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Functional Characterization of a New Type of Satellite associated with Cassava Mosaic Begomoviruses

Cassava is the largest crop produced in Sub-Saharan Africa, where 57% of the global total is produced. Cassava is grown by smallholder farmers and plays an important role in food security across the African continent. With increased demand for biofuel and industrial starch, cassava is also rising in global importance and trade. Cassava mosaic disease (CMD) caused by cassava begomoviruses (CMBs) severely limits cassava production, and can reduce yields by up to 95% in Africa. CMBs have circular, single-stranded DNA genomes that replicate through double-stranded DNA intermediates. A novel DNA sequence, SEGS-2 (sequences enhancing geminivirus symptoms), enhances CMD symptoms. It is thought that the SEGS-2 episome originally came from the cassava genome and acquired the ability to be encapsidated into virions and transmitted by whiteflies through a recombination event with an alphasatellite. We report that SEGS-2 enhances CMD symptoms in wild-type and SEGS-2 transgenic Arabidopsis thaliana when co-inoculated with African cassava mosaic virus (ACMV). SEGS-2 also breaks resistance to geminivirus infection by an immune Arabidopsis accession. Using rolling circle amplification, an episomal form of SEGS-2 was found in Arabidopsis and detected in virions from CMB-infected plants. SEGS-2 replicated in the presence of the ACMV in cultured tobacco cells. An 825-bp region is transcribed from the SEGS-2 epsiosme, which contains an internal promoter. Together these results suggest that SEGS-2 represents a new class of geminivirus associated satellites.

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Discovery of bioactive peptides in high salt acidified and fermented cucumbers by direct analysis IR-MALDESI mass spectrometry

Bioactive peptides possessing therapeutic properties are well documented in lactic acid bacteria fermented foods including milk, sourdough, and cured meats as well as in raw vegetables and grains such as garlic, broccoli, and rice. However, bioactive peptides have not been investigated in fermented vegetables. Cucumber pickles, the most commonly consumed fermented vegetable in the United States, are not amenable to typical peptidomic workflows without extensive sample preparation due to their high salt content. Here, direct analysis infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry (MS) was employed to explore the hypothesis that bioactive peptides are produced during cucumber fermentation. Natural fermentations were conducted in triplicate by brining cucumbers in sodium chloride solutions (648 mM NaCl, equilibrated) and incubating at 28°C for 6 weeks. Acidified cucumber treatments were prepared similarly with the addition of 8mM sodium benzoate to prevent fermentation and 110 mM lactic acid to mimic fermented cucumber acid content. Direct MS analysis was performed on 100µm thick cucumber slices using IR-MALDESI coupled to an orbitrap mass analyzer run in positive ion mode with 140,000 nominal resolving power. Putative matches of known food-derived bioactive peptides were identified using MSiReader imaging and confirmed by targeted MS/MS with 1 m/z isolation windows. Five antihypertensive, angiotensin converting enzyme (ACE) inhibitory peptides were identified in both acidified and fermented cucumbers: lysine-proline, isoleucine/leucine-proline-proline, valine-proline-proline, and arginine-tyrosine. Quantification using labelled standards will provide information regarding their formation or degradation during fermentation. Bioactive peptides, although often present at low concentrations compared to other food constituents, are valuable functional food components due to their high potency. To date, no bioactive peptides have been identified in raw or fermented cucumbers, nor has the potent and most prevalently studied ACE-inhibitory peptide, isoleucine-proline-proline, been previously documented in a vegetable source.