Barley *'orange lemma'* – a mutant in lignin biosynthesis?

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Introduction

- Barley *'orange lemma'* *(rob1)* is visually similar to mutants in maize that have improved digestibility due to mutations in lignin biosynthesis genes
- We investigated *'orange lemma'* to see if it also had a mutation in a lignin gene and whether this had benefits for biofuel production and malting.
- Reduced lignin could have beneficial effects on malting, as is seen with naked barley grains which lack lignin.
- Lignin is also important in biofuel production as it impedes the access of digestive enzymes to wall polysaccharides during saccharification (breakdown to simple sugars). Saccharification yield determines the amount of alcohol that can be produced by fermentation (bioethanol).

Results

• Our results demonstrate that *'orange lemma'* is a lignin mutant. Lignin is reduced in the mutant (Fig. 1), as is the protein level of cinnamyl acohol dehydrogenase (CAD) – the enzyme that catalyses the last step in monolignol biosynthesis (Fig2).

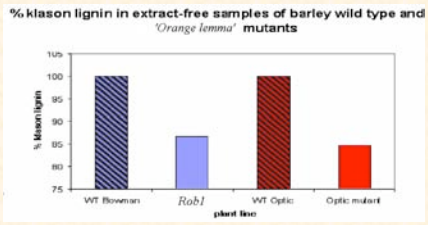

Fig. 1. Substantial reductions in lignin were measured in the *'orange lemma'* mutants in both Bowman (naturally occurring) and Optic (EMS induced) backgrounds.

Fig. 2. Western Blot of proteins from wild type and *'orange lemma'* stems probed with tobacco CAD antiserum showed reduced levels or absence of protein in the mutants.

Fig. 3(a) and (b). The *'orange lemma'* mutation results in an orange colour in the lemma of the grains and in the straw.

Saccharification

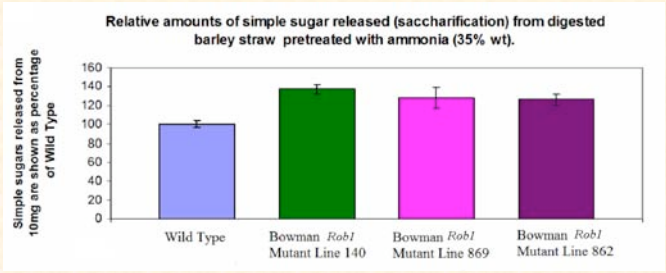

Fig.4. Saccharification of pretreated straw. More sugars were released from the *'orange lemma'* mutants that the wild type ($p<0.05$). The ammonia pretreated straw was saccharified with the Novozyme Biomass Digestion Kit (1). The relative levels of sugar released were measured using the Phenol-Sulfuric Acid Assay.

Malting

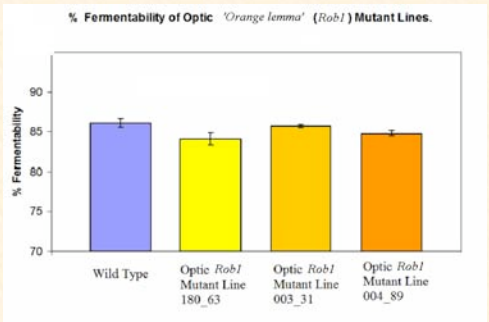

Fig.5. Effect of *'orange lemma'* on malting. There were no significant differences between the wild type and any of the mutants ($P>0.05$). Fermentability was measured according to the Recommended Methods of Analysis from the Institute of Brewing and Distilling.

Conclusions

- Barley *'orange lemma'* is a mutant in the CAD lignin gene
- Our results highlight the potential for using *'orange lemma'* straw as a biofuel feedstock, and the important influence of lignin on saccharification
- The *'orange lemma'* mutation appears to have no effect on malting quality. This indicates that crop residues, such as straw, can be improved for biofuel production without a detrimental effect on the use of the grain for malting.

Future Directions

- Investigate the effect of *'orange lemma'* on digestibility
- Screen TILLING population at SCRI for further mutants in lignin biosynthesis

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Selected References: (1). Tae Hyun Kim and Y. Y. Lee. Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. Applied Biochemistry and Biotechnology. Volume 137-140, Numbers 1-12 / April, 2007