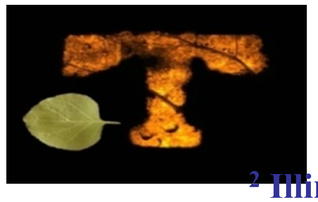
# Dual fluorescence protein tagging in horseweed (*Conyza canadensis*)

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Plant Sciences

## INTRODUCTION

**The evolution of glyphosate resistance (GR) in weedy species places an environmentally-benign herbicide in peril. Horseweed (*Conyza canadensis*) with evolved GR has become an especially problematic weed in crop production across the United States and the world. GR horseweed gene flow research has been undertaken to better understand the evolution of resistance and for practical weed control. Horseweed is a self-pollinating plant with plumed seeds, and most published horseweed gene flow studies have focused on seed spread. There is little knowledge about transfer of GR via pollen as a mechanism of horseweed gene flow. The objective of this study was to produce transgenic GR-horseweed with fluorescence protein-tagging for gene flow research.**

## RESULTS

**The green fluorescence protein (mGFP5-ER) was chosen to tag vegetative portions of horseweed for facile whole plant screening under blue light (Stewart 2001), whereas the orange fluorescence protein (tdTomato-ER) (Shaner et al. 2004) was chosen to tag pollen because it is very bright and easily observable in pollen. The construct (Figure 1) was transformed into a GR horseweed accession from Tennessee (TNR, glyphosate resistant horseweed from Tennessee) by *Agrobacterium*-mediated transformation of leaf disks (Halfhill et al. 2007, Figure 2). GFP was clearly observable in all portions of above ground tissue under blue light in otherwise darkened conditions (Figure 3), which will be helpful in subsequent screening of progeny. The OFP was also clearly observable in transgenic pollen (Figure 4), which will be used to monitor pollen flow.**

## REFERENCES


- **Stewart, C.N., Jr. 2001, *Plant Cell Reports*, 20:376-382.**
- **Shaner, N.C. et al. 2004, *Nature Biotechnology*, 22:1567-1572**
- **Halfhill, M.D. et al. 2007, *Plant Cell Reports*, 26:303-311**
- **Twell, D. et al. 1989, *Molecular Genetics and Genomics*, 217:240-245**


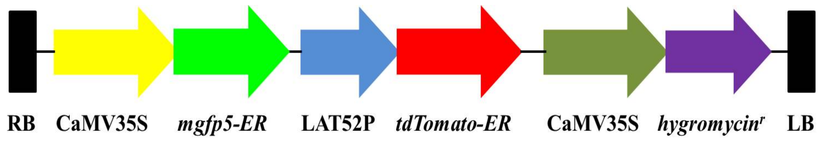


**Figure 1. T-DNA region of the transformation construct.**

**GFP was used for whole plant tagging and pollen tagging using the tdTomato-ER OFP (Shaner et al. 2004) under the control of the tomato LAT52 promoter (Twell et al. 1989). The hygromycin phosphotransferase gene was used as a selectable maker and was under the control of the CaMV35S promoter. LB. Left border of T-DNA region, RB. right border of T-DNA region.**

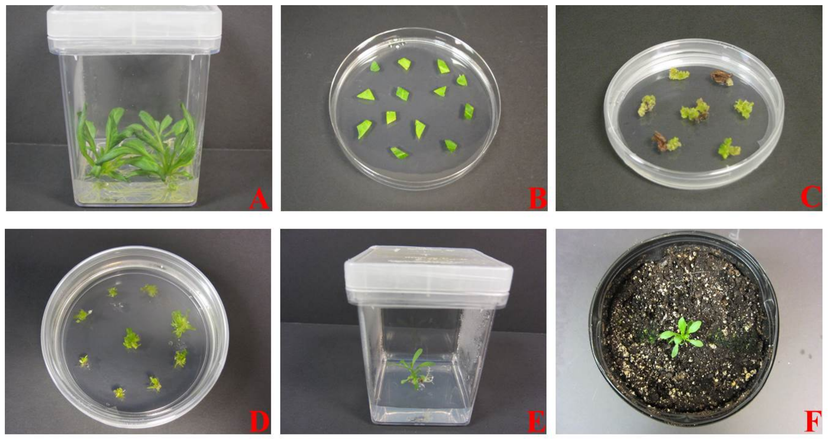


**Figure 2. Stages of *Agrobacterium*-mediated horseweed transformation.**

**A. Aseptic seedlings, B. Co-cultivation, C. Selection, D. Regeneration, E. Rooting, F. In pots**

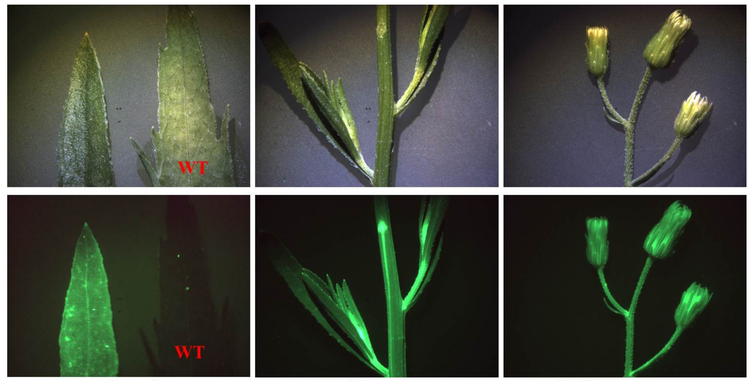


**Figure 3. GFP expression in transgenic horseweed leaves, shoots and inflorescence.**

**These pictures were taken under white light (top row) and blue light (bottom row) with exposure time of 3ms and 1min, respectively. WT. wildtype control.**

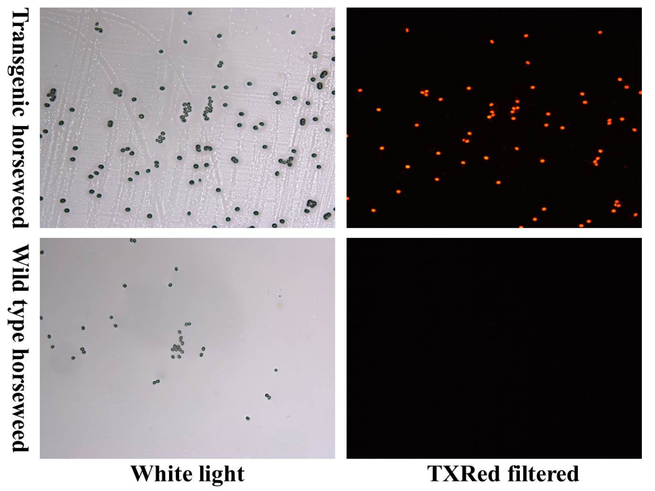


**Figure 4. OFP expression in transgenic horseweed pollen compared with the spectral phenotype of non-transgenic horseweed pollen under epifluorescence microscopy at 100X. The exposure time of white light pictures (left column) and epifluorescent microscopy pictures (right column) are 35ms and 200ms, respectively.**