Scientific Notes

Reaction of lettuce genotypes to *Lettuce mosaic virus*-Most (LMV-Most) and characterization of the translation factor eIF4E

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Abstract – The objective of this work was to evaluate lettuce genotypes for their reaction to *Lettuce mosaic virus* (LMV; Most-type, isolate AF-199) and variations of the eukaryotic translation initiation factor eIF4E. All inoculated genotypes were susceptible to LMV, which was detected by RT-PCR using specific primer pairs. However, the accessions 169501, 169501C, 172918A, and 162499 showed late development of symptoms that appeared only on the inoculated leaves. Sequencing of the coding region of eIF4E showed that these genotypes have an eIF4E^0 (*mol*^{0}) standard typical for their susceptibility to LMV, indicating that the phenotype found is not correlated to nucleotide variations in this translation factor.

Index terms: eIF4E, potyvirus, resistance.

Reação de genótipos de alface ao *Lettuce mosaic virus*-Most (LMV-Most) e caracterização do fator de tradução eIF4E

Resumo – O objetivo deste trabalho foi avaliar genótipos de alface quanto à reação ao *Lettuce mosaic virus* (LMV; Most-type, isolado AF-199) e variações no fator eucariótico de tradução eIF4E. Todos os genótipos inoculados foram suscetíveis ao LMV, que foi detectado por RT-PCR com primers específicos. Porém, os acessos 169501, 169501C, 172918A e 162499, apresentaram sintomas tardios e somente nas folhas inoculadas. O sequenciamento da porção codificadora para eIF4E mostrou que estes genótipos apresentam padrão eIF4E^0 (*mol*^{0}), típico para suscetibilidade ao LMV, o que indica que o fenótipo encontrado não está correlacionado com as variações de nucleotídeos neste fator de tradução.

Termos para indexação: eIF4E, potyvirus, resistência.

Lettuce mosaic virus (LMV; genus *Potyviruses,* family *Potyviridae*) is one of the economically important viruses that affects lettuce (*Lactuca sativa* L.) and causes significant losses due to mosaic symptoms and reduction of plant growth (Pavan et al., 2008). LMV isolates show a high biological variability and can be classified into two large groups known as LMV-Common, which infects the susceptible lettuce cultivars, and LMV-Most, which is capable of being transmitted through seeds and breaking down the recessive alleles *mol*^{1} and *mol*^{2} (Nicaise et al., 2003).

In Brazil, the LMV-resistant lettuce cultivars are in fact tolerant to the LMV-Common isolates, including LMV pathotype II, according to the classification of Dinant & Lot (1992). Owing to the lack of lettuce cultivars that are resistant or tolerant to LMV-Most (Pavan et al., 2008) and to relevance of the lettuce crop in Brazil, it is necessary to search for possible new sources of LMV-Most resistance in lettuce cultivars.

To date, all recessive genes involved in the plant-potyvirus interaction have been characterized as encoding the eukaryotic translation initiation factors (eIFs), including eIF4E and eIF4G and its isoforms (Wang & Krishnaswamy, 2012; Sanfaçon, 2015). Therefore, the absence or alteration of eIF4E or

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eIF4G expression pattern, caused by point mutations or deletions, might lead to total or partial resistance to the species of the *Potyviridae* family (Moury et al., 2014). This factor is usually expressed during all growth periods and in all organs, such as young leaves and roots (Xu et al., 2017). In lettuce, the recessive alleles *mo1*^{1} and *mo1*^{2} encode the eukaryotic translation factor eIF4E (Nicaise et al., 2003). The nucleotide sequence of this factor is conserved, but the presence of small variations or deletions at specific positions characterize the susceptibility to different LMV isolates observed in lettuce cultivars (Nicaise et al., 2003).

The objective of this work was to evaluate the reaction of lettuce genotypes to LMV AF-199, a Brazilian isolate (AF-199), previously classified as LMV-Most (Krause-Sakate et al., 2002), and verify possible variations in the nucleotide sequence of the translation factor eIF4E in these genotypes.

The virus was inoculated in the cultivars Calona and Salinas-88, which are the carriers of the *mo1*^{1} and *mo1*^{2} alleles, respectively. The cultivars Sierra, Robson, Rafaela, Laurel, and Trocadero are all susceptible to LMV (both Common and Most groups), as well as the lettuce introductions 169501, 169501C, 172918A, and 162499 from a collection of FCA/UNESP-Botucatu. The virus-free lettuce seeds were sown, and the LMV-AF199 inoculation was performed thrice consecutively at 1-day intervals, 10 days after transplant, using 0.01 M potassium phosphate buffer (pH 7.0) and abrasive carborundum. Three trials were performed with 3 plants per genotype in each assay.

The total RNA of these plants was extracted using the extraction kits InviTrap Spin Plant RNA Kit (Stratec Molecular, Berlin, Germany) and Total RNA Purification Norgen (Norgen Biotek Crop, Thorold, Canada), following the manufacturer's recommendations, at 15 and 60 days after virus inoculation, corresponding to 30 and 75 days of the plant cycle, respectively. For the amplification of eIF4E, this study used the pair of oligonucleotides previously described by Nicaise et al. (2003), Ls4E3f (+) GGGGGGTGGAAGAAATA and Ls4E813r (-) GCAGAATTGTAGCATAAATCGGG. The reverse transcription-polymerase chain reaction (RT-PCR) was performed in a single step using the Master Mix PCR Kit (Fermentas Life Sciences, Burlington, CA). The following was added to a final volume of 25 µL: 5 µl of RNA extraction product, 12.5 µl of Master Mix buffer, 1 mM of each primer oligonucleotide, 1 unit of AMV reverse transcriptase (*Avian myeloblastosis virus*; Promega, 15 units/µl) and diethyl pyrocarbonate (DEPC) water to make up the volume. The amplification cycle consisted of activation at 42°C for 30 min, followed by initial denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The eIF4E sequences from the lettuce genotypes were analyzed by the software programs Clustal Interactive W and Mega 6 (Tamura et al., 2013). To confirm the viral infection, a RT-PCR was performed to detect LMV as described by Pavan et al. (2008).

The genotypes 169501, 169501C, 172918A, and 162499 were susceptible to LMV-AF199 since the virus was detected by RT-PCR at 20 days after inoculation (DAI); however, the symptoms were observed 14 DAI only on the inoculated leaves, and the young leaves showed no virus symptoms (Figure 1 A). The lettuce cultivars Sierra, Robson, Rafaela, Laurel, Trocadero, Calona, and Salinas-88 showed the initial symptoms of LMV-AF199 on average at 7 days after inoculation in the young leaves, which further evolved to systemic mosaic and leaf and plant deformation (Figure 1 B). The cultivar Salinas-88 developed necrosis followed by plant death as previously verified by Krause-Sakate et al. (2002). Leaves of the genotypes 169501, 169501C, 172918A, and 162499 were used as source inoculums for sap transmission to *Chenopodium quinoa* plants, which showed initial local lesions with posterior systemic mosaic (Figure 1 C), typical of LMV infection, confirming the infectivity of the virus in these plants.

To verify the expression pattern of eIF4E in the lettuce genotypes, a comparative analysis was performed between the nucleotide sequences of the eIFs by observing the positions 228, 299, presence or deletion of nucleotides in the region 344–349, and finally the position 576, all to identify recessive alleles in lettuce cultivars (Nicaise et al., 2003). Thus, the lettuce genotypes 169501, 169501C, 172918 and 162499, and the cultivars Sierra, Robson, Rafaela and Laurel showed an eIF4E^0 (*mol*^{0}) pattern, characteristic of a susceptibility allele (Figure 2). The standard

alleles *mo1*[1] and *mo1*[2] were observed in the Calona and Salinas-88 cultivars, as previously described by Nicaise et al. (2003).

In plant species, the eIF4E sequence is conserved, except for a few mutations involved in the resistance or susceptibility of this species to viruses (Wang & Krishnaswamy, 2012). These genes are related to the limitation of cell-to-cell movement, restriction, and accumulation of the virus (Robaglia & Caranta, 2006). In lettuce, the eIF4E standard clearly defines the susceptibility or resistance of the genotype to LMV isolates (Nicaise et al., 2003). The alleles *mo1*[1] and *mo1*[2] are associated with reduced viral accumulation and absence of symptoms (tolerance) or viral accumulation (resistance) in lettuce (German-Retana et al., 2008). In this study, no variability was observed in the nucleotide sequences of eIF4E in the analyzed genotypes, despite the phenotypic differences in the symptoms caused by LMV-AF199. Although LMV-AF199 was not quantified in the genotypes 169501, 169501C, 172918, and 162499, it could be efficiently recovered by retroinoculation to *C. quinoa*, indicating the replication of LMV-AF199 in these genotypes.

In Brazil, the LMV-Common isolates are prevalent in the lettuce-producing areas (Firmino et al., 2008; De Marchi et al., 2012); therefore, although the eIF4E analysis revealed the presence of a susceptible allele in the genotypes 169501, 169501C, 172918A, and 162499, the observed phenotype, with symptoms prevalent on the inoculated leaves and the young leaves showing no virus symptoms in the first 14 days, might be important in disease management (Figure 1 A). The lettuce crop cycle is around 45 days during the summer season, when lettuce seedlings are transferred to the field 15 days after their emergence. Owing to the absence of resistance sources to LMV-Most, these genotypes should be better evaluated in the field to determine the benefit of this delay in symptoms. The lettuce genotypes 169501, 169501C, 172918A, and 162499 can be classified as promising candidates for breeding programs related to genetic resistance to LMV-Most.

Figure 1. Symptoms observed in the leaves of the lettuce genotype 162499: A,symptoms initially restricted to the inoculated leaves; B, symptom observed in Trocadero (*mol*[0]); and C, systemic symptoms in *C. quinoa* retro-inoculated with the virus from genotype 169501C.

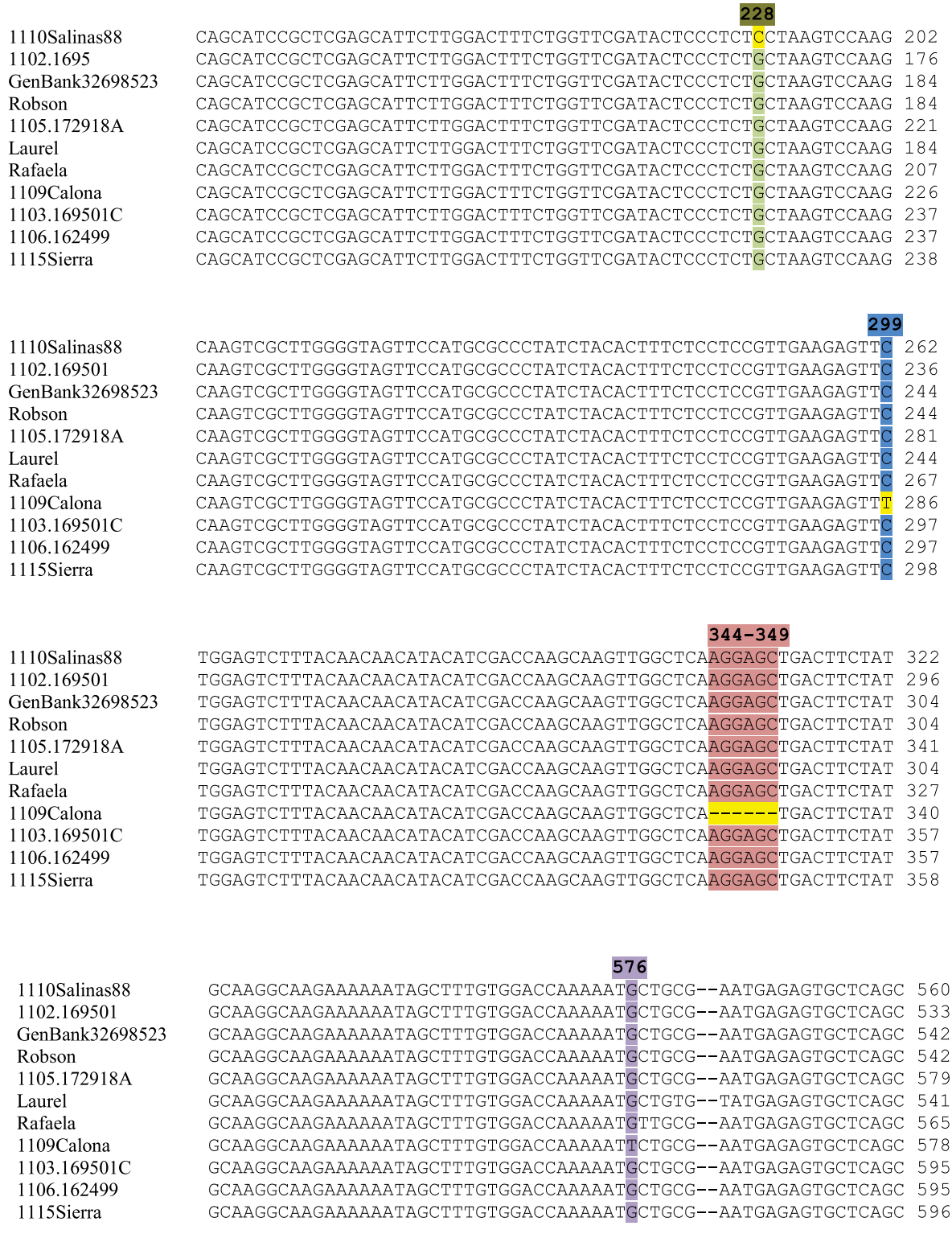

Figure 2. Partial nucleotide alignment of the eukaryotic translation factor eIF4E in lettuce cultivars Calona, Salinas-88, Sierra, Robson, Rafaela, and Laurel, and genotypes 169501, 169501C, 172918A, and 162499. The distinct nucleotide regions among the analyzed lettuce cultivars and genotypes are shown in yellow. Standard NCBI GenBank Accession Number for eIF4E: 32698523.

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