# Evaluating Promoter Expression for HPPD Carryover Resistance using GUS Histochemical Assay

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**Abstract:** 4-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors are a class of herbicides that prevent weed growth during the corn growing season. Although the herbicide provides great residual control, it can cause carryover injury and reduce soybean yield in the next growing season. Covercress is planted between corn and soybean crops, and HPPD herbicide carryover could impact its performance. Preliminary data shows that overexpressing HPPD confers herbicide inhibitor resistance in pennycress, but to provide a non-GMO method of HPPD carryover resistance, pennycress must undergo gene editing to replace the HPPD promoter with a strong pennycress promoter. Determining the appropriate promoter for gene editing remains unclear. We compared 11 pennycress promoter:GUS constructs using a β-glucuronidase (GUS) histochemical assay to perform a qualitative analysis of GUS activity and evaluate promoter strength. Two-week seedlings from each construct were used for histochemical GUS staining**.** Preliminary GUS histochemical assay for pennycress includes vacuum chambering a pretreatment buffer, adding a staining solution, and incubating at 37 °C for 2-3 days. The pretreatment buffer and staining solution contains 0.2% Triton X-100, 50mM sodium phosphate buffer, 2mM ferricyanide, and 2mM ferrocyanide, with the addition of X-Gluc in the staining solution to form the insoluble indigo dye. Using GUS histochemical staining and quantitative GUS activity assay, we found that there is assay variation within lines. In addition, we did not find a correlation between GUS enzyme activity and histochemical GUS staining, suggesting that further optimization is needed for the GUS assay. Constructs TaAP2Mprom_GUS has shown repeated positive results, which can be used as a positive control for further line comparison. Our findings provide a foundation for future GUS histochemical analysis in pennycress, which will provide valuable information on selecting which strong promoter to be used for gene editing-based approach for HPPD carryover resistance.

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