19 Papaya

Maneesh Mishra[1], Ramesh Chandra[1], and Sangeeta Saxena[2]

[1] Central Institute for Subtropical Horticulture, Lucknow 227107, India
e-mail: m_mishra@mailcity.com
[2] N.D. University of Agriculture & Technology, Kumarganj, Faizabad, 224229, India

19.1 Introduction

Papaya belongs to family Caricaceae. This family consists of six genera including *Carica* a monotypic genera, *Jacaratia* with seven species, *Jarilla* with three species, *Cylicomorpha* with two species, *Horovitzia* with one species and *Vasconcellea* with 21 species (Badillo 2000). All members of the Caricaceae examined cytologically are diploid with $2n = 2x = 18$ (Darlington and Ammal 1945). The genus *Carica* is characterized by a unilocular ovary and is represented by a single species *C. papaya,* while the genus *Vasconcellea* comprises the remaining *Carica* species possessing pentalocular origin. *Carica* is the only genus of Caricaceae containing domesticated species *papaya*, which is by far the most economically important having a distribution throughout the tropics and subtropics of the world. Papaya probably originated in the lowland of Central America, between southern Mexico and Nicaragua. However, it is now cultivated in many tropical and subtropical part of world (Storey 1969). 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 the total world production. It thrives well under tropical climate. However, papaya can be grown in frost-free subtropical climate as well.

Intensive papaya improvement program in India, Hawaii, Mexico, Brazil and Philippines gave rise to a large number of improved hybrids and selections such as Kapoho Solo, Sun Rise, Sun Set, Waimanalo, Laie-Gold, Kamiya (USA), Pusa Delicious, Pusa Nanha, Pusa Dwarf, Pusa Majesty, Surya, Coorg Honey Dew, CO 1, 2, 3, 4, 5, 6 and 7, Pant Papaya-1 (India), Cavite Special (Philippines), Sainampueng, Kak Dum (Thailand), and Improved Peterson, Guinea Gold, Sunnybank and Arline-57 (Australia). However, none of these varieties are close to an ideal variety. Moreover, papaya cultivation is hampered severely due to problems like prevalence of papaya ring spot virus (PRSV), papaya leaf curl virus (PaLCuV), fungal diseases such as foot rot and fruit anthracnose, stamen carpelloidy and summer sterility. Papaya is a polygamous plant, and sex forms constitute the basis for papaya breeding program. Some of the prevalent genetic problems are related to sex forms such as summer sterility and stamen carpelloidy (Giacometti 1987). Storey (1984) considered elimination of ambisexual andromonoceous forms that tend to become sterile at certain climatic conditions or show a tendency towards stamen carpelloidy by developing a heterozygous andromonoceous form, M_2M_2 by possible elimination of the zygotic lethal factor. Due to fairly large size of papaya petiole (75–100 cm), high density planting of papaya is not feasible. Papaya improvement program has led to development of the mutant 'Solo' line with short petiole (45–60 cm) that are positioned obliquely upright and trees can be planted at 0.9–1.2 m apart in the row. Breeding program for developing ring spot resistant variety in papaya is going on for long time.

A great deal of work on intergeneric hybridization has been reported from India, Venezuela, Hawaii, Brazil, Taiwan and Australia (Horovitz et al. 1958; Padnis et al. 1970; Gama et al. 1985; Manshardt and Wenslaff 1989a, b; Chen et al. 1991; Drew et al. 1998). In order to transfer the gene conferring resistance to PRSV, *C. papaya* was crossed with *V. cauliflora*, *V. cundinamarcensis, V. quercifoila, V. stipulata, V. goudatiana* and *V. parviflora*. Few hybrids were developed through the embryo rescue technique. However, most of these crosses turned out to be sterile. Incompatibility between C. *papaya* and *Vasconcellea* species has been a major bottleneck in production of useful intergeneric hybrids. Now it is understood that postzygotic barriers, i.e., embryo abortion and lack of endosperm development (Manshardt and Wenslaff

Genome Mapping and Molecular Breeding in Plants, Volume 4
Fruits and Nuts
C. Kole (Ed.)

1989a, b), were mainly responsible for incompatibility between papaya and its wild species. Recent biotechnological investigations revealed that *V. cauliflora* and *C. papaya* are genetically very distant and unfortunately a lot of efforts have gone into hybridization work involving these species (Jobin-Décor et al. 1996). Hybrids between these two species lack vigor, rarely survive till flowering, and if they do, are infertile (Manshardt and Wenslaff 1989a, b). Similarly, hybrids between *papay*a and *stipulata* were reported to lack vigor and viability (Horovitz and Jimenez 1967). Interestingly, intergeneric hybridization program in Australia with *C. papaya* crossed with *Vasconcellea* species *pubescens, quercifolia, parviflora* and *goudotiana* led to the development of vigorous hybrids (Drew et al. 1998). PRSV resistance has been reported often in crosses between *C. papaya* and *V. cauliflora* (Moore and Litz 1984; Vegas et al. 2003). However, the postzygotic barrier in these hybrids has prevented further backcrossing. Interestingly, Khuspe (1980) reported production of viable F_1 and F_2 populations that were resistant to PRSV in the F_1 population and segregated for PRSV resistance in the F_2 population with a 3:1 ratio. However, his work did not confirm development of papaya hybrid resistant to PRSV. Interspecific hybrids between *C. papaya* and other *Vasconcella* species have also demonstrated resistance to PRSV. All *V. pubescens* hybrids were resistant to PRSV when manually inoculated three times at two-weekly intervals in a glasshouse (Drew et al. 1998). A large population of *C. papaya* × *V. quercifolia* hybrids were manually inoculated using the same procedure. Of those tested, two thirds were resistant and the remaining produced symptoms. However, progressing past intergeneric F_1 hybrids has been very difficult, and the only successes have resulted from backcrosses from *C. papaya* × *V. quercifolia* to *C. papaya*. Embryo culture has produced no F_2 progeny to date and only limited progenies in backcrosses with papaya. In Hawaii, F_1 hybrids contributed only unreduced gametes in backcrosses, yielding sequidiploid plants that were very sterile indicating that meiosis did not function normally in those hybrids (Manshardt and Drew 1988). The first fertile backcross plants have been reported from Australia (Drew and O'Brien 2001).

The tolerance-breeding program has enabled farmers to grow papaya with reasonable fruit production despite plants becoming infected with PRSV, and it has also been helpful to farmers for obtaining good quality, reasonably priced papaya seeds. In the same way, other tolerant lines have been developed. In Taiwan, Lin et al. (1989) reported the development of the hybrid Tainung No. 5, from the cross of FL 77-5 (from Florida) and Costa Rica Red, with good level of tolerance and horticultural characteristics. It has a strong trunk and shows early fruit bearing and ripening. The height of the first fruit from the base of trunk is about 56–60 cm. The use of tolerant papayas has not resolved the virus problem in the long term and development of genetically resistant cultivars is considered the only reliable solution to PRSV control. In Thailand, a series of papaya lines developed by crossing the Florida tolerant and local variety 'Khakdum' followed by recurrent selections is the result of an on-going breeding program since 1987 (Prasartsee et al. 1998). 'Khakdum' is a popular Thai cultivar with desirable fruit characteristics, but it is very susceptible to PRSV (Nopakunwong et al. 1993). Previous trials at the Khonkaen Horticultural Experiment Station (Prasartsee et al. 1998) showed that Florida tolerant papaya produced acceptable amounts of fruit despite being infected with PRSV. Thus, reciprocal crosses were made between the Florida tolerant variety and 'Khakdum' in an effort to produce hybrid lines that are PRSV-tolerant and have acceptable horticultural characteristics. Only one cross was made initially between 'Florida Tolerant' and 'Khakdum'. Subsequent crosses were made from within the progeny population. The first priority was to maintain a high level of PRSV tolerance followed by selection of desirable horticultural characteristics. These progenies were named Thapra 1, Thapra 2 and Thapra 3. In 1997, 'Thapra.2' was released as 'Khakdum Thapra' tolerant to PRSV in Thailand.

19.2 Molecular Characterization

Molecular markers are being used in phylogenetic studies of various taxa adding new dimension to evolutionary pathways. Different molecular markers (RAPD, RFLP, AFLP, IISR-PCR) have been employed to measure genetic variation within and between species, varieties and related genera of *Carica*. Badillo (2000) has rehabilitated *Carica* as a monotypic genera consisting one species and elevated *Vasconcellea* as a separate genus with 21 species. Many studies pertaining to molecular characterization are clearly indicating the wide genetic distance between *Carica* and *Vasconcellea*. Sharon et al. (1992) used mi-

crosatellite and minisatellite probes to evaluate genetic relationship among *Carica* species. Genetic analysis of DNA finger-print bands revealed no linkage or allelic relationship among the bands analyzed indicating that these loci are not clustered in *Carica* genome. The phylogenetic relationship of 12 wild and cultivated species of *Carica* was analyzed by Aradhya et al. (1999) using restriction fragment length variation in a 3.2 kb-PCR amplified intergeneric spacer region of cpDNA. The evolutionary split in *Carica* strongly suggests that *C. papaya* diverged from the rest of the species in the early period of evolution of the genus and evolved in isolation probably in Central America. The chloroplast and mitochondrial DNA diversity of 61 genotypes belonging to 18 *Vasconcellea* species using PCR-RFLP revealed higher level of interspecific variation in two cpDNA regions than analysis with mtDNA which supported the monophyly of *Carica*. Further, cpDNA analyses showed two basic evolutionary lineages within the genus *Carica*, one defined by cultivated *C. papaya* and another consisting of the remaining wild species from South America in a well resolved but poorly supported monophyletic assemblage. This may indicate a higher level of inter-fertility for *Vasconcellea* species from the latter clade in interspecific crossing with papaya. A reticulate evolution for *Vasconcellea* has therefore been suggested. Finally, intraspecific cpDNA variation was detected in *V. microcarpa* thus providing molecular evidence for the high diversity previously indicated by morphological observation (Droogenbroeck et al. 2004). AFLP markers have been used to study the genetic relationship among 71 papaya accessions and related species with nine *Eco*RI/*Mse*I primer combinations. Genetic diversity among *papaya* cultivars derived from the same or similar gene pool was smaller such as Hawaiian solo hermaphrodite cultivars and Australian dioecious cultivars with genetic similarity at 0.921 and 0.912, respectively. Self-pollinated hermaphrodite cultivars were as variable as open-pollinated dioecious cultivars. *C. papaya* showed the least genetic similarity with these species. AFLP markers supported the notion that *C. papaya* diverged from the rest of *Carica* species early in the evolution of this genus (Kin et al. 2002). This theory was further strengthened by the study on phylogeny of *Vasconcellea* and *Carica* species native to Ecuador using AFLP markers (Droogenbroeck et al. 2002). A total of 95 accessions belonging to three genera were evaluated. Both cluster and PCO analysis clearly separated the species of three genera and illustrated the large genetic distance between *C. papaya* accessions and *Vasconcellea* group. The specific clustering of highly diverse group of *Vasconcellea* × *heilbornii* accessions also suggests that these genotypes may be the results of bi-directorial introgression events between *Vasconcellea stipulata* and *V. cundinamarcensis*. Papaya is also grown widely in India. Many improved papaya varieties from various parts of India have been developed. Recently, Saxena et al. (2005) have measured genetic diversity among 10 commercially important papaya cultivars using three SPAR techniques namely RAPD, IISR and DAMD and concluded that IISR-PCR is probably the best technique for assessing papaya germplasm. Papaya germplasm was found to be quite narrow. However, least genetic variation was observed between CO 2 and CO 3, whereas CO 4 and CO 7 and Coorg Honey Dew and Red Fleshed were found to be genetically distant.

19.3 Marker-Assisted Selection

Papaya is conventionally propagated by seeds and dioecious papaya varieties do not ensure the right sex type. As a result, 50–60% of seeds produce male plants which need to be uprooted after six to seven months of planting. The resources (fertilizer, water, weeding, land, labor and time) used in development and weeding out male plants makes papaya cultivation cumbersome and uneconomical. Efforts to distinguish sex of papaya at juvenile stage through morphological and biochemical markers have not met with success. A loose linkage between flower morphology and sex type has been identified, but sex determination based on flower morphology is not possible until four months. In an open-pollinated species such as papaya, the selection of the appropriate sex type of the progeny for commercial planting would be beneficial, since only the female and hermaphrodite plants are grown for fruit. Knowledge of the sex type of papaya is important in selecting parents for use in hybridization work. Crosses between females and hermaphrodites will give all fruit-bearing progenies. Sex expression in papaya is controlled by a single gene with three alleles which have a pleiotropic effect (Hofmeyer 1941; Storey 1953). The sex homologues were designated as M for male, MH for hermaphrodite and m for female. All combinations of dominant alleles, such as

MM, MHMH and MMH, are lethal to the zygote. This makes all males and hermaphrodites into enforced sex heterozygotes. One-fourth of the seeds in their fruits are nonviable. The genotypes for sex are MM for male, MHm for hermaphrodite and mm for female. Using these sex genotypes, there are eight possible cross combinations that could be made with various segregation ratios. Self-pollination in males, cross-pollination between males and females and cross-pollination between male and hermaphrodites can all be done using the sexually ambivalent males (SAMs) that produce perfect flowers during certain periods of the year. Male and hermaphrodite trees undergo various degrees of sex reversal, depending on seasonal changes and climate (Awada 1958). To make the cultivation profitable it is necessary to grow more female:male plants. To discriminate between male and female plants, sex specific molecular markers have been identified in a few dioecious species such as *Silene* and *Pistachio*. In papaya, RAPD and microsatellite markers linked to sex have been reported (Soundur et al. 1996; Parasnis et al. 1999). Two RAPD markers, T12 and T1C each mapped 7 cM apart from the SEX 1 locus (Soundur et al. 1996). Parasnis et al. (2000) have also reported sex diagnostic. They have developed a male specific SCAR marker in papaya by cloning a male-specific RAPD (831 bp) fragment and designing longer primers. The potential of this SCAR marker is further exploited to develop a simplified and highly accurate sex diagnostic assay by including an internal PCR control following a single step DNA extraction procedure, optimizing the PCR condition to simultaneously amplify male-specific and control bands from the crude leaf extract. This diagnostic approach is of great commercial significance of papaya growers as well as to seed companies and plant nurseries for early identification of female seedlings of dioecious species. In principle, this experimental design could easily be applied to molecular analysis of any agriculturally important trait for which specific DNA probes could be identified and hence opens new avenues of research in the field of genetic diagnostics of plants. In 2002, a group in Japan (Urashaki et al. 2002 a, b) has reported that the random amplified polymorphic DNA (RAPD) technique was used to determine the sex of a dioecious species, *Carica papaya* L. with three sex types, male, female and hermaphrodite. A 450 bp marker fragmented PSDM (Papaya Sex Determination Marker) was used for all male and hermaphrodite plants but not in the female plants so far analyzed. Recently embryo induction of papaya by anther culture has been reported and identification of the sex of plantlets derived from embryos using a sex diagnostic PCR was done. Anthers, containing approximately 80% pollen, were collected from 10 to 14 mm long male flower buds. They were pre-treated with agar (0.8%) or in liquid medium for 1 – 5 days at 25-35^{0}C, then transferred to agar medium with 0.1 mg l^{-1} BA and 0.1 mg l^{-1} NAA. Agar and liquid media used for the pre-treatment contained only water or MS nutrients with or without Sucrose (2.0%). On the agar medium, no embryos were induced. At 35 °C embryo induction rate tended to increase up to about 4% when anthers were treated in water for 1 day or MS medium with Sucrose for 3 or 5 days. The sex of plantlets established through anther culture was analyzed using a sex-diagnostic PCR. All plantlets were determined as female. From these results it was suggested that all plantlets established through anther culture were of microspore origin and then the anther culture technique is useful for breeding of female papaya.

19.4 Construction of Genetic Maps

Papaya is an ideal fruit crop for genomic research because of its relatively small genome size (372 Mbp) (Arumugunathan and Earle 1991). However, not much information has been generated so far. High-density genetic maps are prerequisite for isolation and cloning of genes of interest, genomic dissection, marker-assisted selection etc. Genetic mapping of many crops has been accomplished. However, genetic map of papaya has only recently been developed. Ma et al. (2004) constructed a high-density genetic map of papaya using 54 F_2 plants derived from Kapoho and Sun Up cultivars with 1,501 markers including 1,498 AFLP markers, PRSV cp markers, morphological sex types and fresh fruit color. These markers map to 12 linkage groups at a LOD score of 5.0 and recombination fraction of 0.25. The 12 major linkages groups covered a total length of 3,294.2 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 methylation-insensitive markers (Fig. 1).

BACs are the most commonly employed vectors for carrying large DNA fragments. Ming et al. (2001) reported construction of bacterial artificial chromosome (BAC) library from papaya. The BAC library consists of 39,168 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× papaya genome equivalents, excluding the false positive and chloroplast clones (Table 1). High-density filters were made containing 94% or 36,864 clones of the library with 12.7× papaya-genome equivalents. Eleven papaya cDNA and 10 *Arabidopsis* cDNA probes detected an average of 22.8 BACs per probe in the library.

Liu et al. (2004) fine-mapped the sex determination gene (Table 2) with the help of 4,380 informative chromosomes, two SCAR markers (W11 and T12), three cloned sex linked AFLP markers (cpsm10, cpsm31 and cpsm54) and one BAC end (cpbe 55). No recombinants were detected. They 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 papaya sex-determining genes indicate 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.

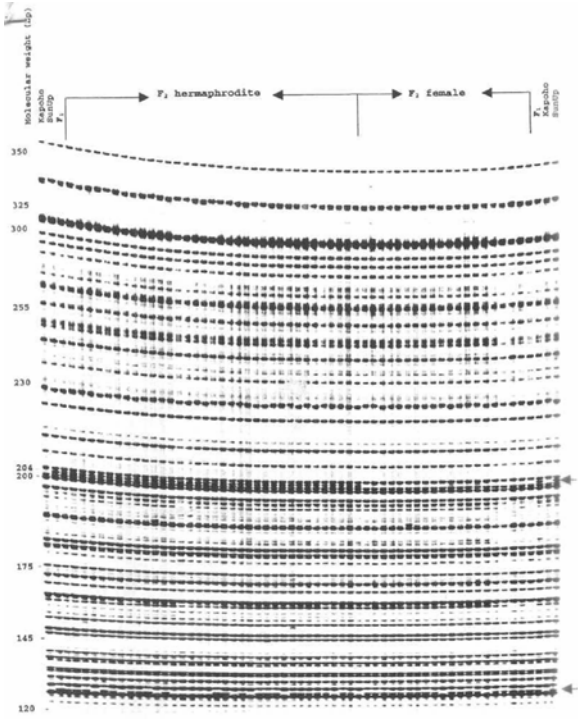

Fig. 1. AFLP products amplified by the primer pair E-GCT/M-AG. The Sun Up dominant marker between 200 and 204 bp is cosegregating with sex (Source: Genetics 166:419–436)

19.5 Recombinant DNA Technology

Recently, DNA recombinant technology has opened up new vistas for development of virus resistant papaya. Pathogen derived resistance (PDR) has been proved to be an effective tool in combating plant viruses. Genetic engineering for virus resistance has been found effective whereby transgenic plants expressing virus genome sequence resist attack by corresponding viruses. Coat protein mediated resistance (CPMR) was first reported in 1986 in tomato. Subsequently, a large number of transgenic lines (citrus, papaya, potato, peanut, squash, sugar beet) containing CP transgene have been produced. There are several mechanisms involved in CPMR. However, it is largely believed that resistance is RNA mediated *via* post-transcriptional gene silencing.

PRSV resistant transgenic papaya has been developed and commercialized in 1998 in Hawaii, USA by Dr. Dennis Gonsalves and his team (Gonsalves 1998). SunUp and Rainbow cultivars of transgenic papaya have been developed by cloning CP gene of mild strain of PRSV from Hawaii. Dennis Gonsalves and his group used PDR concept in 1986 by cloning the CP gene of PRSV HA 5-1 (a mild strain) from Hawaii. Because of various technical difficulties and the requirement that the gene be expressed as a protein, the gene was engineered as a chimeric protein containing 17 amino acids of cucumber mosaic virus at the N terminus of the full-length CP gene of PRSV HA 5-1 (Ling et al. 1991). Dr. Maureen Fitch, Scientist

Table 1. Results of screening of papaya BAC library with homologous and hetrologous cDNA, rDNA and cpDNA. Source: Ming et al. (2001) Theor Appl Genet 102:892–899

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

Table 2. Fine mapping with SCAR markers in MSY region of papaya. Source: Liu et al. (2004) Nature 427:348–352

Population	Progeny	Hema-phrodite	Female	SCAR markers	Recom-binant
Kapoho × SunUp	F_2	335	150	W11	0
Kapoho × SunUp	F_2	335	156	W11, T12, cpbe55	0
Kapoho × SunUp	F_2	481	274	W11, T12, cpbe55	0
Kapoho × Saipan Red	F_2	175	49	Cpsm31, cpsm 10	0
AU0 × SunUp	F_2	170	65	W11, T12, cpsm54	0
Total	2,190	1,496	694		0

at USDA, Hawaii took the challenge of transforming papaya in 1987. The red-fleshed Sunrise, Sunset (a sib selection of Sunrise), and the yellow-fleshed Kapoho, were chosen as target cultivars. The embryogenic tissue was bombarded with tungsten particles coated with engineered DNA construct of the PRSV HA 5-1 CP gene using the gene gun. Fifteen months later, transgenic plants were obtained and grown in the greenhouse (Fitch et al. 1990, 1992). R_0 transgenic lines were screened for virus resistance against severe strain of PRSV HA from Hawaii at greenhouse at Cornell University. R_0 micropropagated plants of the first line were further characterized as it showed excellent resistance to PRSV HA (Fitch et al. 1992) among all R_0 lines. Line 55-1 was female and thus progenies could not be obtained directly from the R_0 plants, unlike a hermaphrodite. In order to know whether 55-1 is resistant to PRSV, a trial was laid down using R_0 plants.

R_1 plants were obtained by crossing line 55-1 with nontransgenic Sunset under greenhouse conditions. 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. This confirmed that transgenic plants had one insert of the *npt*II gene and, the CP gene. Field experiment was conducted to evaluate transgenic plants under natural field condition. The transgenic papaya showed excellent resistance throughout the two-year trial (Lius et al. 1997). Nearly all (95%) of the nontransgenic plants and those of a transgenic line that lacked the CP gene showed PRSV symptoms. The homozygous line 55-1 was later named SunUp. The hybrid made from the cross of the transgenic SunUp and the non-transgenic Kapoho was named Rainbow.

Efforts are being made to generate PaLCuV resistant transgenic papaya using both sense and antisense *Rep* gene as well as CP gene of PaLCuV in India. The first report of papaya leaf curl disease in India to be caused by a geminivirus was published by Saxena et al. (1998a, b). Recently the first report of papaya leaf curl virus (PaLCuV) infecting papaya plants in Taiwan is also published (Chang et al. 2003). Papaya cultivation is severely threatened by PaLCuV. The disease is transmitted by the vector whitefly (*Bemisia tabaci*), and characterized by severe curling, downward cupping and crinkling of leaves. Early infection leads to severe reduction in yield and management of disease is urgently required. Developing transgenic papaya plants resistant to PaLCuV seems to be most promising considering several points as discussed above regarding genetics and breeding program of papaya. Nucleotide sequence and intergeminiviral homologies of the DNA-A of PaLCuV from India have already been reported by Saxena et al. (1998c) and further molecular characterization of papaya leaf curl gemini virus (PaLCuV) and its isolates is currently under studies.

Transformation of plants with viral genes has been proven in many cases to produce resistance to the virus from which the genes were derived. The technology has been successfully used to produce resistance in papaya with respect to PRSV and trials with PaLCuV are undergoing. The benefit of transgenic virus resistance includes increased yield, reduced pesticide use to control the vectors of viruses, i.e. whitefly in case of PaLCuV and improved crop as well as food quality. The coat protein (CP) gene is most often used to confer resistance. In some cases, the expression of CP correlated with resistance, and strong evidence for prevention of uncoating was shown. However, reports indicate that coat protein mediated resistance is not successful in case of geminiviruses, and most of the strategies for genetically engineered resistance to geminivirus involve the replication-associated protein (*Rep*) sequences (Sinistera et al. 1999; Yang et al. 2004). For some viruses there can be both CP and RNA mechanism that can confer resistance in transgenic plants. In case of PaLCuV, it is being speculated that high levels of resistance can be produced in papaya plants transformed with a viral replicase gene, which includes the full length gene as well as various deletions or sequence modifications. The mechanism of resistance in replicase-expressing plants is complex and may involve expression of a protein that blocks virus replication and/or movement, as well as post-transcriptional gene silencing. Further, development of transformation technique in different commercially significant papaya cultivars broadens the possibility of use of engineered virus resistance in papaya breeding.

It has been observed in case of several viruses including geminivruses that resistant plants did not confer resistance to other isolates (if present) of the same virus. Resistance to virus on transgenic plants expressing CP or *Rep* gene was shown to be dependent on the sequence homology between the CP or *Rep* transgene expressed in the plant genome and the CP or *Rep* gene from the incoming virus. Therefore, knowledge of the degree of homology among the CP and *Rep* gene from the distinct PaLCuV isolates which are present in a given area is important to guide the development of transgenic papaya for the control of PaLCuV. To address this problem a comprehensive plan is to be developed in case of PaLCuV and already work on genetic variability of PaLCuV isolates is currently going on in India (S Saxena, personal communication). The CP and *Rep* genes from different isolates of PaLCuV (collected from different geographic locations from North India) were cloned and sequenced. The sequences revealed a substantial amount of variability in different PaLCuV isolates implicating the need to look for most homologous region in CP or *Rep* gene to be used as putative transgene and also the need for using genes from local isolates in generation of PaLCuV resistant transgenic papaya. If successful the technology would improve the yield and quality of papaya fruit.

Acknowledgement. MM gratefully acknowledges contribution of Dr. Savarni Tripathi, Researcher, PBARC, Hilo, Hawaii, USA for sharing information on the subject and Dr. Dennis Gonsalves and Dr. Maureen Fitch, USDA-ARS, PBARC, HI, USA for imparting practical knowledge on transgenic papaya research.

References

Aradhya MK, Manshardt RM, Zee F, Morden CW (1999) A phylogenetic analysis of the genus *Carica* based on restriction fragment length variation in a cp-DNA intergeneric spacer region. Genet Resour Crop Evol 46:579–586

Arumuganathan E, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 93:208–219

Awada M (1958) Relationship of minimum temperature and growth rate with sex expression of papaya plants (*Carica papaya*). Hawaii Agri Exp St Bull 38:16(10):11–19

Badillo VM (2000) *Carica* L. Vs. *Vasconcellea* St. Hil. (Caricaceae) con La rehabilitation de else ultimo. Ernstia 10:74–99

Chen MH, Chen CC, Wang DN, Chen FC (1991) Somatic embryogenesis and plant regeneration from immature embryo of *Carica papaya* × *Carica cauliflora* cultured *in vitro*. Can J Bot 69:1913–1918

Darlington CD, Ammal EKJ (1945) Chromosome Atlas of Cultivated Plants. George Allen & Unwin Ltd, London, UK

Drew RA, O'Brien CM (2001) Progress with *Carica* interspecific hybridization to develop plants with resistance to PRSV-P. Paper presented at 2nd Intl Symp on Biotechnology of Tropical and Subtropical species at Taipei, Taiwan

Drew RA, O'Brien CM, Magdalita PM (1990) Development of interspecific *Carica papaya* hybrid. Acta Hort 461:285–292

Droogenbroeck BV, Kyndt T, Maerten I, Romeijn-Peeters E, Schedeman X, Romero-Monochi J P, Van Damne P, Goerghebeur P, Gheysen G (2004) Phylogenetic analysis of the highland papaya (*Vasconcellea*) and allied genera (Caricaceae) using PCR-RFLP. Theor Appl Genet 108:1473–1486

Droogenbroeck BV, Breyne P, Goerghebeur P, Romeijn-Peeters E, Kyndt T, Gheysen G (2002) AFLP analysis of genetic relationship among papaya and its wild relatives (Caricaceae) from Ecuador. Theor Appl Genet 105:289–297

Fitch MMM, Manshardt RM, Gonsalves D, Slightom JL, Stanford JC (1990) Stable transformation of papaya via microprojectile bombardment. Plant Cell Rep 9(4):189–194

Fitch MMM, Manshardt RM, Gonsalves D, Slightom JL, Stanford JC (1992) Virus resistant papaya derived from tissue bombardment with coat protein gene of papaya ringspot virus. Bio/Technology 10:1466–1472

Grant S, Houben A, Vyskot B, Siroky J, Pan W, Macas J, Saedler H (1994) Genetics of sex determination in flowering plants. Dev Genet 15:214–230

Gama MI, Giacometti DC, Castro LAB (1985) Cruzamento De Espec's De Carica para Resistencia Ao Virus Do Mosico Do Memoeiro. Fitopatologia Brasileria

Giacometti DC (1987) Papaya breeding. Acta Hort 196:53–60

Gonsalves D (1998) Control of papaya ring spot virus in papaya: A case study. Annu Rev Phytopathol 36:415–437

Hofmeyr JDJ (1941) Genetics of *Carica papaya* L. Chron Bot 6:245–247

Horovitz S, Jimenez H (1967) Cruzmientos interspecificosE intergenericos En Caricaceae Y Sus implicaciones Fitotecnicas. Agron Trop 17:323–343

Jobin-Décor MP, Graham JC, Henery RJ, Drew RA (1996) RAPD and isozyme analysis of genetic relationship between *Carica papaya* and wild relatives. Genet Resour Crop Evol 44:1–7

Khuspe S, Hendre R, Mascarenhas A, Jagannathan V, Thombre M, Joshi A (1980) Utilization of tissue culture to isolate interspecific hybrids in *Carica* L. In: Rao PM, Hebel MR, Chadha M (eds) Natl Symp on Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization of Plant Cells. Bhabha Atomic Research Center, Bombay, India, pp 198–205

Kim MS, Moore PH, Zee F, Fitch MMM, Steiger DL, Manshardt RM, Paull RE, Drew RA, Sekioka T, Ming R (2002) Genetic diversity of *Carica papaya* as revealed by AFLP markers. Genome 45:503–512

Lin CC, Su HJ, Wang DN (1989) The control of papaya ringspot virus in Taiwan ROC. Food & Fertilizer Technology Center Tech Bull 114:1–13

Ling K, Namba S, Gonsalves C, Slightom JL, Gonsalves D (1997) Protection against detrimental effects of potyvirus infection in transgenic tobacco plants expressing the papaya ringspot virus coat protein gene. Bio/Technology 9(8):752–758

Lius S, Manshardt RM, Fitch MMM, Slightom JL, Stanford JC, Gonsalves D (1997) Pathogen derived resistance provide papaya with effective protection against papaya ring spot virus. Mol Breed 3:161–168

Liu Z, Moore PH, Ma H, Ackerman CM, Ragiba M, Qingyi Yu, Heather MP, Kim MS, Charlton JW, Stiles JI, Zee FT, Paterson AH, Ming R (2004) A primitive Y chromosome in papaya makes incipient sex chromosome evolution. Nature 427:348–352

Ma H, Moore PH, Liu Z, Kim MS, Yu Q, Fitch MMM, Sekioka T, Paterson AH, Ming R (2004) High density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. Genetics 166:419–436

Magdalita PM, Drew RA, Adkins SW, Godwin ID (1997) Morphological, molecular and cytological analysis of *Carica papaya* × *C. cauliflora* interspecific hybrids. Theor Appl Genet 95:224–229

Manshardt RM, Drew RA (1998) Biotechnology of papaya. Acta Hort 461:65–67

Manshardt RM, Wenslaff TF (1989a) Interspecific hybridization of papaya with other *Carica* species. J Am Soc Hort Sci 114:689–694

Manshardt RM, Wenslaff TF (1989b) Zygotic polyembryonic in interspecific hybrids of *Carica papaya* and *C. cauliflora*. J Am Soc Hort Sci 114:468–479

Ming R, Moore PH, Zee F, Abbey CA, Ma H, Paterson AH (2001) Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree fruit genome. Theor Appl Genet 102:892–899

Moore G, Litz RE (1984) Biochemical markers for *Carica papaya, C. cauliflora* and plants from somatic embryos of their hybrids. J Am Soc Hort Sci 109:213–218

Nopakunwong U, Sutchaponges S, Anupunt P, Prompan S, Rattanakusol S (1993) Papaya selections tolerant to papaya ringspot virus diseases. Srisaket Horticultural Research Center Yearly Report, pp 172–175

Padnis NA, Budrukkar ND, Kaulgud SN (1970) Embryo culture technique in papaya (*Carica papaya* L). Poona Agri Coll Mag 60:101–104

Parasnis AS, Gupta VS, Tamhankar SA, Ranjekar PK (2000) A highly reliable sex diagnostic PCR assay for mass screening of papaya seedling. Mol Breed 6:337–344

Parasartsee V, Chiakiatiyos S, Palakom K, Cheuychoom P, Kongpolprom W, Wichainum S, Fungkiatpaiboon A, Gonsalves C, Gonsalves D (1998) Development of papaya lines that are tolerant to papaya ring spot virus disease. Proc JIRCAS-ITCAD Seminar, March 24, 1998 at Khon Khen, Thailand, pp 27–33

Parasnis A, Ramakrishna W, Chowdari V, Gupta V, Ranjekar PK (1999) Microsatellite $(GATA)_4$ identifies sex specific differences in papaya. Theor Appl Genet 99:1047–1052

Purseglove JW (1968) Papaya. In: Tropical Crops, Vol 1. Dicotyledons. John Wiley, New York, USA

Saxena S, Hallan V, Singh BP, Sane PV (1998a) Leaf curl disease of *Carica papaya* from India may be caused by a bipartite geminivirus. Plant Dis 82:126

Saxena S, Hallan V, Singh BP, Sane PV (1998b) Evidence from nucleic acid hybridisation tests for a Geminivirus infection causing leaf curl disease of papaya in India. Ind J Exp Biol 36:229–232

Saxena S, Hallan V, Singh BP, Sane PV (1998c) Nucleotide sequence and intergeminiviral homologies of the DNA-A of papaya leaf curl geminivirus from India. Biochem Mol Biol Intl 45(1):101–113

Saxena S, Chandra R, Srivastava AP, Mishra M, Pathak RK, Ranade SA (2005) Analysis of genetic diversity among papaya cultivars using Single Primer Amplification Reaction (SPAR) methods. J Hort Sci Biotechnol 80(3):291–296

Sinistera XH, Polston JE, Abouzid AM, Hiebert E (1999) Tobacco plants transformed with a modified coat protein of tomato mottle begomovirus show resistance to virus infection. Virology 89(9):701–706

Stiles JI, Lemne C, Sondur S, Morshidi MB, Manshardt RM (1993) Using randomly amplified polymorphic DNA for evaluating genetic relationship among papaya cultivars. Theor Appl Genet 85:697–701

Sharon D, Hillel J, Vainstein A, Lavi U (1992) Application of DNA fingerprints for identification and genetic analysis of *Carica papaya* and other *Carica* species. Euphytica 62:119–126

Storey WB (1953) *Carica papaya*. In: Simmonds NW (ed) Evolution of Crop Plants. Longman, London, UK ; New York, USA, pp 21–24

Storey WB (1969) Papaya. In: Ferwerda FP, Wit F (eds) Outline of Perennial Crop Breeding in the Tropics. H Veenman and Zonen, NV, Wageningen, The Netherlands, pp 389–408

Storey WB (1976) Papaya. In: Simmonds NW (ed) Evolution of Crop Plants. Longman, London, UK; New York, USA, pp 389–408

Sondur SN, Manshardt RM, Stiles JI (1996) A genetic linkage map of papaya based on randomly amplified polymorphic DNA marker. Theor Appl Genet 93:547–553

Vegas A, Trujillo G, Sandrea Y, Mata J (2003) Obtencion, regeneracion,Y Evaluacion De Hibridos Intergenericos Entre *Carica papaya* Y *Vasconcellea cauliflora*. Interciencia 28:710–714

Urasaki N, Tokumoto, Tarora, K, Ban Y, Kayano T, Tanaka H, Oka H, Chinen I, Terauchi R (2002a) A male and hermaphrodite specific RAPD marker for papaya (*C. papaya* L.). Theor Appl Genet 104:281–285

Urasaki N, Tarora K, Uehara J, Chinen I, Terauchi R, Tokumoto M (2002b) Rapid and highly reliable sex diagnostic PCR assay for papaya (*C. papaya*). Breed Sci 52:333–335