DEPLEÇÃO DO QUADRO DE LEITURA *UPSTREAM* COMO NOVA ESTRATÉGIA PARA MANIPULAR A TRADUÇÃO DE *GMPR10* USANDO CRISPR/CAS9 PARA AUMENTAR A TOLERÂNCIA DA SOJA A FITONEMATOIDES.

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Abstract:
The root-knot nematode (RKN), *Meloidogyne spp.*, is considered one of the most economically important plant pathogens, impacting both the yield and quality of soybean. In previous transcriptomic and proteomic studies on contrasting soybean genotypes (BRS133- susceptible and PI595099- highly tolerant), we searched for candidate genes that might be directly related to the increased tolerance of soybean to RKNs. Among these candidates, we identified genes encoding proteins that inhibit or degrade the digestive tract and cuticle enzymes of these pathogens, such as the pathogenesis-related class 10 protein (*GmPR10*). Overexpression of *GmPR10* in transgenic tobacco plants resulted in a reduction in the number of galls per gram of root (51.6- 57.8%), number of eggs per gram of root (41.9-43.5%), and in nematode reproduction factor (40.4-48.7%) compared to wild-type plants. Likewise, tests on transformed hairy soybean roots showed a 40 % reduction in galls. These results suggest that the *GmPR10* gene is a promising candidate for engineering modification. Editing upstream open reading frames (uORFs) has recently emerged as a strategy to increase mRNA translation using CRISPR/Cas9 technology. uORFs affect the translation of associated downstream primary ORFs (pORFs), not always in a positive way. Therefore, their identification and editing could improve the translation of the *GmPR10* gene. In this study, we identified two uORFs in the 5'-UTR sequence of this gene. The entire 5' leader sequence was amplified, cloned, and site-directed mutated to delete its uORFs start codons (-ATG). The validation of these predicted uORFs was carried out in transformed *Nicotiana benthamiana* protoplasts using a dual-luciferase reporter vector, in which it is possible to analyze the effect of mutated and non-mutated uORFs by dividing the expression of luciferase by that of Renilla-luciferase (LUC/REN). As expected, expression analyses of mRNA levels showed no statistically significant differences between mutated and non-mutated uORF variants. As for the results of the expression of the reporter proteins (LUC/REN), we found that single mutations at the uORF 1 and 2 did not result in significant differences compared to non-mutated sequences. However, double mutations (uORFs 1 and 2) increased LUC/REN activity by approximately 3.5-fold, suggesting that both uORFs should be edited using CRISPR/Cas9 to increase *GmPR10* protein levels in soybean plants. The uORF depletion strategy using CRISPR/Cas9 for gene translation is innovative in soybean and enables the production of non-transgenic plants tolerant to |RKNs.
Key-words: *Glycine max*; *Meloidogyne incognita*; uORF; genome editing;

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