SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

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ENERGY

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Deliverable 1.13
"Maize digestibility QTL fine-mapped"

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SUNLIBB deliverables

Del No: 1.13	Deliverable Name: Maize digestibility QTL fine mapped			
WP: 1	**Lead partner:** INRA	**Dissemination level:*** PP/PU/RE/CO	**Delivery date (proj month):** Month 40	**Actual delivery date:** 11/2013

Objective:

In a Recombinant Inbred Line population of maize (RIL F288xF271; 131 lines), a major QTL for digestibility has been detected on the lower arm of chromosome 6 (bin6.05; Roussel et al., 2002). In order to validate the presence and to fine map this QTL a cross between two "contrasted" RIL from the population was carried out. The objective of this approach is to select lines bearing novel recombination events within the QTL locus. Fine mapping is performed by analyzing the co-segregation of the phenotypic and allelic values of the selected lines. The RIL 5 and 35 (Figure 1) were selected from the population because (i) they have different allelic values at the QTL in an otherwise similar genetic background and (ii) they showed contrasted *in vitro* digestibilities of neutral detergent fiber (IVDNDF = 25% and 35%, respectively).

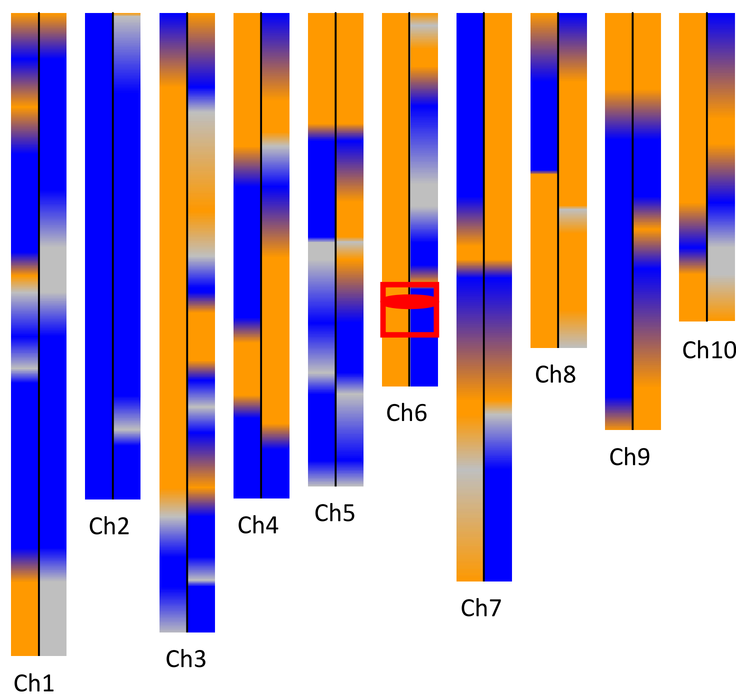

Figure 1: Genotype of RIL5 and RIL35 selected for the validation and fine mapping of the major QTL for digestibility detected on chromosome 6 (encircled in red; the peak of the QTL is represented by the red oval). Individual bars represent the genotype of one chromosome with the left and right sides corresponding to the genotype of RIL 5 and RIL35, respectively. Blue fill: F288 alleles; orange fill: F271 alleles; gray fill: not determined.

Results:

The initial cross between these two RILs (RIL5xRIL35) was performed at the beginning of the SUNLIBB project, during the summer 2011 (Lusignan, France). The resulting F1 line was then selfed during the winter 2011 (counter season in Chile). A total of 2000 seeds (F2 generation) were sown during the summer 2012 in a nursery (Gif sur Yvette, France). In order to determine the different recombination events that had occurred inside the QTL locus, each individual sprouted plant (1775 in total) was genotyped using 4 SSR markers covering the confidence interval of the QTL. Of these, 1336 lines were unequivocally genotyped and represented 34 independent genotypic classes depending on the location of the recombination event (Figure 2A). Overall, a total of 140 independent lines were

selected and selfed during summer 2012 (Figure 2B). Amongst these, 5 genotypic classes (i.e.12 individual lines) presented introgressions in a homozygous state for the tested markers. Genotypes were confirmed through a second round of genotyping. All plants selected were again sent to Chile in counter season for multiplication.

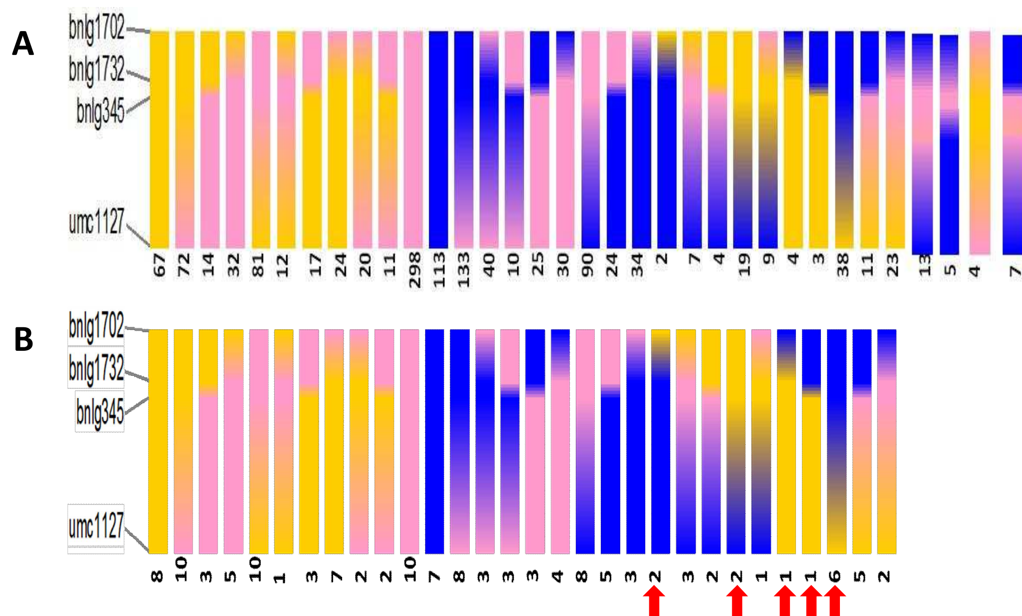

Figure 2: Genotypes of RIL5xRIL35 progenies in the region of the major QTL for digestibility found on chromosome 6 (bin6.05). **A)** Genotypes of the 1336 lines in the F2 progeny. Numbers below each bar represent the number of lines in each genotypic class. **B)** Genotypes of the 130 lines (F2 progeny) selected and selfed. On the left SSR marker names and position are indicated, red arrow represent the lines baring recombination events with alleles homozygous for the markers tested. Blue, yellow and pink represent alleles from F288 parent, F271 parent or heterozygous, respectively.

In summer 2013, two different assays were carried out on the F4 progeny.

1) For the lines having homozygous recombination events in the F2 progeny (figure 2B red arrows), field trials were achieved (45 seeds sown per line, one line per genotype and per block, two replicated blocks). A total of 8 markers were used to genotype and to refine the position of the recombination events. Results from the genotyping of the F4 progeny are presented in Figure 3A. Using NIRS predictive equations, *in vitro* digestibility of neutral detergent fiber (IVDNDF) values were estimated on stover harvested at silage stage (25-30% of dry matter; Figure 3B). A linear regression model analysis was used to explain the variance of IVDNDF by the allelic variation at the markers used to genotype this set of selected lines. Two markers significantly explained the variation observed for the digestibility trait (Table 1) located on the upper part of the QTL region.
2) For the remaining lines (still heterozygous in the F2 progeny), seeds from 6 independent ears were sown in individual lines (25k per line) in a nursery (Gif sur Yvette, France). In both cases, every single field line was genotyped by pooling leaves from 12 plants. The same set of 8 markers was used to genotype and to refine the position of the recombination events. Only a subset of lines was selected for further analyses. These included lines that were homozygous with a recombinant event and lines homozygous without any recombinant event as controls. This subset of selected lines was further separated in two groups according to the allelic context in the region (i.e. genotypic values at the bottom of the region). Genotyping results from this set are presented in Figure 3C and 3E. Here again, using NIRS predictive equations, *in vitro* digestibility of neutral detergent fiber (IVDNDF) values were estimated on stover

harvested at silage stage (25-30% of dry matter; Figure 3D and 3F). All values for IVDNDF were expressed in percentage relative to the control. This preliminary data, from plants cultivated in nursery, confirmed the effect of the QTL on the same marker positions and allowed to fine map the QTL to the upper region of the detected QTL locus (Table 1).

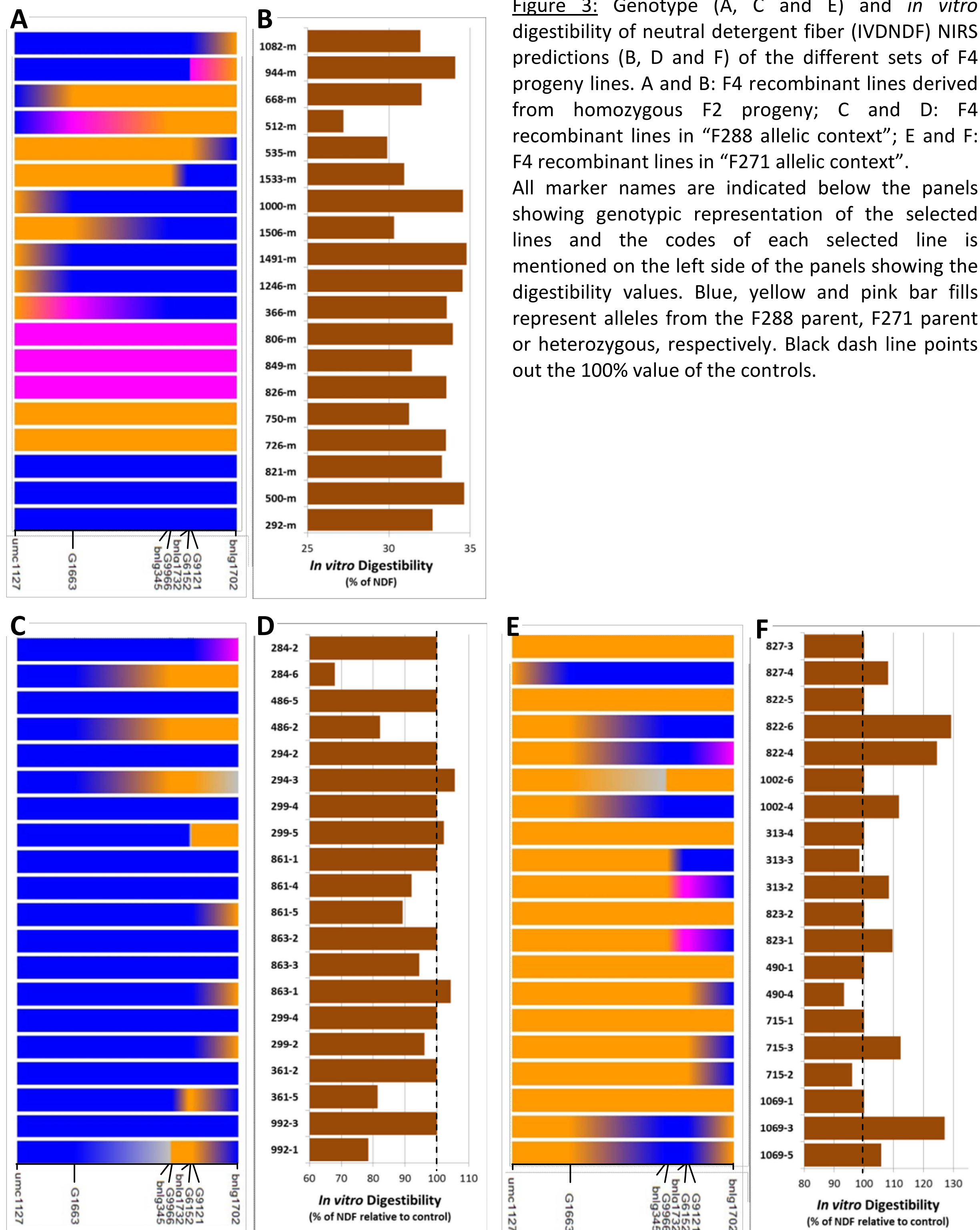

Figure 3: Genotype (A, C and E) and *in vitro* digestibility of neutral detergent fiber (IVDNDF) NIRS predictions (B, D and F) of the different sets of F4 progeny lines. A and B: F4 recombinant lines derived from homozygous F2 progeny; C and D: F4 recombinant lines in “F288 allelic context”; E and F: F4 recombinant lines in “F271 allelic context”.
All marker names are indicated below the panels showing genotypic representation of the selected lines and the codes of each selected line is mentioned on the left side of the panels showing the digestibility values. Blue, yellow and pink bar fills represent alleles from the F288 parent, F271 parent or heterozygous, respectively. Black dash line points out the 100% value of the controls.

Markers	Homozygous recombinants	Segregating recombinants (F288 context)	Segregating recombinants (F271 context)
bnlg1702	0,891	0,043	0,090
G9121	0,607	0,001	0,010
G6152	0,072	0,001	0,010
bnlg1732	0,072	0,001	0,064
bnlg345	0,211	0,543	0,241
G9966	0,211	0,543	0,241
G1663	0,304	NA	NA
umc1127	0,193	NA	NA

Table 1: Statistical analysis results of linear regression depicting the effect of the markers on the IVDNDF trait. P-value scores are indicated. Red, brown and yellow cells indicate p-values inferior to 0,001, 0,05 and 0,1 respectively.

Discussion /Conclusion:

All the data obtained from the F4 progenies confirmed the previously detected effect of this QTL on the *in vitro* digestibility of neutral detergent fiber trait (Roussel et al., 2002). The alleles of the F271 parent remained unfavorable for the IVDNDF trait in this population. Furthermore the data presented enabled us to further reduce the initially detected confidence interval from 21Mb (Roussel et al., 2002) to 1,8Mb. The new confidence interval spans from the markers G9121 and G9966. Interestingly the SSR marker initially detected closest to the QTL peak (bnlg1732) remains present in this interval.

*PU = Public ; PP = Restricted to other programme participantts (including the Commission Services); RE = Restricted to a group specified by the consortium (including the Commission Services); CO= Confidential, only for members of the consortium (including the Commission Services).