



SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

Grant Agreement no. 251132

**Collaborative Project
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ENERGY**

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Deliverable 5.5

“Miscanthus varieties with more digestible biomass identified”

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Workpackage: **5**

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SUNLIBB deliverables

Del No: 5.5	Deliverable Name: Miscanthus varieties with more digestible biomass identified			
WP: 5	Lead partner: P1	Dissemination level: PU	Delivery date (project month):36	Actual delivery date: 36

Objective:

The objective of this deliverable is to identify the most digestible Miscanthus lines and the variability of the saccharification trait within a population of genotypes selected according to biomass yield and growth cycle by P13 in a previous project. 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 the Miscanthus lines with high or contrasting digestibility, P1 CNAP set up the conditions for the analysis of saccharification potential in Miscanthus (task 5.4) and from months 6 to 12 of the project analysed the Miscanthus population provided by P13 Wageningen University. The population provided by P13 is an outcome of a breeding program, coordinated by Plant Research International Wageningen, to improve the burning properties and heat generation from Miscanthus using biotechnology (BIOMIS, 1998-2002). P13 grew 120 genotypes



Figure 1: Field trials of the BIOMIS population in Wageningen

from the BIOMIS population under field conditions (Figure 1), and harvested the biomass at dry stage. Each sample included all plants in a plot, where leaves were removed. The stems were ground in a hammer mill, using a 1 mm screen to determine the final particle size.

The saccharification potential was determined using the platform described in deliverable 5.2. The pre-treatment conditions for the HT analysis were established in 30 minutes at 90°C in the

presence of a 0.5 N NaOH solution. The 120 genotypes that were hydrolyzed during 8 hours with a commercial enzyme cocktail from Novozymes, where the two components, Celluclast and Novozyme 188, were mixed in a 4:1 proportion. Each sample was incubated with the equivalent of 7 FPU/g of biomass. The release of sugars from each material was evaluated using MBTH, as previously described in the present project. Each phenotype was measured in 4 biological repetitions and each of them was measured in 3 technical repetitions.

Figure 2 presents a summary of the results obtained across the population. The genotypes exhibited a significant amount of diversity in their saccharification potential. The average value for sugar release under the conditions assayed for the whole populations is 33.99851nmols of reducing sugars/ mg of biomass.h. The average percentage deviation in the determinations was 12%. The maximum saccharification potential corresponded to line 10, with 50.2 nmols/mg.h, with 15.2 nmols above the average of the population. Conversely, line 40 showed a potential of 18.5 nmols/mg.h, 15.5 nmols below the general average (Table1).

Table 1: Saccharification potential in the BIOMIS population

Line ID	Average (nmol of reducing sugars/h.mg of biomass)	STD	Line ID	Average (nmol of reducing sugars/h.mg of biomass)	STD
001	26.23689	2.143299	021	31.18052	3.05427
002	31.46821	2.909339	022	39.48415	4.946099
003	33.75644	2.039448	024	35.75687	5.033372
004	40.25323	3.564961	025	32.71074	2.419635
005	38.09937	2.445303	026	33.48574	1.447671
006	38.74433	5.956184	027	37.90071	7.909693
008	39.36877	3.670145	028	30.23734	5.875236
009	38.87735	3.97208	029	37.67025	3.520455
010	50.19818	1.456241	030	41.99702	4.207908
011	35.28183	5.435346	031	49.16274	1.973987
012	47.65017	7.717235	032	35.45586	2.006792
013	34.77362	4.956687	033	37.61027	3.482682
014	34.52823	3.128226	034	37.88377	2.352586
015	38.99116	4.585945	035	30.58856	4.267176
016	32.05705	3.628113	036	27.702	2.988434
017	42.35379	3.656575	037	44.06985	3.613936
018	37.09615	8.30091	038	42.71435	1.53612
019	31.80662	4.472622	039	31.53336	4.214272
020	36.82911	6.124835	040	35.75687	3.070284
Line ID	Average (nmol of reducing sugars/h.mg of biomass)	STD	Line ID	Average (nmol of reducing sugars/h.mg of biomass)	STD
041	18.50681	5.12442	081	23.64696	4.055049
042	24.8867	2.801672	082	34.80327	1.712298
043	30.88477	2.773526	083	37.1361	3.777703
044	35.42886	5.813613	084	30.97906	4.485705
045	32.86771	2.479236	085	36.70179	5.652827
046	29.44148	1.101272	086	27.7687	2.148662
047	30.16538	4.648285	087	29.35776	6.125104
048	29.45484	3.670225	088	29.02875	2.304174
049	24.86678	2.872224	089	23.85974	5.764028
050	28.81441	5.85393	090	30.61949	7.068105
051	28.48999	2.406104	091	34.25777	6.734256

052	32.76664	2.467315	092	38.39861	0.866757
053	26.60345	2.382545	093	38.00153	4.345041
054	29.6593	0.959116	094	40.17639	6.979264
055	26.32198	1.850556	095	39.93151	2.51468
056	33.99736	4.141997	096	36.3309	3.590729
057	32.84436	3.818695	097	40.97666	3.382862
058	33.47807	0.744069	098	41.68615	3.015497
059	32.63512	2.672279	099	35.3719	3.499794
060	34.19278	4.304955	100	41.05622	0.840433
060	37.30772	4.577235	101	39.08515	7.848179
061	27.97933	4.251447	102	29.12487	5.588338
062	40.42801	6.612489	103	28.5477	3.617033
063	24.58471	4.932945	104	30.43408	4.211897
064	25.02467	2.889744	105	35.18306	2.511787
065	38.27097	4.444943	106	37.54439	4.727213
066	28.69051	2.311254	107	38.18768	2.863947
067	24.17141	3.892537	108	38.90236	5.554281
068	25.74226	2.45418	109	31.81206	4.604779
070	22.11204	3.051114	110	29.57923	17.57769
071	31.43248	3.410405	112	32.62066	2.309644
072	28.94356	2.967042	113	29.5119	1.9451
074	35.0815	3.926734	114	35.00765	1.019253
075	35.17274	3.924365	115	40.52354	6.579135
076	33.39131	1.227601	116	40.05176	4.682908
077	33.31121	3.391561	117	42.75695	9.030361
078	30.64596	5.288104	118	35.57131	1.690757
079	30.57963	5.632827	120	41.39257	7.53355
080	33.45401	4.406942			

Although the BIOMIS population has not got a marker density that allows mapping of the regions defining the saccharification potential, the profile of saccharification within the population will allow (outside the present project) to associate traits responsible for the determination of saccharification potential.

During the SUNLIBB project, P6 has developed a new population of Miscanthus called SUNLIBB. This is a larger set of genotypes (160) and, although the screening of this population is outside the scope of SUNLIBB, P1 will determine the saccharification potential in this second population and the results will be reported at the end of the project.

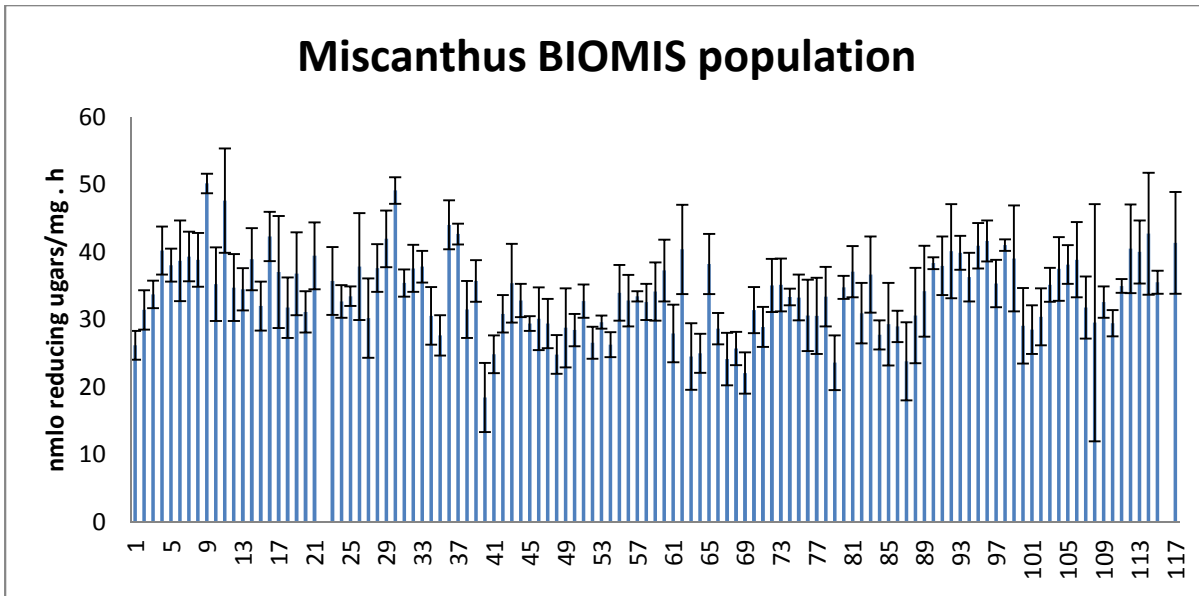


Figure 2: Digestibility across the BIOMIS population