Genetic Marker For LMV Resistance Scored using Agarose Gels

Description:
The single nucleotide difference between the protein-coding regions of the DNA sequences of wild-type *eIF4E* from *L. sativa* cv. Salinas, conferring susceptibility to LMV, and the *mo1*^{2} allele from *L. sativa* cv. Salinas 88, conferring resistance to LMV (Nicaise et al., 2003), was exploited to create co-dominant genetic markers that can be used to identify the two alleles. The sequences used to design forward and reverse primers to amplify the *mo1*^{2} diagnostic markers are shown in Figure 1.

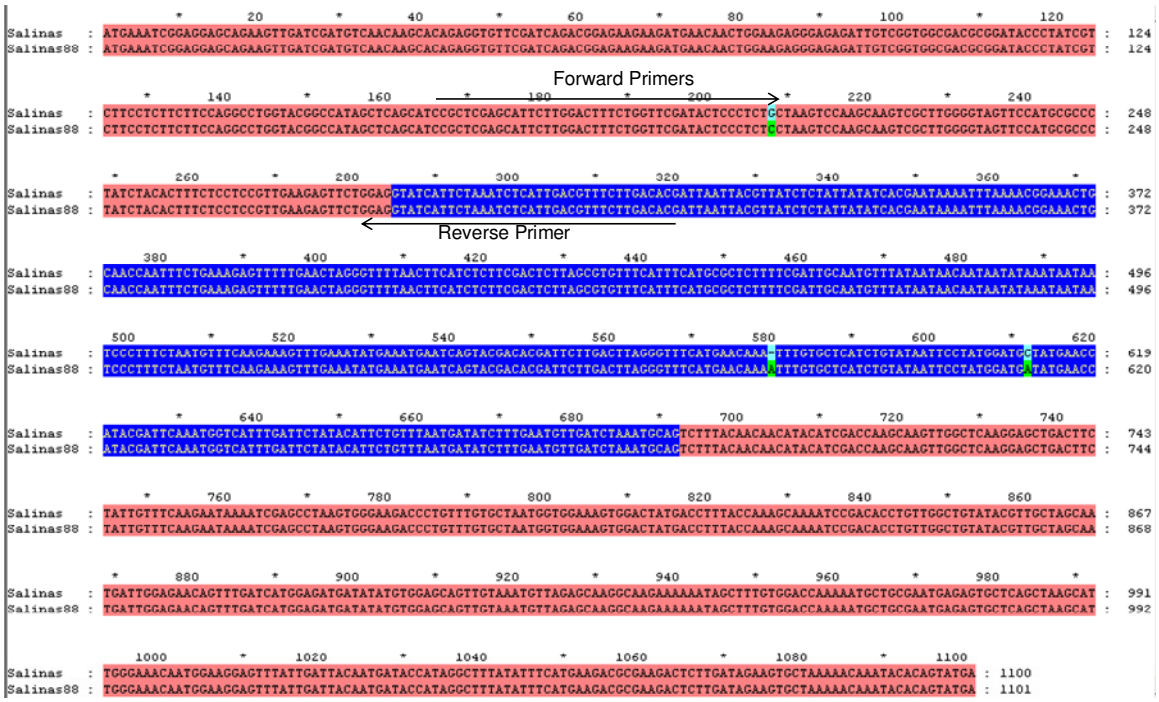

Figure 1. Sequences of *eIF4E* conferring susceptibility (Salinas) and resistance (Salinas 88) to LMV. Intron (non-protein-coding) sequences are indicated in blue. Sequence differences are highlighted. Positions of the sequences used to design forward and reverse primers to amplify the *mo1*^{2} diagnostic markers are indicated by arrows.

Two nucleotides of the sequence of the forward primer for identifying the resistant *mo1*^{2} allele were altered so that the product of a successful polymerase chain reaction (PCR) amplification of the *eIF4E* resistant allele would contain a site that would be recognized and cut by the *Eco*R1 restriction endonuclease enzyme (see Figure 2). Two nucleotides of the sequence of the forward primer for identifying the susceptible *eIF4E* allele were also altered but only to ensure that conditions for PCR amplifications of the two alleles would be identical; the primer for identifying the susceptible allele contains no recognition site for *Eco*R1 enzyme (Figure 2).

Figure 2. Forward primer sequences for distinguishing LMV-susceptible from LMV-resistant alleles. Allele-specific forward primers are shown in aqua and green. Primer nucleotides altered from the sequences of the respective cultivar are underlined. The sequence

difference responsible for resistance versus susceptibility (a single nucleotide) is boxed in red. The *Eco*RI restriction site created in Forward primer2 is indicated in red.

Methods:

Genomic DNA (1 ng/µl) isolated from each lettuce line was used as template in 20-µl volume PCR amplifications containing: 1 mM $MgCl_2$, 0.05 µM forward_primer1, 0.05 µM forward_primer2, 0.05 µM reverse_primer (Table 1), 0.2 mM dNTPs, 1x PCR buffer, and 1 unit Taq DNA polymerase. Cycling parameters are: initial denaturation for 5 min at 95°C, then 35 cycles of 30 sec at 94°C, 30 sec at 57°C and 1 min at 72°C, and a final extension for 5 min at 72°C. Amplification products are then digested with *Eco*RI (New England Biolabs, Ipswich, MA) according to the manufacturer's instructions and visualized following electrophoresis in a 2% agarose gel and staining with ethidium bromide. *Eco*RI digestion cleaves the 5' terminal 20 bases from the PCR product representing the resistant allele and this length difference between the 100bp resistant and the 120bp susceptible products is clearly distinguishable on a 2% agarose gel (Figure 3).

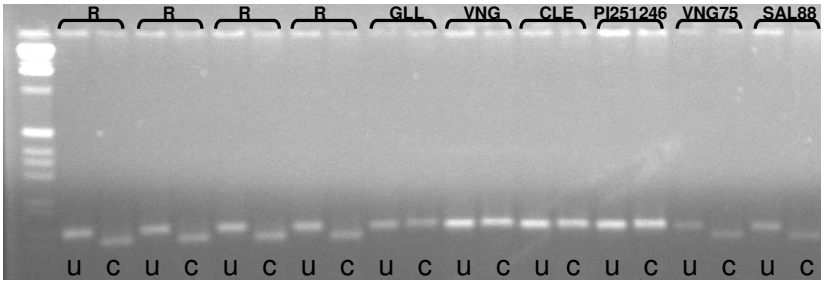

Figure 3. Agarose-based *mo1*^{2} diagnostic assay on breeding lines and controls. 'U' indicates PCR product not subjected to enzymatic digestion (uncut). 'C' indicates PCR product subjected to *Eco*RI-digestion. The first eight lanes contain PCR product amplified from DNA isolated from advanced breeding lines into which *mo1*^{2} had been introgressed. Lanes 9 through 16 contain PCR product from DNA isolated from donor and recurrent parents of breeding lines that do not carry the *mo1*^{2} allele. Lanes 17 through 20 contain PCR products from DNA isolated from Vanguard 75 (VNG75) and Salinas 88 (SAL88), cultivars that are sources of *mo1*^{2}-conferred LMV resistance.

Forward_primer1	CCGCTCGAGCATTCTTGGATATTCTGGTTCGATACTCCCTCTG
Forward_primer2	CCGCTCGAGCATTCTTGGAATTCCTGGTTCGATACTCCCTCTC
Reverse_primer	CGTGTCAAGAAACGTCAATGAGATTTAGAATGATACCTCC

Table 1. **Oligonucleotides used for the agarose-based *mo1*^{2} assay.**

Reference:

Nicaise V, German-Retana S, Sanjuan R, Dubrana MP, Mazier M, Maisonneuve B, Candresse T, Caranta C, LeGall O (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the Potyvirus Lettuce mosaic virus. Plant Physiol 132:1271-1282

(Marker developed by Leah McHale)