

Plant Chemistry/Biochemistry

Red Clover PPO: PPO Activity in Different Tissues

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Introduction

Red clover contains significant amounts of polyphenol oxidase (PPO), an enzyme that appears to have a role in decreasing proteolytic losses during the ensiling of red clover. Not a great deal of information is known about the red clover PPO enzyme so we have undertaken several small studies to determine some of its basic physiological characteristics. Here we report on the PPO activity within different tissues of red clover.

Materials and Methods

Red clover plants were grown in the greenhouse under mercury lamps with a 14:10 hour day:night cycle. All plants were asexually propagated and maintained in four inch clay pots containing growth media (soil:sand:vermiculite 1:1:1) with fertilization (20:20:20, N:P:K) every other week. Plant tissues (ca 3 g) were harvested, quick frozen in liquid nitrogen, and ground to a fine powder in a cold mortar and pestle. Ground samples were quantitatively transferred to a 50 mL beaker with the aid of 10 mL of 100 mM Tris buffer (pH 7.0, 2mM DTT, 5mM ascorbic acid). Samples were gently stirred on ice for 15 min before filtering through 2 layers of Miracloth. Extracts were centrifuged at 20,000 xg to pellet remaining insoluble material. Polyphenol oxidase activity was measured in extracts using caffeic acid (10 mM) as the primary substrate and reduced 5,5-dithiobis(2-nitrobenzoic acid) (TNB) as quinone trap. Activity of red clover PPO was measured as the loss of absorbance at 412 nm due to the reaction of quinones with TNB over a five minute time interval.

Results and Discussion

Since there seems to be a strong connection between loss of proteolytic activity in red clover silages and

PPO activity it is important to know the distribution of PPO within red clover tissues. We have compared the PPO activity in the four major tissues of red clover. All tissues were harvested from the same plant and the experiment was replicated three times.

All tissues tested positive for PPO activity. This is not surprising as at least certain forms of PPO are ubiquitously found in plastids of plants. A comparison of red clover tissues indicates that leaves contain a much higher level of PPO activity on a per gram fresh weight basis (Table 1). The total PPO activity mirrors total protein concentrations within red clover plants. Leaves contain 25 to 33 times as much PPO activity as petioles and stems respectively. The high level of PPO in red clover leaves along with abundant levels of soluble phenolics probably accounts for the rapid discoloration of red clover under normal field drying conditions.

For a comparison we evaluated the PPO activity in leaves of alfalfa and tomato. Tomatoes, as well as other members of the Solanaceae family, have been reported to have high levels of PPO. It is clear from the data in Figure 1 that red clover leaves have markedly higher levels of PPO activity on a gram fresh weight basis. Alfalfa leaves contain PPO activity but at levels 2500 times less than in red clover. We do not know at this time if this difference in activity is due to increased expression of the PPO gene(s) or a more active form of the enzyme. Current work is pursuing the answers to these questions.

Table 1. PPO activity in different tissues of red clover plants.

Plant Tissue	A/min/g fresh weight
Flower	0.005
Stem	0.066
Petiole	0.086
Leaves	2.280

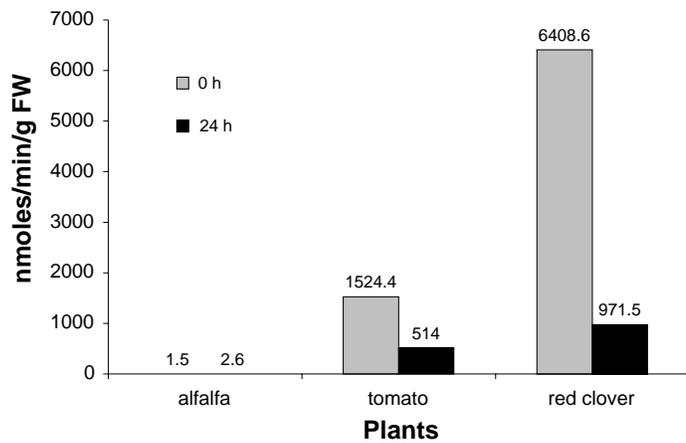


Figure 1. Comparison of PPO activities in leaves of alfalfa, tomato and red clover. Activities were measured immediately after extraction and after 24 hours at 4 °C.