Artocarpus altilis, also known as breadfruit, has recently begun to be used to increase food security in tropical countries. Incentives of growing breadfruit include its high yield and capacity to grow in a variety of conditions. Areas where breadfruit can be cultivated, like Africa and Southeast Asia, also have pernicious problem with Vitamin A deficiency which can potentially lead to blindness in children and increases the risk of disease and death from contaminations (world health organization()). Our goal is to increase beta-carotene, an antioxidant and a metabolic precursor to Vitamin A that is biosynthesized by breadfruit, to help address this deficiency problem.

Our method for increasing vitamin A content involves silencing genes that are responsible for degrading beta-carotene into organic products other than vitamin A. CHY 1, CHY 2 and CYP97A are the enzymes that perform a hydroxylation reaction on beta-carotene molecules in the biosynthetic pathway (Giuliano, G. et al., 2008). Suppressing the expression of these enzymes will slow the breakdown of beta-carotene resulting in a higher vitamin A content in the fruit. The genetic sequence responsible for hydroxylase expression in Artocarpus can be identified through comparison to analogous sequences for the same enzymes in well studied organisms. To do this, we must assay CYP97A, CHY1, and CHY2 (genes that cause beta-carotene degradation) expression in a breadfruit variety that shows high yield, relatively quick growth, and at least some carotenoid production. The Artocarpus Altilis Otea variety satisfied these criteria. Once the genes have been identified in our variety of interest, we can develop a method for genetically engineering the plant. Our decided method will involve using CRISPR-Cas9 carried by a agrobacterium vehicle to produce double stranded breaks in the targeted DNA coding regions for our enzymes. These breaks will be repaired by the cell. During the repair process, mutations will occur that will produce an ineffective coding sequence, thus silencing the gene and the production of the enzymes. First we must test to verify the modified Agrobacterium tumefaciens with extended host range which is compatible with our breadfruit host. If the bacterium is a competent transmission vehicle, we can build a plasmid vector with CAS9 and a gRNA sequence specifically programmed to locate and silence the gene responsible for the expression of hydroxylase enzymes. Agrobacterium containing this construct will be introduced into breadfruit protoplasts after treating the breadfruit cells with electroporation. This procedure makes holes in the membranes of the target cells that allow the transfer of our constructs. Normally a portion of this plasmid will be transferred into the plant genome, which is normal plant transformation, but this is not our intention. In those protoplasts where DNA is not transferred into the genome, the guide RNA and cas9 protein will be expressed for a few days

before the plasmid degrades. During this time they will be able to make edits to our chosen gene. At this point, we will regenerate the protoplasts and select for the plantlets containing properly edited genes but no transfer of DNA material from the agrobacterium. This would be done using PCR. From there, we grow selected plants into full breadfruit trees and analyze the effects of our edits on Vitamin A content. After isolating the plantlets of choice, we will regenerate our breadfruit into full trees through tissue culture and eventually outdoor cultivation. Once our breadfruit trees produce fruit, we will analyze the fruit for beta-carotene content to evaluate the if our gene editing was sufficient.

We chose CRISPR because other methods of crop improvement would be too slow due to the slow growth rate and reproductive cycle of a tree like breadfruit. Our application of CRISPR also intends to cultivate products that are not transgenic. Other methods would pose problems with consumer acceptance and regulation. Our method is considered 100% non-GMO since we are selecting for plantlets that do not have any transgene incorporation. Our goal is for a 220 gram serving of breadfruit to provide at least 10% of the daily recommended intake of vitamin A (roughly 1 mg/serving) (mayo clinic ()). We expect our breeding strategy to take about six years, the first three assaying for expression, tissue culture performance and developing our transformation methods, and the last three growing the trees and analyzing their beta-carotene content.

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Breadfruit Carotenoid Production