

Barley 'orange lemma' is a mutant in the CAD gene



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Introduction

Lignin is an important component of the plant cell wall. It provides support and structure as well as acting as a defence barrier against external stresses.

Most of the genes in the lignin biosynthesis pathway have been characterized in many species including *Arabidopsis*, tobacco and maize.

Genetic manipulation of lignin genes can lead to structural and compositional changes in lignin. Some of these changes affect the extractability or digestibility of the lignin. Commercially, this is beneficial to the paper and pulp industries and also the animal feed industry.

Barley (*Hordeum vulgare*) is the second most important arable crop in the UK with an annual production of approximately 7 million tonnes. Currently this is utilized mainly by the brewing and distilling industries and as animal feed. Barley also has the potential to be an important source for biofuels.

The barley orange lemma (*rob1*) natural mutant was first described over 80 years ago and resembles the brown midrib (*bmr*) mutants in maize.

We have characterized a number of natural and ethyl methane sulfonate (EMS) orange lemma mutants and have now identified them as mutations in the cinnamyl alcohol dehydrogenase (CAD) gene. This enzyme catalyzes the final step in the lignin biosynthesis pathway.

Expression profile of CAD in barley

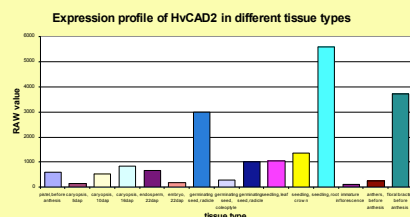


Figure 1. Expression profile of Barley CAD (HvCAD2) from microarray analysis showing CAD is highly expressed in seedling roots and floral bracts.

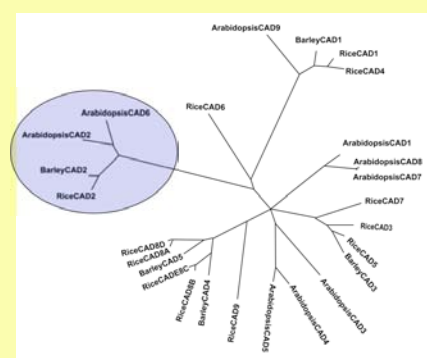


Figure 2. Phylogenetic tree of all known CADs in Arabidopsis, Rice and Barley highlighting the CAD genes involved in lignin biosynthesis (blue) which all cluster together.

Orange lemma phenotype

A number of orange lemma mutants have been identified following screening of the EMS-mutant TILLING population at SCRI.

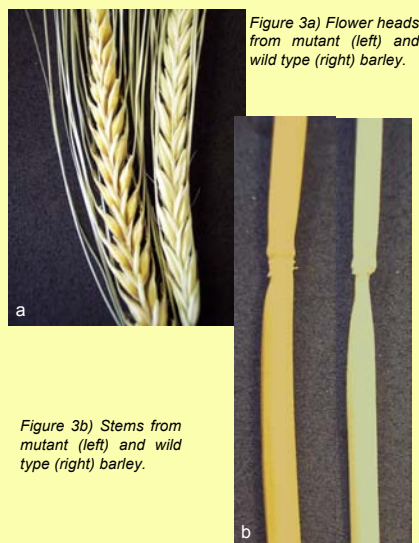


Figure 3b) Stems from mutant (left) and wild type (right) barley.

Characterization of orange lemma mutants

Biochemical analysis of barley stem tissue from natural mutants (Bowman and Emir) and EMS mutants (Optic) show reduced activity of CAD when compared to their wild type counterparts.

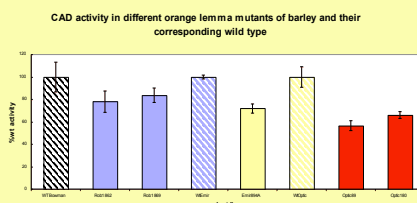


Figure 4. CAD enzyme activity in wild type (shaded) and orange lemma mutants showing reduced activity in the mutants.

Western analysis shows reduced levels of CAD in orange lemma mutants when probed with antiserum raised against the tobacco CAD protein.

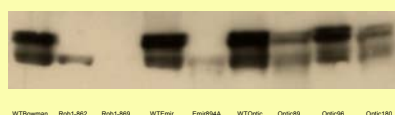


Figure 5. Western blot of proteins from wild type and orange lemma stems probed with tobacco CAD antiserum showing CAD is less abundant in the mutants.

Lignin analysis

Chemical analysis of lignin, which has been extracted from cell walls by thioacidolysis shows that the orange lemma mutants have reduced lignin content (10 to 15% less) when compared to wild type barley.

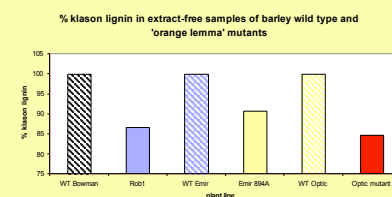


Figure 6. Amount of klason lignin in extract-free samples from wild type and mutant stems shown as a percentage of wild type lignin.

Lignin is mainly composed of two types - syringyl (S) and guaiacyl (G) lignin. A reduction in the S/G ratio was found in the orange lemma mutants when compared to the wild type. It has been reported that a decrease in the S/G ratio can result in lignin with greater digestibility.

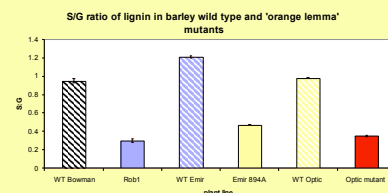


Figure 7. Ratio of S/G lignin in extract-free samples from wild type and mutant stems.

Conclusion

We have identified the first lignin mutant in barley. The detected mutations altered the sequence of the gene encoding cinnamyl alcohol dehydrogenase (CAD). This is the same gene that was mutated in one of the brown midrib maize mutants.

The barley orange lemma mutants have a reduction in lignin content and a reduction in the ratio of S/G lignin.

These changes in lignin may aid digestibility of the plant fibres and help promote its usage as a biofuel source.

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