

A Gem for the Queen of Forages: *RUBY* in Alfalfa

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The reporter construct *RUBY*, a noninvasive marker for monitoring gene expression and plant transformation, converts tyrosine into the stable red compound betalain. Betalain is a water-soluble, nitrogen-rich pigment known for its bright colors and powerful antioxidant activity. Betalain and anthocyanins protect the plant by reducing reactive oxygen species within cells. Both compounds, which are red in color, are not found together naturally in plants. Anthocyanins are derived from phenylalanine whereas betalain is derived from tyrosine. Plants, like alfalfa, that utilize anthocyanins lack the enzymes to convert tyrosine to betalain. We inserted the *RUBY* construct containing three enzymes involved in betalain synthesis into alfalfa (*Medicago sativa*). Twenty-five different transgenic lines of *RUBY*-alfalfa were produced and analyzed for betalain (betacyanin and betaxanthin) concentration using spectroscopic and HPLC-MS-MS analysis of leaf and stem tissue. RNA expression rates of reporter *RUBY* were compared with the betalain concentrations recorded in leaves and stem tissue. Some *RUBY* plants were found to contain a 2-fold difference in betacyanin concentration compared to beet root and remained stable over time within the plant. Therefore, alfalfa may be a new more environmentally sustainable betalain source in food applications as color additives and nutraceuticals than beets.

Figure 1. Transgenic *RUBY* alfalfa. The amount of betalain present in the tissue corresponds with the visual coloring of the plant.

