**Abstract**

Vitamin D deficiency affected more than 15% of the worldwide population from 2000 to 2022. Vitamin D deficiency is often linked to a wide range of chronic conditions such as osteoporosis, muscle weakness, and fatigue. Hardly any foods naturally produce adequate vitamin D concentrations and vitamin D supplements do not fully alleviate the symptoms of the deficiency. In recent years, scientists have used biofortification to enhance vitamin D levels in various vegetables such as tomatoes. Last year a team of WRA students proposed a CRISPR-based knockout method to produce vitamin D in Bell Peppers emulating a similar study done in tomatoes, which belong to the same *Solanaceae* family. Our team now has focused on the experimentation of the mentioned methods. We began by extracting the plasmid pHEE401E from the E. coli bacteria through the Qiagen plasmid miniprep. Our team also designed the gRNA targeting the 7-DR2 gene of bell peppers. Using Golden-gate cloning, the gRNA sequence is inserted into the pHEE401E plasmid. Three different bell pepper cultivars are used and grown in the lab to determine the vitamin D level before the CRISPR knock-out experiment. Finally, a Vitamin D ELISA kit is used to quantify vitamin D levels in the fruit of bell peppers.

*Keywords:* Vitamin D deficiency, Bell Pepper, *Agrobacterium*-mediated Transformation, Golden-gate cloning, ELISA