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Papaya

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Introduction

Papaya is a major tropical fruit grown commercially in India, Brazil, Mexico, Australia, Hawaii, Thailand, South Africa, Philippines, Indonesia and Taiwan. India is the largest producer of papaya contributing 25% of total world production. India is among top ten countries in the world growing 50,000 hectares or more under transgenic crops. In addition to India, other developing countries are China, Indonesia, Argentina, Brazil, Mexico and South Africa has commercialized transgenic crops. It is now being realized that the genetic base on which the conventional breeding is dependent is either shrinking or is not available because of crossability barriers. Hence, search for alternate strategies has become mandatory if the pace of horticultural growth has to be matched with the ever-increasing demand for fruits. Fortunately, advancements made in recent years in the area of recombinant DNA technology and genomics have provided an altogether new dimension to horticultural research. Research on marker assisted selection and transgenics in fruit crops has created interest globally. A large number of economic genes have been mapped, tagged, cloned, sequenced, or characterized for expression and are being used for genetic tailoring of plants through molecular breeding. An array of markers in the arsenal from RFLP to SNP; tools such as BAC, YAC, ESTs and microarrays; local physical maps of target genomic regions; and the employment of bioinformatics contributing to all the "-omics" disciplines are making the journey more and more enriching (Kole, 2007). Papaya entered in the genomics business little late. However, a rough draft of papaya genome is available now. Genetic engineering research in papaya took lead and transgenic plants expressing virus genome sequence resist attack by corresponding viruses is a reality.

Papaya cultivation is hampered severely due to problems like prevalence of papaya ring spot virus (PRSV) and papaya leaf curl virus (PaLCuV). PRSV is spread all over the country. However, PaLCuV is confined to North India. Resistance breeding has not been successful in

tackling papaya ring spot virus. PRSV resistant transgenic papaya has been developed and commercialized in 1998 in Hawaii, USA by Dr. Dennis Gonsalves and his team. Papaya is also susceptible to pathogen *Phyophthora palmivora* which causes root, stem and fruit rot. Papaya cultivation is severely affected by frost particularly in Northern part of India. A frost tolerant papaya variety can boost papaya production in Subtropical region of India. Post-harvest losses are high in papaya due to its perishable nature. There is an urgent need to enhance shelf life of papaya by using antisense technology. This chapter describes important tools of biotechnology such as allele mining, genomics and transgenics in papaya

Gene Mining and Genomics

There are three distinct types of *C. papaya* plants: (1) dioecious papayas that have male and female flowers on separate trees, (2) gynodioecious papayas bare female flowers on some trees and bisexual (hermaphrodite) flowers on others, and (3) trioiceous papayas have male, female, and hermaphrodite flowers in different plants. Many landraces and cultivars present hermaphrodite plants, bearing perfect flowers and producing fruits shaped from long-cylindrical to ellipsoidal, which are preferred for commercial production. Papaya is an ideal fruit crop for biotechnology research because of its relatively small genome (372 Mbp) size (Arumugunathan and Earle, 1991). However, not much information has been generated so far with respect to allele mining and genome mapping. Recently, Oliveira *et al*. (2010) used 81 new microsatellite markers for *Carica papaya* L. previously identified by data mining for polymorphism using 30 germplasm accessions and 18 landraces. Most microsatellites were polymorphic (73%), with an observed number of alleles per locus ranging from one to eleven. The levels of observed and expected heterozygosity for 51 polymorphic loci varied from 0.00 to 0.85 and from 0.08 to 0.82, averaging 0.19 and 0.59, respectively. Forty-four percent of microsatellites showed polymorphism information content (PIC) higher than 0.50. The compound microsatellites seem to be more informative than dinucleotide and trinucleotide repeats in average alleles per locus and PIC. Among dinucleotides, AG/TC or GA/CT repeat motifs exhibited more informativeness than TA/AT, GT/CA and TG/AC repeat motifs. The neighbor-joining analysis based on shared allele distance could differentiate all the papaya accessions and landraces as well as differences in their genetic structure. This set of markers will be useful for examining parentage, inbreeding and population structure in papaya.

High-density genetic maps are prerequisite for isolation and cloning of gene of interest, genomic dissection, marker assisted selection etc. Genetic mapping of many crops has been accomplished (Mishra *et al*., 2007a). Ma *et al.* (2004) constructed a high density genetic map of papaya using 54 F2 plants derived from Kapoho and Sun Up cultivars with 1501 markers including 1498 AFLP markers, PRSV *cp* markers, morphological sex types and fresh fruit colour. These markers are mapped in to 12 linkage groups at a LOD score of 5.0 and recombination frequency of 0.25. The 12 major linkages groups covered a total length of 3294.2 cM, with an average distance of 2.2 cM between adjacent markers. This map revealed severe suppression of recombination around the sex determination locus with a total of 22.5 markers co segregating with sex type. The cytosine bases were found to be highly methylated in this region on the basis of distribution of methylation sensitive and insensitive markers (Fig 4.1).

BACs are the most commonly employed vectors for carrying large DNA fragments. Ming *et al.* (2004) reported construction of bacterial artificial colony (BAC) from papaya. The BAC library consists of 39168 clones from two separate ligation reactions. The average insert size of library is 132 kb. The entire BAC library was estimated to provide 13.7 x papaya genome equivalents, excluding the false positive and and chloroplast clones. High-density filters were made containing 94 % or 36864 clones of the library with 12.7 x papaya-genome equivalents. Eleven-papaya cDNA and ten *Arabidopsis* c DNA probes detected an average of 22.8 BACs per probe in the library (Table 4.1). Liu *et al* (2004) reported the discovery of an incipient Y chromosome in papaya of which 10 % is a non-recombining, rapidly evolving, sex-determining region flanked by normal autosome-like regions that comprise the remaining 90 % of chromosomes. This proves that sex chromosome evolve from autosome. The severe suppression of recombination and excessive divergence between homologues in the region containing the

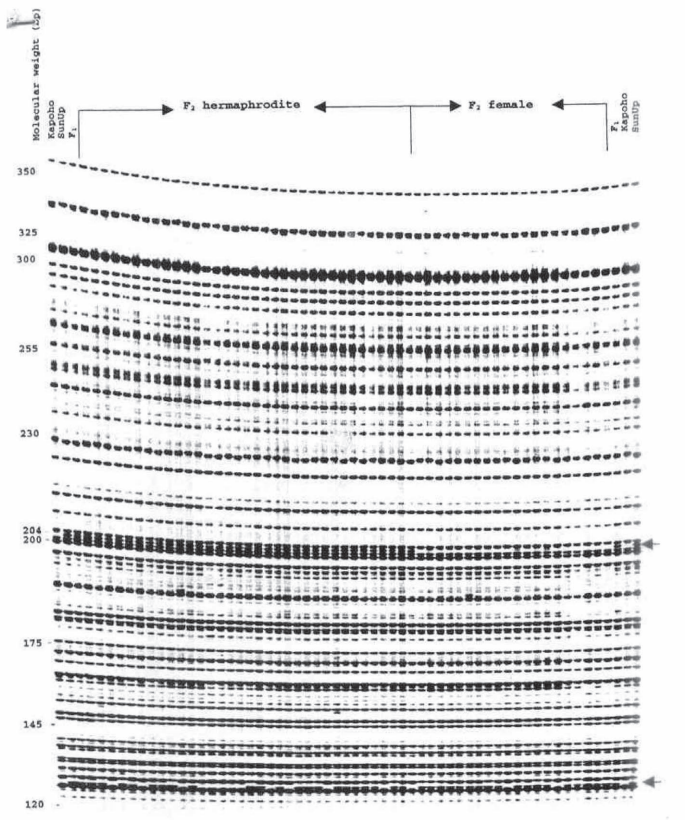

Fig. 4.1: AFLP products amplified by the primer pair E-GCT/M-AG. The Sun Up dominant marker between 200 and 204 bp is cosegregating with sex

Source: Genetics 166:419-436

papaya sex-determining genes indicates that this is an incipient sex chromosome. On the basis of size of present contig map (2.5 Mb) and 57 % of cpsm markers that have been accounted for, the physical size of MSY is estimated at 4-5 Mb or 10 % of papaya's primitive chromosome. The incipient sex chromosomes of papaya may yield insights about earlier stages of sex chromosome evolution. The small physical size of MSY region and the mosaic arrangements of sequence degradation indicate a recent origin of the papaya sex chromosomes (Table 4.2).

Table 4.1: Results of screening of papaya BAC library with homologous and hetrologous cDNA, rDNA and cpDNA

Probes	*No. of bands*	*Ligation 1*	*Ligation 2*	*Total*
AEST9	2	5	8	13
AEST18	2	5	7	12
AEST36	2	6	14	20
AEST37	6	0	0	0
AEST47	2	10	17	27
AEST48	3	6	17	23
AEST63	4	8	17	25
AEST64	2	9	9	18
AEST69	4	16	47	63
AEST127	2	19	29	48
CPF9A1	5	8	7	15
CPF9A2	3	0	0	0
CPF9A3		6	5	11
CPF9A4		9	11	20
CPF9A5		4	6	10
CPF9A6		3	25	28
CPF9A7		10	11	21
CPF26A3		3	3	6
CPF26A7		18	19	37
CPF26A4&5		19	27	46
Total	44	154	279	433
18sPxp108		18	43	61
rop B and *teunk*		211	293	504

Source: Ming *et al*., 2001

The rough draft genome of transgenic papaya has also been worked out (Ming *et al*.,2008) which revealed significant information pertaining to papaya genomics. The comparison of available sequenced genomic data of papaya and grape is given in Table 4.3.

Table 4.2: Fine mapping with SCAR markers in MSY region of papaya

Population	*Progeny*	*Hermaphrodite*	*Female*	*SCAR markers*	*Recombinant*
Kapoho x SunUp	F_2	335	150	W11	0
Kapoho x SunUp	F_2	335	156	W11. T12, cpbe55	0
Kapoho x SunUp	F_2	481	274	W11.T12,cpbe55	0
Kapoho x Saipan Red	F_2	175	49	cpsm31,cpsm10	0
AU0 x SunUp	F_2	170	65	W11,T12,cpsm54	0
Total	2,190	1,496	694		0

(Source: Liu *et al.*, 2004).

Table4.3: Comparison of papaya genome with grape

Name of species	*Genome Size (Mbp)*	*Number of chromosomes*	*G1C content total (%)*	*Gene number*	*Average gene length (bp per gene)*	*Average intron length (bp)*	*Transposons %*
Caricapapaya	372	9	35.3	24,746	2,373	479	51.9
Vitisvinifera	487	19	36.2	30,434	3,399	213	41.4
Arabidopsis thaliana	125	5	35	31,114	2232	165	14
Populus trichocarpa	485	19	33.3	45,555	2300	379	42
Oryza sativa	389	12	43	37,544	2821	412	34.8

Source: Ming *et al.* (2008).

Ming *et al*. (2008) reported 2.8 million whole-genome shotgun (WGS) sequencing reads generated from a female plant of transgenic papaya cultivar SunUp. Papaya genome is largely euchromatic. However, highly condensed heterochromatin knobs were observed on most chromosomes, concentrated in the centromeric and pericentromeric regions. The lengths of the pachytene bivalents that are heavily stained only account for approximately 17% of the genome. However, these cytologically distinct and highly condensed heterochromatic regions could represent 30–35% of the genomic DNA . A large portion of the heterochromatic DNA was probably not covered by the WGS sequence. The 271Mb of contig sequence should represent about 75% of the papaya genome and more than 90% of the euchromatic regions, which is similar to the 92.1% of the EST and 92.4% of genetic markers covered by the assembled genome and the theoretical 95% coverage by 33 WGS sequence . A total of 21,784 (76.1%) of the predicted papaya genes with average length of 1,057 base pairs (bp) have similarity to proteins in the non-redundant database from the National Center for Biotechnology Information, with 9,760 (44.8%) of these supported by papaya unigenes. Among 6,845 genes with average length 309 bp that had no hits to the nonredundant proteins, only 515 (7.5%) were supported by papaya unigenes, implying that the number of predicted papaya-specific genes was inflated. If the 515 genes with unigene support represent 44.8% of the total, then 1,150 predicted papaya-specific genes may be real, and the number of predicted genes in the assembled papaya genome would be 22,934. Considering the assembled genome covers 92.1% of the unigenes and 92.4% of the mapped genetic markers, the number of predicted genes in the papaya genome could be 7.9% higher, or 24,746, about 11–20% less than Arabidopsis (based on either the 27,873 protein

coding and RNA genes, or including the 3,241 novel genes). Comparison of the papaya genome with that of *Arabidopsis* sheds new light on angiosperm evolutionary history in several ways. Considering only the 200 longest papaya scaffolds, Ming *et al.* (2008) found 121 colinear blocks. The papaya blocks range in size from 1.36Mb containing 181 genes to 0.16Mb containing 19 genes (a statistical, rather than a biological, lower limit); the corresponding *Arabidopsis* regions range from 0.69Mb containing 163 genes to 60 kilobases (kb) containing 18 genes. The fact that many papaya segments show co-linearity with two to four *Arabidopsis* segments is most parsimoniously explained if either one or two genome duplications have affected the *Arabidopsis* lineage since its divergence from papaya. Although it was suspected that the most recent *Arabidopsis* genome duplication, might affect only a subset of the Brassicales, previous phylogenetic dating of these events had suggested that the more ancient b-duplication occurred early in the eudicot radiation, well before the *Arabidopsis–Carica* divergence. In contrast, individual *Arabidopsis* genome segments correspond to only one papaya segment, indicating that no genome duplication has occurred in the papaya lineage since its divergence from *Arabidopsis* about 72 million years ago. The lack of relatively recent papaya genome doubling is further supported by an L-shaped distribution of intra-EST correspondence for papaya. However, multiple genome/subgenome alignments reveal evidence in papaya of the ancient 'c' genome duplication shared with *Arabidopsis* and poplar that is postulated to have occurred near the origin of angiosperms. Indeed, both papaya (with no subsequent duplication) and poplar (with a relatively low rate of duplicate gene loss) suggest that c was not a duplication but a triplication, with triplicated patterns evident for about 25% of the 247Mb comprising the 200 largest papaya scaffolds. Despite a closer evolutionary relationship to *Arabidopsis*, papaya shares with poplar an increased number of genes associated with cell expansion, consistent with larger plant size; and lignin biosynthesis, consistent with the convergent evolution of tree-like habit. Amplification of starch-synthesis genes in papaya relative to *Arabidopsis* is consistent with a greater need for storage in leaves, stem and developing fruit of this perennial. Tremendous amplification in papaya of genes related to volatile development implies strong natural selection for enhanced attractants that may be key to fruit (seed) dispersal by animals and which may also have attracted the attention of aboriginal peoples. This also foreshadows what we might expect to discover in the genomes of other fragrant fruited trees, as well as plants with striking fragrance of leaves (herbs), flowers or other organs. Arguably, the sequencing of the genome of SunUp papaya makes it the best-characterized commercial transgenic crop. Because papaya ringspot virus is widespread in nearly all papaya-growing regions, SunUp could serve as a transgenic germplasm source that could be used to breed suitable cultivars resistant to the virus in various parts of the world. The characterization of the precise transgenic modifications in SunUp papaya should also serve to lower regulatory barriers currently in place in some countries.

Transgenics

Gene Delivery System

A robust gene delivery system is prerequisite for obtaining a transgenic plant. Somatic embryos are considered best explants for efficient delivery of gene of interest. Several workers

from USA, China, Taiwan, Brazil and India have reported somatic embryogenesis in papaya. Regeneration is also possible through other somatic tissues such as hypocotyls and root explant. Most of these protocols are highly genotype specific and hence it is mandatory to develop high frequency somatic embryogenesis protocol in commercially important cultivars of papaya. In India, somatic embryogenesis protocols are available on papaya cultivar (s) Washington, Coorg Honey Dew, CO2 and Pusa Delicious. Attempts are being made to develop high frequency somatic embryogenesis in new cultivars like Surya and Red Lady. Both methods of transformation *viz.*, *Agrobacterium* mediated and microprojectile transformation has been reported in papaya. Microprojectile bombardment employs high velocity metal particles to deliver biologically active DNA in to plant cell. The concept has been described by Sanford (1988). Majority of reports indicate that microprojectile (tungsten or gold particles) coated with gene of interest has been preferred pathway in papaya. Scientists at USDA and University of Hawaii, USA reported for the first time transformation of papaya using ballistic gene gun. Generally, tungsten particles of different sizes viz., 0.6,1.2, 2.4µ have been tried. The size of particles depends on the size of gene construct. Microprojectile transformation sometimes gives rise to high copy number of transgene in the tissue which may switch off other genes.

Particle Bombardment Technology

A system for the production of transgenic papaya plants using zygotic embryos and embryogenic callus as target cells for particle bombardment was described (Cabrera-Ponce *et al*., 1995). Phosphinothricin *(bar)* and kanamycin *(npt I1)* resistance genes were used as selectable markers, and the gus gene *(uidA)* as a reporter gene. Selection with 100 mg/l kanamycin and 4 rag/1 phosphinothricin (PPT) yielded a total of over 90 resistant embryogenic colonies from three independent experiments using embryogenic callus as a target tissue. This represents an efficiency of 60 transgenic clones per gram of fresh weight callus bombarded. The efficiency of genetic transformation using zygotic embryos was lower, as only 8 independent resistant clones were recovered out of 645 bombarded zygotic embryos, giving a efficiency of 1.24%. Subsequent subculture of transgenic somatic embryos both from zygotic embryos and embryogenic callus led to the development of plants with apparently normal morphology. Histological, fluorimetric assay for GUS, NPT II assay and DNA analysis (Southern hybridization) showed that kanamycin/PPT resistant plants carried and expressed the transgenes.

The selectable marker gene phospho-mannose isomerase (*pmi*), which encodes the enzyme phosphomannose isomerase (PMI) to enable selection of transformed cell lines on media containing mannose (Man), was evaluated for genetic transformation of papaya (Yun *et al*., 2005). It was found that papaya embryogenic calli have little or no PMI activity and cannot utilize Man as a carbon source; however, when calli were transformed with a *pmi* gene, the PMI activity was greatly increased and they could utilize Man as efficiently as sucrose. Plants regenerated from selected callus lines also exhibited PMI activity but at a lower specific activity level. Transformation efficiency with Man selection was higher than that reported using antibiotic selection or with a visual marker. For papaya, the PMI/Man selection system for producing transgenic plants is a highly efficient addition to previously published methods for selection and may facilitate the stacking of multiple transgenes of interest. Additionally, since the PMI/

Man selection system does not involve antibiotic or herbicide resistance genes, its use might reduce environmental concerns about the potential flow of those genes into related plant populations.

A reproducible and effective biolistic method for transforming papaya was developed with a transformation-regeneration system that targeted a thin layer of embryogenic tissue (Cai, 1999). The key factors in this protocol included: 1) spreading of young somatic embryo tissue that arose directly from excised immature zygotic embryos, followed by another spreading of the actively growing embryogenic tissue 3 day before biolistic transformation; 2) removal of kanamycin selection from all subsequent steps after kanamycin-resistant clusters were first isolated from induction media containing kanamycin; 3) transfer of embryos with finger-like extensions to maturation medium; and 4) transferring explants from germination to the root development medium only after the explants had elongating root initials, had at least two green true leaves, and were about 0.5 to 1.0 cm tall. A total of 83 transgenic papaya lines expressing the nontranslatable coat protein gene of papaya ringspot virus (PRSV) were obtained from somatic embryo clusters that originated from 63 immature zygotic embryos. The transformation efficiency was very high: 100% of the bombarded plates produced transgenic plants. This also represents an average of 55 transgenic lines per gram fresh weight, or 1.3 transgenic lines per embryo cluster that was spread (Cai, 1999).

Fitch *et al.* (1990) reported stable transformation of papaya following DNA delivery via high velocity microprojectiles. Three types of embryogenic tissues, including immature zygotic embryos, freshly explanted hypocotyl sections, and somatic embryos derived from both, were bombarded with tungsten particles carrying chimeric NPT II and GUS genes. All tissue types were cultured prior to and following bombardment on half-strength MS medium supplemented with 10 mg 1^{-1} 2,4-D, 400 mg 1^{-1} glutamine, and 6% sucrose. Upon transfer to 2,4-D-free medium containing 150 mg 1^{-1} kanamycin sulfate, ten putative transgenic isolates produced somatic embryos and five regenerated leafy shoots. Leafy shoots were produced six to nine months following bombardment. Tissues from 13 of these isolates were assayed for NPTII activity, and 10 were positive. Six out of 15 isolates assayed for GUS expression were positive. Three isolates were positive for both NPT II and GUS.

Hraska *et al.* (2006) reported use of green fluorescent protein (GFP), as a visual selectable marker to produce transformed papaya plants following microprojectile bombardment of embryogenic callus. GFP selection reduced the selection time from 3 months on a geneticin (G418) antibiotic containing medium to 3–4 weeks. Moreover, GFP selection increased the number of transformed papaya plants by five-to eightfold compared to selection in the presence of antibiotics. Overall, the use of GFP for selecting transgenic papaya lines improved the throughput for transformation by 15- to 24-fold while avoiding the drawbacks associated with the use of antibiotic resistance based selection markers.

Agrobacterium Mediated Transformation

Fitch *et al*. (1993) reported *Agrobacterium* mediated transformation of papaya. Transgenic papaya plants were regenerated from embryogenic cultures that were co cultivated with a

disarmed C58 strain of *Agrobacterium tumefaciens* containing one of the following binary cosmid vectors: pGA482GG or pGA482GG/cpPRV-4. The T-DNA region of both binary vectors includes the chimeric genes for neomycin phosphotransferase II (NPTII) and l,3-glucuronidase (GUS). In addition, the plant expressible coat protein *(cp)* gene of papaya ringspot virus (PRV) is flanked by the NPTII and GUS genes in pGA482GG/cpPRV-4. Putative transformed embryogenic papaya tissues were obtained by selection on 150 gg-m1-1 kanamycin. Four putative transgenic plant lines were obtained from the *cp* gene- vector and two from the *cp* gene + vector. GUS and NPTII expression were detected in leaves of all putative transformed plants tested, while PRV coat protein expression was detected in leaves of the PRV *cp* gene + plant. The transformed status of these papaya plants was analyzed using both polymerase chain reaction amplification and genomic blot hybridization of the NPTII and PRV *cp* genes. Integration of these genes into the papaya genome was demonstrated by genomic blot hybridizations.

Cheng *et al*. (1996) described an efficient *Agrobacterium-mediated* transformation method based on wounding of cultured embryogenic tissues with carborundum in liquid phase. Embryogenic tissues were obtained from cultured immature zygotic embryos collected 75-90 days after pollination. The expressible coat protein (CP) gene of a Taiwan strain of PRSV was constructed in a Ti binary vector pBGCP, which contained the NPT-II gene as a selection marker. The embryogenic tissues were vortexed with 600 mesh carborundum in sterile distilled water for 1 min before treating with the disarmed A. *tumefaciens* containing the pBGCP. Transformed cells were cultured on kanamyein-free medium containing 2,4-D and carbenicillin for 2-3 weeks and then on the kanamycin medium for 3-4 months. The developed somatic embryos were transferred to the medium containing NAA, BA and kanamycin and subsequently regenerated into normal-appearing plants. Presence of the PRSV CP gene in the putative transgenic lines was detected by PCR and the expression of the CP was verified by Western blotting. The transgene was nuclearly inherited as revealed by segregation analysis in the backcrossed R 1 progeny. From five independent experiments, the average successful rate of transformation was 15.9% of the zygotic embryos treated (52 transgenic somatic embryo clusters out of 327 zygotic embryos treated), about 10-100 times higher than the available methods previously reported. Thus, wounding highly regenerable differentiating tissues by carborundum vortexing provides a simple and efficient way for papaya transformation mediated by *Agrobacterium* (Cheng *et al.*, 1996).

An efficient method for the production of transgenic papaya was developed via Sonication assisted *Agrobacterium*-mediated Transformation (SAAT) of somatic embryos. The plasmid pGA482G was modified to contain gene PTi-Epj-TL-PLDMV with CP coding sequence of PLDMV Japan strain and chimeric gene PTi-NP-YKT with multiple CP coding sequences from PRSV Taiwan strain, PRSV Hawaii strain and PRSV Thailand strain, respectively. Disarmed *Agrobacterium tumefaciens* strain LBA4404 carrying the binary plasmid pGA482G with the CP genes and nptII gene was used to transform embryo calli of papaya variety Sunset to produce transgenic papaya plants. The experiment was focused on the screening of effective transformation method. The engineered *Agrobacterium* grown overnight was diluted with an infection media of high osmotic pressure (1/2 MS medium contain 6% sucrose and 1% glucose,

pH 5.7) and adjusted to optical density OD600nm = 0.15-0.20, embryonic calli were immerged in it for 30 min and treated with 5 s, 15 s, and 20 s sonication respectively during the infection. Results indicated that 15 s sonication treatment improved the transformation efficiency dramatically. After 15 s sonication treatment on embryo calli loaded in 15 ml sterile plastic tubes, 21 putative transgenic lines were produced from 80 pieces embryonic calli (26.3%) transformed by *Agrobacterium* [pGA482G/CPG] and 8 putative transgenic lines was produced from 48 pieces embryonic calli (16.7%) transferred by *Agrobacterium* [pGA482G/CPB], while only a single line came out of 64 pieces embryonic calli (1.6%) transformed by *Agrobacterium* [pGA482G/CPG] and none from 25 pieces embryonic calli transformed by *Agrobacterium* [pGA482G/CPB] in the non-treatment control. Results also showed that the best concentration of selection antibiotic was 120 mg 1^{-1} kanamycin. A total of 42 resistant shoots were produced from 421 pieces of original embryonic calli in 9 months. The presence of the CP genes in the transgenic plants and their integration into the papaya genome were confirmed by PCR and Southern hybridization respectively (Jiang *et al*., 2004).

Some of the problem that is being faced during regeneration of transformed plants is poor growth of transformed plantlets and poor rooting in selective medium. The embryogenic cultures maintained for 6 months or more in media containing 2,4-D may produce tetraploid or may be difficult to regenerate readily. Kanamycin selection limits the efficiency of regeneration of papaya, however recent studies indicate that this may be overcome by judicious use of alternative selective agents or the elimination of antibiotic selection by use of GFP as a visual marker. It has been observed that better roots could be induced in transformed plantlets under ventilated vessels.

Development of Transgenic Papaya for Different Traits

Cold tolerance

Chilling sensitivity is common in tropical and subtropical plant species and the injury is a result of destabilization of cell membranes. Many plant species show increased freezing tolerance in response to low temperature, a phenomenon known as cold acclimation (Sakai and Larcher, 1987). The genes associated with freezing tolerance are either involved with the synthesis of cryoprotective proteins or are induced in response to cold, which in turn activate the other class of genes. The C-repeat binding factor (CBF) genes (Thomashow, 1999) are transcriptional activators that bind to the promoter regions of cold tolerance genes (Gilmour *et al.*, 1998). CBF transcripts begin to accumulate soon after exposure to low temperature in Arabidopsis thaliana (Gilmour *et al.*, 1998), which is followed by expression of cold-regulated (COR) genes. Papaya production is affected by low temperatures that occur periodically in the subtropics. The C-repeat binding factor (CBF) gene family is known to induce the cold acclimation pathway in *Arabidopsis thaliana*. Embryogenic papaya cultures were induced from hypocotyls of “Sunrise Solo” zygotic embryos on semisolid induction medium (Dhekney, *et al.*, 2007). The CBF 1/CBF 3 genes along with the neomycin phosphotransferase (NPT II) gene were placed under the control of the CaMV 35 S promoter and introduced into a binary vector pGA 643. Embryogenic cultures were transformed with *Agrobacterium* strain GV 3101

harboring pGA 643. After selection of transformed embryogenic cultures for resistance to 300 mg l^{-1} kanamycin, somatic embryo development was initiated and transgenic plants were regenerated. The presence of the CBF transgenes in regenerated plants was confirmed by Southern blot hybridization. The papaya and the related cold-tolerant *Vasconcella* genomes were probed for the presence of cold inducible sequences using polymerase chain reaction (PCR). Possible cold inducible sequences were present in the *Vasconcella* genome but were absent in the *Carica* genome (Dhekney *et al*., 2007).

Phytophthora Resistance

The phytoalexin resveratrol (trans-3,5,4¢-trihydroxy-stilbene), a natural component of resistance to fungal diseases in many plants, is synthesized by the enzyme trihydroxystilbene synthase (stilbene synthase, EC 2.3.1.95), which appears to be deficient or lacking in susceptible plants. A stilbene synthase gene (Vst1) from grapevine (*Vitis vinifera* L.), when introduced as a transgene into a range of species showed increased resistance of hosts to pathogens to which they were originally susceptible. Papaya is susceptible to a variety of fungal diseases, including root, stem, and fruit rot caused by the pathogen *Phytophthora palmivora*. Since resveratrol at 1.0 mM inhibited mycelium growth of *P. palmivora in vitro*, papaya resistance to this pathogen might be increased by transformation with the grapevine stilbene synthase construct pVst1, containing the Vst1 gene and its pathogen inducible promoter. Multiple transformed lines were produced, clonally propagated, and evaluated with a leaf disk bioassay and whole plant response to inoculation with *P. palmivora*. RNA transcripts of stilbene synthase and resveratrol glycoside were induced in plant lines transformed with the grapevine pVst1 construct shortly after pathogen inoculation, and the transformed papaya lines exhibited increased resistance to *P. palmivora*.

Mite Resistance

Mite infestation causes major damage to papaya plantations in Hawaii. The transgenic PRSV-resistant cultivar Rainbow is, however, susceptible to both the leafhopper and mites since its female parent, SunUp, and male parent, Kapoho, are very susceptible to the leafhopper and mites, respectively. To enhance papaya resistance to the carmine spider mite, McCafferty *et al.* (2006) transformed a commercial variety of papaya with the gene for chitinase from *Manduca sexta* (msch). A chitinase gene was previously introduced into tobacco resulting in reduced feeding damage and stunted growth of the larvae of the tobacco budworm. Embryogenic calli of papaya were bombarded with the plasmid pBI121 containing the msch gene under the control of CaMV 35S promoter and the nptII gene under the control of the nopaline synthase promoter as selectable marker. Nineteen independent lines were identified after selection with geneticin (G418) and confirmed to be transgenic by PCR. The presence and expression of the msch gene were likewise confirmed by RT-PCR. Chitinase activity was higher by up to 52% in the transgenic leaf extracts compared to control. Bioassays performed in the laboratory showed that the plants expressing the msch gene significantly inhibited the multiplication of the mites. Under field conditions, the number of mites on most transformed lines was significantly lower than the control Kapoho. Two lines, T-23 and T-14 had significantly higher mite counts than control. However, by the end of 10 weeks, the control plants died while lines T-23 and T-14

had grown new leaves. These results indicate a greater tolerance of the transgenic lines to the mites.

Shelf life

Several strategies have been adopted to prolong the shelf life of papaya or delay ripening by genetic engineering: (1) suppressing the production of ethylene by blocking the synthesis of key enzymes such as ACC synthase (ACS) or the ACC oxidase (ACO) and (2) suppressing the synthesis and activity of cell wall degrading enzymes like polygalacturonase (PG). Gene constructs were prepared using pGTVa as the primary vector containing acs2 in antisense orientation and pGTVb as the secondary vector which contains the selectable marker nptII expression cassette (Laurena *et al*., 2002). Based on molecular and phenotypic analysis, twelve papaya trees were selected. The fruits of the selected transgenic papaya lines exhibited similar number of days from color break to full color of 6–7 days compared with 5–6 days for control non-transgenic fruits. However, the number of days from full yellow to fully ripe stage was more pronounced and significant: 4–14 days for selected transgenic lines compared with 2 days for control non-transgenic papayas. The fruits of the selected transgenic papaya lines exhibited similar number of days from color break to full color of 6–7 days compared with 5–6 days for control non-transgenic fruits. However, the number of days from full yellow to fully ripe stage was more pronounced and significant: 4–14 days for selected transgenic lines compared with 2 days for control non-transgenic papayas (Mendoza *et al*., 2008).

Production of Pharmaceuticals Vaccines

The use of transgenic papaya as a new antigen delivery system for the production of a vaccine against cysticercosis has recently been reported by Hernandez et al. (2007). Cysticercosis is an infectious disease that affects humans through pigs which serve as host for the parasite *Taenia solium*. The vaccination of pigs could reduce or eliminate the transmission of this disease to humans. Three peptides, KETc1, KETc12 and KETc7, consisting of 12, 8 and 18 amino acids, respectively, were originally identified in *T. crassiceps* and have been shown to have high protective capacity in piglets under endemic field conditions. The development of an oral edible vaccine in plants could provide a better delivery system for both pigs and humans since both acquire *T. solium* eggs through ingestion. Embryogenic papaya cells were co-transformed with the pUI 235-5.1 vector containing either of three inserts for the above-mentioned peptides and the pWRG1515 plasmid containing GUS-A, hph gene (providing hygromycin resistance) or nptII gene (providing kanamycin resistance) using particle bombardment (Hernandez et al., 2007). KETc1 and KETc12 were modified to contain additional six histidine residues to increase their size and aid in their identification. Embryogenic transgenic papaya clones were selected using hygromycin and kanamycin. Forty-one transgenic clones were obtained and the presence of the transgenes in the genome confirmed using RT-PCR and real-time PCR. Soluble extracts of the transgenic and control embryogenic calli were used to immunize female mice (BALB/cAnN). Antibodies induced by the transgenic extracts were histochemically detected in *T. crassiceps* tissues. Subcutaneous immunization with the soluble extracts of transgenic clones provided complete protection in about 90% of immunized mice.

Development of Virus Resistant Transgenic Papaya

The strategy now commonly referred to as "coat protein mediated protection" is based on insights in the conventional phenomenon of "cross protection". Cross-protection is the term used for phenomenon that a plant, when first inoculated with a mild strain of a given virus, becomes protected against infection with a second, more sever strain of this virus. The mechanism of cross-protection is not yet fully understood. In a few cases, the coat protein plays a crucial role. Coat protein-mediated protection refers to the resistance caused by the expression of the viral CP gene in transgenic plants. Accumulation of the CP in transgenic plants has been shown to counter resistance to infection and/or disease development by the virus from which the CP gene derived and by related viruses. There is little or no genetic resistance to PRSV and PLCV in papaya germplasm. Large collections of papaya germplasm and cultivars representing the World's major production have been screened, but resistance has not been found. Pathogen derived resistance (PDR) via coat protein (cp) has proved to be effective tool in combating plant viruses. The particular gene-silencing strategies have been shown to be effective. For CP-mediated protection, complementary (c) for CP-DNA that represents the CP gene must be synthesize and cloned. This is relatively simple for viruses with CPs that are encoded by subgenomic (sg) RNAs. However, for viruses in which the CP is part of a polyprotein (eg., potyviruses and comoviruses), the CP ORF must be artificially provided with extra AUG start codon. Because of the genetic structure of most plant (RNA) viruses, which encode their most abundant structural protein (CP) at the 3-terminal part of the genome, clones of these genes were the first available for genetic studies. Introduction of the CP gene into the plants is mostly done by *Agrobacterium*-mediated gene transfer. Resistance is in all cases recorded as a significant delay in, or an escape from, disease symptom development and a disease symptoms and virus accumulation.Studies on CP-mediated protection have revealed the following information:

1. CP-mediated protection works at the protein level. Expression of CP RNA only greatly diminishes protection.
2. In most cases, there is no protection against viral RNA inoculation (exception : PVX).
3. The protection is not absolute; it can be overcome by (very) high virus inoculum concentrations. For TMV/tobacco the resistance level is approximately 10^4. This means that transgenic CP plants become diseased at an inoculum concentration 10,000 times higher than needed for infection of control plants.
4. Protection is rather specific; it works for the corresponding virus or very close relatives that have more than 60 percent amino acid sequence homology in their CPs.
5. CP-mediated resistance is a genuine form of resistance, not tolerance. Resistance segregates as a conventional, single, dominant resistance gene.
6. Resistance works under greenhouse and field conditions

PRSV Resistant Transgenic Papaya

Papaya ringspot virus (PRSV) is a major limiting factor for cultivation of papaya in tropical

and subtropical areas throughout the world. Papaya was transformed via *Agrobacterium*-mediated transformation with four constructs containing either the unmodified or modified coat protein (CP) gene of Florida isolate H1K of PRSV. The CP genes were in the sense orientation (S-CP), antisense orientation (AS-CP), sense orientation with a frame-shift mutation (FS-CP), or sense orientation mutated with three-in-frame stop codons (SC-CP). In all, 256 putative transgenic lines with the CP constructs were inoculated mechanically with PRSV H1K (Davis and Ying, 2004). None of the lines was immune to PRSV; however, highly resistant lines were found in each CP transgene group. For breeding purposes, 21 PRSV-resistant lines representing the four transgene constructs were selected and crossed with six papaya genotypes. The lines from the FS-CP and SC-CP transgene groups were highly fertile, but those from the S-CP and AS-CP transgene groups were practically infertile. Plants derived from 54 crosses and representing 17 transgenic lines were planted in the field. After 1 year in the field, 293 of the 1,258 the plants (23.3%) became naturally infected with PRSV; whereas, 29 of 30 of the nontransgenic control plants (96.7%) became infected. The incidence of PRSV infection varied in the R1 progeny depending on both the transgenic line and the nontransgenic parent (Davis and Ying, 2004).

Local varieties of papaya grown in the Andean foothills of Mérida, Venezuela, were transformed independently with the coat protein (CP) gene from two different geographical PRSV isolates, designated VE and LA, via *Agrobacterium tumefaciens*. The CP genes of both PRSV isolates show 92 and 96% nucleotide and amino acid sequence similarity, respectively. Four PRSV-resistant R0 plants were intercrossed or selfed, and the progenies were tested for resistance against the homologous isolates VE and LA, and the heterologous isolates HA (Hawaii) and TH (Thailand) in greenhouse conditions. Resistance was affected by sequence similarity between the transgenes and the challenge viruses: resistance values were higher for plants challenged with the homologous isolates (92 to 100% similarity) than with the Hawaiian (94% similarity) and, lastly, Thailand isolates (88 to 89% similarity). Results showed that PRSV CP gene effectively protects local varieties of papaya against homologous and heterologous isolates of PRSV.

Although the coat protein (CP) gene of PRSV has been transferred into papaya by particle bombardment and transgenic lines with high resistance to Hawaii strains have been obtained, they are susceptible to PRSV isolates outside of Hawaii. This strain-specific resistance limits the application of the transgenic lines in other areas of the world. The CP gene of a local strain isolated from Taiwan, designated PRSV YK, was transferred into papaya via *Agrobacterium*-mediated transformation. A total of 45 putative transgenic lines were obtained and the presence of the transgene in papaya was confirmed by polymerase chain reaction amplification (Bau *et al.*, 2004). When the plants of transgenic lines were challenged with PRSV YK by mechanical inoculation, they showed different levels of resistance ranging from delay of symptom development to complete immunity. Molecular analysis of nine selected lines that exhibited different levels of resistance revealed that the expression level of the transgene is negatively correlated with the degree of resistance, suggesting that the resistance is manifested by a RNA-mediated mechanism. The segregation analysis showed that the transgene in the immune line 18-0-9 has an inheritance of two dominant loci and the other four highly resistant lines have a

single dominant locus. Seven selected lines were tested further for resistance to three PRSV heterologous strains that originated in Hawaii, Thailand, and Mexico. Six of the seven lines showed varying degrees of resistance to the heterologous strains, and one line, 19-0-1, was immune not only to the homologous YK strain but also to the three heterologous strains. Thus, these CP-transgenic papaya lines with broad-spectrum resistance have great potential for use in Taiwan and other geographic areas to control PRSV (Ferreira *et al*., 2002).

Four transgenic papaya lines expressing the coat protein (CP) gene of PRSV were evaluated under field conditions for their reaction to PRSV infection and fruit production in 1996 to 1999. Plants were exposed to natural virus inoculation by aphids in two adjacent fields in four different plantings at the same sites. None of the transgenic lines showed severe symptoms of PRSV whereas control nontransgenic plants were 100 % severely infected 3 to 5 months after planting. In the first and second trials, 20 to 30% of the transgenic plants showed mild symptoms consisting of confined mottling or chlorotic spots on leaves. The number of transgenic plants with mild symptoms fluctuated according to the season and weather conditions, with a tendency to increase in the winter or rainy season and decrease in the summer. Also, the incidence of the mild symptoms in the third trial increased significantly due to infection by root rot fungi during the rainy season. Interestingly, there was no apparent adverse effect on fruit yield and quality in transgenic plants with mild symptoms. In the first and second experiments, transgenic lines yielded 10.8 to 11.6 and 54.3 to 56.7 times more marketable fruit, respectively, than controls. All transgenic plants produced fruit of marketable quality with no ring spots or distortion (Bau *et al*., 2004).

Kung *et al.* (2009) reported generation of transgenic papaya with double resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus. They (Kung *et al.*, 2010) also reported generation of hermaphrodite transgenic papaya lines with virus resistance via transformation of somatic embryos derived from adventitious roots of *in vitro* shoots.

With the development of concept of pathogen derived resistance to combat plant viruses effectively, lot of research was diverted towards developing PRSV resistant papaya using coat protein gene. The efforts made in this direction are being summarized below.

Hawaii

Transgenic papaya resistant to PRSV was commercialized in the US in 1998 (Gonsalves *et al*., 2004). It represents one of three virus resistant crops to be commercialized, with the first being the transgenic squash that was resistant to WMV II and Zucchini yellow mosaic virus. However, virus resistant crops have not been commercialized since the papaya in 1998 and potato in 1999.The concept of parasite-derived resistance (PDR), conceived in the mid-1980s (Sanford and Johnston, 1985), offered a new approach for controlling PRSV. Parasite-derived resistance is a phenomenon whereby transgenic plants containing genes or sequences of a parasite are protected against detrimental effects of the same or related pathogens. The application of this concept, first demonstrated by Beachy's group (Abel *et al*., 1986) has been successfully applied to developing virus resistant crops to numerous viruses. The task of transforming papaya was taken up in 1987 by Fitch. The target cultivars were the red-fleshed

Sunrise, Sunset (a sib selection of Sunrise), and the yellow-fleshed Kapoho, which was by far the most grown cultivar grown in Puna island of Hawaii (USA). Embryogenic tissue was bombarded with tungsten particles coated with DNA of the PRSV HA 5-1 CP gene using the gene gun in Sanford's laboratory. Embryogenic calli were screened on kanamycin. Resistant embryos gave rise to few transformants. These plants were screened in the greenhouse for resistance to PRSV isolates from around world. Analysis clearly showed that 50% of the progenies were transgenic, with the rest nontransgenic. This confirmed that transgenic plants had one insert of the *npt-II* gene and, presumably, the c*p* gene.

The resistance of Rl plants of line 55-1 against 3 PRSV isolates from Hawaii and 13 isolates from different parts of the world was tested (Tennant *et al*., 1994). It should be noted that the transgenic R1 plants were hemizygous for the transgenes. The results of the tests clearly showed that R1 plants were resistant to PRSV HA and other Hawaiian isolates of PRSV. Rather surprisingly, however, the plants were largely susceptible to the strains of PRSV from other regions (Australia, Bahamas, Brazil, China, Ecuador, Florida, Guam, Jamaica, Mexico, and Okinawa). Strains from Bahamas, Mexico, and Florida infected 24 to 72 percent of the inoculated plants, however, the symptoms were not as severe as the control plants and symptom expression was delayed as compared to the nontransgenic controls. Guam, Brazil, Thailand, Ecuador, and Okinawa isolates infected all of the inoculated plants with severe symptoms and milder symptoms were caused by the Australia, China, and Jamaica isolates. In parallel experiments, the effectiveness of PRSV mild strain HA 5-1 for protecting nontransgenic papaya against infection by the above strains was also tested. Interestingly, the effectiveness of cross protection was similar to the results obtained with the transgenic line 55-1. That is, cross protection was complete against the Hawaiian PRSV strains, not complete against the strains which produced milder symptoms on the transgenic papaya, and did not give any protection against those strains that produced severe symptoms on the transgenic papaya. Overall, the results with transgenic papaya reinforced the information previously obtained with cross protection: Rl plants of line 55-1 would not be effective against all isolates of PRSV. However, the results clearly showed that line 55-1 had potential to control PRSV in Hawaii. Shyi-Dong Yeh *et al.* (2004) reported that the coat protein (CP) gene mediated transgenic resistance is found to be the best approach for protecting papaya plants against the destructive disease caused by Papaya ring spot viruses (PRSV). In order to study the variability of PRSV and the potential threat to the CP- transgenic resistance, five virus isolates were collected from transgenic plants of papaya line 16-0-1, which carry the CP gene of the typical mosaic strain of, Taiwan. PRSV YK, in an approved test field and fourteen from untransformed papaya plants in different areas of Taiwan. The results of biological, serological and molecular characterization indicated that all isolates are related to PRSV YK. Among them, the isolate 5-19 from the transgenic line and the isolates CS and TD2 from untransformed papaya were able to overcome the YK CP gene mediated resistance of papaya lines which provide high degrees of resistance to different geographic PRSV trains of Hawaii (HA) and Thailand (TH). These three isolates were also able to cause symptoms on untransformed papaya plants more severe than those induced by YK. In addition to the host reactions, the variability of the collected 19 isolates was also analyzed and compared with YK and other geographic strains by hetero duplex mobility assay (HMA) and sequence analysis. The result of HMA indicated that the CP genes of isolates 5-19 and

TD2 are more divergent than those of other isolates when compared of the transgenic resistance overcoming isolates 5–19, CS and TD2 revealed that their CP coating regions and the 3' untranslated regions (UTRs) share nucleotide identities of 93.9–96.6% and 94.2–97.9% with those of YK, respectively; whereas the other geographic strains of HA, MX and TH that could not overcome the transgenic–resistance share lower nucleotide indentation of 89.8–92.6% and 92.3–95.3% with those of YK, respectively. These results indicate that the ability for overcoming the transgenic resistance is not solely correlated with higher degrees of sequence divergence from the transgenic. Transgenic papaya was released for cultivation in Hawaii and it cover 70% area under papay cultivation

Brazil

Brazil started its transgenic programme in 1992. The CP gene was obtained from a PRSV isolate from the southeast region of the state of Bahia. Translatable or nontranslatable CP gene constructs were used in the transformation experiments. The resulting R0 plants appeared to be resistant to the homologous virus as well as to the Hawaii strain PRSV.HA and to an isolate from Thailand (Souza *et al.*, 1999).

Jamaica

In Collaboration with Cornell University University of the West Indies and the Jamaica Agricultural Development Foundation initiated its papaya transgenic programme to control PRSV by cross protection was in 1990. A virus isolate from the island of Cayman was used in the construct. Two versions of the transgene were made: one with a translatable CP gene and the other a non-translatable CP gene. Transgenic plants were obtained following bombardment into Sunrise (solo type) papaya somatic embryos. Under greenhouse conditions with manual inoculation, high (78%) resistance was found for the translatable cp construct compared to only 10% for the non-translated construct. Resistance to field sources of PRSV of the homologous type was similar to mechanical inoculation in the greenhouse with 80% of the transgenic papaya carrying the translatable CP resistant compared to 44% for the non-translated construct

Thailand

In 1986 Thiland started papaya transgenic programme. Two cultivars, the popular 'Khakdum' and 'Khaknuan' varieties were targeted for transformation using the nontranslatable CP gene of a Thailand isolate of PRSV from Northeast Thailand. In comparative tests, the transgenic line showed that 97% of the progeny were resistant under intense disease pressure and yielded 63 kg fruit per tree in the first year, whereas nontransgenic papaya yield only 0.7 kg per tree per year. Crosses between independent lines of 'Khakdum' have recently shown good resistance under greenhouse and field conditions.

Venezuela

In 1992, the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT, now FONACIT) through a joint venture with the University of Los Andes (ULA) worked

together to develop PRSV-resistant transgenic papayas in collaboration with Cornell University. Two local Venezuelan isolates (LA and EV) of PRSV were collected from domestically and commercially grown papaya. The CP gene of above isolates was cloned in a plant transformation vector in the sense/translatable, sense/untranslatable and antisense forms (Fermin, 1996). The plant transformation vectors with the cloned genes were sent to Venezuela where they were used to transform a local papaya 'Thailandia Roja' via *A. tumefaciens* and analyzed few transgenic lines molecularly and for their resistance against PRSV strains from Venezuela, Hawaii, and Thailand (Fermin *et al*., 2004). The resistance appeared to be RNA mediated, and R1 and R2 plants showed a promising level of resistance not only to local isolates but also to different geographic isolate of PRSV such as isolates from Thailand and Hawaii (Fermin *et al*., 2004).

Taiwan

Transgenic papaya resistant to PRSV were successfully developed by Dr. Shyi-Dong Yeh's team by use of CP-mediated resistance. The CP gene of a local PRSV isolate, YK was inserted as a transgene in the Taiwanese papaya cultivar, Tainung No. 2. the PRSV isolate YK is a severe virus strain found in Taiwan. Transgenic Tainung No. 2 papaya were obtained by *Agrobacterium*-mediated transformation. None of the transgenic lines showed severe symptoms of PRSV infection whereas 100% of the non-transgenic plants were severely infected 3 to 5 months after planting. However, 20-30 % of the transgenic plants exhibited mild symptoms in the first and second field trials but fruit yield and quality were not affected. The transgenic lines were not only protected from virus infection, but also produced 11-56 % more marketable quality papaya compared to nontransgenic papaya (Bau *et al*., 2004)**.**

Australia

PRSV was described in Australia as late as 1991 in tropical North Queensland Based on CP nucleotide sequence data comparisons of isolates from within and outside Australia have shown that domestic PRSV isolates to vary by only 2%. The PRSV isolate used for transgenic studies was obtained from Southeast Queensland. The transgene was designed with a premature stop codon in the PRSV CP sequence, thus it was expected that a functional CP would not be expressed. Transformation was facilitated by biolistic transformation of secondary somatic embryos of cultivars GD3-1-19 and ER6-4, local cultivars that are also used for production and breeding. Copy number also appeared to play a role in the level of resistance, as those with single copies were more susceptible. This observation of gene copy number dependence is consistent with that found for RNA-mediated silencing and PRSV resistance of the original Hawaiian transgenic papayas (Lines *et al*., 2002).

Hongkong (China)

Researchers in China reported the first use of the PRSV replicase gene for the production of PRSV-resistant papaya. The replicase gene was cloned by PCR from RNA from pumpkin leaves infected with PRSV. For the papaya replicase construct, the 3' end of the gene was deleted and additional codons were added to the 5' end of the gene. Transformants were obtained by Agrobacterium-mediated transformation of embryogenic calli of the cultivar Tainung

No. 2. The resulting transformants showed varying levels of resistance in response to mechanical inoculation, including apparent complete immunity in the greenhouse (Chen *et al*., 2001).

Bangladesh and Africa

Bangladesh has recently been established the transformation protocol for papaya transformation *via Agrobacterium* mediated transformation (Rabbani,2000). Several regions in Africa is affected by PRSV infection. Researchers teams is actively working to molecularly characterize and study the sequence diversity of these isolates and to develop a transgenic resistant papaya for these countries.

India

India relatively came late in the field of papaya transgenic. Central Institute of Subtropical Horticulture, Lucknow in collaboration with Advance Center of Plant Virology, Indian Agricultural Research Inistitute (IARI), New Delhi has started research on development of transgenic papaya conferring resistance to both papaya ring spot and papaya leaf curl viruses. A robust regeneration system in papaya through somatic embryogenesis pathway has already been been developed (Mishra *et al*., 2007b). Genetic transformation system in papaya using both methods *viz*., *Agrobacterium* and microprojectile were standardized (Chandra *et al*., 2010; Mishra *et al*., 2010). Few T_0 plantlets have been developed which are under evaluation. The gene construct for this work was developed by ACPV, IARI, New Delhi who shared a dual gene construct (having truncated cp gene from PRSV and truncated replicase from PLCV) in pBinAR binary vector. TNAU, Coimbtore is also engaged in development of transgenic papaya for PRSV resistance . Coat protein mediated resistance against PRSV has been fairly successful approach as on today in papaya. In case of Gemini virus (PaLCuV), replicase mediate resistance will be a better approach.

Genome Squencing of Transgenic Papaya

The 3X draft genome sequence of 'SunUP' variety of papaya, the first virus resistant commercial transgenic fruit tree was reported (Ming *et al.,* 2008). The papaya genome is three times the size of the *Arabidopsis* genome, but contains fewer genes, including significantly fewer disease resistance gene analogues. Comparison of the five sequenced genomes suggests a minimal angiosperm gene set of 13,311. A lack of recent genome duplication, atypical of other angiosperm genomes sequenced so far, may account for the smaller papaya gene number in most functional groups. Nonetheless, striking amplification in gene number within particular functional groups suggest roles in the evolution of tree like habbit, deposition and remobilization of starch reserves, attraction of seed dispersal agents, and adaptation to tropical daylengths. Transgenesis at three locations closely associated with chloroplast insertions into the nuclear genome, and with topoisomeerase I recognition sites. Papaya offers numerous advantages as a system for fruit tree functional genomics, and this draft genome sequence provides the foundation for revealing the basis *Carica*'s distinguishing morpho-physiological, medicinal and nutritional properties.

Future Perspectives

Plant regeneration is not easy in Papaya. Still, significant progress has been accomplished in genetic engineering of this crop. Both biolistic process and *Agrobacterium*-mediated transformation have been proved to be quite efficient for papaya transformation and transgenic papaya were regenerated using either process. The transgenic resistance conferred by the virus Coat Protein gene has become the most effective method to prevent papaya from infection by the noxious PRSV. PRSV- CP gene transgenic papaya Rainbow and SunUp were deregulated and granted approval for commercialization, representing the first successful application of a transgenic fruit tree in the world. The transgenic lines obtained showed various levels of resistance, ranging from delay of symptom development to complete immunity. Use of chimeric constructs targeting multiple viral genes, such as the genes of the CP, the replicase and the gene silencing suppressor HC-Pro of a potyvirus, may minimize the chances of the emergence of a super virus strain that can overcome the transgenic resistance, and provides more durable resistance against different viruses.

Development of transgenic papaya resistant to pathogens need to be intensified. Resistance to insects, tolerance to herbicides, and other important fruit traits like shelf life should be the next targets of research.

Advances in understanding the molecular biology of papaya are still at an early stage. Although some genes of latexenzymes andfruit ripening have been reported, molecular analyses related to essential traits such as sweetness, flesh color, hardness, shape and ripening control of fruits remain to be further explored. Further advances in the functional genomic of papaya will surely benefit this unique tropical fruit, one of the shining stars in the world market for fresh fruit.

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