Detection of Bitter gourd Yellow Mosaic Virus (BGYMV) in Bitter gourd (*Momordica charantia*) through Molecular Markers technique.

1. BGYMV infected Bitter gourd leaves and healthy Bitter gourd leaves sample Collected from field.

2. Total DNA was extracted from BGYMV infected bitter gourd leaves and healthy bitter gourd leaves.

3. Used BGYMV specific degenerate primer for Gemini virus to detection of viral DNA.

4. Transmission vectors of the family Geminiviridae.

 Geminiviridae is the family that contains the greatest number of viruses. General symptoms of diseases caused by Geminivirus are curling of leaves, yellowing of veins, yellow mosaic patterns & dwarfing of leaves. Virus belonging to the family Geminiviridae are plant viruses that are obligate intracellular parasites, having no machinery to replicate themselves. The family comprises four genera: Begomovirus, Curtovirus, Mastrevirus & Topocuvirus. Begomovirus is one the biggest genera of the family (Medina-Ramos et al 2008). It comprises about 200 species that are found woldwide. Begomovirus principally affected dicotyledonous plant species. These viruses are transmitted by whitefly (Bemisia Tabaci).

Detection of BGYMV in bitter gourd by PCR using Gemini virus degenerate primer

BGYMV-1 degenerate primer Sequence are as below,

BGYMV-1 Forward: TAATATTACCKGWKGVCCSC

BGYMV-1 Reverse: TGGACYTTRCAWGGBCCTTCACA

Detection of BGYMV in bitter gourd by PCR using Begomo virus degenerate primer

BGYMV-2 degenerate primer Sequence are as below,

BGYMV-2 Forward: GCCHATRTAYAGRAAGCCNAGRAT

BGYMV-2 Reverse: GGRTTDGARGCATGHGTACANGCC

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| --- |
| **Details of PCR set up** |
| **Sr. No.** |  **Component (details)** | **20 uL reaction volume** |
| 1 | PCR buffer(10x) | 2 ul |
| 2 | dNTPs (10mm) | 0.2 ul |
| 3 | Forward Primer (10 pmole) | 0.6 ul |
| 4 | Reverse Primer (10 pmole) | 0.6 ul |
| 5 | DNA Taq Polymerase (3U/uL) | 0.3 ul |
| 6 | Water (Mol. grade) | 16.3 ul |
| 7 | DNA Template | 2 ul |
|   | **Total** | **22 uL** |

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| **PCR Program for degenerate primer:** |
| **Sr. No.** | **Step** | **Temp.** | **Time** | **Number** |
|  **of Cycle** |
| 1 | Initial Denaturalization | 94 °C | 2 min. | 1 |
| 2 | Denaturalization | 94 °C | 1 min. | 2 |
| 3 | Annealing | 58 °C | 1 min. |
| 4 | Extension | 72 °C | 2 min. |
| 5 | Denaturalization | 94 °C | 1 min. | 35 |
| 6 | Annealing | 56 °C | 1 min. |
| 7 | Extension | 72 °C | 2 min. |
| 8 | Final extension | 72 °C | 30 min. | 1 |
| 9 | Hold | 4 °C | ∞ | 1 |

Polymerase chain reaction technique using BGYMV-1 & BGYMV-2 degenerate primer for gemini virus amplified 560 bp & 575 bp fragment from BGYMV infected Bitter gourd plants.

PCR amplified products were detected through agarose gel electrophoresis (2.5 %) using Low range DNA ladder (Genei) as a reference marker. The amplified DNA size was approximately 560 bp & 575 bp in the BGYMV infected Bitter gourd samples while there was no amplification in the healthy samples.

**Result :**

Table: 1

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr. No.** | **Description**  | **Marker** |  |
| **BGYMV-1** | **BGYMV-2** |
|  |  |  |  |
| 1 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 2 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 3 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 4 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 5 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 6 | Healthy Virus Free Plant | No amplification of 560 bp Fragment | No amplification of 575 bp Fragment |
| 7 | BGYMV infected Plant | 560 | 575 |
| 8 | BGYMV infected Plant | 560 | 575 |
| 9 | BGYMV infected Plant | 560 | 575 |
| 10 | BGYMV infected Plant | 560 | 575 |
| 11 | BGYMV infected Plant | 560 | 575 |
| 12 | BGYMV infected Plant | 560 | 575 |

**Report :** The specificity of the PCR is based on the use of oligonucleotide primers that are complementary to the regions flanking the DNA sequence to be amplified. Degenerate PCR primers for amplification of portions of the DNA-A or DNA-B components of Whitefly Transmitted Gemini viruses (WTGs) were designed from highly conserved regions which may have functional significance, therefore the primer should have general application for the amplification of DNA-A or DNA-B. The bands were of expected size of 560 bp & 575 of Markers BGYMV-1 & BGYMV-2 in the samples from BGYMV infected and healthy bitter gourd plants, thus confirming that all the above was infected only with Gemini virus.