Use of *M. truncatula* EST collections to isolate glucosidases hydrolyzing isoflavonoid 7-O-glucose conjugates.

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Medicago truncatula accumulates malonylated glucose conjugates of formononetin and medicarpin in roots, nodules, and cell cultures. These same conjugates are also found in alfalfa (*Medicago sativa*) and many other legumes. These conjugates are rapidly hydrolyzed by endogenous beta-glucosidases when cells are crushed or treated with fungal elicitors. Hydrolysis of the medicarpin conjugate following pathogen attack releases the fungitoxic medicarpin aglycone (Figure 1). Other roles in plant-environment interactions have also been proposed for the glucosides and aglycones. Isoflavonoids are gaining interest as important nutritional components of legumes, and conjugation to glucose can affect both the stability and uptake of phenolic compounds from the diet. The beta-glucosidases responsible for hydrolysis of some isoflavonoid conjugates had been partially characterized decades ago, but clones for enzymes specific to the hydrolysis of these compounds have not been reported.

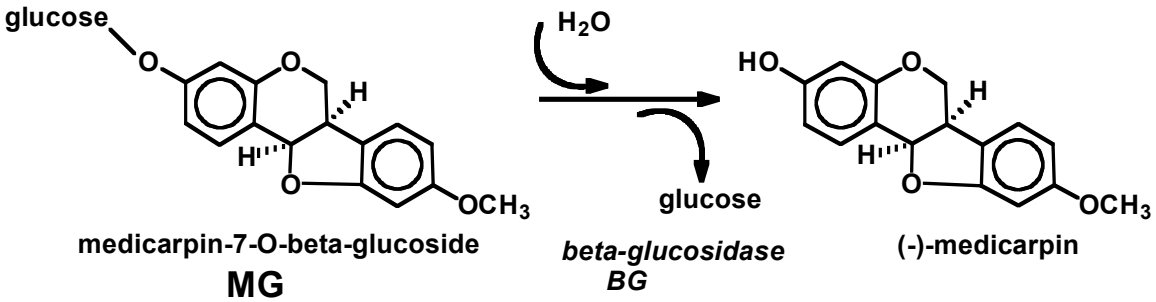

Figure 1: Hydrolysis of medicarpin glucoside by beta-glucosidase.

We isolated *Medicago truncatula* clones encoding beta-glucosidases active on the native isoflavonoid conjugates using a bioinformatics approach. Using Medicago Genome Initiative (MGI) software and database (www.ncgr.org/research/mgi/ or www.noble.org to Medicago links, a joint effort between the National Center for Genome Resources, Santa Fe, New Mexico, and the Noble Foundation Center for Medicago Genomics Research; Nucl. Acids. Res.2001 **29:** 114-117), several ESTs with high similarity to known beta-glucosidases from other species were identified and sorted into groups based on sequence homology. Representative clones from 3 groups were completely sequenced and expressed in *E. coli*. Recombinant proteins encoded by 2 groups hydrolyzed 4-methyl-umbelliferyl-beta-D-glucoside (a synthetic substrate) and formononetin glucoside, while only one of the groups hydrolyzed medicarpin glucosides. Southern blot analysis showed the two groups probably each have a single copy in the genome of *M. truncatula*. The beta-glucosidases are highly expressed in roots where the isoflavonoid conjugates accumulate, but not in leaves and petioles which contain no isoflavonoids, and appear to be inducible in leaves by *Phoma medicaginis* inoculation. Further characterization of these clones is underway, and may provide important new tools to study and manipulate phenolic glycosides in plants.