Virus Amplification

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Abstract

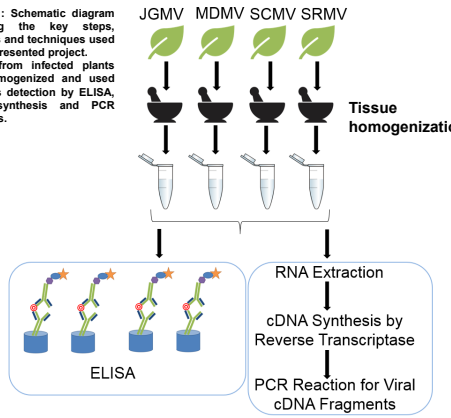
Sorghum is the fifth most produced cereal in the world and a source of nutrients for humans, feeding more than 500 million people in Africa and Asia[1]. It is grown commercially for food, animal feed, fiber and fuel in roughly 100 countries including U.S. [2]. Worldwide, feeding by 150 insect species causes substantial economic damage to sorghum[3]. Besides feeding damage, aphids such as the Corn Leaf Aphid vector Sugarcane mosaic virus (SCMV), Sorghum mosaic virus (SRMV), Maize mosaic virus (MDMV) and Johnsongrass mosaic virus (JGMV)[4]. These are Potyviruses, the largest group of the Potyviridae family with 176 members [5] and cause substantial yield losses to sorghum, sugarcane, and maize. SCMV, SRMV, and MDMV are closely related whereas JGMV is more distantly related to them [6]. All have an average 9.7 kb positive-sense single-stranded RNA genome encoding 10 mature proteins in a single large ORF [7].

Objectives

The predominant detection method for JGMV, SRMV, SCMV and MDMV is ELISA. However, a quantitative method such as qPCR could provide insights into virus accumulation and replication rates in plant tissues. Therefore, the aim of this project was to design and test 4 primer sets for JGMV and 4 primer sets for MDMV, SCMV and SRMV CP protein in order to provide the basis for further research.

Experimental Flowchart

Figure 1: Schematic diagram depicting the key steps, methods and techniques used for the presented project. Tissue from infected plants was homogenized and used for virus detection by ELISA, cDNA synthesis and PCR reactions.



Results

PCR primer design for viruses

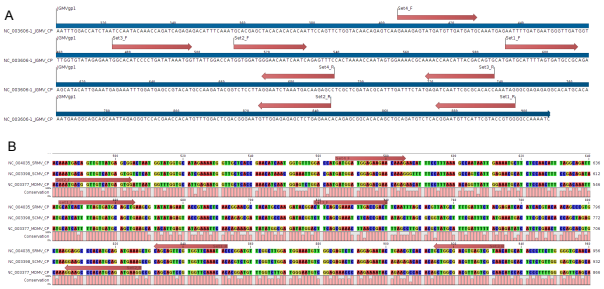


Figure 2: PCR primer design for JGMV (A), MDMV, SCMV and SRMV (B) CP protein gene. Primer regions were chosen based on the G/C content and sequence conservation.

cDNA test and immunological potyvirus detection

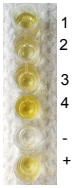


Figure 3: ELISA test for presence of 1: JGMV, 2: MDMV, 3: SCMV,4: SRMV in individually infected plants.

Negative control(-) contained extract of uninfected sorghum plant. Positive control (+) was provided in the Potyvirus ELISA kit.

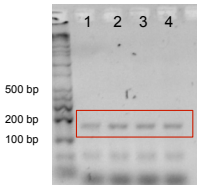


Figure 4: cDNA quality test with primers for *Sorghum bicolor* Eukaryotic Initiation Factor 4A reference gene 1: JGMV;2: MDMV; 3: SCMV; 4:SRMV. Specific amplicons highlighted in red boxes.

PCR based potyvirus detection

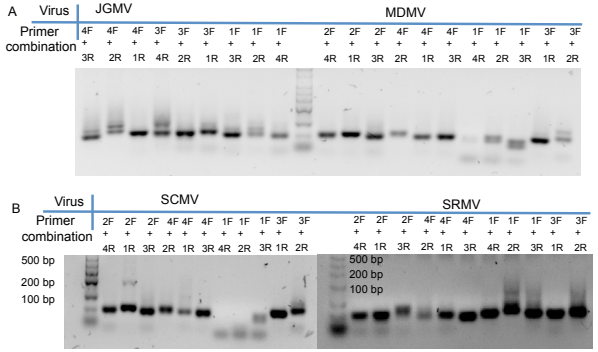


Figure 5: Detection of viral CP gene via standard PCR on 2% Agarose gels. Primer combinations for each virus are shown above the gels. A: JGMV and MDMV amplicons for each primer pair. B: Gel separated SCMV and SRMV PCR products. Intense bands in the range of 50 bp indicate low primer specificity resulting in unspecific products.

Conclusion

The initial step to verify the presence of JGMV, MDMV, SCMV and SRMV in the infected *Sorghum bicolor* plants was done by sandwich ELISA targeting the Potyvirus CP protein. All four samples including the positive control resulted in clear color development at 405 nm in contrast to the negative control confirming the presence of the viruses in plant tissue [Fig. 3].
The successful RNA conversion to cDNA by reverse transcriptase reaction using random oligonucleotides was verified by a subsequent PCR utilizing the *S.bicolor* reference gene (Eukaryotic Initiation Factor 4A) primer set [Fig. 4].
Attempts to amplify viral CP gene fragments using different primer combinations failed for all 4 viruses.
None of the viral primer combinations resulted in specific and calculated amplicon lengths between 100- and 300 bp [Fig. 5].
Considering that only a single isolate genome for each virus is available on NCBI, the design of PCR primers can be difficult and result in less specificity for an isolate from another geographic region. Subsequently, this results in sub-optimal primer annealing and unspecific amplicons during the PCR reaction.

Future Directions

The goal of a method to obtain suitable PCR primer sets to detect Potyvirus isolates requires further optimization. This will be accomplished using two approaches. Attempts will be made to optimize PCR reaction settings for each primer set, or the entire virus genome may be cloned into cloning vector and sequenced to acquire the exact sequence(s) for primer design.

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