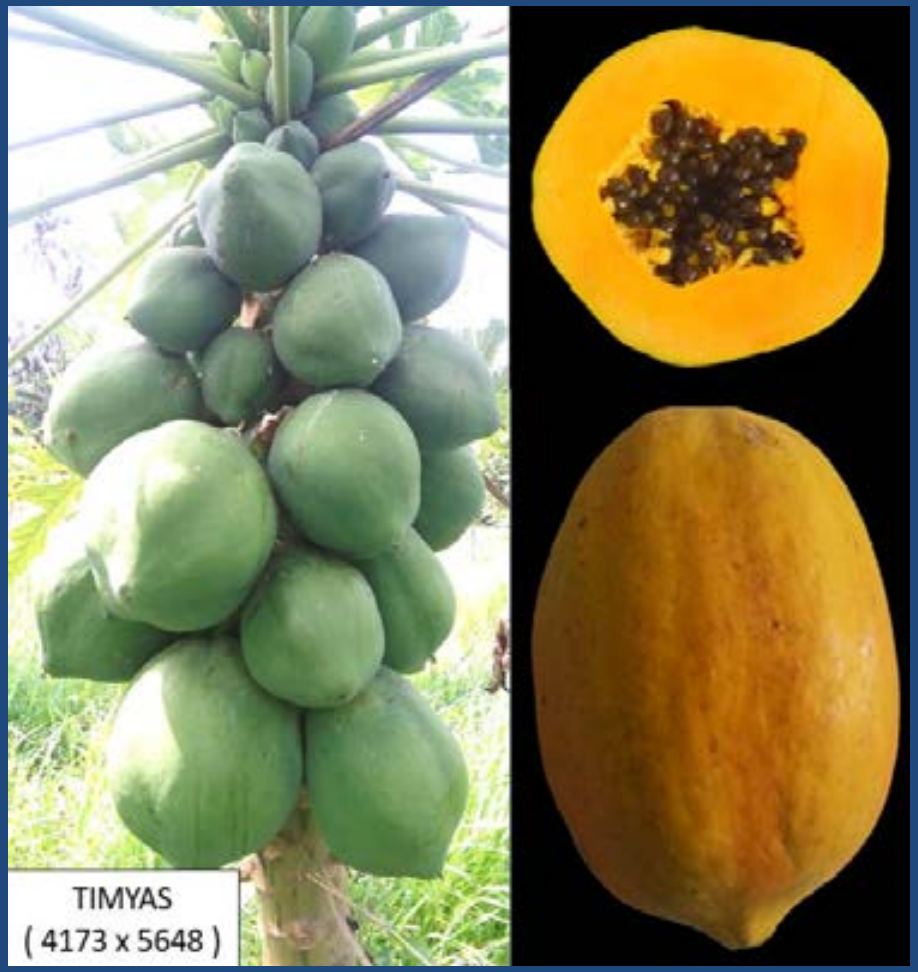
F_1 Hybrid Breeding for Tolerance to PRSV in New Papaya Varieties

- 'Hirang' F_1 hybrid
- Tree is gynodioecious, semi-dwarf & prolific bearer
- Fruit is red, sweet (9-15ºBrix), firm, weighs an average of 1.5 kg/fruit
- EP is 85%, flesh is 1.72-3.36 cm thick, juicy



- Moderately tolerant to papaya ringspot virus
- Marketable yield of 18 fruits per tree per year or 45 t/ha

- 'Timyas' F_1 hybrid
- Tree is gynodioecious, semi-dwarf & prolific
- Fruit is yellow, sweet (8-14ºBrix), firm & juicy
- EP is 87%, flesh 1.9-3.1 cm thick, weighs an average of 1 kg/fruit
- Moderately tolerant to papaya ringspot virus (PRSV)

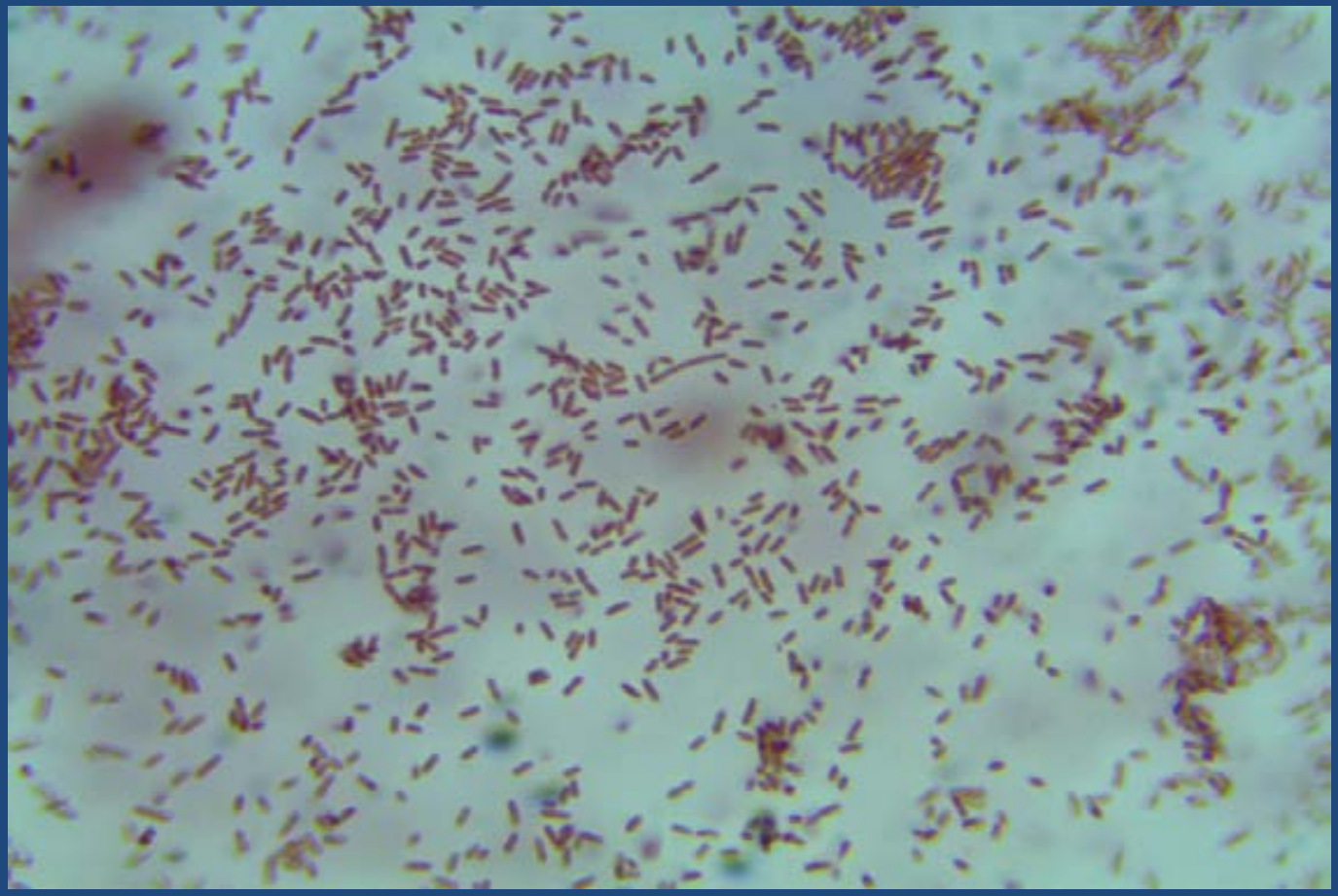


- Marketable yield of 25 fruits per tree per year or 42 t/ha

Breeding & Selection for Tolerance to Bacterial Crown Rot in Papaya

The unproductive papaya tree infected with Bacterial Crown Rot (A) and the regrowth tree previously infected with the BCR (B).

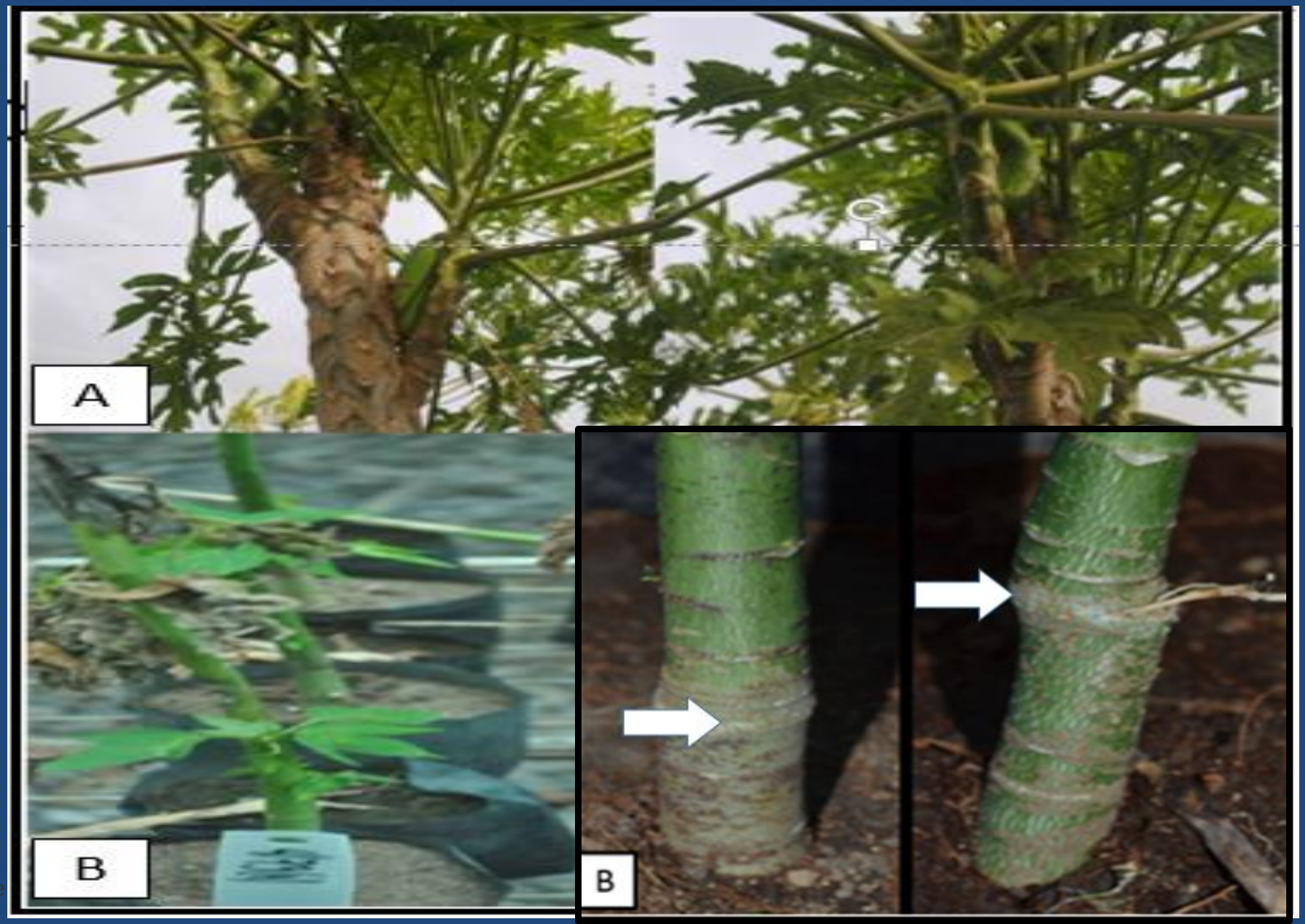
Bacterial cells of *Erwinia mallotivora,* causal organism of bacterial crown rot of papaya through light microscopy



Gram staining reaction of bacillus-shaped *Erwinia mallotivora* cells.

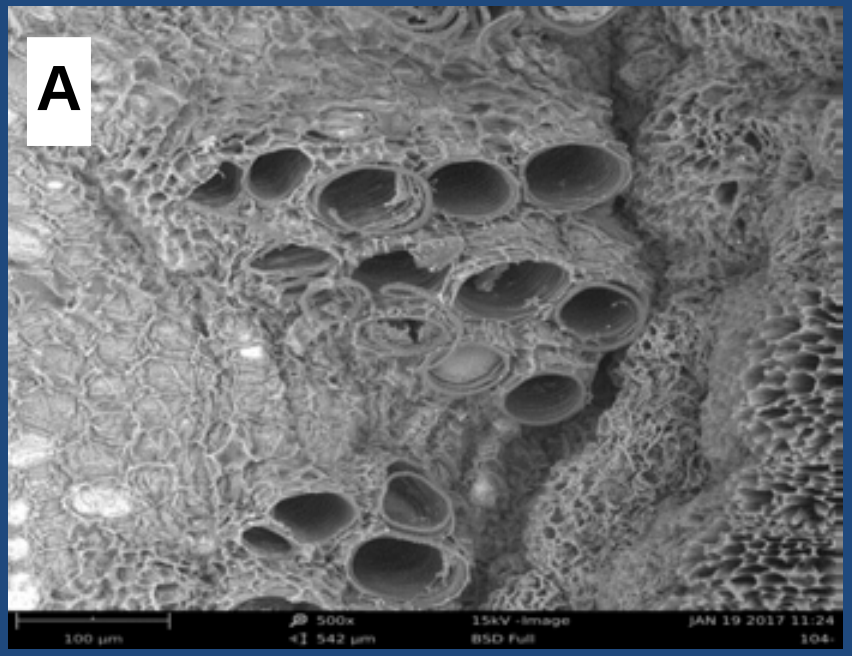
The experimental set-up for the evaluation of different papaya genotypes and the artificial rain system that provided the conditions for bacterial infection of the test plants (A), and the state of the test plants 2 weeks after inoculation (B).

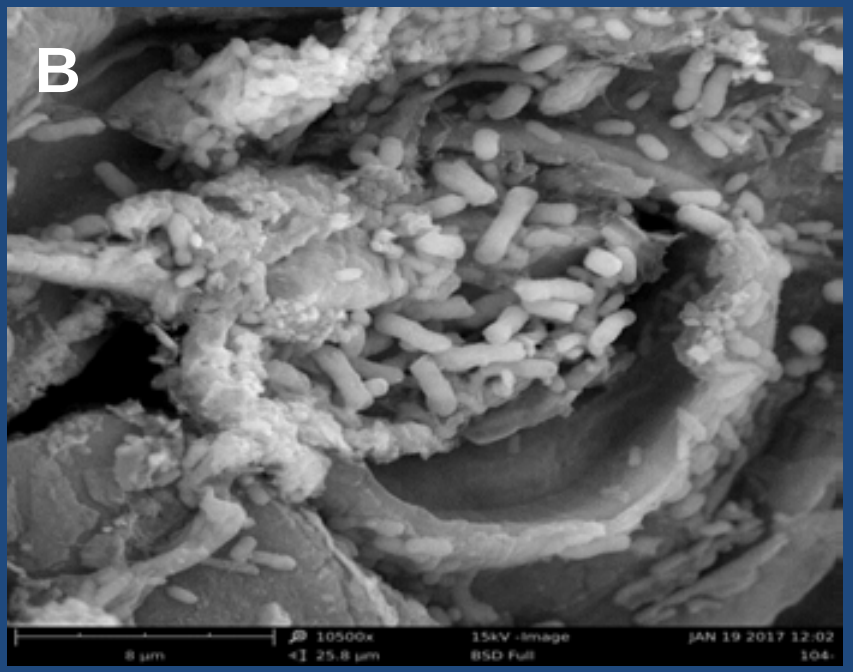
The regrowth in BCR naturally infected adult trees in the field (A), and the regrowth in seedlings that were artificially inoculated with BCR (B).

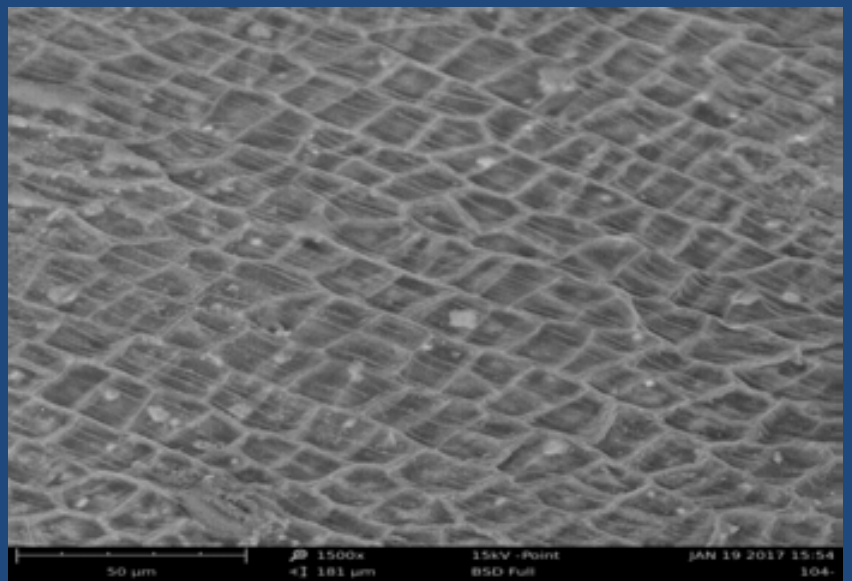


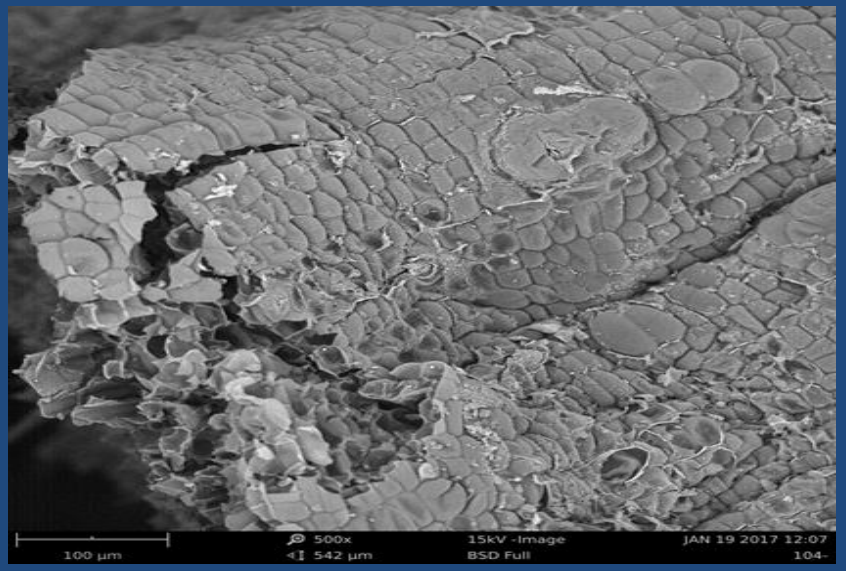
BCR-tolerant regrowth selections growing inside the screenhouse, their prolific fruiting habit of F_1 hybrids 5893x234, 5648x336, and the ripe fruits of 234x5648, 4174x5648 and 382-S_1.

Scanning electron microscopy of the conducting tissues of papaya BCR tolerant selection (A) and susceptible genotype (B).

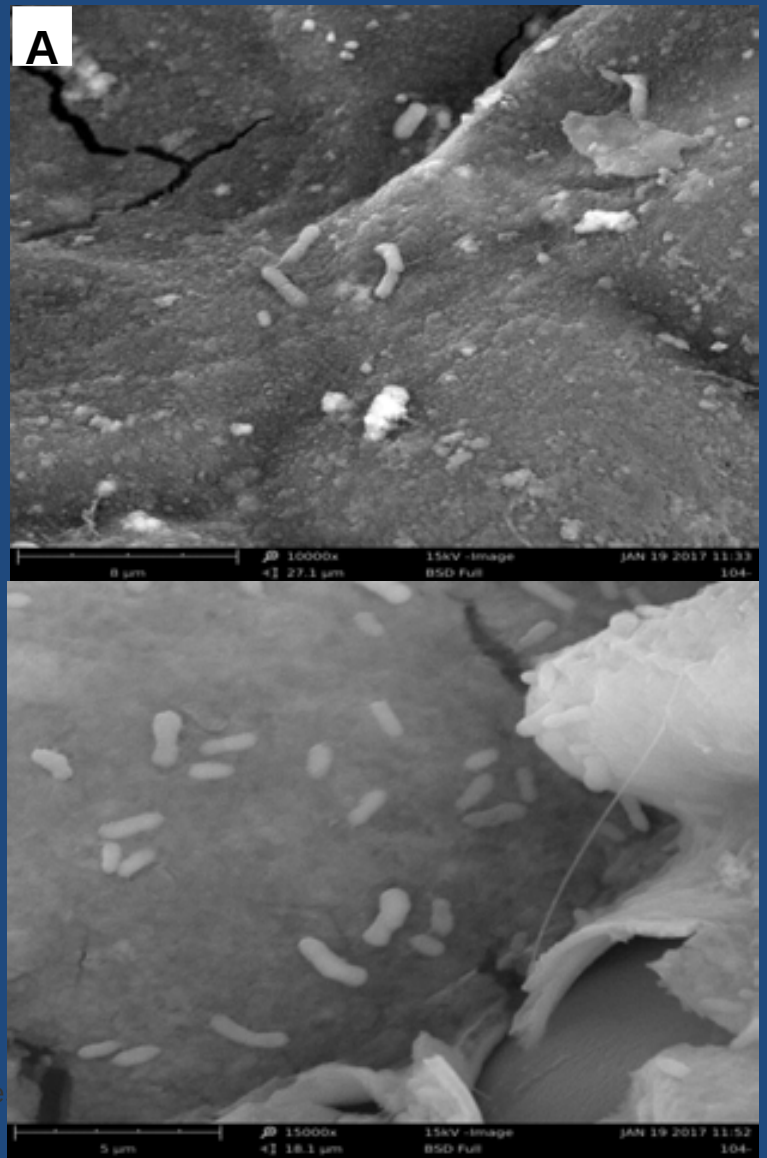








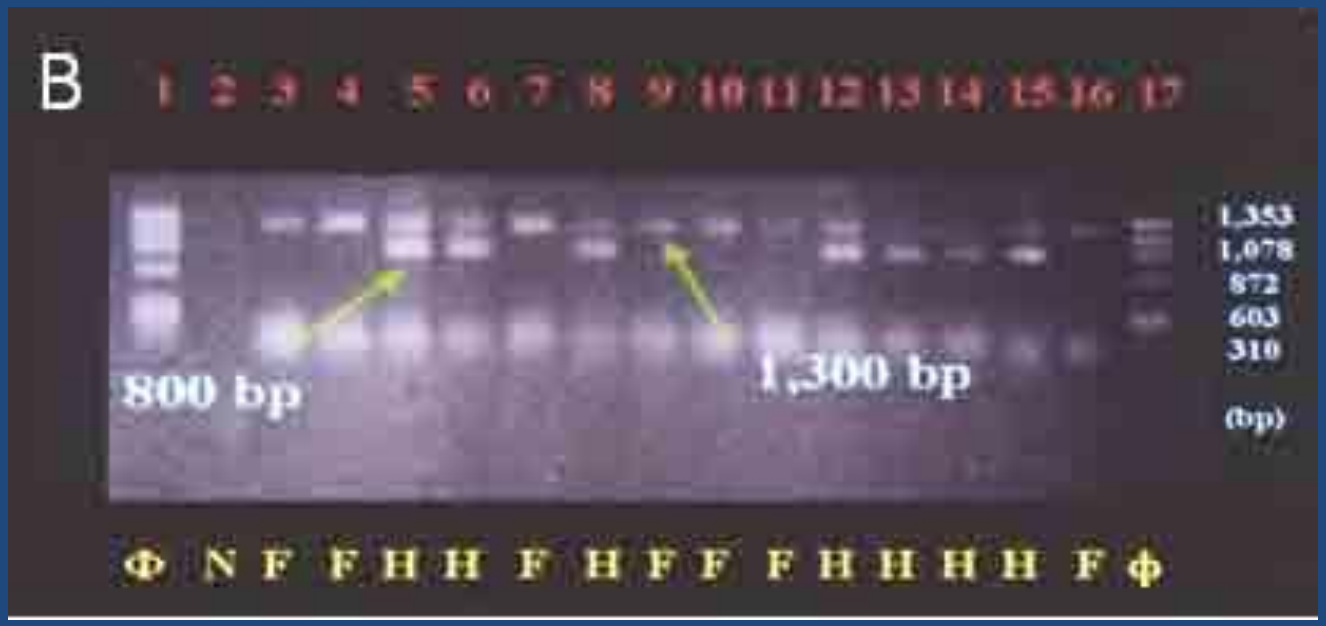
Scanning electron microscopy of susceptible genotype showing surface adherence and ingress of bacterial cells via wound (A) and cuticular layer degradation plus exopolysaccharide production on surface of bacterial cells on the host tissues (B).



Replicated field trial of regrowth selections and hybrids between BCR- and PRSV-tolerant lines in Tranca, Bay, Laguna.

BCR-tolerant x PRSV-tolerant hybrids with semi-dwarf nature and the prolific fruiting habit of the trees.

Male or pistillate (A), female and hermaphrodite or bisexual/perfect (B) and sexually ambivalent male (SAM) (D).



The PCR markers for female, male and hermaphrodite papaya plants generated by primers T1-F, T1-R, W11-F and W11-R.

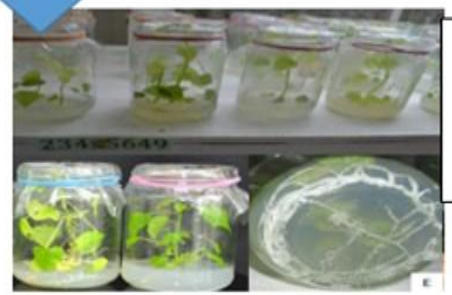
Protocol for Micropropagation of Papaya

Identify proven trees

Induce side shoots or select trees with natural side shoots

Culture side shoots and allow to grow.

Cut into single nodal cuttings



Allow cuttings to fully developed shoot and root before potting out.

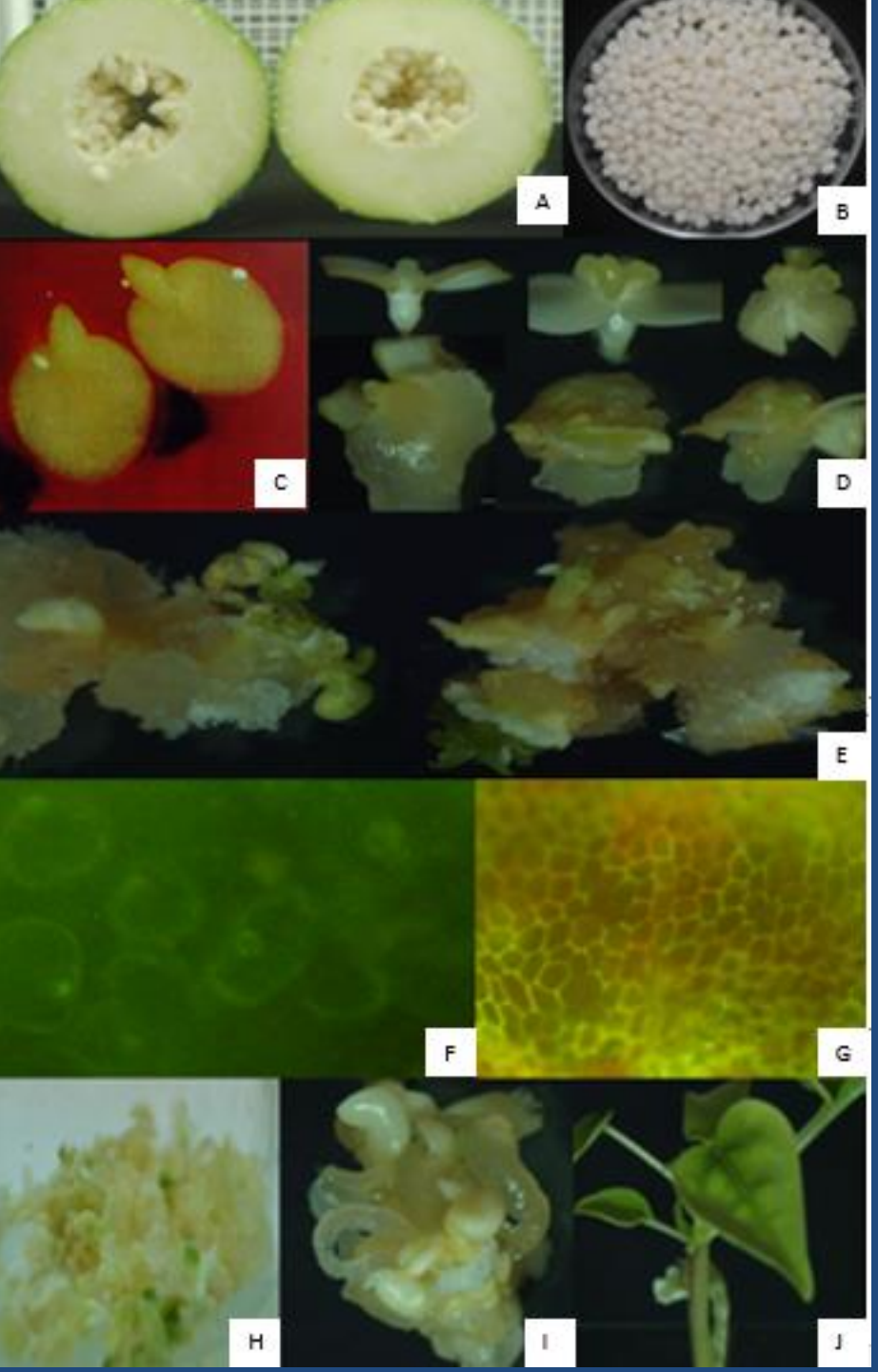
Harden plants before potting out

Pot out. Plant in sterilized medium. Put in a chamber or put plastic cups on top to prevent moisture loss

Remove cover and expose plants to partial sunlight

Transfer plants in benches in the screenhouse. After 1-2 months, plants are ready for field planting

Protocol for Tissue Culture of Papaya F_1 Hybrids



- Immature fruit (A)
- Ivory-colored seeds (B)
- Zygotic embryos (C)
- Somatic embryos and callus (D)
- Embryogenic callus and somatic embryos (E)
- Globular cells (100x) (F)
- Normal cells (100x) (G)
- Highly embryogenic callus (H)
- Torpedo embryos (I)
- Plantlet (J)

- Regenerated plants from somatic embryos and calli (A)
- Somatic embryo-derived plants (B)
- Germinating somatic embryos (C)
- Back-up cultures (D)
- Newly potted-out plants (E)
- Further acclimatization inside the humidity box (F & G).

Field trial of micropropagated papaya F_1 hybrids in UPLB Experiment Station, Tranca, Bay, Laguna.

Adoption of Sinta F1 hybrid by farmers in Mayor Federico's Farm, Sto. Tomas, Batangas

Fruiting habit of some varieties in a farmer's field trial (ACS Farm, Batangas)

On-farm trial in Montealegre's Farm in Silang, Cavite

Fruiting hermaphrodite trees of F_1 papaya hybrids growing in Montealegre's Farm in Silang, Cavite




Fruiting female trees of F_1 papaya hybrids growing in Montealegre's Farm in Silang, Cavite

Fruiting papaya F_1 hybrids intercrop to banana and pineapple in Magdalita's Farm in Boac, Marinduque

Fruiting F_1 papaya hybrids growing in Magdalita's Farm in Boac, Marinduque

Protected cultivation (netting) of papayas for seed F_1 hybrid seed production in Ilocos Norte trial

Fruiting habit of dwarfed papayas (done by bending) inside the nethouse in Ilocos Norte trial

Fruiting habit of dwarfed papayas inside the nethouse in Ilocos Norte trial

Technology Piloting of New Papaya F1 hybrid in Laguna trial 2015

Fruits harvested from Laguna trial (C&D) and Batangas trial (E&F)

Fruits harvested from Laguna trial

Fruit colors of harvest from Laguna trial

Breeding for Fruit Qualities of Selected Fruit Crops

- On-site selection and evaluation of promising trees
  - -preliminary assessment on-site
  - -evaluation of fruit characters in the laboratory
  - -selection of best 3 genotypes out of 100 trees
  - -re-evaluation for 3 seasons to assess stability
  - -propagation
  - -registration

‘Roja’ rambutan has attractive bright red leathery peel.

•Fruits weigh 45.85 g/fruit and are sweet (21°Brix), juicy &, thick flesh.

•The fruit has 56% edible portion.

•Strong and semi-erect tree produces 70-80 kg of fresh fruits (44 tons/ha/season).

Registered with NSIC

Named after its attractive golden yellow peel, ‘Amarillo’ is the first yellow known variety of rambutan.

•Oblong fruit, weighs 34 g, long spines, & smooth flesh.

Edible portion is 84%, juicy & sweet (22°Brix).

•Tree produces about 50-60 kg of fresh fruits (31 t/ha/season).

Registered with NSIC

‘Aguinaldo’ guayabano is for processing.

- **Fruit is well-formed**
- **Weighs about 1.2 kg**
- **It has less seeds, less fibrous & juicy**
- **TA is 65 meq/100 ml juice, TSS is 17°Brix**
- **High edible portion (78%)**
- **Tree produces 20-26 fruits/tree (1.9 t/ha/season)**
- **Tree is a regular bearer**

Registered with NSIC

‘Juan Luna Supersweet’ guayabano

- Well-formed, weighs 0.8 kg & has less seeds
- Very sweet (20 °Brix), juicy & less fibrous
- Mild aroma & fine texture
- High edible portion (80%)
- Regular bearer (18-22 fruits/tree or 5.8 tons/ha/season)

‘Mapino’ Chico

- Very fine flesh, very sweet (21ºBrix) & very juicy
- Very high edible portion (90%), thick flesh (3.0 cm)
- Fruit weighs 122 g
- Pleasant flavor & mild aroma
- Tree is very prolific, has semi-erect growth habit, strong
- High-yielding (500 fruits/tree or 4.3 tons/ha/season)
- Registered with NSIC

‘Mahitik’ chico

- Very prolific tree & regular bearer
- Fruit weighs 150 g
- Very sweet (22 °Brix), no gritty taste & juicy
- Very high edible portion (92%)
- Very high-yielding (700 fruits/tree or 11 tons/ha)

‘Primera’ avocado

- Prolific tree & regular bearer
- Sweet (10°Brix), nutty & buttery
- Fruit weighs 400 g
- Fine texture & non-fibrous
- High edible portion (82%)
- High-yielding (200 fruits/tree or 12.5 tons/ha/season)

‘Quezon’ pummelo

- Prolific tree & regular bearer
- Fruit weighs 1.2 kg
- Sweet (9.6°Brix), sub-acid & juicy
- Seedless & attractive red color
- Edible portion is 60%
- No astringent taste
- High-yielding

(70 fruits/tree or
13 tons/ha/season)

‘Jacinto’ pummelo

- Prolific tree & regular bearer
- Fruit weighs 1.4 kg
- Very sweet (10 °Brix) & juicy
- Edible portion is 62%
- Fine texture
- Less seeds
- No astringent taste
- High-yielding

(65 fruits/tree or
14.2 tons/ha/season)

Selection of Climate Change Resilient Hardy Fruits
(Drought tolerant selections)

Sg-41

Mc-41

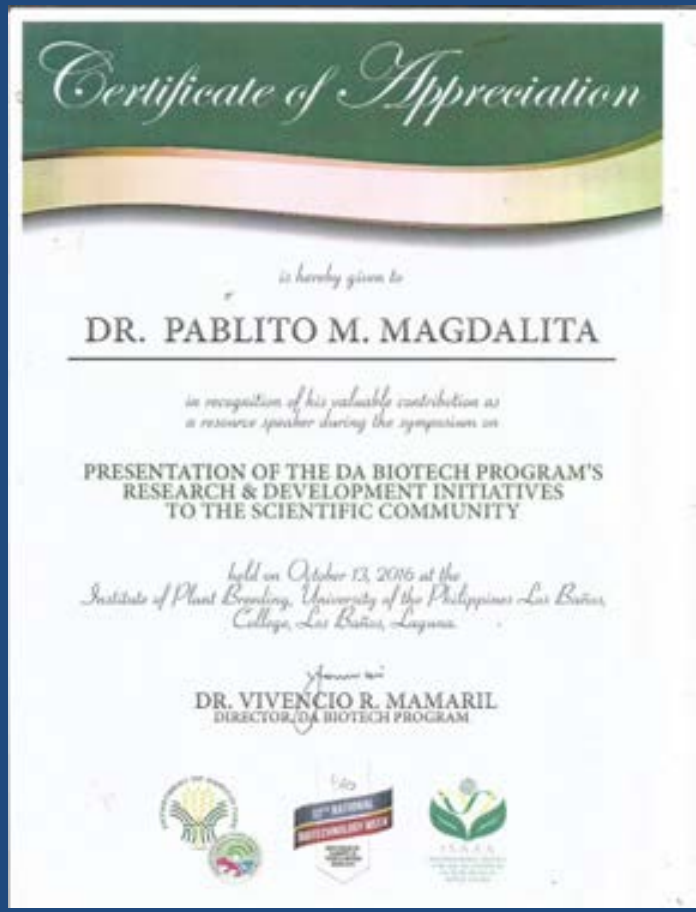
Sn-47

Cm-18

Different fruit varieties and their quantities that were propagated and disseminated to the fruit growers.

Drought Tolerant Fruit Species	Quantity Disseminated to Growers
Magallanes pummelo	60
Aguinaldo guayabano	50
Seedless duhat	150
Hongkong Pink guava	50
Primera avocado	30
Sweet tamarind	10
Mapino chico	50
Lobo atis	50
Lipote	10
Bignay	100
Surinam cherry	50
Pomogranete or granada	10
Papaya hybrids (PRSV-tolerant)	1,430

Recognition for Techno-Innovations on Papaya F_1 Hybrid Production in the Countryside



Recognition for presenting to the Community DA Biotech's Promising Technology on Papaya F_1 Hybrid Tissue Culture Propagation

Support to Agricultural Development in Sto. Tomas, Batangas

Republic of the Philippines
Province of Batangas

The Municipal Government of Sto. Tomas

Awards this

CERTIFICATE OF RECOGNITION

to

Dr. Pablito M. Magdalita

In gratitude of a long standing partnership, support and commitment to the advancement of agricultural development and for unselfishly devoting the time, resources and knowledge to the FARMERS of Sto. Tomas throughout these years

Given this 18th day of September 2015 at the Municipal Hall Grounds, Sto. Tomas, Batangas in the celebration of **STO.TOMAS FARMERS WEEK 2015**

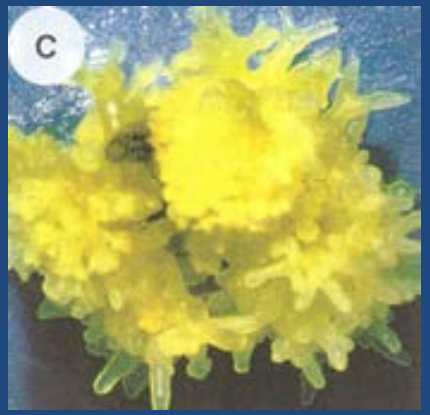
MS. OFELIA B. MALABANAN, MPA
Municipal Agriculturist, MGDH

ATTY. ARTH JHUN A. MARASIGAN
Municipal Administrator

HON. EDNA P. SANCHEZ
Municipal Mayor

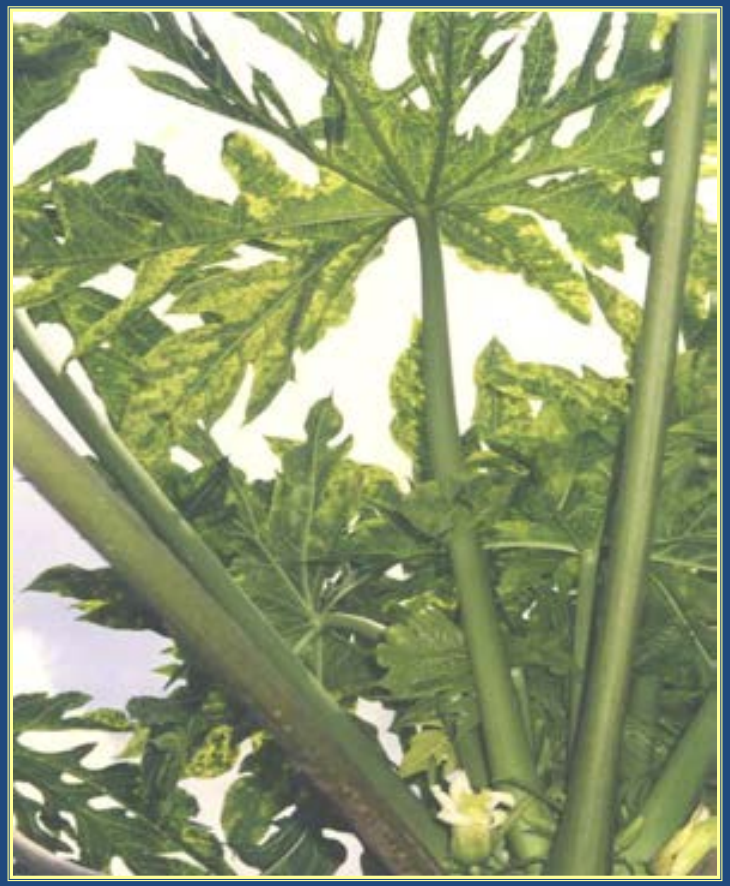
Biotechnology-assisted breeding methods

- Gene discovery and cloning
  - RNA extraction and cDNA synthesis
  - RT-PCR
  - Sequencing
  - Cloning of viral gene/s and development of gene construct
    - Amplify viral gene using specific primers
    - Clone the gene into a plasmid vector
    - Transform the gene construct into *Agrobacterium*
- Plant transformation and regeneration
  - Embryo isolation
  - Somatic embryogenesis
- Transformation of somatic embryos
  - Prepare bacterial suspension and OD reading
  - Co-cultivate somatic embryos in *Agrobacterium*
  - Incubate tissues in plasmolysis medium
  - Incubate tissues in selection medium
- Regeneration of transformed tissues
  - Proliferation of tissues
  - Rooting
  - Acclimatization & potting-out in soil



Breeding for Disease Resistance

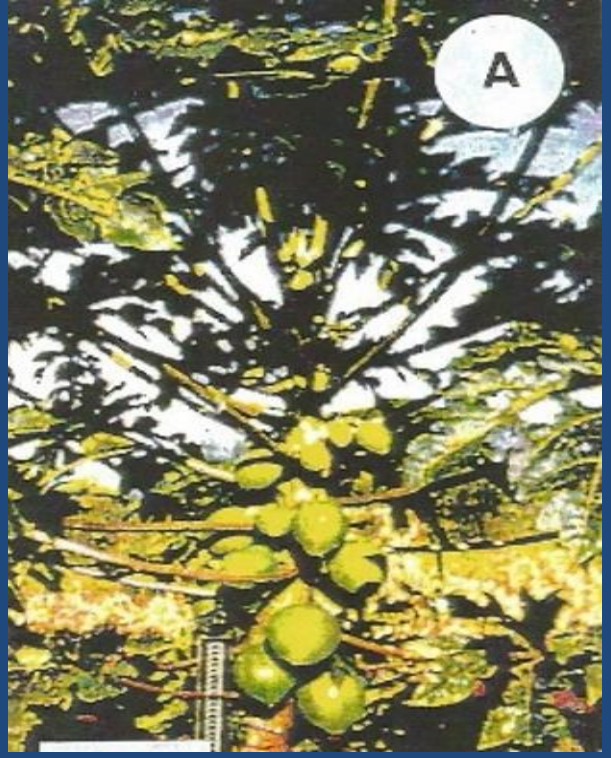
- Intergeneric hybridization & embryo rescue to develop PRSV resistant papaya



Chlorosis on the leaves

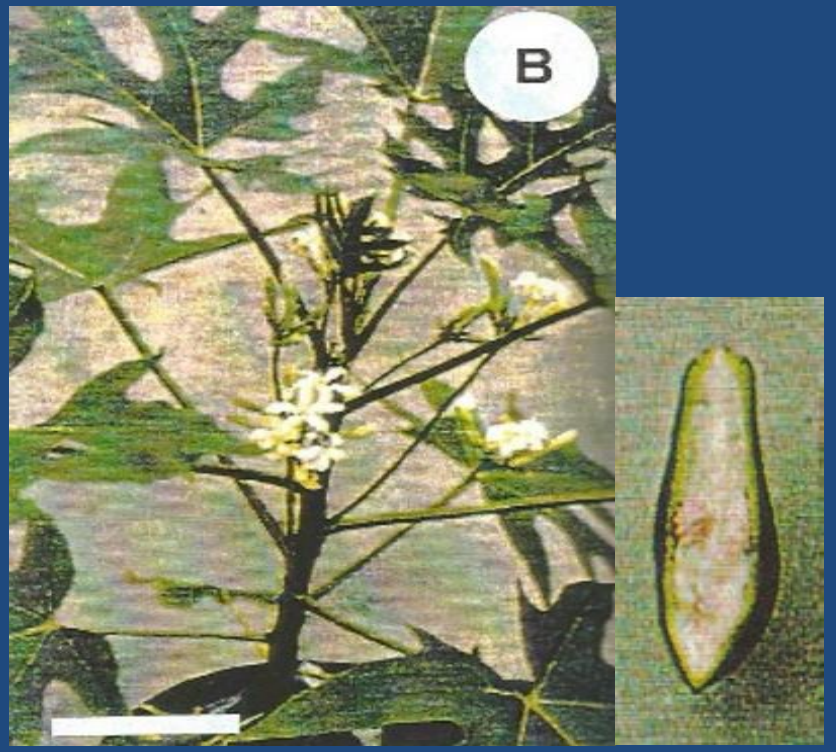
Concentric rings of the fruit

Symptoms of PRSV

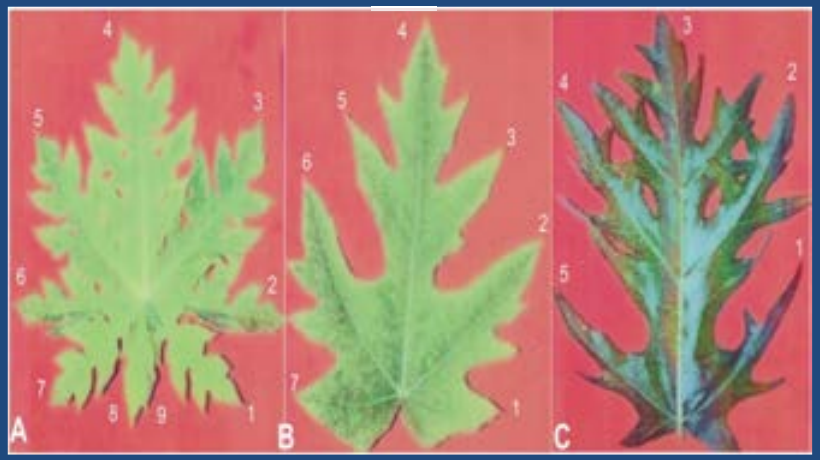


Carica papaya L.

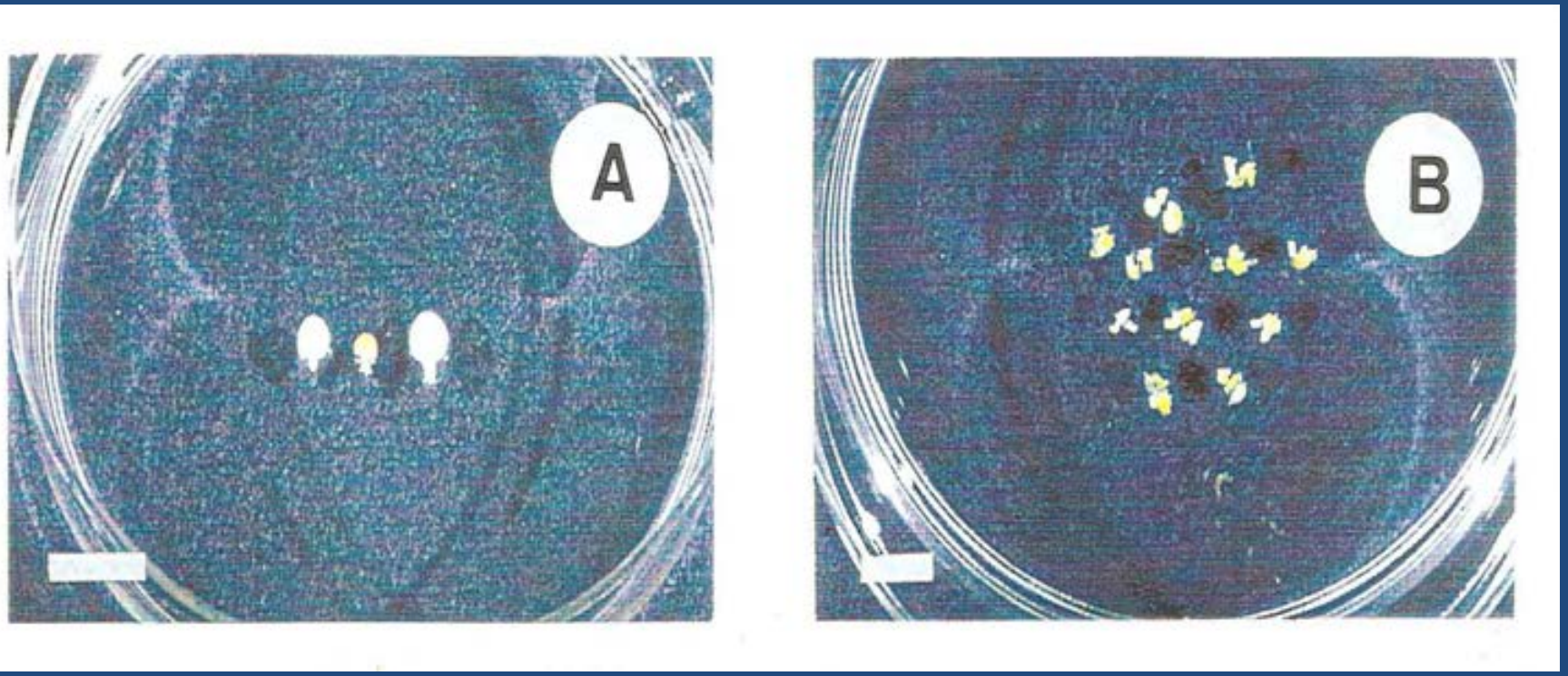
X



Vasconcellea cauliflora



F1 intergeneric hybrid



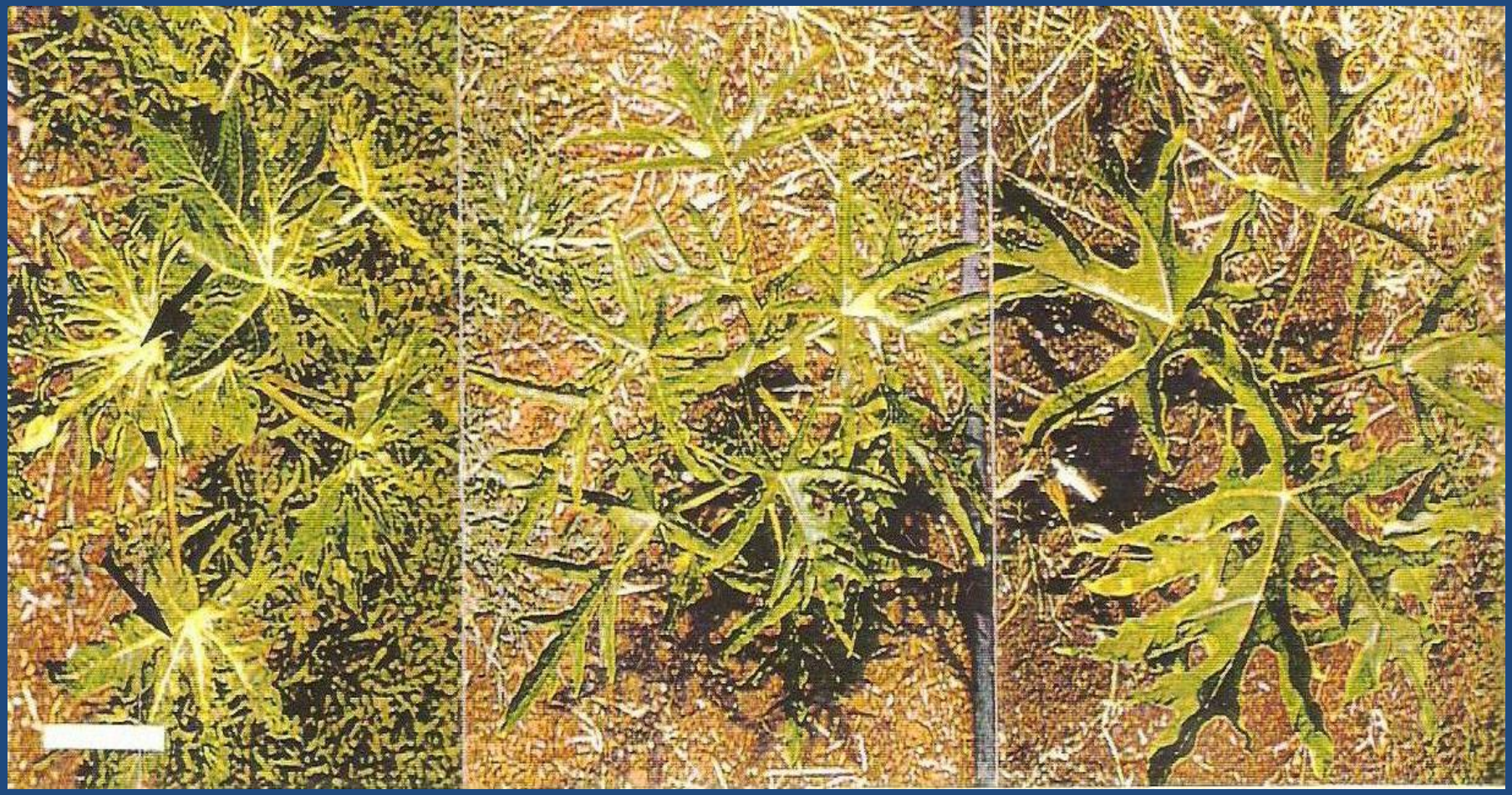
***Carica papaya* x *Vasconcella cauliflora* intergeneric hybrid embryos**

Single embryos of *V. cauliflora*, intergeneric hybrid & *C. papaya* (A)

Multiple embryos of *C. papaya* x *V. cauliflora* intergeneric hybrid (B)

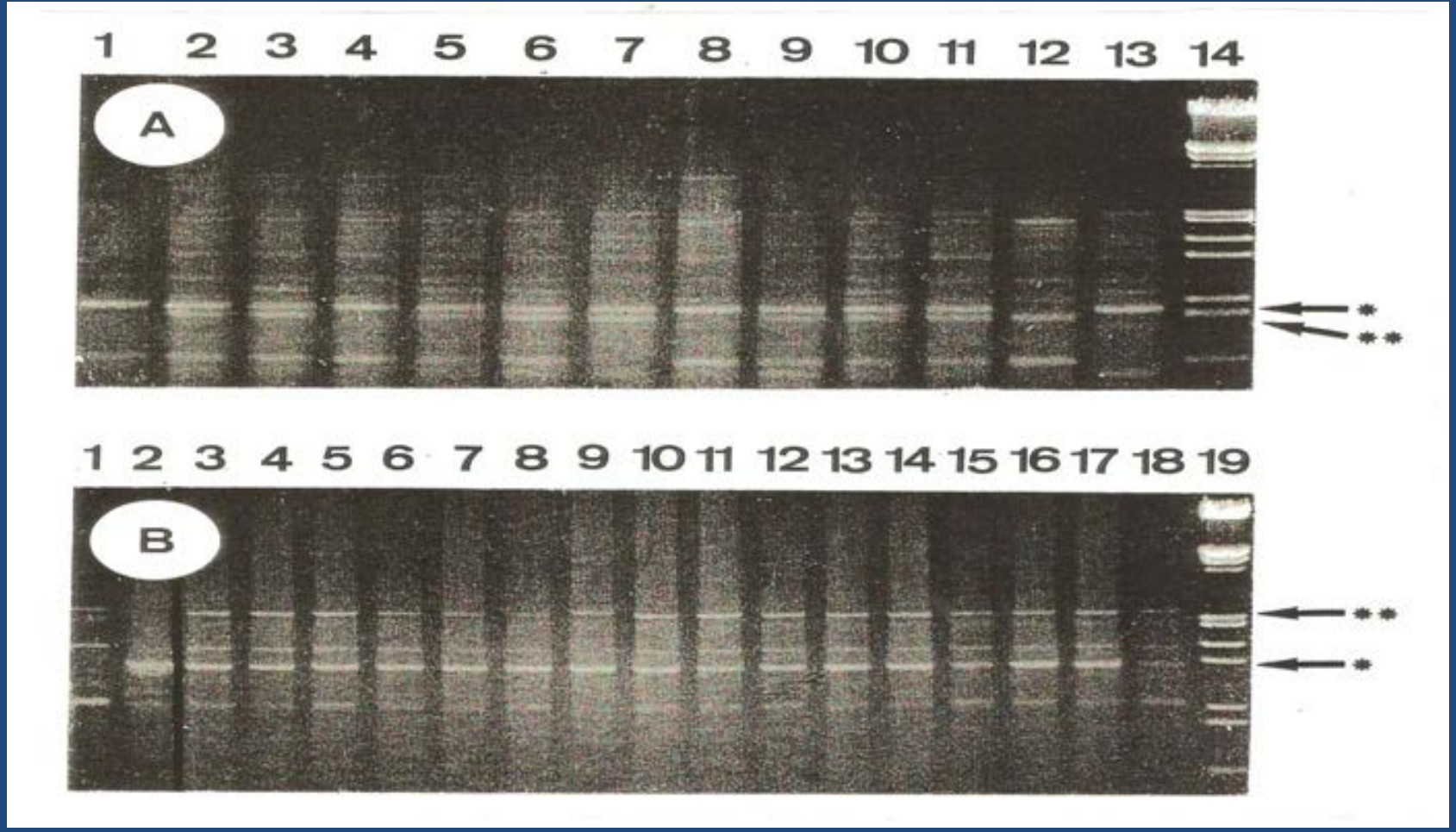
Germinating embryos of *C. papaya* x *V. cauliflora* intergeneric hybrid
Single embryo 5 days after germination on DF+BAP, NAA & GA_3 (liquid & solid) (A&B)
Multiple embryos germinating on liquid DF+BAP, NAA & GA3 (C)
Intergeneric hybrid seedlings 30 days after germination (D)

***C. papaya* x *V. cauliflora* intergeneric hybrid plants growing in the glasshouse**



***C. papaya* x *V. cauliflora* intergeneric hybrid undergoing screening for resistance to PRSV in the field. The plants have been previously inoculated with the virus 33 days earlier.**

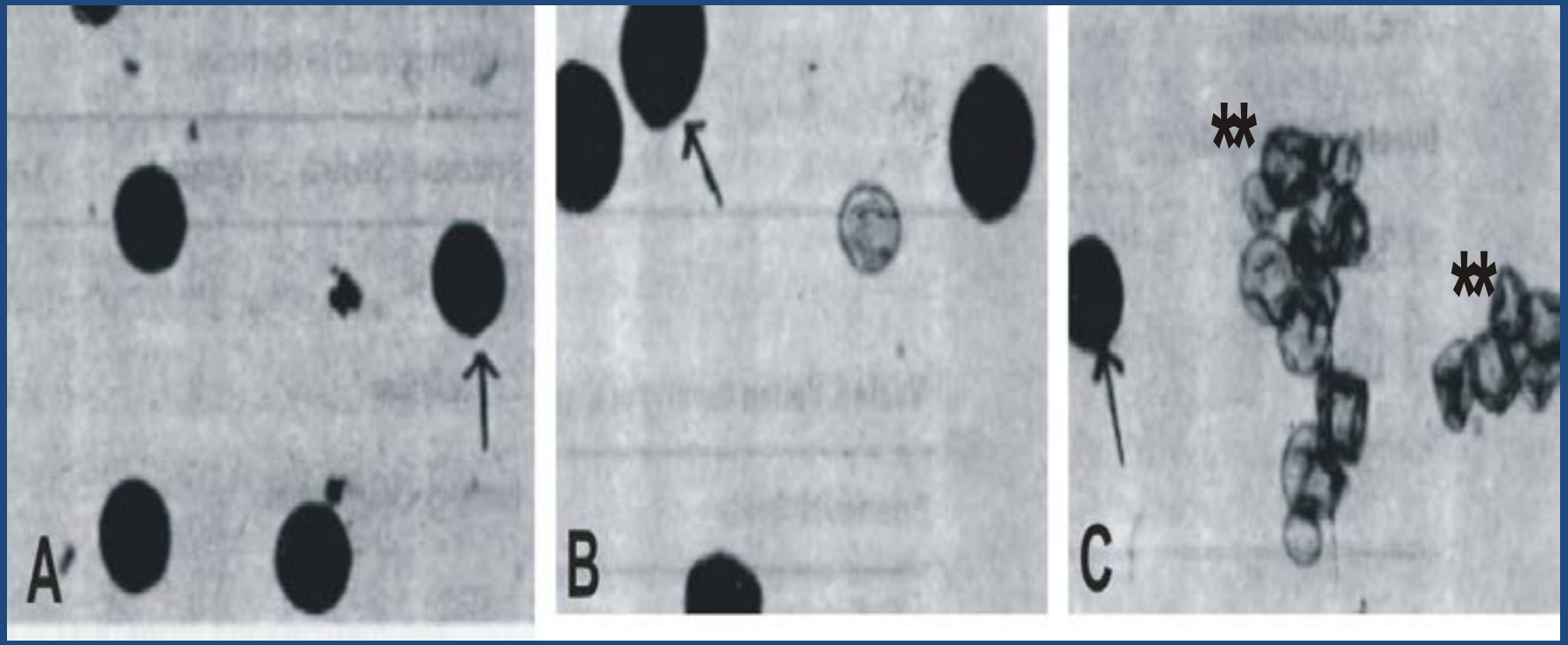
***C. papaya* exhibits typical PRSV symptoms like chlorosis and leaf distortion (A)**
***C. papaya* x *V. cauliflora* intergeneric hybrid without PRSV symptoms (B)**
V. Cauliflora with no PRSV symptoms (C)



Band profiles of *C. papaya* x *V. cauliflora* intergeneric hybrids generated by RAPD primers OPA-09 and OPA-07 resolved in 1.2% agarose gel and strained with ethidium bromide.

A) OPA-09: Lanes 1-12: *C. papaya* x *V. cauliflora* (Lanes 2, 3, 5,7, 91-11)
Lane 12: *V. cauliflora* (800 bp)**
Lane 13: *C. papaya* (850 bp*)
Lane 14: MW marker

B) OPA-07: Lane 1: V. *cauliflora* (1600bp)**
Lane 2: *C. papaya* (1200 bp*)
Lane 3-18: *C. papaya* x *V. cauliflora*
Lane 19: MW marker



Pollen fertility of *C. papaya* (A), *V. cauliflora* (B), and integeneric hybrid (C).

Papaya chromosome number 2n=18

Methodology

Gene cloning

↓

Transformation and regeneration

↓

Greenhouse contained trial

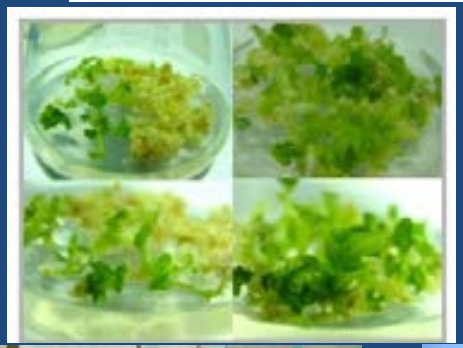
↓

Limited field trial

↓

Multi-location trial

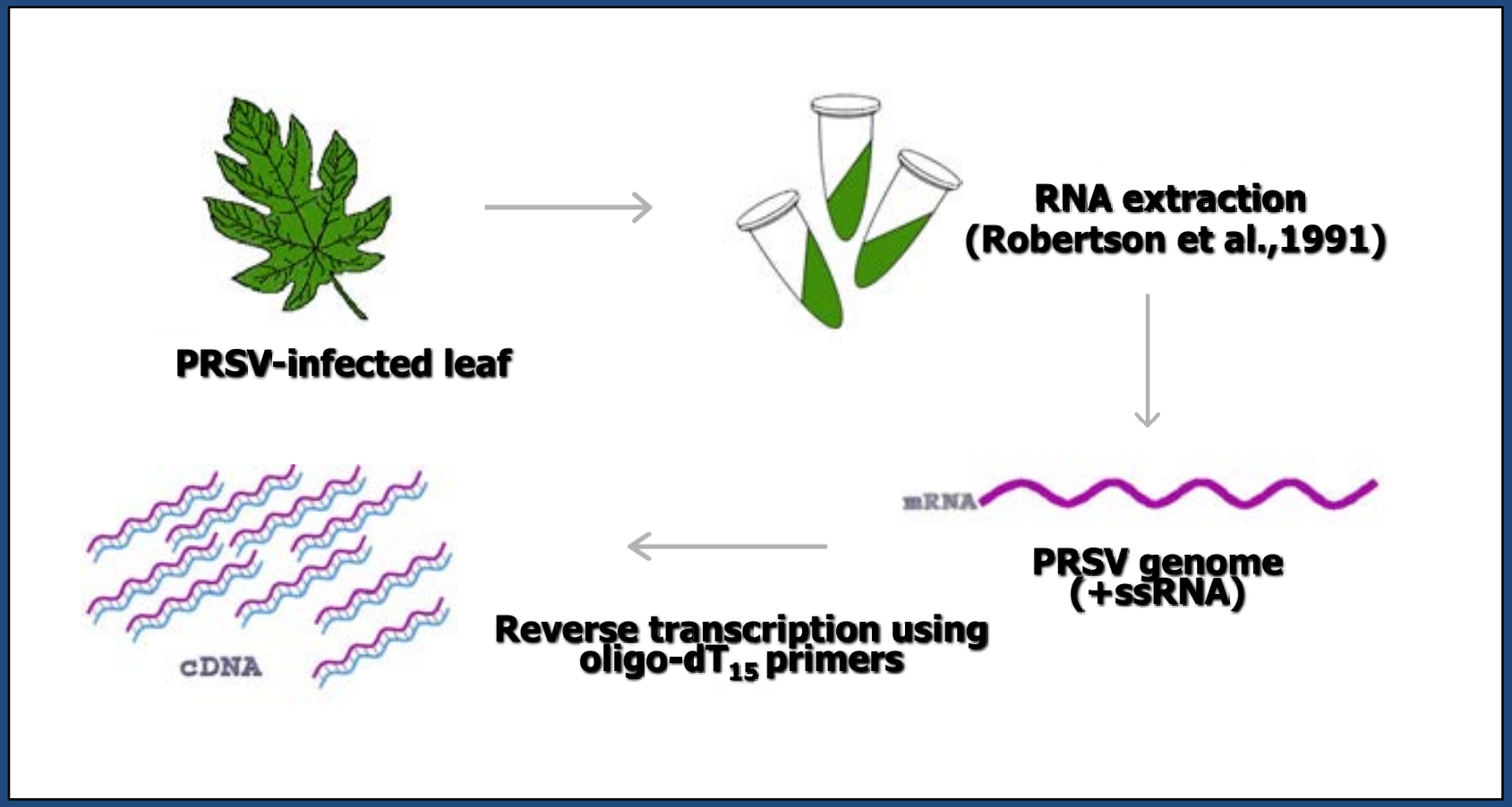
pMon 65306



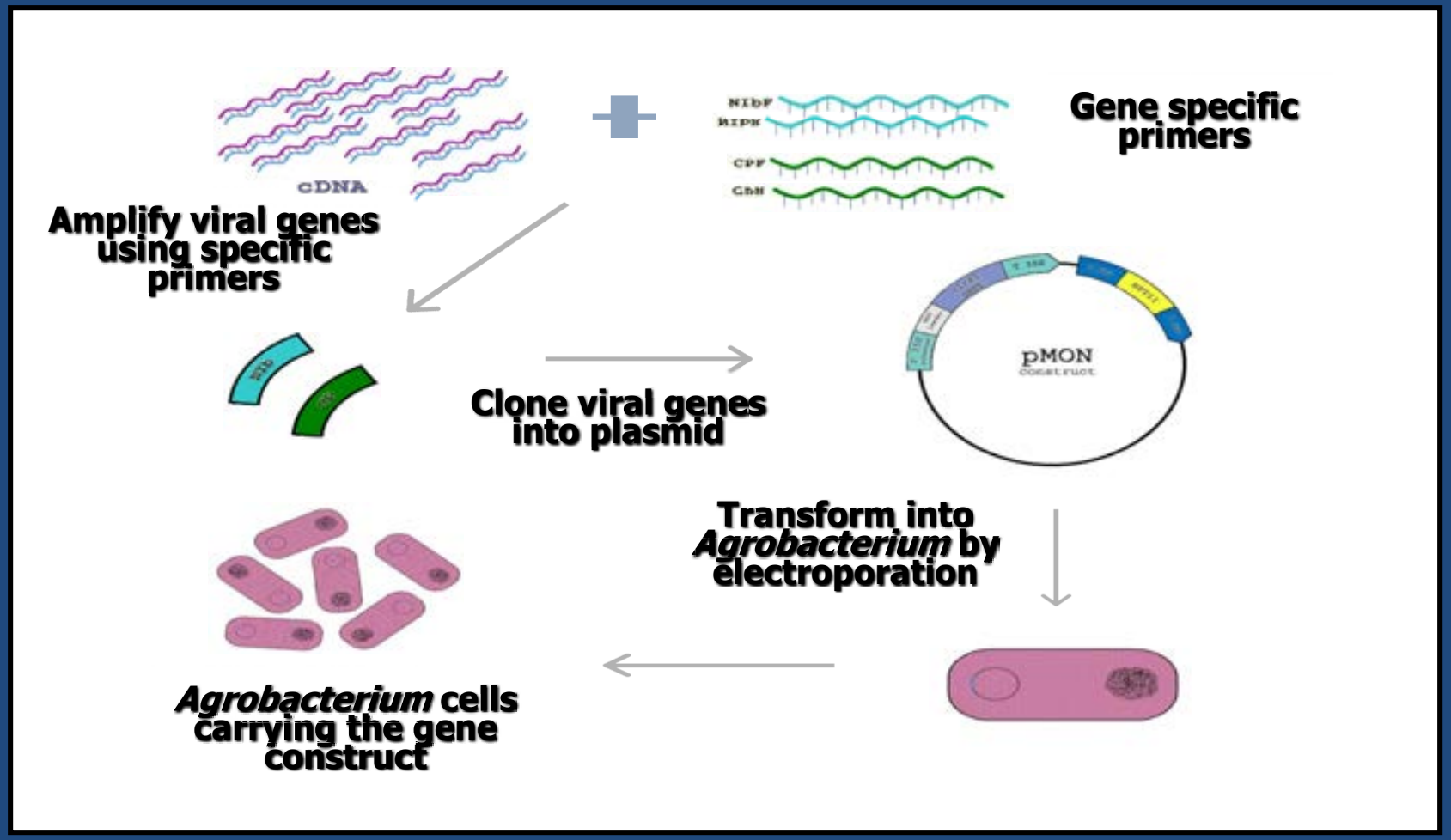
Coat Protein Gene-mediated Protection via *Agrobacterium* Transformation to Develop PRSV-Resistant Papaya

Cloning of Coat Protein Gene

RNA extraction and cDNA synthesis



Cloning of viral genes and generation of constructs



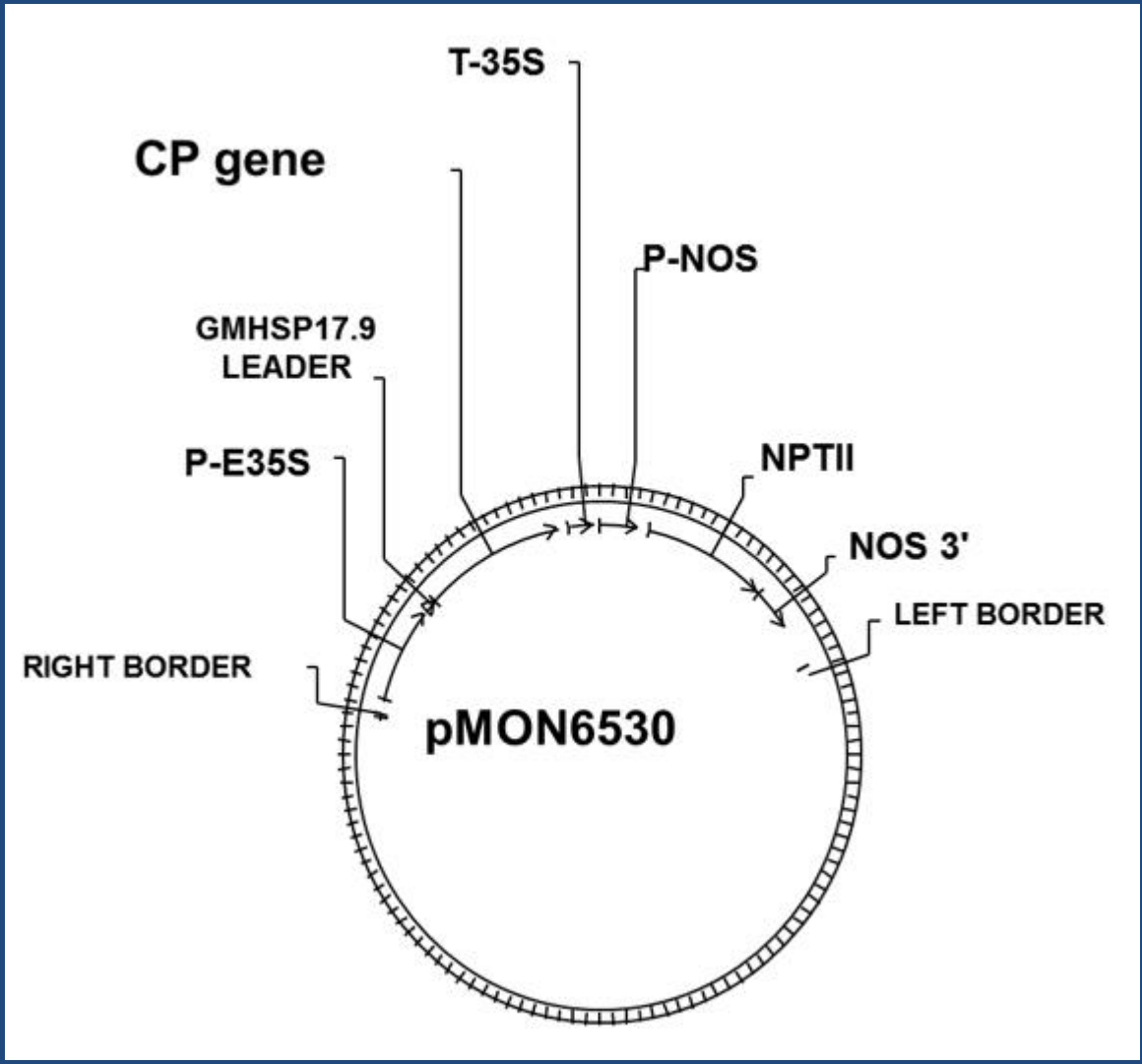
Cloning of PRSV-PH Coat Protein clones 1-7

Sequence of the cDNA of PRSV coat protein gene provided by Monsanto Co., St Louis, Missouri, USA

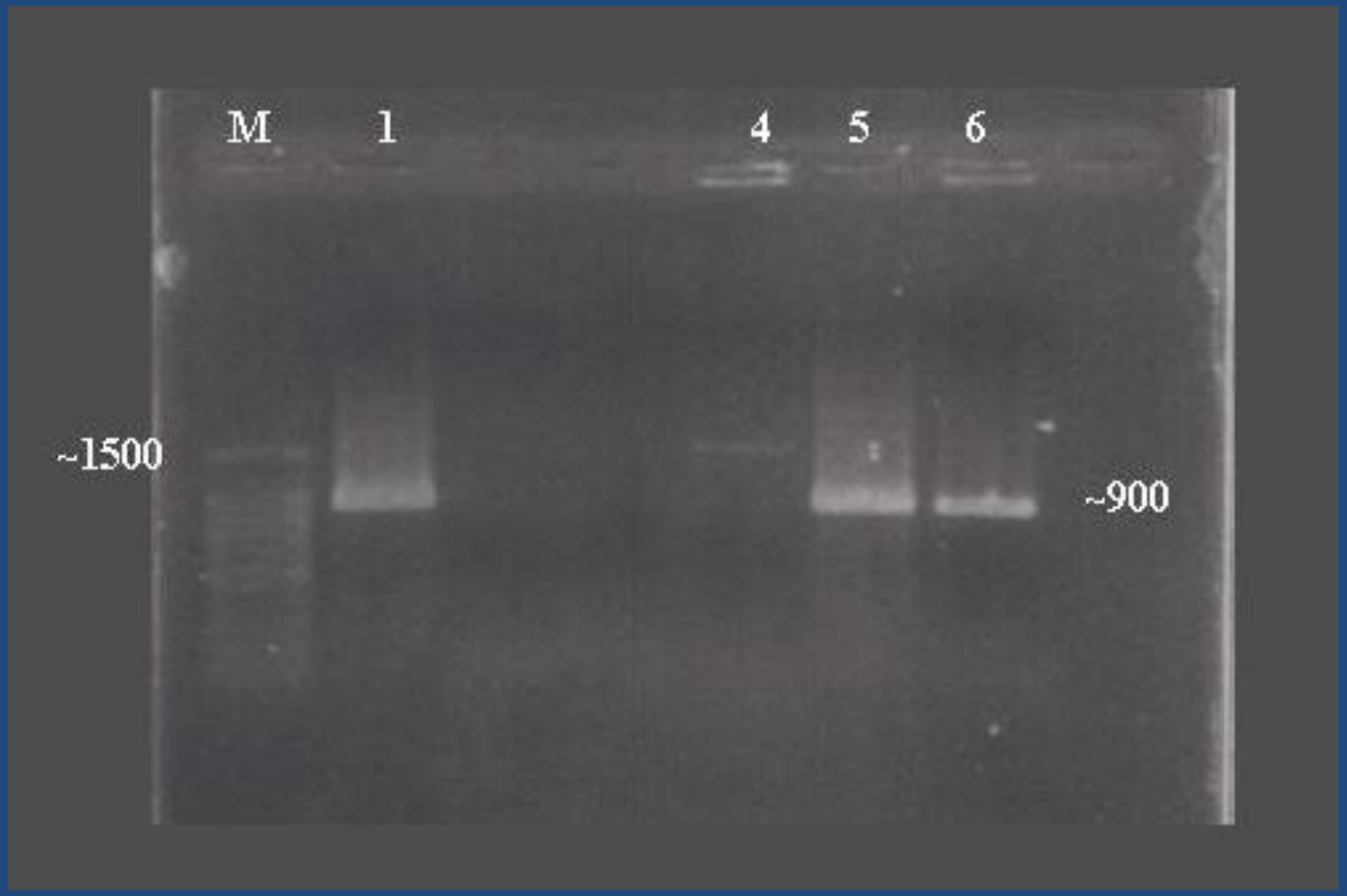
GCTGGTTTGAATGAGAAGCTCAAAGAGAAAGAAAAACAAAAAGAAGAAAAAGATAAACAAAA
A
GATAAGATTAATGAAGGTGCTAGTGACGGAAACGATGTGTCAACTGGCACAAAAACTGGAGA
G
AGAGATTAATGAAGGTGCTAGTGACGGAAACGATGTGTCAACTGGCACAAAAACTGGAGAG
CTGACAAGATGATTTTACCAAAAATAAGGGAAAAACTGTCCTTAATTTAAATCATCTTCTTCAG
TA
TAATCCGCAACAAATTGATATCTCAAACACTCGTGCCACTCAAACTCAATTTGAAAAGTGGTA
TG
AGGGAGTGCGAAATGATTACGGTCTTAACGATAACGAAATGCACGTAATGTTAAATGGTTTG
AT
GGTTTGGTGCATCGAAAATGGTACATCTCCAGACTGTCTGGTGTTTGGGTGATGATGGATGG
GG
AAATACAGGTTGATTATCCCATCAAGCCTTTAATTGAACATGCAACTCCCTCGTTTAAGCAAA
TC
ATGGCTCACTTCAGTAACGCGGCAGAAGCATACATCAGCAAAGAGGAATGCAACTGAAAGG
TAC
ATGCCGCGGTATGGAATAAAGAGGAATTTGACTGATATTAGCCTCGCCAGATATGCTTTCGA
TTT
CTATGAAGTGAATTCAAAAACACCTGCTAGAGCTCGTGAAGCTCATACGCAGATGAAAGCTG
CA
GCACTGTGTAACGCTGGCCGCAGAATGTTTGGCATGGACGGCTCTGTCAGCAACAAAGAAG
AA
AACACAGAACGCCACACAGTGGAAGACGTAAACAGAGACATGACATCT

Development of PRSV coat protein (cp) gene construct

CP clone



Electroporation of gene construct into *Agrobacterium*



PCR on *Agrobacterium* confirming the presence of gene inserts (lane 1=strain 65306 and lanes *5 & 6=strain 65310 for CP gene*; lane 4=strain 65307 for the replicase gene).

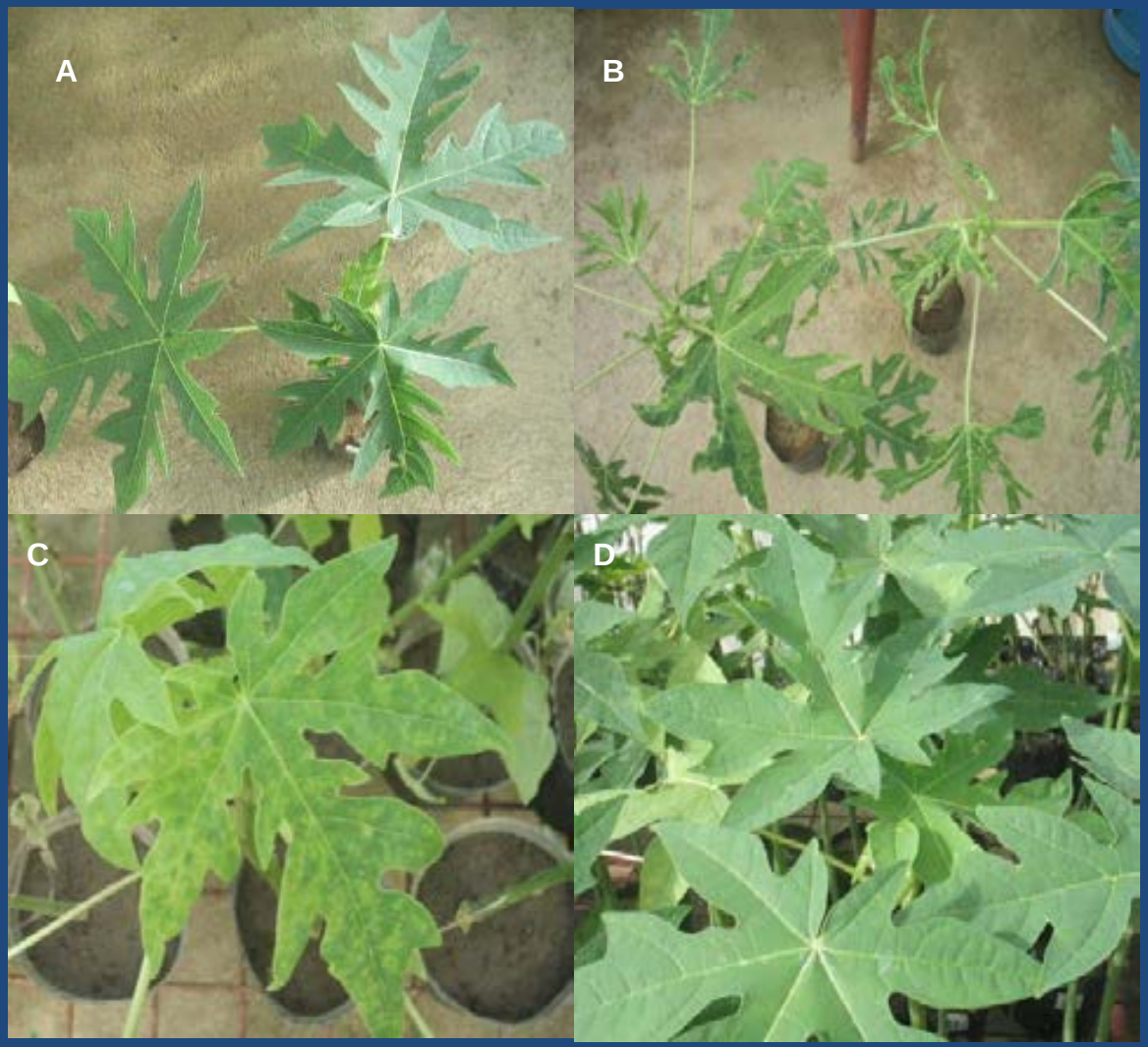
Fruiting habit of T_0 transgenic plants (~F_1)

Harvested fruits from T_0 transgenic plants (~F_1)

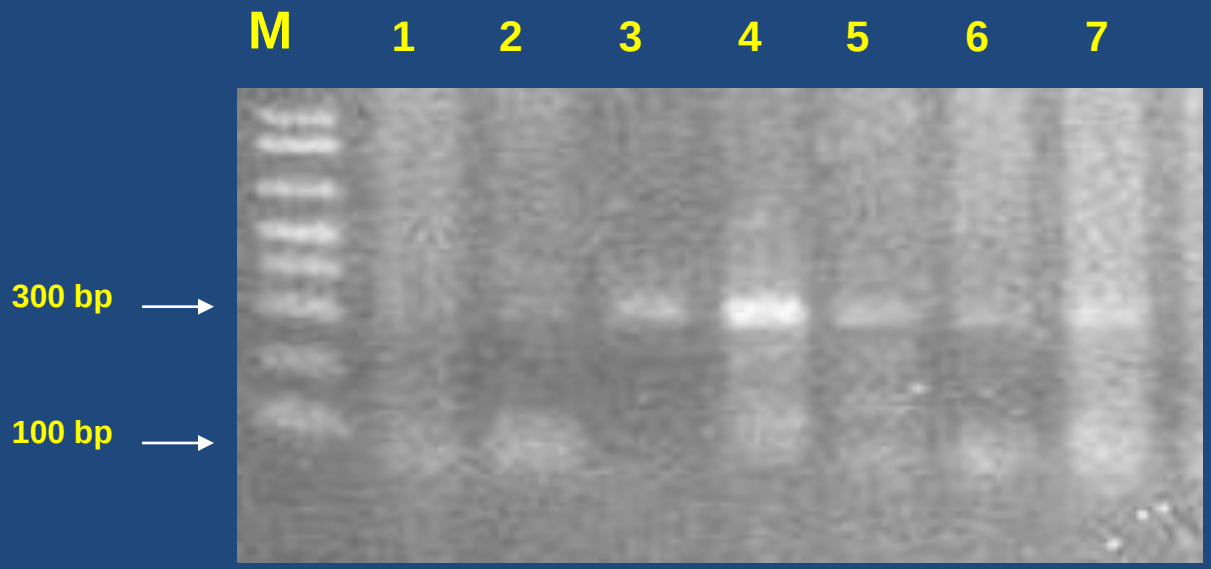
- 172 fruits were harvested
- Most are sweet (TSS 11-13°B)
- Average fruit weight: 337.5 gms

- T_1 fruiting trees (~F_2) inside the BL2

Reaction of T1 lines (~F_2) to virus challenge



- A. Uninoculated T1 plants;
- B. Inoculated untransformed control plants showing severe leaf deformation and mottling;
- C. Inoculated T_1 plant showing mosaic and mottling (susceptible);
- D. Inoculated T_1 plants, healthy and without symptoms (resistant)



Touch down PCR analysis of DNA samples. Lanes 1 &2 = untransformed 'Davao Solo'; 3 = Plasmid; 4 to 7 = T_1 lines.

PCR detection of cp gene inserted to papaya genome in the T_1 generation (~F_2).

The selected transgenic lines (T2 generation ~F3) and non-transgenic 'Davao Solo' control plants at flowering stage growing in the confined trial site.

Concept

Antisense ACC Oxidase Gene Technology to Develop Papaya with Extended Shelf-life Using *Agrobacterium* System

- **Papaya, a climacteric fruit contains the ACC oxidase (1-amino acid cyclopropane carboxylic acid) gene.**
- **This gene is responsible for the formation of ACC oxidase enzyme, which is the precursor of ethylene, the hormone responsible for the ripening of papaya.**
- **Hence, ACC oxidase gene was targeted for genetic engineering to delay the ripening process.**

Concept

- **If the ACC oxidase gene is isolated from the papaya peel and cloned in a vector or carrier and placed in antisense or reverse orientation (mis-hybridization of antisense RNA and normal mRNA), hence no ACO enzyme produced for ethylene production).**

- **If this antisense construct is then inserted into the papaya genome via *Agrobacterium*, the resulting transgenic papaya will produce reduced amount of ethylene, hence delaying the ripening.**

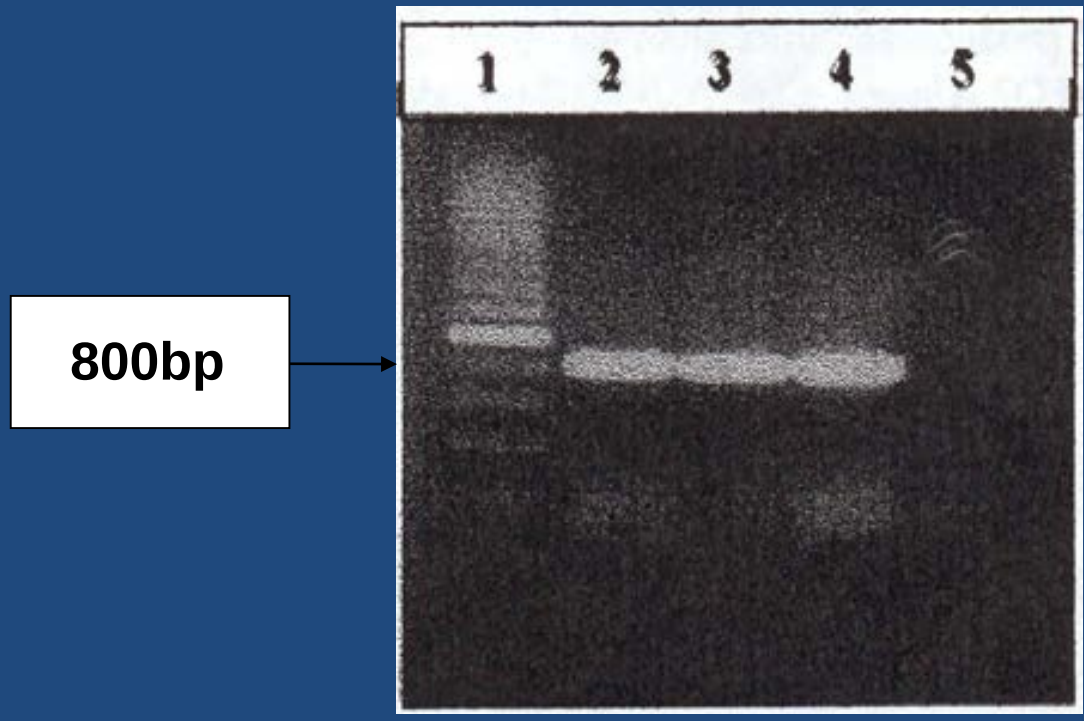
- **Before this happens, the ACC oxidase gene must be first isolated and cloned from the donor organism, the papaya.**

THE TECHNOLOGY

- Good quality total RNA from fully ripe samples was used in cloning the *ACC oxidase* gene using RT-PCR.

- A 800 bp partial cDNA sequence of the *ACC oxidase* gene of the yellow 'Solo' papaya was obtained.

The 800 bp amplicons generated by RT-PCR using primers VF01 and VF02.
(1) 200 bp DNA ladder
(2-3) *ACC oxidase* gene of 'Solo' at full ripe stage
(4) *ACC oxidase* gene of 'Eksotika'
(5) no DNA template



NCBI Sequence Viewer

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ASN.1 Summary FASTA TinySeq XML GenBank GBSeq XML INSDSeq XML

GenBank (Full) GI List Graphics XML default Show: 1 2 5 10 20 50 100 200 500 File Text Clipboard

1: AY570545. Carica papaya 1-a...[gi:48527657] Links

LOCUS AY570545 834 bp mRNA linear PLN 01-JUL-2004
DEFINITION Carica papaya 1-aminocyclopropane-1-carboxylate oxidase-like mRNA, partial sequence.
ACCESSION AY570545
VERSION AY570545.1 GI:48527657
KEYWORDS .
SOURCE Carica papaya (papaya)
ORGANISM Carica papaya
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; rosids; eurosids II; Brassicales; Caricaceae; Carica.
REFERENCE 1 (bases 1 to 834)
AUTHORS Perez,M.T.M., Magdalita,P.M., Laurena,A.C., Comia,R.L. and Bakar,U.K.A.
TITLE Development of transgenic papaya with delayed ripening characteristics containing the acc oxidase gene via agrobacterium-mediated transformation

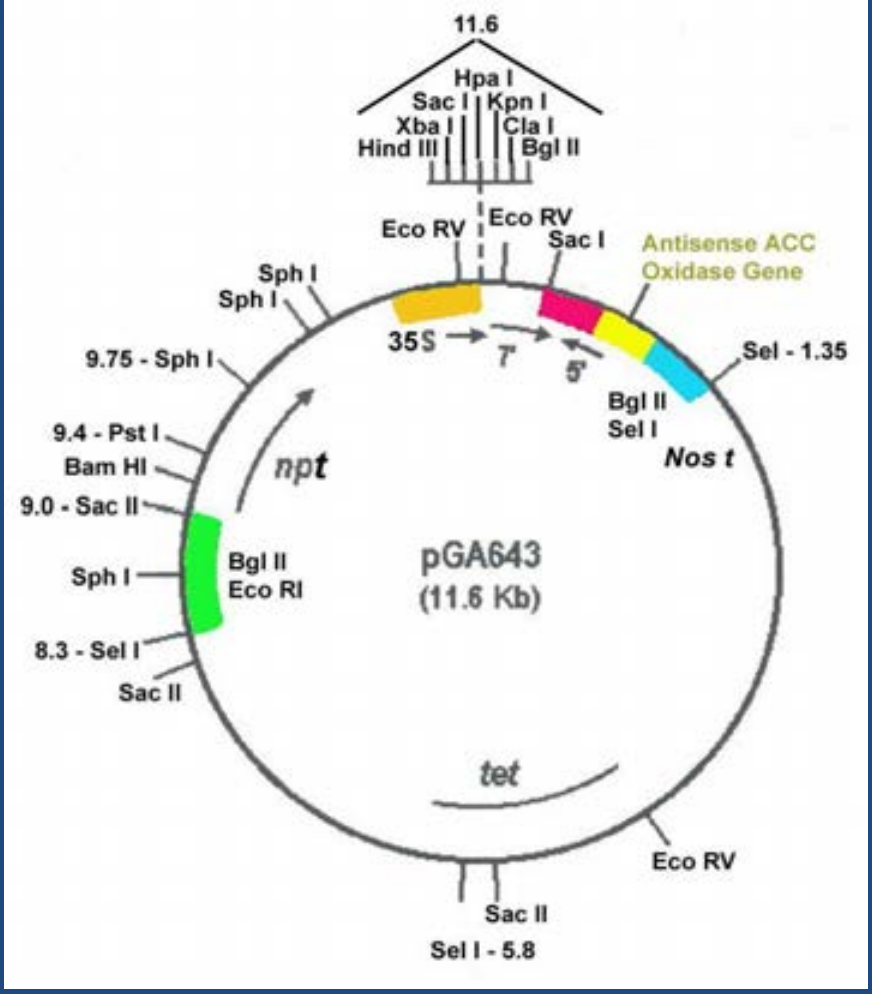
ACC oxidase cDNA gene sequence deposited to the National Center for Biotechnology Information (NCBI), part of the US National Library of Medicine, a branch of the National Institute of Health (NIH), Bethesda, Maryland, USA.

ORIGIN

1 gtgaattcgc ctgtgagaat tggggtttcc ttgagctggt gaatcatggg atcccaattg
61 agctgctgga cactgtcgaa agattgacaa aagggcacta cagaaaatgc atggagcaga
121 gattcaagga aataatggcg agcaaggggct tagatggtat ccaaacagag gtcactgata
181 tggactggga aagcaccttt ttcatacgcc atctccctga gcctaacata gctgagattc
241 cagatctcga cgatgaatac aggaaagtga tgaaagaatt tgctctgaaa ctggagaaaa
301 tagcagagga gcttcttgat ttgttatgcg agaatctcgg gctggaaaaa gggtatttga
361 aaaaagcatt ttacgggtcg agaggtccaa ctttcggcac caaagtcagc aactaccctc
421 catgccctaa accaaacttg atcaaagggc tccgggcaca caccgacgcc ggcggcatca
481 tcttgctctt ccaggacgac aaagtcagcg gcctccaact cctcaaagac ggcaaatggg
541 ttgatgttcc accaatgcgc cactccattg tcgtcaacct cggcgaccaa ctcgaggtga
601 ttaccaacgg gaaatacaag agcgtggagc acagagtggt ggcacaaacc gacgggacga
661 ggatgtcgat agcttctttc tacaccccgg aagcgacgcc gtgatttatc cggcgccgat
721 attggtggag aagaacagag gagaagaaaa cagcgtaccc gaaattcgtg ttcgaggatt
781 acatgaagct gtatgctggg ttgaagttcc aggcgaagga ctctagacga aagg

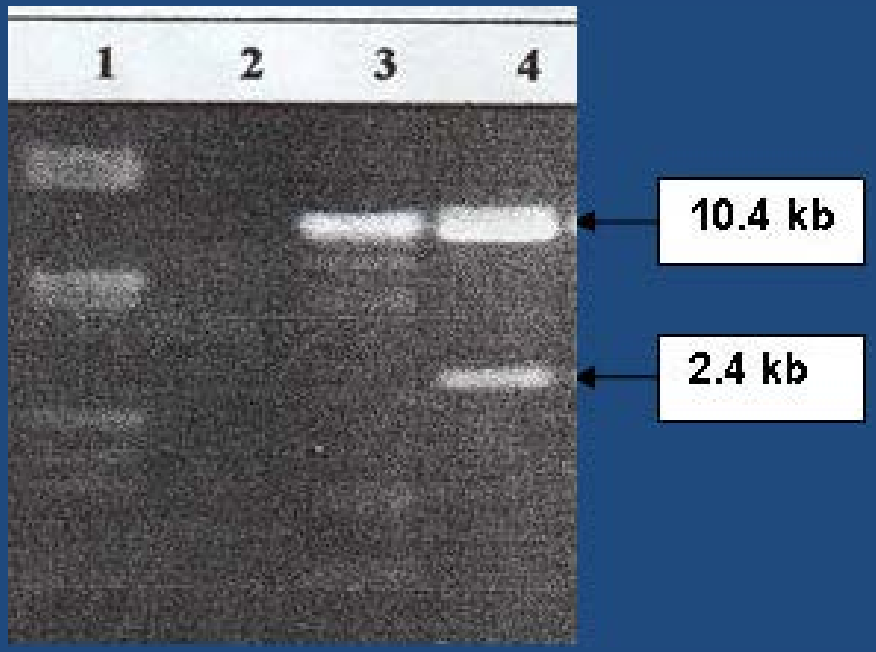
The 834 bp cDNA of *ACC oxidase* gene of Solo papaya represents a partial sequence of the entire *ACC oxidase* gene which is >1 kb.

The construction of *p*GA643 with antisense *ACC oxidase* and *Agrobacterium* transformation



- Plasmid map of expression vector *p*GA643 transformed with the antisense *ACC oxidase* gene
- The left and right borders, *npt II* and *tetracycline* resistance genes, CaMV 35s promoter, nos terminator, and the different restriction sites.
- Source: An et al., 1990, plasmid provided by MARDI, Malaysia

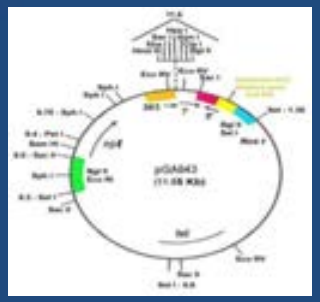
- **The construct *p*GA01 containing the insert obtained from *p*DvSI-15, has two major fragments namely, 10.4 kb and 2.4 kb in size.**
- **Hence the insert fragment in the construct is in the antisense orientation.**



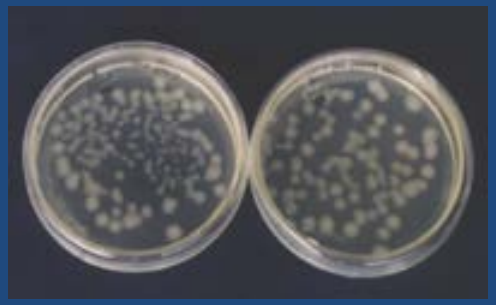
Restriction digests of *p*GA643 antisense *ACC oxidase* positive clones showing the fragments generated by the different restriction enzymes namely:
(1) *Hin*d *III/Eco* RI
(2) *p*GA643 *Hin*d III/*Eco* RI
(3) *p*GA01 *Hin*d III/*Eco* RI and
(4) *p*GA01 *Xba I/Bam HI*

Agrobacterium transformation

Transgenic papaya



*p*GA643 w/ ACO2



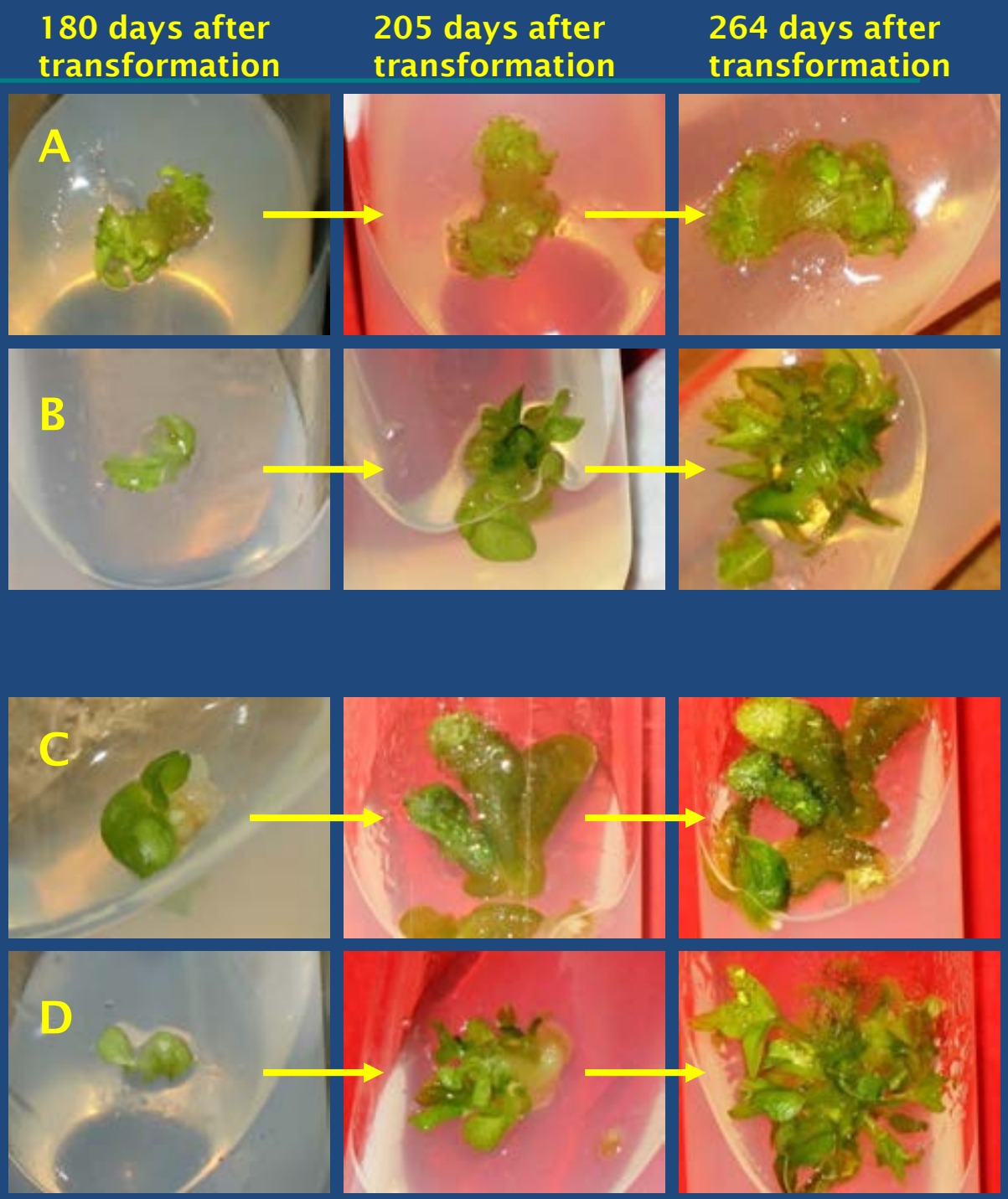
Agrobacterium w/ *p*GA643 w/ ACO2

Somatic embryos

Agrobacterium suspension (w/ ACO2)

Transformed somatic embryos undergoing selection

Regenerated plantlets



DFH + BL LOW

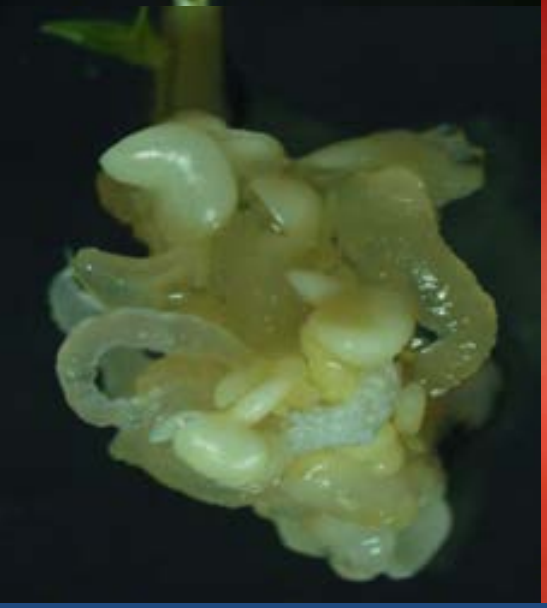
2 weeks old transformed tissues

Regeneration using brassinolide

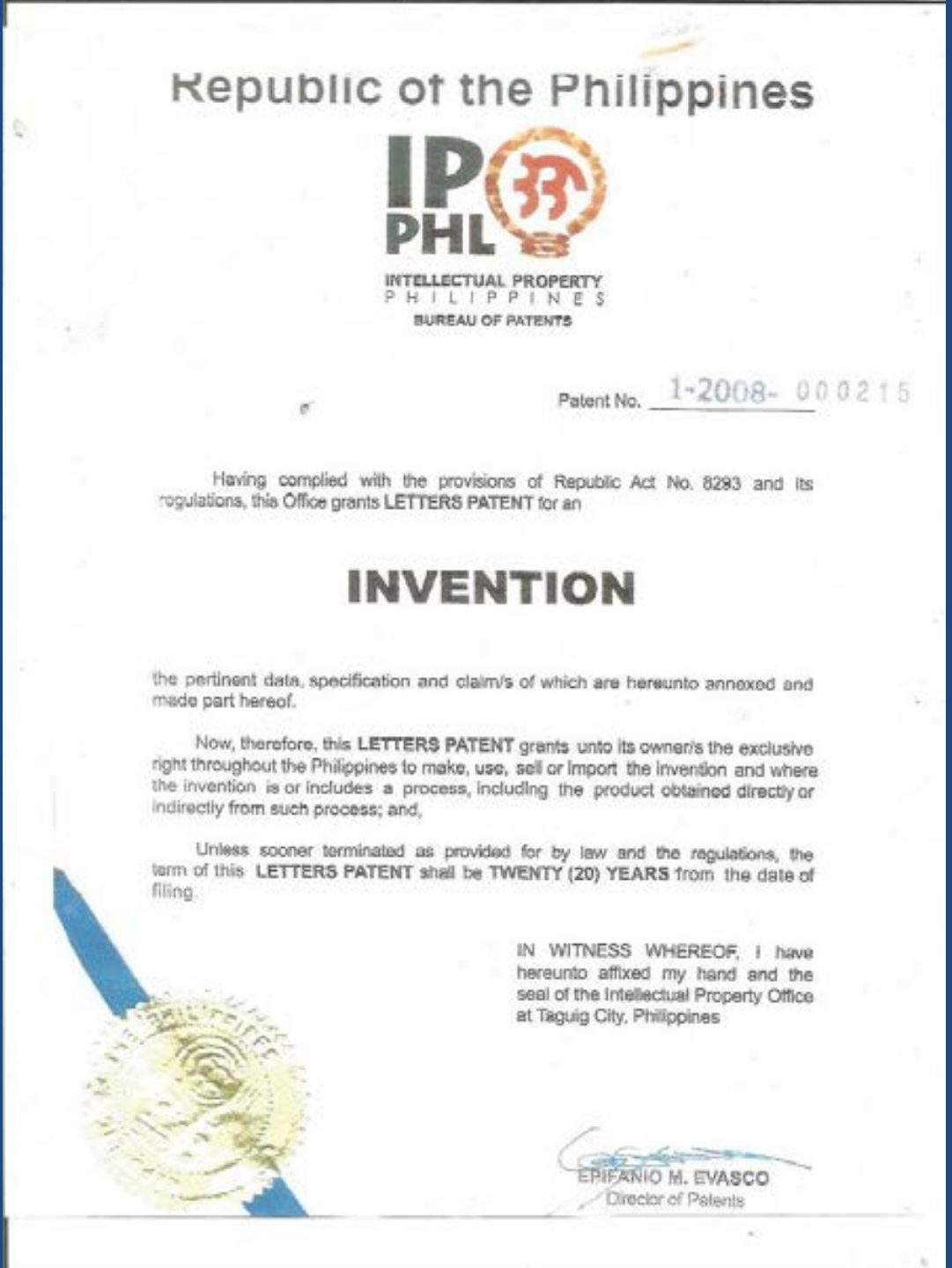
DFH + BL LOW

3 weeks old transformed tissues

Transgenic plants containing the antisense ACC oxidase gene



Transgenic papayas with antisense ACC oxidase gene under BL2 Greenhouse conditions



Republic of the Philippines

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Patent No. 1-2008-000215

Having complied with the provisions of Republic Act No. 8293 and its regulations, this Office grants **LETTERS PATENT** for an

INVENTION

the pertinent data, specification and claim/s of which are hereunto annexed and made part hereof.

Now, therefore, this **LETTERS PATENT** grants unto its owner/s the exclusive right throughout the Philippines to make, use, sell or import the invention and where the invention is or includes a process, including the product obtained directly or indirectly from such process; and,

Unless sooner terminated as provided for by law and the regulations, the term of this **LETTERS PATENT** shall be **TWENTY (20) YEARS** from the date of filing.

IN WITNESS WHEREOF, I have hereunto affixed my hand and the seal of the Intellectual Property Office at Taguig City, Philippines

EPIFANIO M. EVASCO
Director of Patents

Patent Application No: 1/2008/000215

Date of filing: June 27, 2008

Publication Date: Nov. 15, 2010

Letters Patent Issued: March 11, 2015

Application of Antisense ACC Oxidase Gene Technology to Extend Shelf-Life of Export Winner Fruits

FUTURE TECHNOLOGY FOR BIOTECHNOLOGY-ASSISTED BREEDING OF FRUIT CROPS

New Breeding Techniques (e.g. Gene Editing) for Developing Insect Resistant Papaya

Example: Developing papaya resistant to fruit fly, aphids and mites using Crispr-Cas 9 technology

SUMMARY

- Development of fruit varieties with good fruit qualities can be hastened by on-site selection and evaluation.
- Papaya F_1 hybrids tolerant to PRSV can be developed by conventional hybridization.
- Genetic markers can be used for early detection of sex forms in papaya in the seedling stage.
- Micropropagation is used to multiply proven papaya F_1 hybrid trees.
- Direct conduct of on-farm trials and technology piloting of PRSV-tolerant papaya F_1 hybrids in farmer cooperators' fields can showcase performance of a variety in the rural areas.

SUMMARY

- Intergeneric papaya hybrid resistant to PRSV is difficult to develop due to pollen infertility.
- Transgenic papaya resistant to PRSV and with extended shelf-life is difficult to develop due to regulatory problems associated with GMOs.
- Papaya hybrids with double resistance to PRSV and BCR can be developed.
- Because of problems associated with GMO's, new breeding techniques like gene editing can be used to develop insect resistant papaya varieties in the future.

ACKNOWLEDGEMENTS

Classical Breeding Projects:

- Papaya variety development for climate change adaptation (2013-2018)
- Field trial of new papaya ringspot virus tolerant papaya F_1 hybrids (2016-2019)
- Sinta Technology: Further Improvements for Commercial Seed Production (2008-date)
- Breeding and selection of hardy fruit crops for climate change adaptation (2013-2018)
- Selection of drought- and disease-resistant species for climate change adaptation (2012-2015)
- Development of Bacterial Crown Rot Tolerant Papayas (Part of ACIAR Project) (2014-2018)
- European Union-SEA Partnering: Regional Network for Research and development (2016-2017)

Biotechnology-assisted Breeding Projects:

- Development of PRSV-resistant papaya by interspecific hybridization and embryo rescue (2000-date)
- *Agrobacterium*-mediated transformation of papaya for papaya ringspot virus resistance (2004-2008)
- Development of papaya with delayed ripening characteristics containing the antisense ACC oxidase gene via *Agrobacterium* system (2002-2008)
- Micropropagation and artificial seed production of PRSV-tolerant papaya F_1 hybrids for field trials (2012-2018)

ACKNOWLEDGEMENTS

Funding Agencies:

- DA-BAR
- DA-BIOTECH
- PCAARRD-DOST
- EWSC & UPLB-FI
- ACIAR
- AusAID
- ABSP II
- EU-SEA Cooperation

Farmer-Cooperators in the Countryside:

- Montealegre's Fruit Farm - Silang, Cavite
- ACS Farm – Sto. Tomas, Batangas
- Federico's Farm – Sto. Tomas, Batangas
- Solsoloy's Farm – Batac, Ilocos Norte
- EWSC Research Farm – Solsona, Ilocos Norte

UPLB Research Institutions:

- CTTE
- BIOTECH
- IPB, CAFS

Papaya and Hibiscus Research Team
Motto: *"Scientia ac Labore"*

MARAMING SALAMAT PO AT MABUHAY!!!