ENGENHARIA GENÉTICA DE PRECISÃO PARA TOLERÂNCIA À SECA EM SOJA E SEU EFEITO NA VIA DE MORTE CELULAR PROGRAMADA DO RETÍCULO ENDOPLASMÁTICO

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Abstract:
Understanding plant molecular pathways associated with drought tolerance has become essential for crop genetic improvement in a climate change scenario. Considering the different drought tolerance mechanisms, the endoplasmic reticulum (ER), responsible for protein synthesis and processing, is one of the main targets of severe stresses in plants. The efficiency of ER performance is linked to the activity of molecular chaperones such as *GmBiP* (binding protein). In severe and prolonged stress, *GmBiP* plays a crucial role as a negative regulator of the NRP/DCD-mediated cell death response, attenuating the modulation of expression and activity of the signaling components of this circuit. This integrated ER stress response pathway converges on N-rich proteins (NRPs) containing the cell death and development domain (DCD) to induce activation of the vacuolar processing enzyme promoter and the programmed cell death. Thus, in this study, we applied two strategies to suppress senescence induced by the DCD-NRP circuit to increase drought tolerance. Firstly, we investigated the potential of CRISPR/dCas9 system fused with transcription activators (VP64) in positively modulating the expression of *GmBiP*. For this, the efficiency of three single guide was tested in transient tobacco transformation assays. We found that the sgRNA position affects the transcription modulation, being the best sgRNA, the closest to the transcription start site. These results were replicated in a soybean transient transformation system confirming that overexpression of endogenous *GmBiP* leads to the repression DCD-NRP circuit genes. After validation tests, we obtained stable transformant soybean plants overexpressing *GmBiP*. As expected, DCD-NRP circuit genes were repressed in these plants and *GmBiP* modulation remained stable over generations. Drought stress trials are ongoing. To develop a non-transgenic strategy for DCD-NRP regulation, we identified a potential downstream gene (*GmNAC030*) that positively regulates this circuit to be knocked out via CRISPR/Cas9 technology. We designed and validated a sgRNA in a soybean hairy root system. The selection of CRISPR-edited plants is ongoing. Our results confirm the potential of DCD-NRP circuit regulation and CRISPR technology for developing drought tolerance crops.
Key-words: CRISPR/Cas9; CRISPR/dCas9; *Glycine max*; *GmBiP*; *GmNAC30*

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