SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

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ENERGY

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Deliverable 5.4

"Identification of QTL for cellulose digestibility in maize"

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Workpackage: **5**
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Dissemination Level: **PU**

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Deliverable 5.4: Identification of QTL for cellulose digestibility in maize

This deliverable was accomplished in a close collaboration with Work Package 1, and is also reported in the context of that WP.

The objective of this research was to identify physical positions in the genome of maize that alter the saccharification potential. In the SUNLIBB project we used Recombinant Inbred Lines to define these positions. The phenotyping of the population was performed by determining the saccharification potential of each of the lines under exactly the same conditions in an automated protocol.

P6 delivered biomass samples from a mapping population (Recombinant Inbred Lines derived from the cross F288xF271) to P1. The set-up of the saccharification analysis was performed as described in Deliverable 5.2. Saccharification potential was quantified by P1 with different pre-treatments on this population (alkaline and water). One single QTL for saccharification was detected on chromosome 1 on maize. The physical positions of this QTL were also sent to P4 in order to identify the genes present at this locus (according to the reference maize genome from the accession B73). QTL for silage digestibility have been previously detected on the F288xF271 RIL population. P6 selected a set of NILs in order to validate the effects of these QTL and to see their impact on saccharification potential. These selected lines have been cultivated in field trials and harvested. These materials were sent to P1 for further phenotyping.

SUNLIBB deliverables

Del No: 5.4	Deliverable Name: Identification of QTL for cellulose digestibility in maize			
WP: 5	**Lead partner:** P6	**Dissemination level:** PU	**Delivery date (project month):30**	**Actual delivery date:** 30

Objective:

The objective of this research was to identify physical positions in the genome of maize that alter the saccharification potential. In the present project we use Recombinant Inbred Lines to define these positions. The phenotyping of the population was performed by determining the saccharification potential of each of the lines under exactly the same conditions in an automated protocol.

Discussion /Conclusion:

To identify genomic regions involved in the release of sugars from maize stover, High-Throughput (HT) saccharification assays were conducted using a maize recombinant inbred line (RIL) population: F288xF271. These populations have been previously characterised according to their digestibility (Figure 1a). One hundred and nineteen RILs were grown in a field trial in the western part of France (Lusignan) during the summer 2010 (Figure 1b). 40 plants from each line were cultivated in two independent blocs. Stover was harvested at silage stage (corresponding to 30% of dry matter).

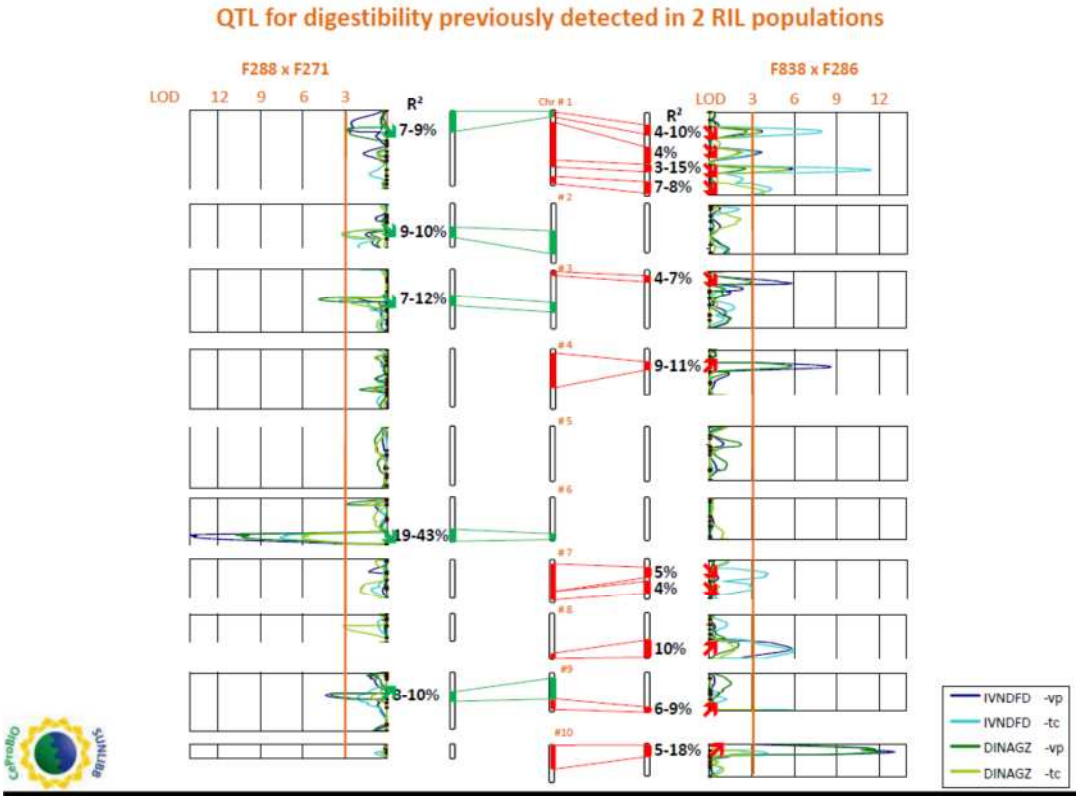

Figure 1(a): Digestibility QTL previously characterised in the RIL populations

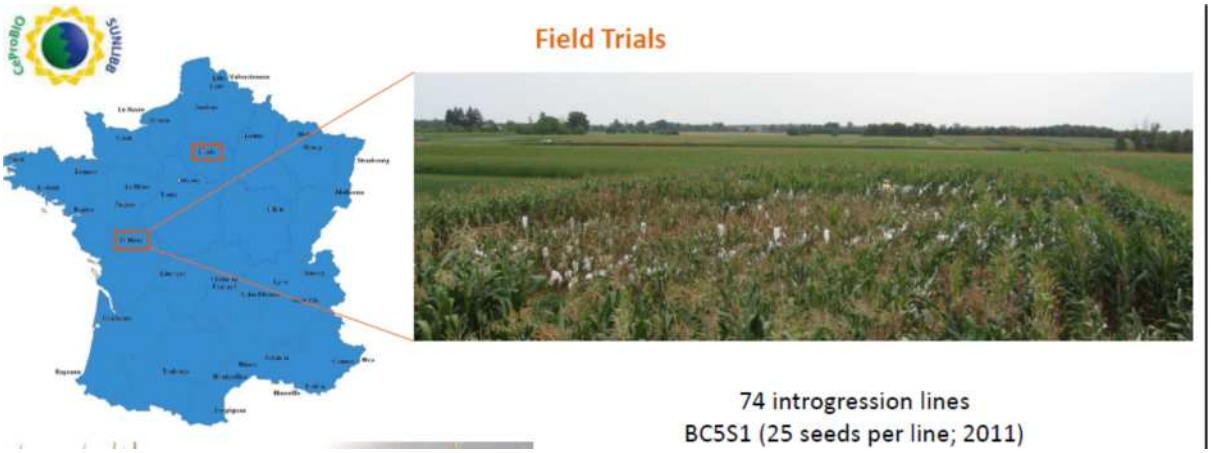

Figure 1(b): Location of the field trials

High-Throughput saccharification assay conditions developed in D5.2 were used to estimate sugar release of the RIL population. P6 delivered biomass samples from a mapping population (Recombinant Inbred Lines derived from the cross F288xF271) to P1. 8 and 16 technical replicates were carried out per line for the alkaline and the water pre-treatments, respectively. A statistical analysis of the data obtained using a mixed model approach was conducted in collaboration with the group of Antonio Augusto Franco Garcia (USP, Brazilian partner of the joint CeProBIO project). This analysis revealed a significant genotypic effect with a heritability of the trait ranging from 19 to 46%, according to the pre-treatment used (alkaline or water, respectively) and taking into account the block, the plate and the position effects. Block effect was never significant in any of the experiments with the different pre-treatments. Finally BLUPs (Best Linear Unbiased Predictions) were performed, allowing us to estimate the sugar release potential of each of the lines and for each of the pre-treatments selected.

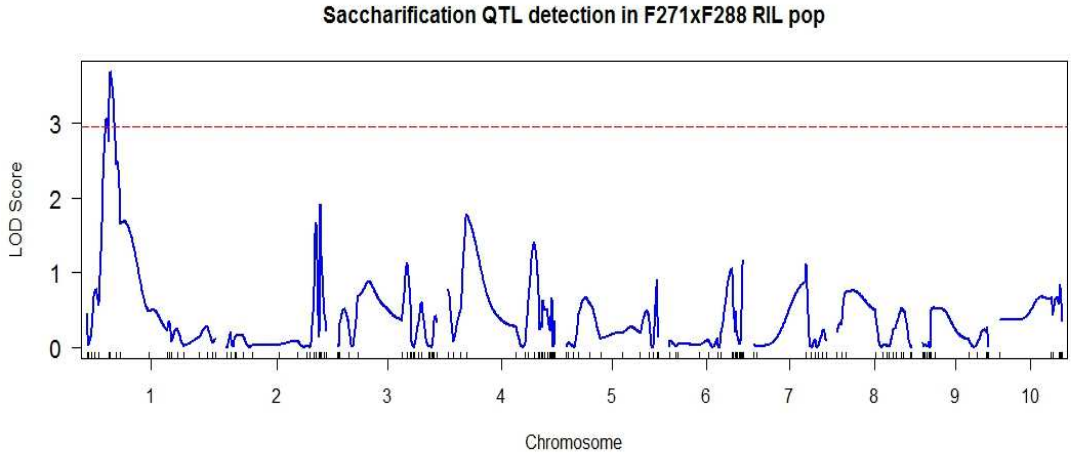

Figure 2: QTL detection for saccharification potential in the F288xF271 RIL population using water pre-treatment.

Using the R-qtl package a simple interval mapping was conducted to map significant QTL (Quantitative Trait Loci) for saccharification (LOD>2.9; α=0.01 following a permutation test n=500). As presented in figure 3 (see below), a main effect QTL with a LOD score of 3.41 was detected on the upper arm of chromosome 1 of the maize genome. This QTL was significantly mapped using the water pre-treatment condition only. However, under alkaline pre-treatment conditions, a putative QTL (LOD score=1.7) for saccharification could also be noted in the same genomic region. These two QTLs partially overlap and their peaks are found in an interval of approximately 12cM.

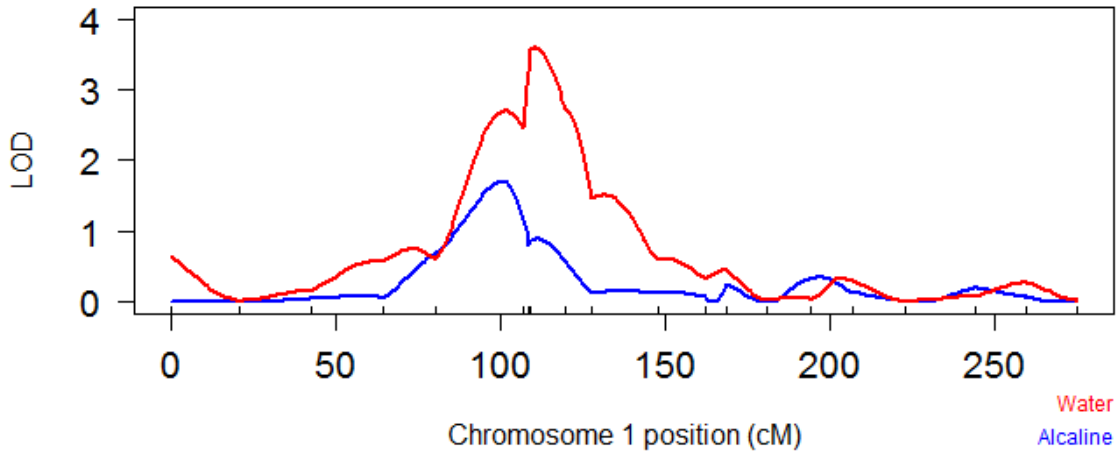

Figure 3: Mapping of QTL for saccharification on chromosome number 1. Red and blue curves represent the LOD traces of the QTL detection for saccharification assays using a water and alkaline pre-treatment, respectively.

The confidence interval for the main effect QTL was determined using two different approaches: a LOD support interval and a Bayes credible interval method. Both methods recovered an interval of approximately 35cM. Adjacent primers were identified and a search of candidate genes inside this interval was undertaken in WP1 (P6) in collaboration with Biogemma (P4).

QTL for silage digestibility have been previously detected on the F288xF271 RIL population. P6 selected a set of NILs in order to validate the effects of these QTL and to see their impact on saccharification potential. These selected lines have been cultivated in field trials and harvested. These materials were sent to P1 for further phenotyping.