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Introduction Harvesting and storing high quality forage in the cool humid regions remains a challenge due to the potential for protein degradation during ensiling. Red clover is an exception as high protein levels are maintained during ensiling. Decreased proteolytic activity in red clover is due to polyphenol oxidase (PPO) activity and appropriate *o*-diphenol substrates (Jones *et al*., 1995, Sullivan *et al*., 2004). This project was undertaken to determine if PPO activity is present in a range of grasses and the potential role in proteolytic inhibition in the presence of the *o*-diphenol caffeic acid.

Methods Fifteen grass species were established in the greenhouse under a 14/10 h (day/night) lighting regime. Leaf blades were harvested from plants at the vegetative stage, frozen in liquid nitrogen and stored at -80°C. Individual samples were processed and analysed following modified methods of Sullivan *et al*. (2004). The PPO activity was determined in duplicate using *o*-diphenol substrates (caffeic acid, chlorogenic acid and catechol; 2mM final concentration). To determine the potential impact of PPO and *o*-diphenols on proteolytic activity leaf blades were prepared similar to above. Two samples were prepared with one processed through a G-25 sephadex spin column to remove low molecular weight materials and the other clarified by centrifugation and removal of the supernatant. At time zero, caffeic acid (3 mM final concentration) was added to the eluted protein (spin column). Aliquots were removed from each sample at specific time intervals (t_0, t_1, t_2, t_4 and t_{24} h) and soluble amino acids and small peptides were quantified to assess the degree of proteolysis.

Results and discussion Both species and the specific type of *o*-diphenol significantly altered PPO activity (Table 1). Orchardgrass, meadow fescue, ryegrass, and smooth bromegrass exhibited the highest PPO activities. Chlorogenic acid and/or caffeic acid were the preferred substrates, although there were differences among the most active grasses as to which was the best utilised. This suggests potential differences among the individual PPO enzymes. Generally, the addition of caffeic acid to isolated grass extracts resulted in proteolytic inhibition in grasses with substantial PPO activity. Such results suggest that several important grass species contain PPO activity, but may lack the appropriate *o*-diphenol substrates to effectively inhibit proteolysis. Initial results suggest that proteolytic inhibition can be achieved with the addition of caffeic acid.

Table 1 PPO activity in 15 grass species in the presence of three representative *o*-diphenol substrates and the percent reduction in *in vitro* proteolysis with the addition of caffeic acid after 24 h

	Polyphenol Oxidase Activity (μmoles/μg/min)			Reduction in proteolysis
Grass Species	Caffeic Acid	Chlorogenic Acid	Catechol	
Tall fescue (soft)	3.2 E-4	2.9 E-4	8.9 E-5	5%
Meadow fescue	3.5 E-3	4.1 E-3	9.0 E-4	90%
Timothy grass	1.5 E-4	2.2 E-4	1.1 E-4	6%
Smooth bromegrass	8.2 E-4	4.1 E-3	8.7 E-4	99%
Quackgrass	1.0 E-4	1.6 E-4	2.1 E-4	20%
Tall fescue	1.1 E-4	5.6 E-5	4.5 E-5	55%
Reed canary grass	5.1 E-5	3.4 E-5	3.1 E-5	49%
Ryegrass	2.6 E-3	1.3 E-2	7.9 E-4	78%
Spring wheat	2.1 E-5	1.0 E-5	2.6 E-5	8%
Winter wheat 1	2.5 E-5	1.8 E-5	2.5 E-5	16%
Winter wheat 2	1.1 E-5	1.0 E-5	2.1 E-5	15%
Rye	2.6 E-5	1.7 E-5	4.2 E-5	0%
Oat	2.6 E-5	1.7 E-5	1.9 E-5	0%
Orchardgrass	1.1 E-2	2.6 E-2	1.7 E-3	60%
Kentucky bluegrass	6.0 E-5	3.3 E-5	7.0 E-5	24%

References

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