Manipulating the phenolic acid content and digestibility of forage grasses by targeted expression of fungal cell wall degrading enzymes

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Introduction Grass cell walls constitute 30-80% of forage dry matter, representing a major source of energy for ruminants. Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) and other hydroxycinnamic acids are ester linked to arabinosyl residues in arabinoxylans of grass cell walls and undergo oxidative coupling reactions resulting in the formation of a variety of dehydrodiferulate dimers which cross-link cell wall polymers. Although such cross-links have a number of important roles in the cell wall, they also hinder the rate and extent of cell wall degradation by ruminant microbial and fungal enzymes. We have shown previously the expression of a ferulic acid esterase gene from *Aspergillus niger* in *Festuca arundinacea* and the potential of the expressed FAE to break phenolic cross-links and release monomeric and dimeric ferulic acids on cell death in vacuole targeted FAE plants. This was enhanced several fold by the addition of exogenous recombinant xylanase (Buanafina *et al*., 2002). We propose to decrease the level of phenolic cross-linking of cell wall carbohydrate by inducible expression of FAE to the apoplast, ER and golgi and by co-expressing FAE and endo-ß-1,4-xylanase from *Trichoderma reesei* to the apoplast and vacuole.

Material and methods Young *F.a.* suspension cultures were bombarded with plasmid DNA using a Particle Inflow gun (PIG) as in Dalton *et al*. (1999). FAE activity was determined as the amount of FA released under incubation with EF (ethyl 4-hydroxy-3-methoxycinnamate) as substrate for 24hr. Xylanase activity was determined measuring the absorbance at 590nm of plant extract after incubation with Azo-Xylan as substrate for 22 hrs. Ester bound compounds were extracted with NaOH under N_2, acidified and analysed by HPLC.

Results and discussion We found that targeting FAE to the apoplast and ER/golgi system resulted in a significant reduction in the levels of monomeric and dimeric cell wall phenolics in leaves of some plants when expressed constitutively. Apoplast targeting might be expected to directly affect cell wall composition by removing ferulic acids, whereas ER/golgi targeting may affect cell wall composition, indirectly, by reducing feruoylation of the arabinoxylans in the ER/golgi destined to the cell wall. We also show the potential of expressed FAE to break phenolic cross-links in vacuole targeted FAE plants, leading to increased initial rates of fermentation. We have now produced *F. a.* plants expressing endo-ß-1,4-xylanase from *Trichoderma reesei* co-transformed with FAE to determine whether co-expression of xylanase will increase further release of ferulates and increase digestibility.

References

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