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Biochemical and Antiviral Study of Peptides Isolated from Amaranthus hypochondriacus on Tomato Yellow Leaf Curl Virus Replication

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Abstract : Agroindustrial plants such as cereals and pseudo cereals offer a substantial source of biomacromolecules, as they contain large amounts per tissue-gram of proteins, polysaccharides and lipids in comparison with other plants. In particular, Amaranthus hypochondriacus seeds have high levels of proteins in comparison with other cereal and pseudo cereal species, which makes the plant a good source of bioactive molecules such as peptides. Geminiviruses are one principal class of pathogens that causes important economic losses in crops, affecting directly the development and production of the plant. One such virus is the Tomato yellow leaf curl virus (TYLCV), which affects mainly Solanacea family plants such as tomato species. The symptoms of the disease are curling of leaves, chlorosis, dwarfing and floral abortion. The aim of this work was to get peptides derived from enzymatic hydrolysis of globulins and albumins from amaranth seeds with specific recognition of the replication origin in the TYLCV genome, and to test the antiviral activity on host plants with the idea to generate a direct control of this viral infection. Globulins and albumins from amaranth were extracted, the fraction was enzymatically digested with papain, and the aromatic peptides fraction was selected for further purification. Six peptides were tested against the replication origin (OR) using affinity assays, surface resonance plasmon and fluorescent titration, and two of these peptides showed high affinity values to the replication origin of the virus, dissociation constant values were calculated and showed specific interaction between the peptide Ampep1 and the OR. An in vitro replication test of the total TYLCV DNA was performed, in which the peptide AmPep1 was added in different concentrations to the system reaction, which resulted in a decrease of viral DNA synthesis when the peptide concentration increased. Also, we showed that the peptide can decrease the complementary DNA chain of the virus in Nicotiana benthamiana leaves, confirming that the peptide binds to the OR and that its expected mechanism of action is to decrease the replication rate of the viral genome. In an infection assay, N. benthamiana plants were agroinfected with TYLCV-Israel and TYLCV-Guasave. After confirming systemic infection, the peptide was infiltrated in new infected leaves, and the plants treated with the peptide showed a decrease of virus symptoms and viral titer. In order to confirm the antiviral activity in a commercial crop, tomato plants were infected with TYLCV. After confirming systemic infection, plants were infiltrated with peptide solution as above, and the symptom development was monitored 21 days after treatment, showing that tomato plants treated with peptides had lower symptom rates and viral titer. The peptide was also tested against other begomovirus such as Pepper huasteco yellow vein virus (PHYVV-Guasave), showing a decrease of symptoms in N. benthamiana infected plants. The model of direct biochemical control of TYLCV infection shown in this work can be extrapolated to other begomovirus infections, and the methods reported here can be used for design of antiviral agrochemicals for other plant virus infections.

Keywords : agrochemical screening, antiviral, begomovirus, geminivirus, peptides, plasmon, TYLCV
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