

ABSTRACT - APPLICATIONS OF BIOTECHNOLOGY IN ENVIRONMENTAL
AND AGRICULTURAL AREAS;

**SOYBEAN DNA-FREE GENOME EDITING VIA CRISPR/SACAS9
RIBONUCLEOPROTEIN DELIVERY**

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CRISPR/Cas systems have emerged as powerful tools for precise genome editing in molecular breeding. However, conventional delivery methods in plants often involve DNA integration, raising regulatory and consumer concerns in many countries. To address this, DNA-free approaches—such as the delivery of pre-assembled ribonucleoproteins (RNPs) offer a safer alternative that circumvents the use of foreign DNA, enhancing regulatory acceptance. Despite their potential, these strategies remain underexplored in soybean (*Glycine max* L.). In this work, we developed a DNA-free genome editing system for soybeans using *Staphylococcus aureus* Cas9 (SaCas9) RNP delivery to embryonic axis via gold particle-assisted bombardment, targeting the shoot apical meristem

(SAM). The heterologous expression of SaCas9 was optimized in *Escherichia coli* BL21 (DE3) pLysS for maximum yield. Single-guide RNAs (sgRNAs) were designed and synthesized in vitro to knock out phytoene desaturase (GmPDS), which leads to an albino phenotype. Firstly, RNP activity was validated through in vitro cleavage assays. RNPs were then introduced into the shoot apical meristem of embryonic axes (cultivar Williams 82) via gold particle bombardment, evaluating variations in shot pressure and number of shots per plate. Also, we introduced an additional sonication step of embryos prior to bombardment. Regenerated plants exhibiting green-white variegated leaves were genotyped by PCR and Sanger sequencing of the target region. Analysis with ICE software (Synthego) revealed an editing efficiency of 8%. Encouraged by these results, we extended the approach to target GmAIP10 (ABAP1 Interaction Protein 10), a gene associated with increased biomass. Editing of GmAIP10 was detected in 20% of regenerated plants, and preliminary findings suggest heritability of the CRISPR-induced mutations in the T1 generation. In conclusion, the RNP-based genome editing system developed here offers an efficient, DNA-free method for targeted mutagenesis in soybean, avoiding genetically modified organism regulations and supporting crop improvement. Future work will aim to optimize editing efficiency and expand the approach to other key crops, such as cotton (*Gossypium hirsutum*), broadening its impact on sustainable and accepted agricultural innovation.

Palavras-chave: glycine max transgene-free rnp biolistic.