Improving plant digestibility by in-planta expression of fungal enzymes.

Marcia M. de O. Buanafina[1], Tim Langdon[2], Barbara Hauck[2], Sue Dalton[2] and Phillip Morris[2].

[1]*Department of Biology, 208 Mueller Laboratory, Pennsylvania University, University Park, PA 16802, USA*

[2]*Plant Animal and Microbial Science Department, Institute of Grassland and Environmental Research Plas Gogerddan, Aberystwyth, SY23 3EB, Wales UK*

Cell walls play important roles in the life of plants. Moreover, as a major sink for photosynthates they factor heavily in the nutrition of farm animals and - potentially – in the future replacement of petroleum with a renewable, domestic source of fuel for transportation needs.

Ferulic acid (4-hydroxy-3-metoxy-cinnamic acid) is the most abundant hydroxycinnamic acid (HCA) in grass cell walls and is intracellularly esterified to arabinosyl residues attached to arabinoxylans. These HCAs are important components of the cell wall because they can be oxidatively coupled to form a variety of dehydrodiferulate dimers, cross-linking hemicellulose polysaccharide chains[1] and contributing to the integrity of the cell wall. These feruloyl polysaccharide esters also anchor lignin in the cell wall of grass, generating a ferulate-polysaccharide-lignin complex[2]. Such cross-linking is among the factors most inhibitory to the biodegradation of grass cell wall carbohydrates.

We have demonstrated previously the expression of an *Aspergillus niger* ferulic acid esterase gene (*faeA*) in *Lolium multiflorum*[3] and the potential of the recombinant FAEA to break phenolic cross-links and release monomeric and dimeric ferulic acids on cell death in plants with FAEA targeted to the vacuole. We have also attempted to manipulate ferulate cell wall cross-linking further by targeting expression of FAEA to the apoplast and ER/Golgi system in order to achieve more complete degradation of cell wall polymers. We report the efficiency of gene targeting and the effect of FAEA expression on the level of cell wall ester linked HCAs. We also show the synergistic effect of *Aspergillus* FAEA and exogenous *Trichoderma* xylanase on the release of esterified HCAs from grass cell walls. Our findings also indicated the positive effect of FAEA on increasing digestibility and the kinetics of cell wall degradation under rumen conditions.

We have now produced *F. arundinacea* FAEA plants expressing endo-ß-1,4-xylanase (XYN2)[4] from *Trichoderma reesi* and we report whether expression of xylanase will increase the release of ferulates further.

In conclusion we show that it is possible to genetically modify the levels of cell wall phenolic cross-link in grasses and its digestibility by inducible expression of FAEA to different cell wall compartments. These results also provide further understanding of how grass cell wall composition affects digestion rates generating novel material with altered levels of cell wall hydroxycinnamic acids.

References

[1] Ralph, J., Quideau, S., Grabber, J. H. and Hatfield, R. D. (1994) *Journal of the Chemical Society-Perkin Transactions 1*, 3485-3498.
[2] Jacquet, G., Pollet, B. and Lapierre, C. (1995) *Journal of Agricultural and Food Chemistry*, **43,** 2746-2751.
[3] Buanafina, M. M., Langdon, T., Hauck, B., Dalton, S. J. and Morris, P. (2006) *Appl. Biochem.Biotechnol.*, **129-132,** 416-426.
[4] Grange, LA D.C., Pretorius, I.S. and van Zyl, W.H.(1996). *Appl. Environ. Microbilo*., **62**:1036-1044.