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Virus Resistant Papaya Plants Derived from Tissues Bombarded with the Coat Protein Gene for Management of Papaya Ringspot Virus Disease

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Abstract

Papaya (Carica papaya) gets damaged by the papaya ringspot virus (PRSV). Transgenic papaya was generated using the coat protein gene isolated from the Papaya ringspot virus Thai isolate. A vector that contains the coat protein gene under the supervision of a 35S promoter has been developed and transformed by microprojectile bombardment into somatic embryos of Khak Dum papaya cultivar. Eight transgenic lines of papaya somatic embryos bombarded by kanamycin were reported from 1980. PCR amplification of the protein gene, GUS research and Southern blot hybridization has checked the incorporation of the transferred genes into kanamycin-resistant papaya calli. Although the coat protein gene was found to be highly resistant to virus in all transgenic lines, out of which only line G2 was found to be resistant. The resistant line showed the inserted coat protein expression cassette to a high level of rearrangement while the coat protein gene itself was removed by 166 bp at the 3 ' end of the series. While RT-PCR was detected in every transgenic line in the transcription of the coat protein gene, only two transgenic papayas were expressing the intact coat protein. In addition, the volume of the truncated protein mRNA in the resistant line G2 has been significantly reduced. These results suggest that the RNA mediated process, which is presumably formed in post-transcriptional gene silencing, is a protein mediated resistance in papaya.

Key words: Microprojectile bombardment, Papaya (Carica papaya), Papaya ringspot virus (PRSV), PCR amplification, Post-transcriptional gene silencing, Somatic embryos, Transgenic papaya, Truncated protein mRNA.

Introduction

Papaya is a member of the Caricaceanic family, and in many tropical and subtropical countries is one of the most economically valuable fruit plants. Papaya is a diploid, polygamous and dicotyledonous family. Southern Mexico and Costa Rica are the geographic origins of Papaya [1]. Papaya was cultivated in USA, India, Malaysia, Peru, Thailand and the Philippines, Brazil, Mexico, Jamaica, Indonesia, China. The fruit of papaya is known for its high nutritional and medicinal value. Papaya, as well as proteolytic enzymes like papain and chymopapain, is a rich source of vitamins A, B, C. It is a good source of beta carotene that can help prevent heart disease, cancer and diabetes. Mature fruits are normally fresh eaten and can be turned to jam, jelly, marmalade and sweets. For fruits, "green" or unripe fruit can be used. Papayas are also used in the pharmaceutical and cosmetics industries [2].

The disease problems of the Papaya Ringspot Virus (PRSV) are currently occurring on plants. PRSV symptom manifests as prominent pattern of mosaic on leaf lamella, wet-oily streaks on the pectioles and upper trunk and young leaves distortion. PRSV is the world's greatest threat for the production of papaya. In many tropical and subtropical areas, including USA, South America, India, Thailand, Taiwan, China and the Philippines, Mexico, Australia, Japan, French Polynesia, and the Cook Islands, the PRSV has been recognized as a destructive disease that results in a reduction in the fruit yield [3]. In some areas, this disease can lead to crop losses of up to 100%. In a process involving coat protein (CP) and helping component proteinase (HC-Pro), PRSV is transmitted in an unpersistent manner by various types of aphids. Papaya and the aphid virus are widely transmitted. Different methods, such as roughing infected plants, barrier cultivation, cross protection and transgenic resistance, monitor PRSV.

Papaya and the aphid virus are widely transmitted. Different methods, such as roughing infected plants, barrier cultivation, cross protection and transgenic resistance, monitor PRSV. PRSV disease management is very difficult to conduct by vector control, though cross defense is not effective globally for controlling PRSV disease [4]. Carica papaya was not found to be immune to PRSV. Genetic plant transformation has allowed the selected genes to be incorporated into the plants in order to combat plant diseases and pests. The idea of pathogen-derived resistance has stimulated work to achieve virus in papaya through gene technology. Pathogenic resistance is mediated by the transgenic (protein mediated) proteins or by the transcripts generated from the transgene (RNA-mediated). Recently, research indicated that the RNA-based post-transcriptional gene-silencing mechanism mediates the pathogen-derived resistance. Protein-mediated resistance provides the modest protection against a wide range of associated viruses while RNA-mediated resistance provides a high level of protection against the virus's closely associated strains [5]. The RNAi technology has allowed an immune reaction to PRSV to be induced. In order to develop environment friendly molecular methods, this research has been at the front of a new era, with which specific genes which are responsible for control of diseases can be silenced.

At present in Hawaii, transgenic Papaya is cultivated and comprises over 70% of the acreage of Hawaiian Papaya. In the USA, SunUp and Rainbow were common without any negative human health effects [6]. The CP gene from their geographical region is used for developing region-specified transgenic papayas to control PRSV, in countries such as Australia, Jamaica, Venezuela, Vietnam, Thailand, Taiwan and the Philippines. Several studies have been conducted on the development of PRSV-resistant C. papaya. PRSV Disease Management Papaya is available via genetic technology. According to a researcher, the progress has been summarized in different countries of transgenic papaya technology and research but did not cover all fields of PRSV management. This review paper will therefore review recent PRSV, genomics, PRSV diversity, molecular identity and vector transmission determinants, biosafety and major challenge which are being faced.

1. The Papaya Ringspot Virus (PRSV) genome:

PRSV is Potyvirus genus which belongs to the Potyviridae family [7]. The PRSV genome is made up of about 800–900 nm of versatile filamentous, unenveloped particles with a sequence of approximately 10,324 nuclear ssRNA. The virion contains a weight of 94.5% protein and 5.5% nucleic acid. A large protein (3.344 amino acids) is inserted into the PRSV genome, which is subsequently split into less protein with different functions. Fig. 1 shows the proposed map of PRSV polyprotein [8].

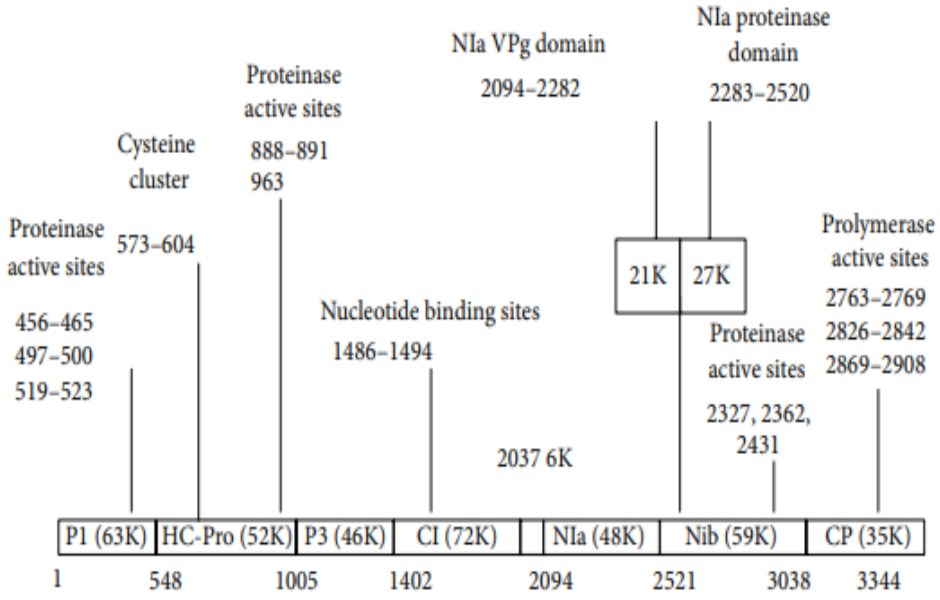

Fig.1: The map of the PRSV polyprotein. Specific motifs are indicated, solid bars indicate cleavage sites in the polyprotein, and the dashed line indicates the potential internal site of the NIa protein.

2. The genetic diversity of PRSV:

For successful evidence-based disease management, information about PRSV's genetic diversity is critical. Throughout different regions of the world, the genetic diversity of PRSV has been seen. For establishing the origin, growth, dispersion and etiological diseases of virus isolates, sequence diversity and their distribution is essential in the pursuit of effective management of the virus disease. The CP genes of the PRSV isolates in the USA and Australia have some sequence variability [9]. The CP genes of PRSV isolates from India and Mexico, however, have a higher sequence variance. The variety of PRSV isolates in the Asian population at amino acid and nucleic acid levels was highest. The isolates of PRSV found in India were different from those of the other countries. A researcher has confirmed that the PRSV originated in South Asia and that PRSV isolates from India have a greater diversity. There were variations in PRSV due to differences in the length of the PRSV gene. In the CP and HC-Pro genes obtained from India the largest diversity of PRSV nucleotide sequences was observed. PRSV could have been transported in the early 18th century from

India to America and spread throughout the 19th and 20th century [10]. Across various parts of the world, differences in the CP PRSV gene sequences were found. PRSV genetic variability is geographically defined. The transgenic CP (HA 5-1) papaya isolated from the United States demonstrated resistance to PRSV infection with the extreme United States isolate (HA), for example but did not exhibit resistance to infection with PRSV isolates of the Australian or Thai Isolates.

3. Host range determinants and vector transmission:

Virus plant propagates from cell to cell by virus and host factors interacting. The plant viruses enter the host cell mechanically or through vector mechanisms through wound sites. Two other ways viruses infect plants: the short distance (cell to cell) and the long distance. In the plant, viral movements rely on different reactions of the host. PRSV has few hosts in the Caricaceae, Chenopodiaceae and Cucurbitaceae families. The PRSV spread hosts are *C. papaya, Cucurbita pepo, Cucumis metuliferus. Chenopodium quinoa* and *Chenopodium amaranticolor* are lesion test hosts of PRSV. Two PRSV strains can be distinguished according to their host selection. The Papayas may be infected by PR SV (PRSV-P), while PRSV-W (PRSV-W) may infect cucurbits only. They are closely related to both strains. According to a researcher, the CP gene is not a determinant of papaya infection. The papaya disease was caused by the NIa and a portion of the NIb gene PRSV. P1 and HC-Pro mutation caused PRSV symptoms to attenuate papaya and *Chenopodium quinoa* for the local lesion formation, while HC-Pro is the leading determinant of lesion formation in *Chenopodium quinoa.* For biological importance and to establish disease management strategies the relationship between the host and PRSV is important [11]. While cross-cutting at molecular level is not clearly understood, post-transcriptional gene silencing (PTGS) in Papaya cultivation is effective in the management of PRSV. Several species of aphids transmit the virus in an unexpected way. For PRSV vector transmission, CP and HC-Pro are required. Transmission happens if aphids feed on infected papaya plants and feed on healthy papaya plants.

4. Molecular diagnostic of PRSV:

The key move is to classify viruses in order to control PRSV effectively. Diagnosis of PRSV is critical because it occurs in various strains. The particulate virus is very fragile and usually adds plant particles. The PRSV is diagnosed primarily through symptom assessment; a rapid but also unreliable visual diagnosis. The effects of micronutrient deficiency in land and a number of weather conditions can be the causes for the symptoms similar to PRSV. Molecular diagnostics such as ELISA, RT-PCR immunocapture, RT-PCR and DIBA could be reported to PRSV. ELISA is widely used as a fast and reliable technology for rapid detection of PRSV in papaya in various parts of the world [12]. The RT-PCR immunocapturing technique for rapid virus identification can detect low PRSV concentrations in papaya and is more active than ELISA, RT-PCR, and DIBA. Reverse transcrits and polymerase chain reaction (RT-PCR) were reported by Ruiz-Castro and Silva-

Rosales to show reliable results for the detection of PRSV in papayan samples. DIBA is an easy and economical tool for large scale viral detection that is useful for indexing viruses.

5. Strategy for PRSV disease management:

PRSV is Papaya's most harmful viral disease. PRSV regulation requires rouging and sprinkling of contaminated plants with aphicides. Nevertheless, the spread of the disease cannot be stopped when it is created. Likewise, aphicide spraying is often unsuccessful, because before the aphids are destroyed, a virus is transmitted to the plants. The management of PRSV disease has been concentrated on cultivating tolerant or resistant papaya, but rarely planted because of the poor fruit quality and intensity. In some wild varieties associated with Carica species, PRSV-resistant genes are available. Nevertheless, it has been difficult for PRSV-resistant varieties to evolve through traditional breeding methods as wild and cultivated papayas are incompatible sexually. This PRSV strategy also restricts the disease resistance in commercial papaya crosses. Cross defense against economic damage caused by severe strains of the same virus was used to monitor the PRSV [13]. The cross-protection strategy of inoculating PRSV somewhat provides resistance to the serious PRSV infections. Cross safety relies on the availability of mild strains to defend against the target virus effectively. Extra agricultural practice and care are required for cross-cutting protection. Nevertheless, the benefits of this method are limited by the strain specificity and the technical difficulties associated with the propagation of the pure strains of mild forms of the virus. Field tests have shown that the cross protection in the field for PRSV management assessment was marginally effective. The two researchers have suggested the idea of pathogen-derived resistance for the production of pathogen resistance. The idea of disease dependent resistance was extended to this research group, which stimulated research into the achievement of viral resistance through gene technology. Protein-mediated as well as RNA-mediated methods control the pathogen dependent resistance. An alternative strategy has been developed using transgenic plants expressing viral genes using RNA-mediated gene silencing. Despite success in this approach, PRSV resistance levels vary from environmental factors and plant development. Broad spectrum resistance to various PRSV Isoli isolates may depend on the homology and genetic heterogeneity of the various PRSV strains that relate to the geographic distribution of transgenes with viral target genes [14]. For various Papaya growing regions, the transgenic papaya varieties PRSV-resistant to various viral strains must be individually produced. Developing PRSV-resistant lines is commonly seen as the best strategy in papaya for efficient control of PRSV diseases.

6. Gene technology for the development of PRSV-resistant transgenic papaya:

Crops resistant to the viral diseases can usually be created through viral sequence genes that provide PDR, genes from various other sources which can interfere with the target virus, and natural resistance genes. A new approach to PRSV management is the principle of pathogen-derived resistance (PDR). Pathogenic related genes in their host plants in various ways interfere with the replication cycle of viruses. Using coat protein (CP, RNA silencing, and the gene replicate technology) PRSV-resistant transgenic papaya was previously developed.

Conclusion

The biggest threat to the production of papaya is PRSV. PRSV disease management has been focused on transgenic papaya through gene technology. In this study it has been analyzed that the CP genes or RNA interfere with pRSV-resistant papayas have been established. PRSV has been recognized in the world as a genetic diversity. The failure is the main challenge facing the production of transgenic papayas. Although the PRSV-transgenic papaya gene flux is small, work should be carried out to minimize this issue. PRSV-resistant transgenic papayas are still slow and are dependent on the demand for papaya, biosafety regulations and technology social acceptability. Recent studies show that PRSV-resistant transgenic papaya does not have adverse environmental effects on human health and is environmentally safe. Post-transcriptional gene silencing technology (PTGS) could be ideal for the future development of transgenic PRSV-resistant papaya. This analysis indicates that papaya producing countries should use their own PRSV isolates to develop post-transcriptional gene silencing technology to produce PRSV Resistant transgenic papaya.

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