Development of metribuzin tolerant chickpea: Proof of concept validation of CRISPR based gene editing tools in chickpea

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SPG Contributions	Project Status	Duration/Timeline of Project (Year to Year)	Total Project Cost
$318,656.00	Completed	March 2017 – April 2019	$318,656.00

Project Description

To initiate a systematic evaluation and use of new genome editing tools for trait improvement in chickpea.

Chickpea (Cicer arietinum), is the world's second most important grain legume after common bean. It is considered one of the most important food legumes because of its nutritional value and symbiotic nitrogen fixation ability. The process of developing new chickpea varieties through conventional breeding methods can take eight to 12 years. Gene editing, a promising new plant breeding technique, can expand the traditional plant breeding tools. Using this technique, one can modify plant traits quicker and with high precision, thus potentially saving years in bringing the new varieties to farmers. The objective of this research was to examine the utility of the Cas9/sgRNA based gene editing technology in chickpeas using Phytoene desaturase (PDS) as a proof of concept and, thereby, building knowledge for manipulating the gene of interest (Photosystem II – PsbA) to improve tolerance to metribuzin in chickpeas. During the second year of the two year research, progress has been made on several fronts: guided-RNA designing for both PDS and PsbA, vector construction, protoplast isolation and transfection, as well as progress in the early detection of the gene editing events in vitro and in vivo.

The major challenge in this work was the assembly of the Homology Directed Repair (HDR) fragment for PsbA gene editing since it is a plastid gene. Plastid gene mutagenesis requires Cas9 protein and sgRNA synthesis with suitable promoters either after plasmid transfection of the protoplasts or by direct delivery of mature Cas9 and gRNA complexes into plastids, otherwise editing cannot be achieved.

Outcome

To develop the gene editing capability in this study, we used five gRNAs designed to target exon 1 of the PDS gene in chickpea cultivar CDC Frontier. From the result of the in vitro cleavage assay, we decided to transform gRNA #2, 4, and 5 to chickpea protoplast. We investigated a number of factors that may affect the delivery of CRISPR/Cas9 reagents into chickpea protoplasts and obtained maximum viability and yield using a combination of 1.5% cellulase and 0.75% macerozyme 4 hours digestion of leaf tissues collected from 10 day old seedlings. Using multiple Cas9 and sgRNA constructs and ribonucleoprotein (RNP) complex, we present the evidence for the seamless introduction of targeted modifications in the chickpea PDS gene. This study demonstrates the value and feasibility of combining protoplasts technology and CRISPR/Cas9-based gene editing for trait discovery and improvement in chickpea and potentially in other pulse crops. This is the first report of an effective CRISPR/Cas9 modification system in chickpea. This editing platform will be very helpful for the future development of herbicide tolerance, disease resistance and enhanced nutritional quality in chickpea.

Research Objective

OBJECTIVE 1

To initiate a systematic evaluation and use of new genome editing tools for trait improvement in chickpea.