SEQUENCES OF THE COAT PROTEIN GENE FROM BRAZILIAN ISOLATES OF *Papaya ringspot virus*

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ABSTRACT

Papaya ringspot virus (PRSV) is the causal agent of the main papaya (*Carica papaya*) disease in the world. Brazil is currently the world´s main papaya grower, responsible for about 40% of the worldwide production. Resistance to PRSV on transgenic plants expressing the PRSV coat protein (cp) gene was shown to be dependent on the sequence homology between the *cp* transgene expressed in the plant genome and the *cp* gene from the incoming virus, in an isolate-specific fashion. Therefore, knowledge of the degree of homology among the *cp* genes from distinct PRSV isolates which are present in a given area is important to guide the development of transgenic papaya for the control of PRSV in that area. The objective of the present study was to assess the degree of homology among the PRSV *cp* genes of several Brazilian isolates of this virus. Papaya and PRSV are present in many different ecosystems within Brazil. Twelve PRSV isolates, collected in eight different states from four different geographic regions, were used in this study. The sequences of the *cp* gene from these isolates were compared among themselves and to the gene used to generate transgenic papaya for Brazil. An average degree of homology of 97.3% at the nucleotide sequence was found among the Brazilian isolates. When compared to 27 isolates from outside Brazil in a homology tree, the Brazilian isolates were clustered with Australian, Hawaiian, and Central and North American isolates, with an average degree of homology of 90.7% among them.

Additional keywords: *Potyvirus*, *Carica papaya*, PRSV, phylogenetic analysis.

RESUMO

Seqüência do gene da proteína capsidial de isolados brasileiros de *Papaya ringspot virus*

O *Papaya ringspot virus* (PRSV) é o agente causal da mancha anelar, principal doença do mamoeiro (*Carica papaya*) no mundo. O Brasil é o maior produtor desta fruteira, sendo responsável por aproximadamente 40% da produção mundial. A resistência a este vírus, obtida em mamoeiros transgênicos expressando o gene da proteína capsidial (cp) do PRSV, mostrou-se dependente do grau de homologia entre a seqüência do transgene expresso pela planta e o gene *cp* do vírus invasor, de forma isolado-específico. Dessa forma, quando se objetiva produzir mamoeiros transgênicos com amplo espectro de resistência ao PRSV, é importante o conhecimento do grau de homologia deste gene entre os diversos isolados presentes em uma área geográfica específica onde o mamoeiro será cultivado. O objetivo do presente estudo foi avaliar o grau de homologia entre o gene *cp* de diversos isolados brasileiros de PRSV. O mamoeiro e o PRSV encontram-se presentes em diversos ecossistemas brasileiros. Doze isolados de PRSV, coletados em oito estados de quatro regiões geográficas, foram utilizados neste estudo. As seqüências do gene *cp* destes isolados foram comparadas entre si e com o gene utilizado para gerar mamoeiros transgênicos para o Brasil. Um grau de homologia médio de 97,3% para as seqüências de nucleotídeos foi observado entre os isolados brasileiros. Quando comparado com 27 isolados de outras regiões, em uma árvore de homologia, os isolados brasileiros foram agrupados com os isolados australianos, havaianos, e os da América Central e do Norte. Um grau de homologia médio de 90,7% foi observado entre os 40 isolados analisados.

INTRODUCTION

Ringspot, a viral disease caused by *Papaya ringspot virus* (PRSV), family *Potyviridae,* genus *Potyvirus* is considered the major limiting factor for papaya (*Carica papaya* L.) production worldwide. Although very difficult, because of its efficient way of natural transmission by different aphid species, and the absence of source of resistance in *C. papaya* (Manshardt, 1992), the control of PRSV is necessary in all the region were papaya is grown. Several strategies have been evaluated to control this disease without satisfactory results. The recent development of transgenic papaya plants expressing the virus coat protein gene (*cp*) has opened up the possibility of solving the problem by using an efficient and

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possibly more durable control method (Fitch *et al*., 1992; Tennant, 1996; Cai *et al*., 1999; Souza Jr., 1999).

The first transgenic papayas with resistance to PRSV were developed in the beginning of the 1990's. The transgenic line expressing the *cp* gene from the mutant isolate HA 5-1 (Yeh & Gonsalves, 1984), named 55-1, was resistant to this and other Hawaiian PRSV isolates (Fitch *et al*, 1992; Tennant, 1996). Rainbow and SunUp varieties, which became the first transgenic papayas commercially produced in the world, were derived from line 55-1 (Gonsalves, 1998). However, when challenged with PRSV isolates from other geographic regions, including Brazil, this transgenic line was susceptible (Tennant *et al*., 1994).

Tennant (1996) demonstrated that the resistance observed in some transgenic papaya lines expressing the *cp* gene is isolate-specific. Additional studies have shown that RNA mediates this resistance through the mechanism of post-transcriptional gene silencing (PTGS), and that resistance is dependent on gene dosage and the degree of homology between the *cp* transgene and the *cp* gene of the challenging PRSV isolate (Tennant *et al*., 1997; Souza Jr. *et al*., 1998; Souza Jr., 1999). In general, as the gene dosage increases, not only does the spectrum of resistance to PRSV isolates get wider, but the resistance also becomes more efficient against a specific isolate (Souza Jr., 1999). Additional factors, such as plant age and inoculum concentration, can also play a role in the fate of the resistance phenotype (Tennant *et al*., 1997; Souza Jr., 1999).

Brazil is a country with a vast territory, and papaya and PRSV are present almost everywhere. The process of developing PRSV-resistant transgenic papaya varieties, currently in place at the Brazilian Corporation for Agricultural Research - Embrapa (Souza Jr. & Gonsalves, 1999a), aims at obtaining varieties with a broad spectrum of resistance to this virus in Brazil. Because resistance is dependent on the degree of homology between the *cp* transgene and the *cp* gene of the challenging PRSV isolate, the process of developing transgenic papaya plants with broad spectrum resistance requires knowledge of the degree of homology among the *cp* gene of distinct Brazilian isolates. The objective of the present study was to assess the degree of homology among the *cp* gene from PRSV isolates obtained in different Brazilian regions, and to compare it to isolates from throughout the world.

MATERIAL AND METHODS

Virus Isolates

Twelve PRSV isolates, ten from biotype PRSV-P and two from biotype PRSV-W, were collected in geographically different areas in Brazil and used in this study (Table 1). The isolates were collected from infected papaya plants in eight different States from four Brazilian geographical regions. All isolates except PE and PB were maintained in a greenhouse on papaya or *Cucumis metuliferus* L. (Yeh & Gonsalves, 1984) prior to RNA isolation. RNA isolation from PE and PB isolates was done directly from infected tissue collected in the field.

The *cp* gene sequence from a Brazilian isolate of PRSV, collected in the State of Bahia (Souza Jr., 1999), was used as standard for comparison with other Brazilian isolates. The *cp* gene from this isolate is present in the transgenic papaya developed for Brazil (Souza Jr. & Gonsalves, 1999a). The sequence of the *cp* gene from an additional 27 PRSV isolates from around the world, available in the literature or from GenBank (http://www.ncbi.nlm.nih.gov), were used to

TABLE 1 - *Papaya ringspot virus* (PRSV) isolates used in this study

Isolate	Type	Origin	Accession number or Literature source
Determined in this study:			
CEW	W	Aracoiaba-Ceará	AF344648
CE	P	Guaiúba - Ceará	AF344647
PB	P	Alhandra - Paraíba	AF344645
PE	P	Camaragibe - Pernambuco	AF344646
BA-CA	P	Cruz das Almas - Bahia	AF344641
BA-IT1	P	Itabela - Bahia	AF344639
BA-IT2	P	Itabela - Bahia	AF344640
DFW	W	Brasília - Distrito Federal	AF344649
DF	P	Brasília - Distrito Federal	AF344650
SP	P	Piracicaba - São Paulo	AF344642
ES	P	Linhares - Espírito Santo	AF344644
PR	P	Paranavaí - Paraná	AF344643
Obtained from the Genbank or from the literature:			
Brazil.Bahia	P	Nova Viçosa, Bahia, Brazil	Souza Jr. (1999)
JAM	P	Jamaica	Tennant (1996)
TAW-YK	P	Taiwan	X97251
THA	P	Thailand	U14743
AUS-BD	P	Bridgeman Downs, Australia	U14736
AUS-BUN	P	Bundaberg, Australia	U14737
AUS-DAY	P	Dayboro, Australia	U14738
AUS-DB1	W	Deception Bay, Australia	S89893
AUS-GAT	W	Gatton, Australia	U14739
AUS-NT	W	Darwin, Australia	U14744
AUS-WP	P	Wellington Point, Australia	U14740
MEX-CHT11	P	Chiapas, Mexico	AJ012650
MEX-VPO28	P	Vera Cruz, Mexico	AJ012099
MEX-VTB6	P	Vera Cruz, Mexico	AJ012649
MEX-Colima	P	Mexico	AF309968
VIET	P	Vietnam	U14742
MAL	P	Malaysia	AB044342
SRI	P	Sri Lanka	U14741
IND	P	India	AF063220
INDW	W	India	AF063221
USA-HA	P	Hawaii, USA	X67673
USA-HA5-1	P	Mutant derived from HA	D00595
USA-H1K	P	Florida, USA	AF196839
USA-FLW	W	Florida, USA	D00594
USA-PR	P	Puerto Rico, USA	AF196838
JAP-OK	P	Okinawa, Japan	AB044339
JAP-S	?*	Japan	D50591
CHI-SM	?	China	X96538

* Type non-specified.

compare their degree of homology to the Brazilian isolates (Table 1).

RNA isolation, reverse transcription-PCR, cloning and sequencing

Total plant RNA from PRSV infected papaya or *C. metuliferus* plants was extracted as described by Napoli *et al*. (1990). The Reverse Transcription (RT) was performed under the following conditions: 1-2 µg of total RNA, 200 ng of the antisense primer (5'-AGCTAACCATGGGCGAGTATTCA GTTGCGC -3'), 0.4 mM of each dNTP, 10 mM DTT, 80 units of RNAsin, 360 mM 2-mercaptoethanol, 1X RT buffer, and 400 units of M-MLV RT (Promega, Madison, WI). Initially, a 10 µl aliquot containing only the total RNA and the antisense primer was heated at 70º C for 5 min, and cooled on ice for 2 min. Then, a 40 µl aliquot containing the other reaction components was added to the initial 10 µl aliquot and incubated for 90 min at 37º C. After that, the sample was incubated at 70º C for 5 min to stop the reaction.

A total of 5 µl of RT solution was used as the template for PCR under the following conditions: 0.4 mM of each dNTP, 1X PCR buffer, 100 ng of each primer (5'-ATCATTCCATG GGCGTGTTCCATGAATCAA-3', sense, and 5'- AGCTA ACCATGGGCGAGTATTCAGTTGCGC-3', antisense), and 2.5 units of Taq DNA polymerase (Gibco-BRL/LifeTechnologies, Rockville, MD), in a 50 µl final volume. A first cycle of 94º C/ 3 min, 50º C/ 1 min, and 72º C/ 3 min was followed by 25 cycles of 92º C/ 1 min, 52º C/ 1 min, and 72º C/ 3 min, and by a cycle of 72º C/ 7 min. The PCR products were separated by 1% agarose gel electrophoresis buffered in 0.5X TBE (Sambrook *et al*., 1989) and stained with ethidium bromide.

The RT-PCR products were cloned using the pGEM-T Vector System I (Promega, Madison, WI) as described by the manufacturer. *Escherichia coli* strain XL1 blue competent cells (Stratagene, La Jolla, California) were used for electroporation–mediated transformation, and recombinants were selected using the blue/white screening system.

Plasmid DNA, purified accordingly to a modified mini alkaline-lysis/PEG precipitation procedure (Taq DyeDeoxy Terminator Cycle Sequencing Kit, PE Applied Biosystems, Foster City, CA), was *Nco* I-digested in order to identify recombinants containing a DNA insert about 900 bp long. A *Nco* I site is present in both the sense and antisense primers used for RT-PCR (Souza Jr., 1999). Plasmid DNA from selected recombinants was sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) at the Nucleic Acid Laboratory, Embrapa Genetic Resources and Biotechnology, Brasília, DF. Data from both strands were used to assemble the *cp* gene sequence from each isolate.

Sequence alignment and analysis

The Contig Assembly Program for Sequence Fragment Alignment and Assembly (CAP3 - http://gcg.tigem.it/ASSEMBLY/assemble.html) was used to assemble the sequencing data generated for all twelve Brazilian isolates. Once assembled, the nucleotide and amino acid sequences were analysed using the DNAMAN 4.0 software (Lynnon BioSoft, Quebec, Canada).

RESULTS

Degree of similarity of the PRSV *cp* gene among Brazilian isolates

All Brazilian PRSV isolates have a 924 bp long *cp* gene, except Brazil.Bahia, BA-CA, and PR, which have a 921 bp long *cp* gene. A deletion of three nucleotides corresponding to the 43º amino acid was observed in the *cp* gene from these three isolates. The aphid transmission motif known as DAG triplet (Atreya *et al*., 1990; Shukla *et al*., 1994) is present in all 13 Brazilian isolates, as well as a stretch of glutamic acid and lysine repeats ("EK region") (Shukla *et al*., 1994), which begins at the third amino acid after the DAG triplet (data not shown).

Although the alignment and sequence homology analyses have considered the entire *cp* gene from the Brazilian isolates, it is important to state that the sense and antisense primers used for RT-PCR already contained four and ten nucleotides respectively from this gene. Therefore, only 98.5% of the sequence of the *cp* gene was actually obtained in this study. If there is any variation among the Brazilian isolates in these 14 nucleotides it was not considered here.

The alignment between the sequence of the *cp* gene from PRSV.Brazil.Bahia and the other twelve Brazilian isolates displayed an average homology of 97.3% and 97.1% for nucleotide and amino acid sequences, respectively. The most distinct pair, BA-CA vs. DFW, shares 93.8% homology at the nucleotide sequence, while the closest pair, BA-IT1 vs. BA-IT2, shares 99.9% of homology (Table 2).

The alignment between the sequence corresponding to the N terminal region (the first 224 nucleotides of the *cp* gene) from PRSV.Brazil.Bahia and the other twelve Brazilian isolates displayed an average homology of 95.0%, while the core (641 nucleotides after the N terminal region) and the C terminal regions (the last 56 nucleotides before the stop codon) displayed an average homology of 97.9% and 99.2%, respectively (data not shown).

The homology tree for the *cp* gene (Figure 1) separates the Brazilian isolates of PRSV into two main branches. The first branch comprises eight isolates (Brazil.Bahia, BA-CA, BA-IT1, BA-IT2, ES, SP, DF, and PR), and the second one contains the remaining five isolates (DFW, CEW, CE, PB, and PE).

Comparison between the *cp* gene from Brazilian isolates of PRSV and isolates from throughout the world

In order to execute the alignment and sequence homology analyses between the group of 13 Brazilian isolates and the one with 27 isolates from around the world (Table 1), it was necessary to perform some modification in the

TABLE 2 - Percent nucleotide sequence homology among the *cp* gene of *Papaya ringspot virus* (PRSV) isolates

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
BrazilBahia	1	100																																							
BA-IT2	2	96,2	100																																						
BA-IT1	3	96,1	99,9	100																																					
BA-CA	4	98,0	96,1	96,0	100																																				
CEW	5	94,1	95,1	95,2	94,0	100																																			
CE	6	94,0	95,2	95,1	94,0	96,3	100																																		
ES	7	95,3	96,2	96,1	95,4	94,4	94,3	100																																	
DFW	8	93,9	94,9	94,8	93,7	96,4	96,4	93,0	100																																
DF	9	95,7	96,4	96,3	95,7	94,8	94,7	95,7	94,6	100																															
SP	10	96,3	97,2	97,1	96,1	95,7	95,3	96,5	95,5	97,8	100																														
PE	11	94,5	95,1	95,0	94,2	95,8	96,4	94,4	96,0	94,7	95,5	100																													
PR	12	98,5	96,5	96,4	99,6	94,2	94,5	95,8	94,1	96,1	96,5	94,7	100																												
PB	13	94,2	95,2	95,1	94,2	96,8	96,8	94,5	96,6	95,1	95,5	97,0	94,7	100																											
AUS-WP	14	93,7	94,2	94,1	93,1	95,0	94,3	93,2	94,7	93,7	94,7	94,3	93,3	95,0	100																										
AUS-BUN	15	93,8	94,3	94,2	93,4	95,4	94,7	93,6	95,0	93,8	95,0	94,7	93,7	95,4	98,5	100																									
AUS-DAY	16	94,3	94,5	94,4	93,7	95,4	94,6	93,6	95,0	94,0	95,0	94,6	93,9	95,4	98,9	98,8	100																								
AUS-DB1	17	93,8	94,6	94,4	93,1	95,1	94,7	93,4	94,9	93,9	94,8	94,6	93,5	95,1	98,3	98,5	98,7	100																							
AUS-GAT	18	93,7	94,2	94,1	93,1	95,0	94,3	93,2	94,7	93,7	94,7	94,3	93,3	95,0	100,0	98,5	98,9	98,3	100																						
AUS-NT	19	93,9	94,4	94,3	93,3	95,0	94,5	93,5	94,9	93,9	94,9	94,3	93,6	95,0	98,1	98,2	98,5	98,1	98,1	100																					
AUS-BD	20	94,0	94,7	94,5	93,4	95,2	94,7	93,5	94,9	94,1	94,9	94,5	93,7	95,2	98,3	98,5	98,7	98,3	98,3	98,1	100																				
CHI-SM	21	88,3	88,4	88,3	87,9	89,5	89,4	88,7	88,4	88,4	88,9	89,3	88,2	89,6	89,2	89,5	89,7	89,4	89,2	90,0	89,6	100																			
IND	22	91,4	92,9	92,8	91,4	93,7	93,2	91,8	92,6	92,5	92,9	92,3	91,8	93,7	94,5	95,1	94,8	94,8	94,5	94,5	94,7	89,0	100																		
MEX-VTB6	23	92,7	92,9	92,8	92,3	94,4	93,1	92,0	93,0	92,2	93,4	93,0	92,2	93,9	95,7	95,8	96,0	95,7	95,7	95,5	95,9	89,1	93,3	100																	
JAM	24	90,8	91,6	91,5	91,2	93,2	92,9	91,0	92,2	91,3	92,2	92,2	91,4	93,1	94,1	94,3	94,6	94,1	94,1	94,4	94,3	87,8	91,9	93,7	100																
JAP-OK	25	89,1	89,9	89,8	88,8	90,2	90,5	89,7	89,5	89,5	89,9	90,2	88,9	90,7	90,9	91,1	91,3	90,9	90,9	91,3	91,2	92,6	89,7	89,7	88,7	100															
JAP-S	26	88,3	89,5	89,4	88,3	89,8	90,0	89,0	89,0	88,8	89,6	89,6	88,5	89,7	90,5	90,7	90,9	90,8	90,5	90,9	90,8	93,6	88,9	89,6	87,8	94,7	100														
MAL	27	88,4	89,0	88,9	88,1	89,6	89,5	88,7	88,7	88,7	89,0	89,2	88,2	89,7	90,1	90,4	90,6	90,3	90,1	90,6	90,5	92,5	89,0	89,2	88,2	97,5	93,8	100													
MEX-CHT11	28	91,6	91,6	91,7	91,1	93,0	91,8	90,6	91,6	91,6	92,0	91,6	91,3	92,5	94,5	94,6	94,8	94,5	94,5	94,5	94,9	88,2	91,9	93,5	92,1	89,9	89,5	89,4	100												
MEX-Colima	29	92,4	92,7	92,6	92,0	94,1	93,2	91,8	93,5	92,7	93,7	93,0	92,2	93,7	95,5	95,8	95,8	95,5	95,5	95,5	95,7	88,8	93,3	95,2	93,7	90,4	89,5	90,0	93,4	100											
MEX-VPO28	30	92,2	92,3	92,2	91,7	94,1	92,8	91,4	92,7	91,8	93,0	92,7	91,6	93,6	95,2	95,3	95,6	95,2	95,2	95,2	95,5	89,1	93,1	98,4	93,0	89,7	89,6	89,2	93,3	94,7	100										
INDW	31	86,0	86,1	85,9	85,5	87,4	86,3	85,8	86,7	85,8	86,1	86,5	85,6	86,6	88,6	89,2	89,2	88,6	88,6	89,0	88,9	85,7	86,4	88,6	85,7	85,9	86,2	85,1	86,7	87,2	88,0	100									
VIET	32	88,3	89,5	89,4	88,3	90,5	90,6	88,7	89,4	89,0	89,5	89,8	88,4	89,8	90,5	90,7	90,7	90,5	90,5	90,7	91,0	92,0	89,7	89,7	88,4	93,6	94,6	92,3	89,1	89,0	90,0	87,4	100								
TAW-YK	33	88,3	89,5	89,4	87,7	89,5	89,8	88,7	89,1	88,6	89,3	89,6	88,1	90,0	90,4	90,6	90,8	90,4	90,4	90,0	91,0	93,2	88,9	89,0	88,3	95,9	95,0	95,1	88,9	89,9	89,0	85,3	93,6	100							
THAP	34	90,0	90,3	90,2	89,6	91,1	90,8	90,1	90,2	89,7	90,6	90,3	89,7	90,3	90,8	91,4	91,4	90,8	90,8	91,4	91,2	93,1	89,9	89,6	89,2	93,8	95,4	92,7	89,3	89,9	89,6	88,4	93,1	93,7	100						
USA-FLW	35	92,8	93,5	93,4	92,5	94,8	94,3	92,9	94,0	93,2	93,8	93,5	92,9	94,5	96,8	96,9	97,1	96,8	96,8	96,8	97,1	88,3	93,8	95,2	93,4	89,7	89,6	88,7	94,3	94,8	95,2	87,1	90,6	89,5	90,8	100					
USA-H1K	36	93,0	93,5	93,4	92,7	94,7	94,7	93,1	93,9	92,9	94,0	93,7	93,0	94,5	95,9	96,1	96,3	96,3	95,9	96,2	96,2	89,7	94,2	95,8	94,0	91,0	91,0	90,2	94,4	95,3	95,3	87,5	89,7	90,4	90,3	96,4	100				
USA-HA	37	92,8	93,7	93,6	92,7	94,7	94,4	92,7	94,0	93,4	93,8	93,6	92,9	94,4	96,6	96,7	96,9	96,5	96,6	96,6	96,8	88,5	93,4	95,5	93,5	90,3	90,3	89,1	95,0	95,2	95,5	87,4	90,7	89,6	91,3	97,6	96,2	100			
USA-HA5-1	38	92,7	93,6	93,5	92,6	94,6	94,3	92,6	93,9	93,3	93,7	93,5	92,8	94,3	96,3	96,4	96,7	96,3	96,3	96,3	96,6	88,4	93,2	95,2	93,4	90,2	90,2	89,2	94,8	95,0	95,2	87,5	90,5	89,8	91,1	97,5	96,0	99,7	100		
USA-PR	39	92,0	92,3	92,2	91,4	92,9	92,6	91,6	92,2	92,4	92,9	92,5	91,8	93,1	95,0	95,2	95,0	94,8	95,0	94,9	95,0	88,4	92,7	93,4	92,7	89,9	89,7	89,4	93,3	93,4	93,0	87,3	89,0	89,5	89,6	94,3	94,8	94,3	94,1	100	
SRI	40	87,6	88,0	87,8	87,0	88,7	88,2	87,1	87,4	87,0	87,8	87,5	87,2	87,8	87,8	88,2	88,2	87,8	87,8	88,1	87,8	87,6	88,8	87,6	87,1	89,3	88,5	88,6	86,1	86,8	87,9	86,1	88,0	88,7	88,5	88,6	88,8	88,3	88,1	86,7	100

sequences obtained from the literature and GenBank. The modification done was the removal of all sequences that were not a part of the *cp* gene. The stop codon of the *cp* gene from each isolate was localized and the stretch of nucleotides 924 long, upstream (and including) this codon, was selected for analysis. When the sequence upstream the stop codon was longer than 924 nucleotides, the sequence upstream this stretch was removed; however, when the sequence was shorter than 924 nucleotides, no further modification was done.

The alignment of all 40 sequences showed an average homology of 90.7% at the nucleotide sequence. An alignment only with 31 known P type sequences (Table 1) displayed an homology of 90.9%. The most distant pair, INDW vs. MAL, shared 85.1% of homology for the nucleotide sequence, while the closest one, AUS-WP vs. AUS-GAT, shared 100% homology (Table 2).

The homology tree (Figure 1) for the *cp* gene separates the 40 PRSV isolates used in this study into two main branches. The first branch comprises 31 isolates (all Brazilian, Australian, American, Mexican, Jamaican isolates and an Indian isolates), while the second one contains seven isolates (all from Asia). Only the isolate from Sri Lanka and a type W isolate from India are not included in any of the two well defined branches (Figure 1).

The alignment of the sequences corresponding to the core region, using all 40 isolates, revealed an average homology of 95.7%, while the C terminal region showed an average homology of 98.5%. The homology tree generated from the alignment of the Core region of all isolates maintained the same organization as the one done with the entire *cp* gene sequence (data not shown).

DISCUSSION

Our results have shown a remarkably high degree of homology on the nucleotide sequence of the *cp* gene of 13 PRSV Brazilian isolates. This very high degree of homology is puzzling, considering that the isolates were obtained from distinctive areas in Brazil, some of them about 2000 miles apart.

Previous reports have shown distinctive results when using isolates from Australia and Mexico. Bateson *et al.* (1994), studying seven Australian isolates (four P-type and three W-type), found that they shared a high degree of homology in the *cp* gene sequence, ranging from 98.1 to 98.9%. However, six out of seven Australian isolates came from Queensland. On the other hand, Silva-Rosales *et al.* (2000), studying three Mexican P-type isolates from geographically close areas, observed a lower degree of homology, ranging from 93.4 to 98.4% at the nucleotide sequence. Regardless of the great distance between the areas where the isolates were collected in Brazil, the lowest degree of homology disclosed between isolates was 93.8%. As expected, the highest degree of homology was observed between the only two isolates collected in the county of Itabela, in the State of Bahia, which showed 99.9% of homology.

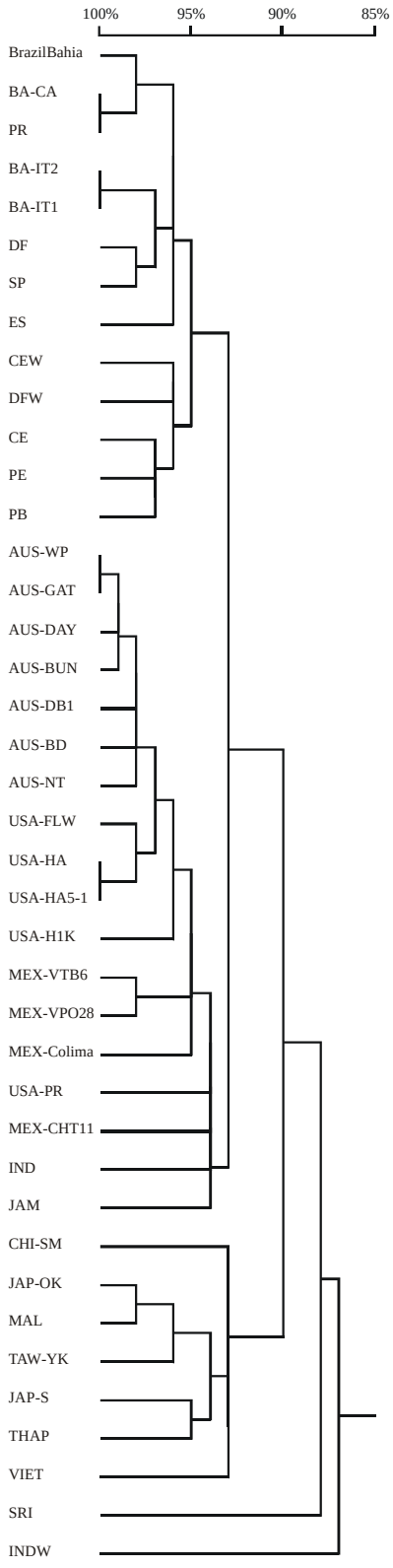

FIG. 1 - Homology tree for the *cp* gene from 13 *Papaya ringspot virus* (PRSV) Brazilian isolates and 27 additional isolates from diverse world locations. Nucleotide sequences were analysed using the DNAMAN version 4.0 software (Lynnon BioSoft, Quebec, Canada).

A closer look at the homology tree shows both Brazilian W-type isolates in the same branch, which also contains all the P-type Brazilian isolates. This result reinforces the hypothesis from Bateson *et al*. (1994), who suggested that P-type isolates are generated from W-type isolates, possibly by mutation.

Bateson *et al*. (1994) compared the nucleotide sequence of 13 PRSV isolates from different parts of the world, although mostly from Australia, and showed that their *cp* genes did not diverge more than 12%. Tennant (1996) compared the coat protein sequences of 17 PRSV isolates and found that this protein did not diverge more than 12%. Souza Jr. (1999), when comparing the *cp* gene sequence from 22 different PRSV isolates, observed that the most distant pair had 84% of homology at the nucleotide sequence when comparing P-type and W-type isolates, while the most distant pair among the P-type isolates showed 86.3% of homology. Silva-Rosales *et al*. (2000), evaluating the *cp* gene sequence from 14 isolates, observed a degree of homology ranging from 81.1 to 99.8% at the nucleotide sequence, and from 89.7 to 99.3% at the amino acids sequence. In preparing the sequences for analysis, we decided to remove all sequences that do not belong to the *cp* gene, a decision apparently not taken before by the groups studying the homology degree of the PRSV *cp* gene. That is probably the reason for some differences observed between our results and the results from other groups for the same pair of isolates. Our results, derived from 40 different isolates from all over the world, showed pairs with degree of homology varying from 85.1% to 100% at the nucleotide sequence. These results support Gonsalves (1998), who stated that the PRSV types P and W cannot be distinguished on the basis of their *cp* gene and coat protein sequences.

Studies with the PRSV resistant 'Rainbow' and 'SunUp' papayas have shown that the resistance in these transgenic lines is RNA-mediated and operates via PTGS (Tennant, 1996; Souza Jr., 1999). When the first transgenic papaya, line 55-1, showed isolate-specific resistance, it was thought that the fastest and safest way to produce a transgenic papaya resistant to an specific isolate would be by using the *cp* gene from that particular isolate. However, as more papaya transgenic lines were generated (Tennant, 1996; Cai *et al*., 1999; Souza Jr., 1999), and more was learned about this resistance system in papaya, it was recognized that a wide spectrum of resistance can be achieved using the *cp* gene from any isolate. For instance, Souza Jr. (1999) obtained transgenic papayas expressing the *cp* gene from a Brazilian isolate, but resistant to the donor isolate and to isolates from Hawaii and Thailand.

It has been shown that factors such as plant age, inoculum concentration, gene dosage, and the degree of homology between the *cp* transgene and the *cp* gene from the incoming virus, play an important role in the outcome of the interaction between the transgenic papaya and PRSV (Tennant *et al*., 1997; Souza Jr. *et al*., 1998; Souza Jr., 1999). It seems that, as the degree of homology is reduced, a higher gene dosage is necessary to permit a plant to be resistant to that specific isolate. The higher the gene dosage, the wider the spectrum of resistance (Souza Jr., 1999). Transgenic plants resistant to isolates sharing 10 to 11% of heterogeneity among their *cp* genes were already observed (Yeh *et al*., 1997; Souza Jr., 1999). The low degree of heterogeneity among the Brazilian isolates, demonstrated in this present study, suggests that the probability of obtaining transgenic papaya plants with broad spectrum resistance to Brazilian isolates, and therefore able to be planted anywhere in Brazil, is higher than initially expected. Even the hemizygous transgenic Ro plants that were only resistant to the Brazilian donor isolate, but not to isolates from other countries (Souza Jr., 1999), could show a broad spectrum of resistance inside Brazil. However, to confirm the prediction that local Brazilian isolates of PRSV will not overcome resistance in transgenic papayas, it is necessary to challenge these plant with these different isolates. This experiment is expected to be done as soon as a population of transgenic papaya, homozygous at the *cp* locus, is available.

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