

DELIVERABLE REPORTING



Multipurpose hemp for industrial bioproducts and
biomass

(Ref n. 311849)

WP1 – Heteroduplex mapping to improve hemp target traits

Deliverable 1.9 “Lignin and pectin detailed analysis of
new fibre varieties from HDM characterised”

Lead partner: UoY

Dissemination level: PU

Delivery date (proj month): 46

Summary of chemical analysis carried out on plants obtained from HDM are reported in Table 1.

Homozygous material has been produced for three targets csMHO41b (CAD1), csMH034b (CsRWA2) and csMH045 (CsCAD2) but not for CsPME1. A summary of all mutations identified is shown in Table 1. CsCAD1 is an important target for mutation breeding of hemp fibre characteristics because homologs in other fibre crops such as flax display a mutant phenotype (Chantreau et al. BMC Plant Biol. 13:159, 2013). One missense mutation MH041b (E308K) has been taken forward and led to a decrease in lignin content in fibres. However, no homozygous seeds are available for further characterisation. A putative splice site mutation was taken forward for

CsCAD2. None of the mutants showed alteration in lignin content. This target was selected because his expression increased with the maturation of the fibres as does lignin in hemp fibres. It is possible that the mutant is altered for the accumulation of non-lignin phenolics such as oligolignols. For CsRWA2 a mutation introducing a premature stop codon (MH034b) has been taken forward and brought to homozygosity. The mutant lines exhibited a reduction in cell wall acetylation associated with a dwarf phenotype. Attempt to introgress the mutation in Futura enabled the identification of heterozygous Futura plants. Three missense mutations (MHO72, MH069 and MH071) were taken forward for CsPME1. MHO72, a potentially non-sense mutant could not be retrieved and further work focused on MHO69. Two sets of segregating population experiments only led to heterozygous plants. The methylation of the pectin was not significantly altered in the plants.

Table 1: Summary of mutant lines available in WP1

Gene	Mutant ID	Mutation	Populations	Backcross	Phenotyping	Work beyond Multi hemp
CsCAD1	MH041b	E308K	Hom sib Cross Het sib cross	BC2 growing	Only two homozygous plants: reduced lignin levels in fibres.	These two plants had no seeds. We could use the BC2 seeds to obtain new homozygous lines and verify the results.
CsCAD2	MH025		Het sib cross		No effect on lignin content	
CsCAD2	MH023		Het sib cross		No effect on lignin content	
CsCAD2	MH045	Intron 2 splice	Het sib cross	BC2 growing	3 homozygous plants analysed: No alteration in lignin content in the fibres	
CsRWA 2	MH034b	W213*	Hom sib cross	BC2 growing Hom M line x Futura7 5	Reduced acetylation in fibres and core of het and hom lines in comparison to WT. Second set of experiment showed an even stronger decrease associated with a small phenotype. No hom Futura lines. Het	Further analysis of the plants to investigate how fibre cell wall has been affected by the decrease in acetylation. Future work to obtain a homozygous mutant in Futura
CsPME1	MH072	Q117*			Allelic mutant could not be retrieved	
CsPME1	MH069	G220E	Het sib cross	BC2 growing	Analyse Heterozygous line, no significant change in the degree of methylation	Two segregating populations led to no homozygous line. Need. to analyse a larger segregating population.

Het=heterologous; Hom=homologous; Het sib crossing=crossing between 2 heterologous lines; Hom sib crossing=crossing between 2 hom lines; BC=back cross to Finola wild type.

***indicates mutation creates a stop codon.**