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Standard Quantification of Gene Expression in Viral Infected Plant by using Real Time PCR

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Abstract: **-- Real time PCR is currently considered as the gold standard method for detection of plant pathogen. Real time PCR aiming at quantifying the level of plant infection by viral pathogens are becoming more and important within microbiology. Real time PCR is frequently used in gene expression studies as it fits perfectly with it wide dynamic range, sensitivity, and ease of automation possibilities. This technique allows the monitoring of the reaction during the amplification process by using of a fluorescent signal that increases proportionally to number of amplicons generated and to the number of target present in the samples. Real time PCR is the reliable and provides high throughput quantification of target viral pathogens DNA in various environmental samples, including hosts tissues, soil, and air. Real time PCR has versatile practical application in diagnostic of plant disease. Monitoring of health and detection of diseases in plants is critical for sustainable agricultural. Till now 85 % of viral diseases have been diagnosed in virus infected plants. Real time PCR plays a significant role in better understanding of the dynamic of plant pathogenic microbes and thereby allows better management of diseases.**

Keywords: **-- Real time PCR, viral pathogens, Quantification, fluorescent, diagnosis.**

I. INTRODUCTION

Plant viruses are widespread plant pathogens. Virtually all plants that humans grow for food, feed and fibre are affected by at least one virus. It is the virus infecting cultivated crop that have been intensively studied because of financial implications of the losses they incur. However, it is also important to recognise that many wild plants are also hosts to viruses. Although plant viruses do not have an immediate impact on human, the damage they do to food supplies has a significant indirect effect. The study of plant viruses has a junction rectifier the overall understanding of viruses in several aspects.

Viruses causing many plant diseases are responsible for huge losses in crop production and quality in all parts of the world. Infected plants may show a range of symptoms depending on the disease; often culminating in leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the total plant, abnormalities in flower or fruit formation).

Sometimes the virus is restricted to sure elements of the plant (e.g. the vascular system; discrete spots on the leaf) but in others it spreads throughout the plant causing a systemic infection. Infection does not always result in visible symptoms (as witnessed by names such as Carnation latent virus and Lily symptomless virus, both members of the genus Carla virus). Occasionally, infection may end up symptoms decorative worth, like 'breaking' of tulips or variegation of dilleniid dicot genus.

Viral Pathogens are estimated to cause around 12.5% of global crop losses, threatening many commercially and socially valuable crops, such as coffee, cassava, oranges, olives, wheat and rice. Bacteria, viruses, and fungi can reduce crop yields, impact on crop quality and in some cases kill their hosts leading to an impact to food security; as diseases in a plants cause major production and economic losses in agricultural industry world-wide. Plant protection and food safety is important because 60% GDP is based on the agronomic area in India. The obvious reason is that everybody needs food. But the quality of delivering enough food to a national population and to the total world's population shows why food security is such a priority for all countries, whether developing or developed. In short, that can be a world challenge as a result of it's not just regard to food and feeding folk however conjointly about much all aspects of an economy and society. Consider in India, agriculture accounts for 18% of the economy's output and 47%o f its workforce. An India the second biggest producer of fruits and vegetables within the world. Yet in keeping with the Food and Agriculture Organization (FAO) of the United Nations, some 194 million Indians are undernourished, the largest number of hungry people in any single country. Agriculture must also be sustainable. Waste is also a problem, with an estimated one third of food consumption, some $750 billion in value, lost. So monitoring of health and detection of diseases in plants is critical for sustainable in agriculture.

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II. MATERIAL AND METHODS

In 2015 researcher Wei-Lin Fu and his co workers worked on the quantification of gene expression using the Real Time PCR. They quantified the viral gene expression in sugarcane. They developed the protocol for the detection of viral gene expression and identified the gene expression virus in the host.

2.1 RNA extraction: Research performed by Wei-Lin Fu used a collection of 35 infected leaf sample from different developing countries and extracted RNA by using TRIZOL

2.2 Primer and probe design: Primers required for the amplification of new DNA strands were collected from Genbank library by using the primer express system version 2 for detection of sugarcane streak mosaic viral infection.

2.3 One-step Real-Time PCR assay perform by Wei-Lin Fu: Standard RNA was developed by using the RNAscript RT-PCR kit and PGEM-cp plasmid.

Particular signals which developed during the amplification on RT-PCR were quantified using reporter dye and quencher on 3' end.

2.4 Standard curve developments: Standard curve was developed for the comparison with the observed data from different samples. Base on which comparison of the gene expression of viral particles were performed.

2.5 Real Time PCR and different types of probes: Real-Time PCR or quantitative PCR (qPCR) is a PCR-based technique that is able to simultaneously amplify and detect changes in the amplicon concentration. Real-time PCR collects data during PCR amplification by utilizing fluorescence signal emitted by either special probes or DNA binding dyes. The ability to detect fluorescence signals is crucial to the proper functioning of qPCR. Proper instrumentation is required for both input of energy to excite fluorescent dyes as well as detection of wavelengths emitted by these dyes. Three main ways an instrument is able to provide excitation energy includes: lamps, LEDs and lasers Data is collected by utilizing photo detectors that only allows passage to desired wavelengths (the peak of the emission spectrum of the fluorophore) in a single reaction tube. The last portion of the instrument consists of a regular thermal cycler for PCR amplification. Appropriate computer software is required for the proper collection and analysis of data.

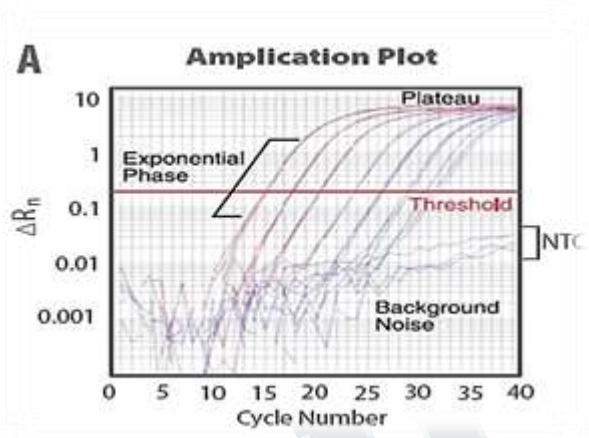

Fig1.Amplification Plot. Cycle Number Is On The X-Axis Plotted Against ΔRn

2.5.1 Taqman probe: The simplest and most commonly used types of probe is the Taqman probe. The Taqman probe method uses a fluorescent labelled probe that hybridizes to an additional conserved region that lies with the target amplicon sequence. These probes are labelled with a fluorescent reporter molecule at one end a quencher molecule at the other. Hence, the reporter and quencher are physically separated and the fluorescence increase fluorescence can be measured throughout the PCR.

2.6 Sequences analysis: Wei-Lin Fu obtain different sequences with individual primers during the quantification of the SCSMV (sugarcane streak mosaic virus) gene expression. They also obtained SCSMV, SrMV and SCMV sequences by BLAST search in NCBI.

2.7. Absolute quantification method: Absolute quantification gives the exact number of target DNA molecule by comparing with DNA standard using a calibration curve.

1. Absolute quantification gives the exact number of target DNA molecule by comparing with DNA standard using a calibration curve.
2. Absolute quantification measures the PCR signal to input copy number using a calibration curve.
3. The calibration curve used in absolute quantification can be based on known concentration of DNA standard molecules.
4. Calibration curves are highly reproducible and allow the generation of highly specific sensitive and reproducible data.
5. Absolute quantification is requires the standard calibration curve using serially diluted standard of known concentration for highly specific, sensitive and reproducible.

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6. In this method the linear relationship between Ct value and initial amount of total using standard curve allows the RNA detection of known concentration based on their Ct value.
7. In this method the standard and target sequence should have the same primer binding site and produce a product of approximately same sequence. Standard can be synthesized by cloning the target sequence into plasmid, purifying the conventional PCR product.
However, DNA standard is generally not feasible as a standard for absolute quantification of RNA because there is no control for the efficiency of reverse transcription. Therefore, RNA molecules are strongly recommended as standard for quantification of RNA where an in vitro transcribe sense RNA transcript is generated. The sample is digested with RNAs free DNase allowing for accurate quantification. A recombinant RNA can be synthesised in vitro by cloning the DNA of GOI into a suitable vector. After in vitro transcribed RNA is synthesised, the standard concentration is measured on a spectrophotometer.
The copy number of sample RNA can be calculated by compared of sample Ct values to that standard curve after Real Time amplification

2.7. Relative quantification

1. Relative quantification is easier to carry out as it does not require a calibration curve as the amount of the studied gene is compared to the amount of a control reference gene.
2. References gene (housekeeping gene): abundantly and constantly expressed gene. Expression level of this gene remains constant.
3. Relative quantification measures the relative change in mRNA expression levels.
4. The main advantages of relative quantification is that standard with known concentration is not required so that there is no need for generating a standard calibration curve.
5. During the relative quantification amount of target and reference gene can be determined with same sample.
6. After reaction the ratio between each target and reference gene is calculated. Gene expression is quantified by using the Delta method.

IIII. RESULT

3.1. Sequences alignment and primer specificity: The phylogenetic tree of the coat protein encoded gene is used for the report the genotype.
3.2. Standard curve of the real time q-RT PCR assay: The standard curve is established using the tenfold serial dilution of the standard ssRNA copies determine from the q-RT PCR.
3.3. Application of the Real Time PCR: Using the different viral infected sample collected from the different countries identification and gene expression for virus infected plants are possible.

IV. DISCUSSION

Real Time PCR technique shows great potential for plant biotechnology and plant pathology for better detection and for quantifying the viral gene expression in infected plants.

V. CONCLUSION

Real Time PCR is used for detection and accurate identification of viruses and is one of the most important strategies for controlling plant diseases. This test is the most accurate and specific test in diagnosing plant disease. It is a suitable technique for multiple pathogen detection in a single assay. Currently, it can be expensive for routine application for diagnostics due to the high cost of machines and reagents. Hopefully, in future this technique may become economically more widely accessible. Real Time PCR technique shows great potential for plant biotechnology and plant pathology for better detection and quantifying the viral gene expression in infected plant.

VI. INFERENCES

6.1. Developing of Real Time PCR for future expansion: Crops can be infected by many types of viruses, which is the cause the different types of diseases in plants. Recently molecular biotechnology is developing the new modified Real Time PCR for the detection of multiplexing viral gene expression for more than one virus infecting the plants. Now more than one viral gene expression can be quantified using Real Time PCR. Real Time PCR is also very helpful for the research work on tomato in fruit biology because normally tomato is used for the research work. So references gene are used for quantify the gene expression during the fruit ripening and also during the infection of viruses.

VII. ACKNOWLEDGEMENT

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