P.12 - RT-PCR amplification and cloning of 2A gene from GFLV isolates from north-west Iran

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We have previously cloned and sequenced movement protein (MP) gene flanking regions from Grapevine fanleaf virus (GFLV) isolates from north-west Iran. Having the 3' end region of 2A gene from these isolates sequenced, designing a reverse primer to detect 2A gene was facilitated. The forward primer was designed on the basis of aligned sequences of previously sequenced isolates of GFLV from other parts of the world as recorded in GenBank. Vineyards in the northwest region of Iran were inspected and leaf samples were taken from the vines expressing symptoms similar to those caused by GFLV.Total RNA was isolated from 200 mg of leaf tissue and then subjected to first strand cDNA synthesis by the use of oligo d(T)18 and followed by PCR with the newly designed 2A-specific primers. As a result, expected DNA fragment corresponding to GFLV 2A DNA was amplified from several samples. The amplified fragments were ligated into pTZ57R and used to transform Escherchia coli competent cells. Screening of the transformed cells and restriction analysis of the recombinant plasmids from the selected colonies proved insertion of the amplified fragment. The clones are now being sequenced for further analysis. This is the first report of amplification and coning of the 2A gene from GFLV isolates from Iran.