Reconstructing the Red Clover Polyphenol Oxidase System in Alfalfa to Evaluate Its Potential for Post-Harvest Protein Protection

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Some forages, including legumes red clover and perennial peanut, express polyphenol oxidase (PPO) and accumulate *o*-diphenol PPO substrates in their leaves. In the case of red clover, the caffeic acid *o*-diphenol derivatives that accumulate are phaselic acid (caffeoyl-malate) and clovamide (an amide formed between caffeic acid and L-DOPA [L-3,4-dihydroxyphenylalanine]). We previously showed that after harvest, PPO-mediated oxidation of *o*-diphenols to reactive *o*-quinones reduces proteolytic losses in these forages [1, 2]. Maintaining true protein following harvest should result in improved protein utilization by ruminant animals and less loss of N to the environment. Unfortunately, both the benefits (improved protein utilization) and any limitations of the PPO system have been difficult to assess, since most studies have relied on a comparison of feeding PPO/*o*-diphenol forages, such as red clover, with forages lacking a PPO system, such as alfalfa. Other differences in the feed characteristics of the forages make interpretation of these studies difficult. We previously transformed alfalfa with a red clover PPO gene [3]. More recently, we recreated a biosynthetic pathway for phaselic acid in alfalfa by the introduction of two transgenes [4]. We have crossed PPO-expressing alfalfa with phaselic acid-accumulating alfalfa to reconstruct the red clover PPO system in alfalfa. We are currently screening the progeny of these crosses for PPO and phaselic acid accumulation, and expect to recover populations of plants that are wild type (no PPO or phaselic acid), PPO-expressing, phaselic acid-accumulating, and both PPO-expressing and phaselic acid-accumulating. These populations should allow us to better evaluate the PPO system in terms of improving protein utilization, initially through small scale ensiling and in vitro protein utilization experiments. The populations will also allow evaluation of the role of the PPO system in resistance to plant diseases and herbivory by insects.

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